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The role of checkpoints in the treatment of GBM

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Abstract

Targeted immunotherapy is founded on the principle that augmentation of effector T cell activity in the tumor microenvironment can translate to tumor regression. Targeted checkpoint inhibitors in the form of agonist or antagonist monoclonal antibodies have come to the fore as a promising strategy to activate systemic immunity and enhance T cell activity by blocking negative signals, enhancing positive signals, or altering the cytokine milieu. This review will examine several immune checkpoints and checkpoint modulators that play a role in cancer pathogenesis, with an emphasis on malignant gliomas.

Keywords

PD-1; CTLA-4; Immune checkpoint; Glioblastoma multiforme

Introduction

Over the past two decades, checkpoint blockade has emerged as a powerful and promising means of treating human cancers [1]. Under normal conditions, immune checkpoints provide stimulatory or inhibitory signals that lead to a stereotyped up- or down-regulation of the immune response (Fig. 1). Ideally, immune activity should increase in times of physiologic and immunologic stress, such as in infection or tumor infiltration. However, tumor cells have been shown to hijack this carefully orchestrated system by activating negative regulatory molecules on tumor-specific immune cells and thereby suppressing the antitumor inflammatory response [1]. Glioblastoma multiforme (GBM), the most common primary malignancy of the central nervous system (CNS), has been shown to induce T cell anergy and lymphopenia, impair antibody synthesis, increase circulating levels of immunosuppressive cytokines (i.e. IL-10 and transforming growth factor beta [TGF- β]), upregulate T cell inhibiting molecules (i.e. Fas ligand [FasL] and programmed death ligand-1 [PDL-1]), and recruit suppressive cells such as regulatory T cells (Tregs) and

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myeloid derived suppressor cells (MDSCs) [1–4]. In this manner, GBM are able to evade the host antitumor response at the levels of antigen recognition and immune activation.

Targeted checkpoint modulators in the form of agonist or antagonist monoclonal antibodies are now considered a promising strategy to activate systemic immunity, protect Tumor infiltrating lymphocytes (TILs) from the locally immunosuppressive effects of immunoinhibitory signals from both brain tumor and circulating monocytes, and enhance T cell activity by blocking negative signals or altering the cytokine milieu [5].

This paper includes a detailed discussion CNS tumors as a potential target for immunotherapy, and a review of two major checkpoint inhibitors, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) that have reached clinical trials for the treatment of GBM. We will also review other checkpoints that are currently in varying stages of preclinical and clinical study and have potential to be significant additions to the antitumor armamentarium.

Brain tumor immunology

Immunogenicity and “immune privilege”

Over the past decade, landmark successes in cancer immunotherapy have included the FDA approval of sipuleucel-T, the first active cellular cancer vaccine for castration-resistant prostate cancer [6], and ipilimumab, the first checkpoint inhibiting antibody therapy for melanoma [7]. Both cancer types are notable for having well-characterized antigens that are immunogenic and may play a biologic role in tumor progression [8]. The inherent immunogenicity of each cancer lends itself to therapeutic immune modulation. Tumors of the CNS, however, are often overlooked as potential targets of immunotherapy, having earned the reputation of being poorly immunogenic cancers that reside in an immune-privileged location. Yet, brain tumors may express and/or respond to the same checkpoint molecules seen in peripheral or non-CNS tumors, including PD-1 and CTLA-4. Several preclinical studies have shown convincing anti-tumor effects of as checkpoint blockade in gliomas, as well as synergy with traditional therapeutics such as chemo- and radiotherapy [9–11]. These findings strongly suggest that GBMs may be more susceptible to immune processes than was once believed.

The traditional conception of the brain as an immune privileged organ also merits re-evaluation. Several pre-clinical studies have demonstrated the variable permeability of the blood–brain barrier (BBB) to immune cells, as seen in the settings of septic encephalopathy, experimental autoimmune encephalomyelitis (EAE), or tumorigenesis [12–14]. In addition to the permeable BBB, lymphatic communications between the CNS and the periphery provides an important challenge to the concept of absolute immune privilege. Afferent lymphatic drainage may travel from the brain parenchyma, along the perivascular (Virchow-Robbins) spaces, through the cribriform plate, and out to the deep cervical lymph nodes [15]. This model is supported by T cell trafficking studies, one of which illustrated how immune cells injected into the entorhinal cortex migrated to the cervical lymph nodes by way of the cribriform plate [16]. A subsequent study found that antigen presenting cells (APCs) could migrate from the brain to the cervical lymph nodes, and that the type and

extent of inflammation in the brain correlated with the frequency of CNS antigens found in those draining nodes [17].

It is important to note that relative immune privilege is compartmentalized within the CNS. In contrast to brain parenchyma, innate and active immune reactivity in the ventricles, leptomeninges, and perivascular spaces is similar to that seen in the periphery [15, 18, 19]. While immune cells may enter these compartments seemingly at random, there is evidence that only antigen-specific lymphocytes remain or cyclically re-enter the CNS; in contrast, non-specific lymphocytes exit within a couple days [20–22]. Thus, in the setting of an inflamed brain, effector T cells are capable of entering the parenchyma to proliferate, home or migrate to the tumor, and initiate an active inflammatory response [15, 19].

These findings combined suggest that the immune system may not only have considerable access to the CNS, but also play a significant role in host antitumor defense. Therefore, while only a few of the growing number of checkpoint inhibitors (namely, CTLA-4 and PD-1) have been studied in GBMs, there is reason to believe that these immune modulators can be effectively used to target tumors of the CNS. Currently ongoing checkpoint trials for GBM will provide better answers regarding feasibility and efficacy in the coming months.

Immune checkpoints

CTLA-4

Biological overview—Identified in 1987, CTLA-4 is generally conceived as the archetypal inhibitory checkpoint [23]. This transmembrane glycoprotein is homologous with the costimulatory CD28 molecule (best known for its participation in “signal 2” of T cell activation) and competes for the same ligands, B7-1 (CD80) and B7-2 (CD86). Whereas the CD28:B7 association leads to increased immune activation, CTLA-4 binds the ligand with nearly 20 times greater affinity and results in immune downregulation [24].

CTLA-4 is expressed constitutively or rapidly induced on naïve CD4⁺, CD8⁺ and plays a critical role in modulating the threshold for immune cell activation and lymphoproliferation [25]. Constitutive expression may also be an absolute requirement for FOXP3⁺ regulatory T cell (Treg) development and activity [26]. CTLA-4 ligation has been shown to decrease CD4⁺ T cell production of pro-inflammatory cytokines (i.e. interleukin-2 [IL-2] and interferon-gamma [IFN γ]), as well as increase production of anti-inflammatory transforming growth factor-beta (TGF β) [27]. In this manner, CTLA-4 regulates and maintains tolerance to both self and foreign antigens. Knockout of the CTLA-4 gene results in uncontrolled T cell expansion and early death secondary to activation by self-antigens [28].

Clinical applications—First studied in a mouse model of B7-1 positive colon cancer, anti-CTLA-4 monoclonal antibodies have been shown to induce tumor regression and durable T cell memory against tumor re-challenge [29]. Similar findings have been noted in murine models of breast, prostate, ovarian, and bladder cancers [30–33].

In the context of brain tumors, CTLA-4's role in maintaining an immunosuppressive tumor microenvironment requires further elucidation. TIL analysis has shown that the ratio of

immunosuppressive Tregs to effector T cells increases in the gliomas, and that CTLA-4 expression is increased on these Tregs [2, 34]. In a subsequent study, treatment of murine glioma with anti-CTLA-4 antibody led to 80 % long-term survival and restored CD4+ effector activity [4]. Combinations of anti-CTLA-4 with anti-CD25 (IL-2 receptor, α chain) [35] or Gvax, a whole cell tumor vaccine [36] has also been employed to successfully increase survival in glioma-bearing mice.

Given the preclinical success of this therapeutic antibody, phase I and II clinical trials of two humanized anti-CTLA-4 antibodies—ipilimumab (BMS) and tremelimumab (Pfizer)—were initiated in 2000. Objective response rates of about 10 % were noted in melanoma patients given either antibody [37, 38]. However, a phase III tremelimumab trial was discontinued after it failed to improve survival compared to standard of care chemotherapies [39].

In 2011, ipilimumab received FDA approval for unresectable and metastatic melanoma following a triple arm clinical trial that demonstrated a 3.5 month survival benefit in patients with metastatic melanoma (including brain metastases) who received ipilimumab (18 % survival) as compared to treatment with a melanoma-specific gp100 peptide vaccine, PMEL (5 % survival). Addition of PMEL to ipilimumab did not further increase survival [7, 40, 41]. Subsequent phase I and II trials have further examined the use of anti-CTLA-4 antibody (as monotherapy or combination therapy) in pancreatic, prostate, and small cell lung cancers, and phase I-IV trials in melanoma, lymphoma, and non-small cell lung, prostate, cervical, pancreatic, and colorectal cancers are ongoing [41, 42] (See Table 1 for summary of CTLA-4 clinical trials). The Radiation Therapy Oncology Group (RTOG 1125) recently received approval for a randomized, phase II and III clinical trial for the treatment of newly diagnosed GBM with ipilimumab plus temozolomide (current standard of care chemotherapy [43]). The results from this and similar studies will help determine the role of CTLA-4 blockade in brain tumor immunotherapy.

PD-1/PDL-1

Biological overview—PD-1 (CD279) is an inhibitory checkpoint that, like CTLA-4, acts as a negative regulator of the immune response. Whereas CTLA-4 inhibits naïve lymphocytes, PD-1 is mostly expressed on activated peripheral lymphocytes and protects host tissues from inflammatory processes. PD-1 is also a marker of mature T cell exhaustion in the setting of chronic inflammation or tumor growth [1]. Its ligands include PD ligand-1 (PDL-1, also known as B7-H1 or CD274) and PDL-2 (also known as B7-DC or CD273) [44]. PD-1 ligation and activation leads to suppressed IFN γ , IL-2, and tumor necrosis factor alpha (TNF α) synthesis, as well as increased IL-10 production. This altered cytokine milieu not only suppresses lymphocyte activity, but also induces anergy and apoptosis of antigen-specific lymphocytes [44]. Furthermore, PD-1 expression on B cells, NK cells, and macrophages is associated with diminished immunoglobulin production, reduced cytotoxicity, and improper activation, respectively [45–47].

Preclinical studies have helped elucidate PD-1's role in induction and maintenance of immune tolerance. Knockout of the PD-1 gene results in loss of self-tolerance and development of local and systemic autoimmunity [44]. Conversely, PD-1 upregulation leads to significant immunosuppression, as seen in chronic infection or tumorigenesis. Though

normally found on immune cells, PDL-1 is also expressed on the surface of several immunogenic tumors such as gliomas, melanomas, and various carcinomas [48]. Binding of tumor PDL-1 by host immune cell PD-1 receptors results in inactivation and even death of antitumor TILs [49].

Anti-PD-1 and anti-PDL-1 antibodies have been successfully employed to block PD-1:PDL-1 ligation and protect TIL activity in preclinical studies of immunogenic tumors. PD-1 blockade has also been shown to increase levels of the immunostimulatory cytokine, IFN γ . Since IFN γ production is associated with TH1 lymphocyte activity and CD8+ activation, these findings suggest that PD-1 blockade may improve effector T cell activity [50]. PD-1 and PDL-1 blockade may also result in restoration of CD8+ T cell function, down-regulated FOXP3 expression, and tumor regression in a variety of murine cancer models [49]. Specifically in an orthotopic GBM mouse model, combination PD-1 blockade and focal radiotherapy has been shown to significantly improve TIL infiltration and activity, immune memory, and long-term survival.

Clinical applications—At this time, numerous monoclonal anti-PD-1 and -PDL-1 antibodies are in the clinical testing stage. Commercially produced anti-PD-1 antibodies include Nivolumab (BMS), Lambrolizumab (Merck), AMP-224 (Amplimmune), and Pidilizumab (CureTech). Anti-PDL-1 antibodies include BMS-936559 (BMS), MEDI4736 (Medimmune), MPDL3280A (Genentech), and MSB0010718C (Merck). The anti-PDL-2 antibody rHigM12B7 (Mayo Foundation) is also available.

In 2013, two influential clinical reports on PD-1 blockade were published. In one, Hamid et al. presented the results of their phase I clinical trial of lambrolizumab monotherapy for melanoma (NCT01295827), which showed that all tested doses of humanized anti-PD1 antibody were safe and resulted in tumor regression with increased TIL presence [51]. In another phase I trial (NCT01024231), Wolchok et al. found that combined Nivolumab (a humanized anti-PD1 antibody) and Ipilimumab (anti-CTLA-4) resulted in significant tumor regression in 53 % of study patients with advanced melanoma [52]. Additional phase I, II, and III trials are currently underway to investigate the utility of anti-PD-1 antibodies in melanoma, lung, colorectal, blood, and other solid cancers [41] (Table 2). A phase I/II trial (NCT01952769) has also begun recruiting participants to investigate the safety and efficacy of Pidilizumab (humanized anti-PD-1 antibody) in the setting of relapsed GBM and diffuse intrinsic pontine glioma (DIPG).

Additional checkpoints: negative regulators

LAG-3

Lymphocyte-activation gene 3 (LAG-3, CD223) is upregulated on the surface of activated T and NK cells and is a negative regulator of T cell expansion during inflammation [53]. LAG-3 also promotes Treg immunosuppressive functions [54] and may induce dendritic cell (DC) production of IL-12 and TNF [55]. Though its precise mechanism is not yet fully understood, Tregs from LAG-3 knockout mice have been shown to have significantly decreased inhibitory activity; effector T cells from these knockout mice demonstrated increased lymphocyte proliferative capacity and immune memory [53]. In murine tumor

models, blocking the checkpoint with anti-LAG-3 antibody has also been shown to diminish Treg inhibition, enhance antitumor CD8+ T cell expansion, and restrict tumorigenesis [56].

IMP321, a chimeric anti-LAG-3 antibody, has undergone phase I testing in combination with gemcitabine for the treatment of advanced pancreatic adenocarcinoma, the results of which suggest that while well tolerated, an efficacious dose was not reached during the course of the study [57]. A phase I/II study of metastatic breast cancer patients treated with IMP321 and paclitaxel reported an increase in APC and NK activation, CD8+ memory cell proliferation, and overall tumor response [58]. A phase 1 study of BMS-986016 (anti-LAG-3) with or without nivolumab is currently underway [41].

TIM-3

Like PD-1, T cell immunoglobulin mucin-3 (TIM-3) is a widely expressed surface receptor and a marker of T cell exhaustion in states of chronic antigen exposure [59, 60]. Once bound to its ligand, galectin-9, TIM-3 triggers aggregation and apoptosis of effector lymphocytes [60]. T cells that co-express PD-1 and TIM-3 may represent a severely exhausted or impaired population of lymphocytes in the tumor microenvironment. Presence of PD-1+/TIM-3+ TILs has been associated with disease progression in a variety of murine cancers including melanoma, sarcoma, colon and breast cancers, and acute myelogenous leukemia [60, 61], and combined treatment with PD-1 and TIM-3 blockade has been shown to prolong survival and cause tumor regression in several of these mouse models [59, 61].

Though there are no ongoing clinical trials of anti-TIM-3 antibody at this time, promising preclinical results suggest a potential role for TIM-3 blockade in cancer immunotherapy.

KIR

Killer immunoglobulin-like receptors (KIRs) encompass a variety of proteins that bind MHCI to inhibit NK activity. NK cells comprise approximately 15 % of peripheral lymphocytes and play an important role in innate immunity against viral infections and cancers. Because they do not have finely tuned antigen specificity as seen with B and T cells, NK cells rely on KIRs to prevent autoimmunity. By recognizing self-HLA molecules, activated KIRs may tolerize NK cells against self-antigens [62]. Early studies suggested that NK cells play an important role in rejecting tumors that lack self MHCI molecules, a supposition that was borne out in adoptive transfer experiments of KIR-ligand mismatched NK cells, which led to increased anti-tumor cytotoxic activity [63]. Furthermore, anti-KIR antibodies have been used to prevent tolerance induction against tumor antigens in various leukemia models [64, 65].

Two human anti-KIR antibodies, Lirilumab (IPH2102, BMS) and IPH2101 (Innate Pharma), are being currently tested in early clinical trials. At present, IPH2101 is undergoing phase I clinical testing for monotherapeutic use in patients with multiple myeloma or AML. Results from a phase I and II trial of Lirilumab (NCT01714739, NCT01750580) in combination with ipilimumab and nivolumab have demonstrated both safety and early efficacy [41].

Additional checkpoints: positive regulators

4-1BB

In contrast to the previously checkpoints, 4-1BB (also known as CD137) represents a costimulatory molecule that is expressed on activated effector T cells, NK cells, neutrophils, and DCs [66]. Ostensibly, its role in T cell regulation is to enhance or salvage inadequate immune responses. Upon binding to the 4-1BB ligand (4-1BBL) on B cells, DCs, or macrophages, the 4-1BB receptor activates pro-survival signaling pathways and upregulates transcription of the anti-apoptotic Bcl-x(L) and Bfl-1 genes. This translates to significant prolongation of CD8⁺ T cell survival and raises the possibility of augmenting recognition and immune response to poorly immunogenic cancer cells [66]. Administering stimulatory anti-4-1BB antibodies has been shown to increase tumor-specific CD8⁺ T cell activity, which corresponds to tumor regression in preclinical animal studies of sarcoma, mastocytoma, breast, and colon cancers [66–68]. Similarly, in a pI trial of BMS-663513 (a humanized anti-4-1BB agonizing antibody), melanoma, renal, and ovarian cancers patients were found to have increased numbers of circulating effector T cells, as well as increased transcription of IFN γ -associated genes [69].

GITR

Glucocorticoid-induced TNFR family related gene (GITR) is a positive regulator that is constitutively or inducibly expressed on CD4⁺, CD8⁺ and regulatory T cells. When bound to GITR ligand (GITRL), which is widely expressed on APCs as well as on endothelial and epithelial tissues, GITR initiates a signaling pathway that results in both Treg suppression and CD4⁺ T cell stimulation [70]. In the setting of an immunosuppressive tumor microenvironment, agonizing antibodies against the GITR receptor have been shown to decrease the number of tumor infiltrating Tregs, FOXP3 expression, Treg activity [71]. As demonstrated in B16 melanoma-bearing mice, these changes in TIL profile are associated with significant tumor regression [71]. Conversely, antagonizing antibodies have been shown to increase CD4⁺ T cell sensitivity to Treg suppression [72].

TRX518 (Tolerx) is a “first in class” humanized agonist antibody that blocks GITR:GITRL interactions and results in enhanced stimulation of peripheral lymphocytes [73]. This non-depleting antibody is currently in phase I studies for the treatment of late stage melanoma and additional solid tumors (NCT01239134). A phase I study of combination therapy with DC vaccines and anti-CTLA-4 or anti-GITR in melanoma has been approved but is currently suspended, pending additional funding (NCT01239134).

Combination therapies

Combination checkpoints

Given the preclinical and clinical successes of anti-checkpoint monotherapy, it has been hypothesized that two or more anti-checkpoint antibodies could achieve additive, if not synergistic, antitumor benefits. TILs can express multiple checkpoints in varying combinations, and expression of two or more checkpoints may denote a more severely exhausted or downregulated T cell phenotype [74]. Curran et al. demonstrated in a B16

melanoma model that simultaneous administration of anti-CTLA-4 and anti-PD-1 antibodies after antitumor vaccination could significantly improve survival compared to vaccination plus a single checkpoint inhibitor, due to a more favorable immune profile [75]. Similar findings have been seen in animal studies of anti-PD-1 and -LAG-3 [76] or anti-PD-1 and -TIM-3 [60]. In a recent phase I clinical trial, Wolchok et al. demonstrated that combination therapy with ipilimumab (anti-CTLA-4) and nivolumab (anti-PD1) was relatively safe and resulted in rapid, durable tumor regression and improved survival in patients with advanced melanoma [52].

Combination with chemotherapy or radiation

Additional studies have explored the efficacy of combining checkpoint therapy with more conventional treatment modalities such as chemotherapy and radiation. As mentioned in the above section on CTLA-4, RTOG 1125 has commenced a phase II/III trial of ipilimumab and temozolomide for the treatment of newly diagnosed GBM [43]. Numerous ongoing clinical trials have also begun integrating checkpoint blockade with traditional chemotherapy such as cisplatin, cyclophosphamide, and paclitaxel in a various solid and liquid cancers [41].

Though lymphocytes are exquisitely sensitive to the lethal effects of ionizing radiation, several studies have demonstrated that radiation may synergize with immunotherapy by exposing tumor-specific antigens and releasing activating cytokines from damaged cells and stroma [77]. Combination of ipilimumab and ionizing radiation is being studied in several phase I and II trials for patients with metastatic melanoma (including brain metastases), lymphoma, and various solid tumors [41].

Caveats for checkpoint blockade

As the search for immunotherapeutic targets broadens, it has become clear that there are a multitude of intrinsic checkpoints that, together, constitute a built-in safety mechanism against immune overstimulation. Whether or not they are constitutively or inducibly expressed, these checkpoints act in a coordinated fashion to maintain tolerance against self-antigens, and fine-tune or temper responses to foreign antigens. Loss of a functioning checkpoint, therefore, may correspond with lymphoproliferation and autoimmunity, as demonstrated by human and animal studies of CTLA-4 [78, 79] and PD-1 [80] deficiency.

Correspondingly, one of the greatest risks of checkpoint blockade is autoimmunity secondary to unrestrained immune activation. The most common adverse events reported in humans include colitis, hypophysitis, vitiligo, pancreatitis and hepatitis. In one noteworthy ipilimumab trial [7], over 60 % of patients experienced immune related adverse events (irAEs); moreover, 10–15 % were reported to have had grade 3 or 4 severe irAEs. Topalian et al. also reported a 14 % grade 3 and 4 irAE rate in patients receiving anti-PD1 therapy (nivolumab), with pneumonitis being a unique toxicity seen in 1 % of patients (3 % for all grades) [5]. Wolchok et al. found that 54 % of patients receiving both nivolumab and ipilimumab experienced grade 3 or 4 toxicities, which were generally reversible. Interestingly, this rate was similar to those seen in the monotherapy arm [52]. In light of these findings, it is critical that the therapeutic benefits are weighed against the significant

toxicity risks, and that dosing and combination strategies are purposefully constructed to ensure that irAEs are minimized, anticipated, and well managed.

Summary

Checkpoint modulating agonist and antagonist antibodies are a powerful addition to the armamentarium of anticancer drugs. Since the discovery of the archetypal negative regulator, CTLA-4, several other checkpoints (i.e. PD-1, LAG-3, TIM-3, KIR, 4-1BB, and GITR) have emerged as promising targets for passive immunotherapies. Preclinical studies of anti-CTLA-4 and anti-PD-1 suggest a role for these antibodies in the treatment of GBMs, and have led to ongoing clinical trials for primary and recurrent brain tumors. Future applications may include synergistic combinations with other checkpoint inhibitors, anticancer vaccines, chemotherapy, or radiation. In the meantime, further clinical testing and development will allow for a more thorough understanding of the limits and therapeutic potential of checkpoint modulation.

References

1. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012; 12:252–264.10.1038/nrc3239 [PubMed: 22437870]
2. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro Oncol*. 2006; 8:234–243.10.1215/15228517-2006-006 [PubMed: 16723631]
3. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009; 9:162–174.10.1038/nri2506 [PubMed: 19197294]
4. Fecci PE, Ochiai H, Mitchell DA, Grossi PM, Sweeney AE, Archer GE, Cummings T, Allison JP, Bigner DD, Sampson JH. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell function. *Clin Cancer Res*. 2007; 13:2158–2167. [PubMed: 17404100]
5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366:2443–2454.10.1056/NEJMoa1200690 [PubMed: 22658127]
6. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010; 363:411–422.10.1056/NEJMoa1001294 [PubMed: 20818862]
7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–723.10.1056/NEJMoa1003466 [PubMed: 20525992]
8. Blankenstein T, Coulie PG, Gilboa E, Jaffee EM. The determinants of tumour immunogenicity. *Nat Rev Cancer*. 2012; 12:307–313.10.1038/nrc3246 [PubMed: 22378190]
9. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro Oncol*. 2006; 8:234–243. [PubMed: 16723631]
10. Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, Durham N, Meyer C, Harris TJ, Albesiano E, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *Int J Radiat Oncol Biol Phys*. 2013; 86:343–349.10.1016/j.ijrobp.2012.12.025 [PubMed: 23462419]
11. Fecci PE, Ochiai H, Mitchell DA, Grossi PM, Sweeney AE, Archer GE, Cummings T, Allison JP, Bigner DD, Sampson JH. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell function. *Clin Cancer Res*. 2007; 13:2158–2167. [PubMed: 17404100]

12. Davies DC. Blood-brain barrier breakdown in septic encephalopathy and brain tumours. *J Anat.* 2002; 200:639–646. [PubMed: 12162731]
13. Rascher G, Fischmann A, Kroger S, Duffner F, Grote EH, Wolburg H. Extracellular matrix and the blood-brain barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin. *Acta Neuropathol.* 2002; 104:85–91.10.1007/s00401-002-0524-x [PubMed: 12070669]
14. Reardon DA, Freeman G, Wu C, Chiocca EA, Wucherpennig KW, Wen PY, Fritsch EF, Curry WT Jr, Sampson JH, Dranoff G. Immunotherapy advances for glioblastoma. *Neuro Oncol.* 2014; 16:1441–1458.10.1093/neuonc/nou212 [PubMed: 25190673]
15. Galea I, Bechmann I, Perry VH. What is immune privilege (not)? *Trends Immunol.* 2007; 28:12–18. [PubMed: 17129764]
16. Goldmann J, Kwidzinski E, Brandt C, Mahlo J, Richter D, Bechmann I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. *J Leukoc Biol.* 2006; 80:797–801. [PubMed: 16885505]
17. van Zwam M, Huizinga R, Melief MJ, Wierenga-Wolf AF, van Meurs M, Voerman JS, Biber KP, Boddeke HW, Hopken UE, Meisel C, et al. Brain antigens in functionally distinct antigen-presenting cell populations in cervical lymph nodes in MS and EAE. *J Mol Med (Berl).* 2009; 87:273–286.10.1007/s00109-008-0421-4 [PubMed: 19050840]
18. Stevenson PG, Hawke S, Sloan DJ, Bangham CR. The immunogenicity of intracerebral virus infection depends on anatomical site. *J Virol.* 1997; 71:145–151. [PubMed: 8985333]
19. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat Rev Immunol.* 2012; 12:623–635.10.1038/nri3265 [PubMed: 22903150]
20. Hersh L, Salzman B. Clinical management of urinary incontinence in women. *Am Fam Physician.* 2013; 87:634–640. [PubMed: 23668526]
21. Prins RM, Shu CJ, Radu CG, Vo DD, Khan-Farooqi H, Soto H, Yang MY, Lin MS, Shelly S, Witte ON, et al. Anti-tumor activity and trafficking of self, tumor-specific T cells against tumors located in the brain. *Cancer Immunol Immunother.* 2008; 57:1279–1289.10.1007/s00262-008-0461-1 [PubMed: 18253732]
22. Odoardi F, Sie C, Streyl K, Ulaganathan VK, Schlager C, Lodygin D, Heckelsmiller K, Nietfeld W, Ellwart J, Klinkert WE, et al. T cells become licensed in the lung to enter the central nervous system. *Nature.* 2012; 488:675–679.10.1038/nature11337 [PubMed: 22914092]
23. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P. A new member of the immunoglobulin superfamily—CTLA-4. *Nature.* 1987; 328:267–270.10.1038/328267a0 [PubMed: 3496540]
24. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity.* 1994; 1:793–801. [PubMed: 7534620]
25. Gajewski TF, Fallarino F, Fields PE, Rivas F, Alegre ML. Absence of CTLA-4 lowers the activation threshold of primed CD8+ TCR-transgenic T cells: lack of correlation with Src homology domain 2-containing protein tyrosine phosphatase. *J Immunol.* 2001; 166:3900–3907. [PubMed: 11238634]
26. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med.* 2000; 192:303–310. [PubMed: 10899917]
27. Chen W, Jin W, Wahl SM. Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor beta (TGF-beta) production by murine CD4(+) T cells. *J Exp Med.* 1998; 188:1849–1857. [PubMed: 9815262]
28. Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu Rev Immunol.* 2001; 19:225–252. [PubMed: 11244036]
29. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science.* 1996; 271:1734–1736. [PubMed: 8596936]

30. Jure-Kunkel M, Masters G, Girit E, Dito G, Lee F, Hunt JT, Humphrey R. Synergy between chemotherapeutic agents and CTLA-4 blockade in preclinical tumor models. *Cancer Immunol Immunother.* 2013; 62:1533–1545.10.1007/s00262-013-1451-5 [PubMed: 23873089]
31. Demaria S, Kawashima N, Yang AM, Devitt ML, Babb JS, Allison JP, Formenti SC. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin Cancer Res.* 2005; 11:728–734. [PubMed: 15701862]
32. Yang YF, Zou JP, Mu J, Wijesuriya R, Ono S, Walunas T, Bluestone J, Fujiwara H, Hamaoka T. Enhanced induction of antitumor T-cell responses by cytotoxic T lymphocyte-associated molecule-4 blockade: the effect is manifested only at the restricted tumor-bearing stages. *Cancer Res.* 1997; 57:4036–4041. [PubMed: 9307290]
33. Mangsbo SM, Sandin LC, Anger K, Korman AJ, Loskog A, Totterman TH. Enhanced tumor eradication by combining CTLA-4 or PD-1 blockade with CpG therapy. *J Immunother.* 2010; 33:225–235.10.1097/CJI.0b013e3181c01fcb [PubMed: 20445343]
34. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE 2nd, Bigner DD, Dranoff G, Sampson JH. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res.* 2006; 66:3294–3302.10.1158/0008-5472.CAN-05-3773 [PubMed: 16540683]
35. Grauer OM, Nierkens S, Bennink E, Toonen LW, Boon L, Wesseling P, Suttmuller RP, Adema GJ. CD4+FoxP3+ regulatory T cells gradually accumulate in gliomas during tumor growth and efficiently suppress anti-glioma immune responses in vivo. *Int J Cancer.* 2007; 121:95–105.10.1002/ijc.22607 [PubMed: 17315190]
36. Agarwalla P, Barnard Z, Fecci P, Dranoff G, Curry WT Jr. Sequential immunotherapy by vaccination with GM-CSF-expressing glioma cells and CTLA-4 blockade effectively treats established murine intracranial tumors. *J Immunother.* 2012; 35:385–389.10.1097/CJI.0b013e3182562d59 [PubMed: 22576343]
37. Camacho LH, Antonia S, Sosman J, Kirkwood JM, Gajewski TF, Redman B, Pavlov D, Bulanhagui C, Bozon VA, Gomez-Navarro J, et al. Phase I/II trial of tremelimumab in patients with metastatic melanoma. *J Clin Oncol.* 2009; 27:1075–1081.10.1200/JCO.2008.19.2435 [PubMed: 19139427]
38. Margolin K, Ernstoff MS, Hamid O, Lawrence D, McDermott D, Puzanov I, Wolchok JD, Clark JI, Sznol M, Logan TF, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *Lancet Oncol.* 2012; 13:459–465.10.1016/S1470-2045(12)70090-6 [PubMed: 22456429]
39. Ribas A, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol.* 2013; 31:616–622.10.1200/JCO.2012.44.6112 [PubMed: 23295794]
40. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012; 12:252–264.10.1038/nrc3239 [PubMed: 22437870]
41. Aranda F, Vacchelli E, Eggermont A, Galon J, Fridman WH, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: immunostimulatory monoclonal antibodies in cancer therapy. *Oncoimmunology.* 2014; 3:e27297.10.4161/onci.27297 [PubMed: 24701370]
42. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, MacRae S, Willman A, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci USA.* 2003; 100:4712–4717.10.1073/pnas.0830997100 [PubMed: 12682289]
43. Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J. Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? *Nat Rev Clin Oncol.* 2013; 10:14–26.10.1038/nrclinonc.2012.204 [PubMed: 23183634]
44. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol.* 2008; 26:677–704.10.1146/annurev.immunol.26.021607.090331 [PubMed: 18173375]
45. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol.* 1996; 8:765–772. [PubMed: 8671665]

46. Brauner H, Elemans M, Lemos S, Broberger C, Holmberg D, Flodstrom-Tullberg M, Karre K, Hoglund P. Distinct phenotype and function of NK cells in the pancreas of nonobese diabetic mice. *J Immunol.* 2010; 184:2272–2280.10.4049/jimmunol.0804358 [PubMed: 20130214]
47. Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, Swan R, Kherouf H, Monneret G, Chung CS, et al. PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci USA.* 2009; 106:6303–6308.10.1073/pnas.0809422106 [PubMed: 19332785]
48. Weber J. Immune checkpoint proteins: a new therapeutic paradigm for cancer—preclinical background: CTLA-4 and PD-1 blockade. *Semin Oncol.* 2010; 37:430–439.10.1053/j.seminoncol.2010.09.005 [PubMed: 21074057]
49. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res.* 2005; 65:1089–1096. [PubMed: 15705911]
50. Blazar BR, Carreno BM, Panoskaltsis-Mortari A, Carter L, Iwai Y, Yagita H, Nishimura H, Taylor PA. Blockade of programmed death-1 engagement accelerates graft-versus-host disease lethality by an IFN-gamma-dependent mechanism. *J Immunol.* 2003; 171:1272–1277. [PubMed: 12874215]
51. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med.* 2013; 369:134–144.10.1056/NEJMoa1305133 [PubMed: 23724846]
52. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med.* 2013; 369:122–133.10.1056/NEJMoa1302369 [PubMed: 23724867]
53. Workman CJ, Vignali DA. The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. *Eur J Immunol.* 2003; 33:970–979.10.1002/eji.200323382 [PubMed: 12672063]
54. Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, et al. Role of LAG-3 in regulatory T cells. *Immunity.* 2004; 21:503–513. [PubMed: 15485628]
55. Andrae S, Piras F, Burdin N, Triebel F. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). *J Immunol.* 2002; 168:3874–3880. [PubMed: 11937541]
56. Grosso JF, Kelleher CC, Harris TJ, Maris CH, Hipkiss EL, De Marzo A, Anders R, Netto G, Getnet D, Bruno TC, et al. LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. *J Clin Invest.* 2007; 117:3383–3392.10.1172/JCI31184 [PubMed: 17932562]
57. Wang-Gillam A, Plambeck-Suess S, Goedegebuure P, Simon PO, Mitchem JB, Hornick JR, Sorscher S, Picus J, Suresh R, Lockhart AC, et al. A phase I study of IMP321 and gemcitabine as the front-line therapy in patients with advanced pancreatic adenocarcinoma. *Invest New Drugs.* 2013; 31:707–713.10.1007/s10637-012-9866-y [PubMed: 22864469]
58. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, Boussetta N, Medioni J, Gligorov J, Grygar C, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med.* 2010; 8:71-5876-8-71.10.1186/1479-5876-8-71 [PubMed: 20653948]
59. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, Murphy WJ, Azuma M, Anderson AC, Kuchroo VK, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood.* 2011; 117:4501–4510.10.1182/blood-2010-10-310425 [PubMed: 21385853]
60. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med.* 2010; 207:2187–2194.10.1084/jem.20100643 [PubMed: 20819927]
61. Ngiew SF, von Scheidt B, Akiba H, Yagita H, Teng MW, Smyth MJ. Anti-TIM3 antibody promotes T cell IFN-gamma-mediated antitumor immunity and suppresses established tumors. *Cancer Res.* 2011; 71:3540–3551.10.1158/0008-5472.CAN-11-0096 [PubMed: 21430066]
62. Yu J, Venstrom JM, Liu XR, Pring J, Hasan RS, O'Reilly RJ, Hsu KC. Breaking tolerance to self, circulating natural killer cells expressing inhibitory KIR for non-self HLA exhibit effector function

- after T cell-depleted allogeneic hematopoietic cell transplantation. *Blood*. 2009; 113:3875–3884.10.1182/blood-2008-09-177055 [PubMed: 19179302]
63. Tam YK, Martinson JA, Doligosa K, Klingemann HG. Ex vivo expansion of the highly cytotoxic human natural killer-92 cell-line under current good manufacturing practice conditions for clinical adoptive cellular immunotherapy. *Cytotherapy*. 2003; 5:259–272.10.1080/14653240310001523 [PubMed: 12850795]
 64. Kohrt HE, Thielens A, Marabelle A, Sagiv-Barfi I, Sola C, Chanuc F, Fuseri N, Bonnafous C, Czerwinski D, Rajapaksa A, et al. Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. *Blood*. 2014; 123:678–686.10.1182/blood-2013-08-519199 [PubMed: 24326534]
 65. Koh PK, Tan BK, Hong SW, Tan MH, Tay AG, Song C, Tan KC. The gluteus maximus muscle flap for reconstruction of sacral chordoma defects. *Ann Plast Surg*. 2004; 53:44–49. [PubMed: 15211198]
 66. Cheuk AT, Mufti GJ, Guinn BA. Role of 4-1BB:4-1BB ligand in cancer immunotherapy. *Cancer Gene Ther*. 2004; 11:215–226.10.1038/sj.cgt.7700670 [PubMed: 14671675]
 67. Martinet O, Divino CM, Zang Y, Gan Y, Mandeli J, Thung S, Pan PY, Chen SH. T cell activation with systemic agonistic antibody versus local 4-1BB ligand gene delivery combined with interleukin-12 eradicate liver metastases of breast cancer. *Gene Ther*. 2002; 9:786–792.10.1038/sj.gt.3301687 [PubMed: 12040460]
 68. Chen SH, Pham-Nguyen KB, Martinet O, Huang Y, Yang W, Thung SN, Chen L, Mittler R, Woo SL. Rejection of disseminated metastases of colon carcinoma by synergism of IL-12 gene therapy and 4-1BB costimulation. *Mol Ther*. 2000; 2:39–46.10.1006/mthe.2000.0086 [PubMed: 10899826]
 69. Sznol M, Hodi FS, Margolin K. Phase I Study of BMS-663513, a Fully Human Anti-CD137 Agonist Monoclonal Antibody, in Patients (Pts) with Advanced Cancer (CA). *J Clin Oncol* (Meeting Abstracts). 2008; 26(Suppl):3007.
 70. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol*. 2002; 3:135–142.10.1038/ni759 [PubMed: 11812990]
 71. Cohen AD, Schaer DA, Liu C, Li Y, Hirschhorn-Cymerman D, Kim SC, Diab A, Rizzuto G, Duan F, Perales MA, et al. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. *PLoS One*. 2010; 5:e10436.10.1371/journal.pone.0010436 [PubMed: 20454651]
 72. Stephens GL, McHugh RS, Whitters MJ, Young DA, Luxenberg D, Carreno BM, Collins M, Shevach EM. Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by CD4+CD25+ T cells. *J Immunol*. 2004; 173:5008–5020. [PubMed: 15470044]
 73. Resenzweig M, Ponte J, Doty D, Guild J, Slavonic M, Ponath P, Vaickus L. Development of TRX518, an aglycosyl humanized monoclonal antibody (Mab) agonist of huGITR. *J Clin Oncol* (Meeting Abstracts). 2010; 28(Suppl):e13028.
 74. Nirschl CJ, Drake CG. Molecular pathways: coexpression of immune checkpoint molecules: signaling pathways and implications for cancer immunotherapy. *Clin Cancer Res*. 2013; 19:4917–4924.10.1158/1078-0432.CCR-12-1972 [PubMed: 23868869]
 75. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci USA*. 2010; 107:4275–4280.10.1073/pnas.0915174107 [PubMed: 20160101]
 76. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, Bettini ML, Gravano DM, Vogel P, Liu CL, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012; 72:917–927.10.1158/0008-5472.CAN-11-1620 [PubMed: 22186141]
 77. Demaria S, Bhardwaj N, McBride WH, Formenti SC. Combining radiotherapy and immunotherapy: a revived partnership. *Int J Radiat Oncol Biol Phys*. 2005; 63:655–666. [PubMed: 16199306]

78. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, Rainbow DB, Hunter KM, Smith AN, Di Genova G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature*. 2003; 423:506–511.10.1038/nature01621 [PubMed: 12724780]
79. Khattni R, Auger JA, Griffin MD, Sharpe AH, Bluestone JA. Lymphoproliferative disorder in CTLA-4 knockout mice is characterized by CD28-regulated activation of Th2 responses. *J Immunol*. 1999; 162:5784–5791. [PubMed: 10229811]
80. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol*. 2007; 8:239–245. [PubMed: 17304234]
81. Di Giacomo AM, Ascierto PA, Pilla L, Santinami M, Ferrucci PF, Giannarelli D, Marasco A, Rivoltini L, Simeone E, Nicoletti SV, et al. Ipilimumab and fotemustine in patients with advanced melanoma (NIBIT-M1): an open-label, single-arm phase 2 trial. *Lancet Oncol*. 2012; 13:879–886.10.1016/S1470-2045(12)70324-8 [PubMed: 22894884]
82. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, Sherry RM, Topalian SL, Yang JC, Lowy I, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010; 33:828–833.10.1097/CJI.0b013e3181eec14c [PubMed: 20842054]
83. O'Day SJ, Maio M, Chiarion-Sileni V, Gajewski TF, Peham-berger H, Bondarenko IN, Queirolo P, Lundgren L, Mikhailov S, Roman L, et al. Efficacy and safety of ipilimumab mono-therapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. *Ann Oncol*. 2010; 21:1712–1717.10.1093/annonc/mdq013 [PubMed: 20147741]
84. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, Suri KB, Levy C, Allen T, Mavroukakis S, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *J Immunother*. 2007; 30:825–830.10.1097/CJI.0b013e318156e47e [PubMed: 18049334]
85. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366:2455–2465.10.1056/NEJMoa1200694 [PubMed: 22658128]

Fig. 1. Summary of co-stimulatory or -inhibitory immune checkpoints receptors expressed on T cells (blue), regulatory T cells (aqua), dendritic cells (green), and natural killer cells (purple), and their associated ligands on tumor cells (red)

Table 1

Clinical study outcomes of ipilimumab (anti-CTLA-4) in solid tumors

ClinicalTrials.gov registration no.	Phase	Cancers	Combination/simultaneous therapies	Status	No. of patients	Outcome
NCT00094653	III	Melanoma	None	Published results	676	3-year survival rate 25 %, BORR 37.5 % [23]
NCT00623766	II	Melanoma with brain metastasis	None	Published results	93	Disease control, 18 % in neurologically asymptomatic patients not on CCS; 5 % in symptomatic patients on CCS [21]
NCT01654692	N/A	Melanoma	None	Published results	27	2-yr survival rate 23.5 % [61]
NCT00112580	II	Pancreatic adenocarcinoma	None	Published results	27	No responders [62]
NCT00289627	II	Melanoma	None	Published results	155	BORR 5.8 % [63]
NCT00289640	II	Melanoma	None	Published results	217	BORR 11.1 % [33]
NCT00057889	II	RCC	None	Published results	40	PR 12.5 % [64]
NCT01711515	I	Locally advanced cervical carcinoma	+RT +cisplatin	Currently recruiting	N/A	N/A
NCT01769222	I/II	CRC, recurrent melanoma, Non-Hodgkin lymphoma	+local RT	Temporarily suspended	N/A	N/A
NCT01860430	Ib	Locally advanced head neck cancer	+RT +cetuximab	Currently recruiting	N/A	N/A
NCT01935921	I	Stage II-IVB head neck cancer	+RT +cetuximab	Currently recruiting	N/A	N/A
NCT01557114	I	Unresectable advanced malignant melanoma	+RT	Currently recruiting	N/A	N/A
NCT01703507	I	Melanoma with brain metastases	+SRS or WBRT	Currently recruiting	N/A	N/A [32]

Outcomes are best outcomes in each study, in patients receiving Ipilimumab only

BORR best overall response rate, RCC renal cell carcinoma, CCS corticosteroids, PR partial response, OR objective response, PSA prostate specific antigen, RT radiation therapy, SRS stereotactic radiosurgery, WBRT whole brain radiation therapy, CRC colorectal cancer

Table 2
Clinical study outcomes of anti-PD-1 or anti-PD-L1 antibody in solid tumors [81–85]

ClinicalTrials.gov registration no.	Phase	Antibody type	Cancer	No. of patients	Outcome
NCT01295827	II	Lambrolizumab	Melanoma	135	RECIST 1.1 RR: 38 % [32]
NCT011176461	I	Nivolumab	Melanoma	90	RECIST 1.1 RR: 25 % [30]
NCT00730639	I	Nivolumab	Advanced solid cancers	296	OR 18–36 % [5]
NCT00729664	I	Anti-PDL1	Advanced solid cancers	207	OR 6–17 % [65]

RECIST response evaluation criteria in solid tumors, *OR* objective response, as defined by partial or complete tumor regression, *RR* response rate, *CRR* cumulative response rate