Blockade of the B7-H1/PD-1 Pathway for Cancer Immunotherapy

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The aim of cancer immunotherapy is to treat malignant disease by inducing or enhancing cancer specific immune responses. With the identification of tumor-associated antigens (TAAs\textsuperscript{†}) in the 1990s, cancer immunotherapy research largely focused on inducing immune responses against TAAs but achieved limited success. More recently, the underlying mechanisms and molecular pathways that cancers manipulate to subvert immune-mediated destruction have been identified, including a set of molecules with potent coinhibitory functions. Coinhibitory molecules are expressed on the surface of immune cells, cancer cells, and stromal cells and negatively regulate immune responses to cancer. In particular, one of these ligand-receptor coinhibitory interactions, B7-H1/PD-1, is critical for modulating immune responses to cancer. This knowledge led to the design of revolutionary new immunotherapeutics based on the manipulation of these molecular pathways. Monoclonal antibodies (mAbs) are the primary immunotherapeutic modality used to promote immune function via antagonism or agonism of inhibitory or stimulatory molecular pathways, respectively. Here, we review current knowledge on the function of the B7-H1/PD-1 pathway in mice and humans, its role in the subversion of immune responses in cancer, and clinical evidence that mAb targeting of this pathway results in profound immune anti-cancer effects.

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\†Abbreviations: PD-1, programmed death-1; B7-H1, B7-homolog-1; CSSMs, cell surface signaling molecules; TCR, T cell receptor; MHC, major histocompatibility complex; APC, antigen presenting cell; CTLA-4, cytotoxic T lymphocyte antigen-4; BTLA, B and T lymphocyte attenuator; Tim-3, T cell immunoglobulin and mucin domain-3; Lag-3, lymphocyte activation gene-3; TNF, tumor necrosis factor; IFN, interferon; TILs, tumor infiltrating lymphocytes; GVHD, graft versus host disease; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; BATF, basic leucine zipper transcription factor of the ATF family; PTEN, Phosphatase and Tensin Homology; NFAT, nuclear factor of activated T cell; RCC, renal cell carcinoma; ADCC, antibody dependent cellular cytotoxicity; CDC, complement dependent cytotoxicity; MBRT, multiple brake release therapy; KO, knockout.

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INTRODUCTION

In The Selfish Gene by Richard Dawkins, the question is posed: “What makes a good gene?” Dawkins proposes that a good gene, from an evolutionary perspective, is defined by longevity, fecundity, and copying-fidelity [1]. He goes further to suggest that a good gene must also be good at making “survival machines,” or in other words, enhance the survival and reproductive capacity of an organism. However, as the incidence of cancer increases in the industrial era, perhaps due to lengthening human life spans, industrial and occupational risks, environmental factors, and other causes, we face the realization that humans have not evolved to deal effectively with cancer [2,3]. Many cases of cancer occur after the reproductive age, limiting the ability of evolutionary processes to select for good anti-cancer genes [2,3].

The evolutionary selection of good anti-cancer genes is particularly interesting with respect to the immune response to cancer. Some components of immunity critical for human survival, ironically, also may be exploited for the survival and progression of cancer [4,5]. With this in mind, we can hardly describe such immune components as dysfunctional, as they are performing precisely as evolutionary design dictates. In fact, some negative regulatory components of the immune system may have enhanced function in the cancer microenvironment, thus promoting cancer progression rather than halting it [4,5]. These genes may act as double-edged swords in the cancer microenvironment to dampen inflammatory responses while simultaneously preventing optimal immune destruction of transformed cells.

Costimulatory and coinhibitory molecules that represent a subgroup of cell surface signaling molecules (CSSMs) are particularly susceptible to manipulation by cancers [6,7]. CSSMs provide cells of the immune system with decision-making input following initial triggering by a primary signal [6,7]. In T cells, the primary signal is generated in an immunological synapse via T cell receptor (TCR) engagement of a major histocompatibility complex molecule (MHC), which presents antigenic peptide on the surface of an antigen presenting cell (APC) (reviewed in [8]). Cosignaling occurs via T cell cosignaling receptor molecules binding to ligand molecules expressed on APCs. These interactions further enhance or dampen primary signaling pathways [8]. Co-signals are involved in all phases of T cell function including priming, activation, expansion, effector function, and contraction [9,10]. Several families of cosignaling molecules have now been identified and characterized as functional modulators of T cell-mediated immune responses [9,10].

CD28 and B7 family molecules are considered the best-characterized sets of cosignaling molecules. These families include both costimulatory and coinhibitory receptors and ligands with CD28-like molecules primarily interacting with molecules of the B7 family [6,7]. The interactions between CD28 and B7 family molecules are critical for immune responses to infection and disease [6,7]. T cell activation, for example, depends on the binding of CD28 to B7-1 (CD80) and B7-2 (CD86) on APCs, while Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4; CD152), another member of the CD28 family, down-regulates T-cell activity when it engages B7-1 and B7-2 [6,7]. A recent study shows that B7-H2 (CD275), a B7 family molecule best known as the ligand for Inducible Costimulator (ICOS), is the third ligand for CD28 and CTLA-4 in humans [11]. The complete set of known CD28 and B7 family members and their interactions are reviewed elsewhere [12-15]. Molecules of the B7-H1/PD-1 pathway are critical modulators of immune responses. Programmed Death-1 (PD-1; CD279) is a member of the CD28 family expressed on activated T cells, B cells, dendritic cells, and macrophages [12-15]. Engagement of PD-1 inhibits function in these immune cell subsets [6,16]. PD-1 has two known counter-receptors or ligands, B7-H1 (CD274, PD-L1) and B7-DC (CD273, PD-L2) of the B7 family. While B7-H1 expression is inducible on a variety of cell types in lymphoid and peripheral tissues, B7-DC is more restricted to myeloid cells, including dendritic cells [12-15]. The B7-
H1/PD-1 pathway has emerged as playing a pivotal role in the negative regulation of T cell activity, including suppression of immune responses against cancer [6,7].

While the major role of the B7-H1/PD-1 pathway is to tune down inflammatory immune responses in tissues and organs, this function is manipulated by cancer as a way to escape from immune destruction. In this review, we illustrate these mechanisms and describe how interventions utilizing mAb immunotherapy to block this molecular pathway may re-awaken the immune system to engender a dramatic counter-attack against cancers. Using these methods, we may reverse a mechanism by which cancers manipulate otherwise good genes.

**OVERVIEW OF THE PD-1/B7-H1/B7-DC PATHWAY**

Regulatory elements of the immune system exist primarily to prevent the damaging immunopathology that accompanies
excessive responses against infection, as well as to block autoimmune responses by maintaining peripheral tolerance of self-antigens. The B7-H1/PD-1 molecular cosignaling axis appears to play a critical role in both of these processes. This premise has been substantiated by analysis of mice that are genetically deficient in various components of this axis [17-20]. Experimental mouse models in which signaling through these pathways are blocked by mAbs, including alloantigen and graft-versus-host-disease (GVHD) models, autoimmune disease models, and fetomaternal tolerance models, demonstrate that this pathway generally functions to prevent excessive peripheral immune responses and associated immunopathology [21,22].

**PD-1 is a Coinhibitory Receptor Expressed on Activated T Cells**

PD-1 and coinhibitory receptors such as CTLA-4, B and T Lymphocyte Attenuator (BTLA; CD272), T cell Immunoglobulin and Mucin domain-3 (Tim-3), Lymphocyte Activation Gene-3 (Lag-3; CD223), and others are often referred to as a checkpoint molecules or, more appropriately, checkpoint CSSMs [6,7]. They act as molecular “tollbooths,” which allow extracellular information to dictate whether cell cycle progression and other intracellular signaling processes should proceed [23]. Ample evidence shows that PD-1 is a coinhibitory receptor expressed on activated T cells that negatively regulates T cell function. For example, a high level of PD-1 expression on antigen-experienced CD8+ T cells is associated with a CD8+ T cell phenotype termed “T cell exhaustion.” This phenotype is defined by impaired effector function and the persistent expression of inhibitory receptors (reviewed in [24]). Exhausted CD8+ T cells gradually lose effector functions, including the capacity to proliferate, the ability to express cytokines like Interleukin-2 (IL-2), Tumor Necrosis Factor-α (TNF-α), and Interferon-γ (IFN-γ), the expression of effector molecules like perforin, and eventually appear to undergo apoptosis [24]. Exhausted PD-1-expressing CD8+ T cells have been identified among tumor-infiltrating lymphocytes (TILs) in cancers, as well as in chronic viral infections (Figure 1) [24]. However, the exact mechanism by which PD-1 expression inhibits CD8+ T cell function is not completely understood.

B7-H1 is the major ligand for PD-1 mediated immunosuppression. It is constitutively expressed on APCs and can be broadly induced on cells in both lymphoid tissues and non-lymphoid peripheral tissues following cellular activation, making B7-H1 ideally suited for peripheral modulation of activated T cells [18,21,25]. The cytokine IFN-γ is particularly effective in upregulating B7-H1 due to IFN-γ response elements in the B7-H1 promoter region [12-15]. The expression of B7-DC, another ligand of PD-1, is largely restricted to myeloid DCs and macrophages in lymphoid compartments and is not broadly expressed in peripheral tissues or on multiple cell types, thus making it less effective at dampening peripheral T cell responses [12-15].

PD-1 engagement on T cells stimulated with anti-CD3 and anti-CD28 mAbs delivers an inhibitory signal that blocks PI3K activity and subsequent downstream activation of Akt [26]. Although the cytoplasmic tail of PD-1 contains both an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM), both of which can have inhibitory function, it was found by mutational analysis that only the ITSM, and not the ITIM motif, is required for the suppression of PI3K/Akt activation and T cell expansion [26]. PD-1 engagement recruits both SHP-1 and SHP-2, SH2-domain containing protein tyrosine phosphatases, which dephosphorylate and deactivate downstream signaling components [26]. However, the role of SHP-1/2 in PD-1 mediated inhibition remains unclear at this time, while the possibility exists that other signaling molecules found to interact with the PD-1 cytoplasmic tail may play an important role in T cell inhibition.

Studies of the regulation of PD-1 expression on CD8+ T cells, using an integrated genomics approach, have identified a number of associated signaling molecules.
BATF, a basic leucine zipper transcription factor of the ATF family, may promote exhaustion following PD-1 ligation by negatively regulating the AP-1 transcription factor by displacing c-Fos/c-Jun dimerization [27-29]. Additionally, the transcription factors Blimp-1 and NFATc1 are associated with exhausted T cells, and both regulate PD-1 expression. By contrast, the transcription factor T-bet may repress PD-1 expression and promote sustained CD8+ T cells responses [21,22]. Nevertheless, correlation studies do not paint a clear picture of the true relation of PD-1 expression and distal signaling events on exhausted CD8+ T cells.

**Phenotypes of Mice Deficient in PD-1, B7-H1 and B7-DC**

Mouse strains deficient in components of the PD-1 pathway display moderate, progressive autoreactive phenotypes, primarily in peripheral tissues. PD-1 knockout (KO) mice develop strain-dependent late-onset autoimmune-like pathologies (including lupus-like arthritis, glomerulonephritis, and cardiomyopathy), while B7-H1 KO mice accumulate seemingly non-functional CD8+ T cells in peripheral organs over time without autoimmune manifestations, likely due to lack of PD-1 dependent T cell apoptosis and clearance [12-15]. Meanwhile, B7-DC deficient mice display no obvious phenotype but develop enhanced immunopathology in asthma models [30]. The phenotype of PD-1/B7-H1/B7-DC KO mice is in contrast to CTLA-4 KO mice, which display a much more acute and systemic autoimmune phenotype [18,21,25]. However, mechanisms have not been fully defined for the differences in autoimmune toxicity in these mouse strains.

**Additional Mechanisms of T cell Regulation by the PD-1/B7-H1 Molecular Axis**

The identification of additional interactions within this molecular axis expands potential mechanisms of action and distorts receptor/ligand designations. First, as discussed previously, not only does B7-H1 deliver a regulatory signal through PD-1, it has been shown that PD-1 is capable of delivering a reverse signal through B7-H1 on cancer cells to induce an anti-apoptotic effect [31]. Second, B7-H1 can engage B7-1 to deliver an inhibitory signal to T cells and induce T cell suppression in vitro and T cell tolerance in vivo [32,33]. Finally, evidence suggests that additional receptors for B7-H1 and B7-DC exist through which these molecules deliver costimulatory signals [6,7]. These findings blur the lines between receptor and ligand and between stimulatory and inhibitory signaling within this molecular axis. Therefore, although the primary function of this molecular axis is clearly negative modulation of effector T cell activity, it will be critical to elucidate how this pathway is involved in regulating immune responses within specific environments.

**Subversion of the PD-1/B7-H1 Pathway by Cancers**

Although it was once unclear if tumor immunosurveillance existed, we now believe the immune system constantly monitors and eliminates newly transformed cells [4,34]. Accordingly, cancer cells may alter their phenotype in response to immune pressure in order to escape attack [4,34]. This “immunoediting” hypothesis largely deals with the relationship between cancer cells and the immune response during the early stages of tumorigenesis. However, ample evidence suggests subsequent progression of primary tumors and metastases employs a wide variety of subversive processes to maintain their own survival [4,5]. Subversion of anti-tumor immunity is believed to be a mechanism by which cancers maintain unbridled growth and metastasis [4,5].

Numerous studies indicate that the cancer microenvironment manipulates the B7-H1/PD-1 molecular pathway and that induction of B7-H1 expression is associated with inhibition of immune responses against cancer, thus permitting cancer progression and metastasis [35,36]. The B7-H1/PD-1 molecular pathway is a primary mechanism of cancer immune evasion for several reasons. First, and most importantly, this path-
way is involved in negative regulation of immune responses, particularly in peripheral tissues where cancers often originate and grow. Second, B7-H1 is upregulated in cancer microenvironments, while PD-1 is also upregulated on activated tumor infiltrating T cells, thus possibly potentiating a vicious cycle of inhibition. Third, this pathway is intricately involved in both innate and adaptive immune regulation through bi-directional signaling. These factors make the PD-1/B7-H1 molecular pathway a central point through which cancer can manipulate immune responses and promote its own progression. In other words, manipulation of the B7-H1/PD-1 molecular axis by cancers results in “good genes” gone bad.

**Tumor Evasion via B7-H1 Expression in the Cancer Microenvironment**

B7-H1 mRNA is found in virtually all tissues and nucleated cells examined to date, while B7-H1 protein expression appears limited [21,22]. Studies demonstrate that B7-H1 can be broadly induced on lymphoid and non-lymphoid cells alike following stimulation with type I (α, β) and type II (γ) IFNs [37,38]. Numerous studies have demonstrated the presence of cell surface B7-H1 in murine and human tumors, and expression of B7-H1 has been linked to poor clinical outcomes in a variety of cancers [16]. These results suggest that B7-H1 expression may be a primary mechanism by which cancers subvert immune responses by suppression of PD-1-expressing CD8+ T cells.

B7-H1 is expressed on some murine tumor cell lines, and mouse models have confirmed that B7-H1 over-expression on transplantable tumors impairs anti-tumor immunity. Using transplantable and systemic tumor models including P815 mastocytoma, SCCVII squamous cell carcinoma, B16 melanoma, C1498 AML, Panc02 pancreatic cancer, and others, it became evident that B7-H1 expression significantly enhances tumor growth and causes apoptosis of tumor-specific T cells [35,36]. Meanwhile, mAb blockade of B7-H1 ligation to PD-1 in these models permits cytotoxic activity and reverses tumor growth.

An analysis of human tumor cell lines found that few have baseline expression of B7-H1. However, immunohistochemical analysis of freshly isolated tumor samples from patients with ovarian, lung, and breast cancers, renal cell carcinoma, squamous cell carcinoma of the head and neck, esophageal carcinoma, glioblastoma, thymoma, colon carcinoma, and melanoma found that the vast majority express B7-H1 [35,36]. In one study, 22 out of 22 melanoma samples examined stained positively for B7-H1, with the majority of samples showing expression in at least 40 percent of cells [37,38]. Importantly, these findings illustrate that B7-H1 is widely expressed in cancer cell and cancer microenvironments, yet expression remains heterogenous [36]. The underlying mechanism for the “compartmentalization” of B7-H1 expression remains unclear.

Cytokines such as IFN-γ in the tumor environment are believed to be a main driver of the expression of B7-H1, though the overall mechanism of B7-H1 upregulation is yet to be elucidated. Most human and mouse tumor cell lines examined are capable of quickly upregulating B7-H1 following incubation with IFN-γ [37,38]. Studies of glioblastoma cell lines have correlated increased B7-H1 expression to the loss of the tumor suppressor gene Phosphatase and Tensin Homology (PTEN), a negative regulator of the intracellular signaling molecule PI3K [39]. Expression of B7-H1 has been correlated with poor clinical outcomes, including decreased survival, in a number of human cancers, including esophageal, ovarian, pancreatic, and renal cell carcinoma (RCC) [16]. For example, patients with RCC tumors expressing B7-H1 were at greater risk of metastatic dissemination, and their risk of death was nearly four times greater than patients with B7-H1-negative tumors [40]. These studies further support a role for the B7-H1/PD-1 pathway in subversion of tumor immunity and point to B7-H1 expression as a useful biomarker to identify patients at greater risk of metastatic disease progression and death. Moreover, this knowledge supports the development of therapies to block this pathway in order to
rouse seemingly dormant immune responses against cancers.

**MONOCLONAL ANTIBODY IMMUNOTHERAPY TARGETING PD-1 AND B7-H1**

Cancer immunotherapy is a rapidly developing field as immunologists continue to elucidate how the immune system responds to and is subverted by cancers. A variety of immunotherapeutic modalities are currently being developed, including mAbs and small molecules. However, advantages of mAb immunotherapy include target specificity and affinity, persistence in vivo, and secondary function of mAbs through the crystalline fragment (Fc) and glycosylation effects [41]. Additionally, engineering for therapeutic optimization, increasing ease of production, and reductions in cost of production of mAbs have progressed rapidly in the past 10 years, as reviewed in detail elsewhere [42]. Mechanisms through which immunotherapeutic mAbs may function include antagonism (physical blockade), agonism (signal induction through antibody cross-linking of receptors), antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), toxin conjugation to Fc portions of mAb, and bi-specific mAbs, which simultaneously target two epitopes. These mechanisms are reviewed in detail elsewhere [41].

CSSMs make ideal targets for mAb immunotherapy because they are broadly accessible for mAb binding and typically have very specific functions in immune modulation. Antagonist mAbs targeting inhibitory CSSMs such as PD-1 and B7-H1 promote immune activation against cancer, which results in the generation of immune memory and, consequently, a durable response against cancer, which is of critical importance in immunotherapeutics [23]. By contrast, small molecule inhibitors targeting intracellular signaling proteins in cancer cells, blood vessels, or stromal components (e.g., vemurafenib, an inhibitor of the serine-threonine kinase B-Raf, a protein found mutated in some cancers) efficiently suppress growth or cause death of cancer cells but often do not eliminate all cancer cells due to acquired or innate resistance and do not directly stimulate adaptive immune responses [43]. Thus, immune escape and cancer progression are likely without adaptive immune memory generation.

**Lessons Learned from Murine Tumor Models**

As discussed previously, expression of B7-H1 on human cancer cells and in cancer microenvironments has been shown to correlate with their growth and progression. Several mouse models confirmed that B7-H1 overexpression by transfection on transplantable tumors impairs anti-tumor immunity, while blockade of B7-H1 by mAb enhanced tumor immunity [35,36]. Moreover, growth of J558L myeloma tumors, which naturally express high levels of B7-H1, was significantly reduced in PD-1 deficient mice, as well as in normal mice following B7-H1 blockade [44]. In other models, it was shown that B7-H1 blockade reversed the suppressive phenotypes of both myeloid and plasmacytoid DCs derived from both human and murine tumor environments [45,46]. It is interesting to note that in the majority of these models, effector CD8+ T cells were required for the anti-tumor effect. As such, it was shown that adoptive transfer of tumor draining lymphocytes to treat established murine tumors was also enhanced by B7-H1 blockade [47].

**Additional Modes of B7-H1 Action**

Interestingly, B7-H1 expression on cancer cells may induce intrinsic resistance to T cell attack upon binding by PD-1, resulting in a so-called molecular shield against CD8+ T cell mediated cytotoxicity [31]. B7-H1 can deliver an anti-apoptotic signal into cancer cells that prevents Fas-mediated apoptosis [31]. In this model, it was shown that cancer cells transfected with the full length B7-H1 molecule conferred cancer cell resistance to lysis by antigen-specific CD8+ T cells, while cancer cells transfected with B7-H1 lacking the cytoplasmic tail lost resistance to CD8+ T cell mediated lysis.
Therefore, B7-H1 may act as a molecular shield on cancer cells to prevent killing by effector immune cells but could possibly also provide resistance to other types of anticancer agents through cell intrinsic mechanisms. Regardless of the mechanisms governing B7-H1/PD-1 bi-directional interactions, blockade by either PD-1 or B7-H1 mAbs should effectively prevent both suppressive signals to T cells through PD-1 and anti-apoptotic signals through B7-H1 on cancer cells. It has been shown that B7-H1 also can bind to B7-1 on activated T cells, thus conferring B7-H1 with additional roles in modulation of immune function [32,33]. In a series of in vitro studies, one group showed that B7-H1 specifically interacts with B7-1 to inhibit T cell activation and proliferation [32,33]. Interestingly, either B7-1 or B7-H1 expressed on the T cells has an inhibitory effect after receiving a signal from plate-bound (cross-linking) B7-H1Ig or B7-1Ig protein, respectively [32,33]. We have recently demonstrated that B7-H1 delivers an inhibitory signal through B7-1 on T cells to induce T cell anergy in a mouse model [32,33]. Specific mAb blockade of the B7-H1/B7-1 interaction was capable of both preventing tolerance induction of naive T cells and restoring antigen responsiveness to previously anergized cells [32,33]. Thus, the role of B7-H1 in T cell function is multi-dimensional.

The effects of B7-H1/B7-1 interactions in the suppression of tumor immunity are not fully understood and will need to be defined by future experiments. However, the discovery of the B7-H1/B7-1 interactions has important implications for designing mAb immunotherapy strategies. The data suggest that PD-1 mAb blockade on T cells alone may not be sufficient to eliminate all the suppressive effects of B7-H1, while B7-H1 mAbs with the capacity to block B7-H1 binding to both PD-1 and B7-1 may be effective in enhancing tumor immunity. However, B7-H1 mAbs will not block B7-DC interactions with PD-1, which is also potentially suppressive for T cell responses, although the biological significance of B7-DC/PD-1 interactions remains unclear at this time [35,36]. The complexity of these molecular interactions suggests that B7-H1 or PD-1 blockade alone may be insufficient to completely block inhibitory pathways and that a combination of both B7-H1 and PD-1 blockades may be optimal for cancer immunotherapy.

Clinical Outcomes with mAbs to Block the B7-H1/PD-1 Pathway

Currently, antagonist mAbs against both PD-1 and B7-H1 are in various stages of development for the treatment of cancer, and recent human trials have shown promising results in cancer patients with advanced, treatment-refractory disease.

The first of the agents blocking the B7-H1/PD-1 pathway to enter phase I clinical trials was MDX-1106 (BMS-936558/ONO-4538), a fully human IgG4 anti-PD-1 mAb developed by Bristol-Myers Squibb [48]. In this multi-center trial, small cohorts of patients were administered a single dose of 0.3, 1.0, 3.0, or 10 mg/kg. Limited re-treatment was allowed for patients with stable disease or with tumor regression suggestive of clinical benefit. Re-treatment was given as two doses spaced 4 weeks apart at intervals of 3 months, for up to six doses of MDX-1106. The study included 39 patients with advanced metastatic melanoma, non-small-cell lung cancer, renal cell carcinoma, castration-resistant prostate cancer, and colorectal cancer. Because anti-PD-1 and other immunotherapies release a brake on the immune system, particular concerns were raised about potential autoimmune adverse events. These concerns came from clinical experience with anti-CTLA-4 mAbs (ipilimumab, Bristol-Myers Squibb; and tremelimumab, Pfizer), which induce moderate to severe autoimmune-type side effects including dermatitis, enterocolitis, hepatitis, endocrinopathies (including hypophysitis, thyroiditis, and adrenalitis), arthritis, uveitis, nephritis, and aseptic meningitis in up to 30 percent of patients [49,50]. In contrast to the anti-CTLA-4 experience, initial trial results for anti-PD-1 showed that it was safe, well-tolerated, and induced a low rate
of autoimmune-type side effects [51]. Only one patient in the initial phase I MDX-1106 trial developed inflammatory colitis, which was treated with steroids and anti-TNF therapy.

Phase I studies are typically performed to assess the safety, toxicity, and pharmacodynamics of a therapy and are generally not designed to determine clinical activity. Nevertheless, antitumor activity was evident in the initial MDX-1106 trial. Two patients (melanoma, non-small cell lung cancer) experienced regression in several metastatic lesions with progression at other sites (“mixed response”), two patients (melanoma and renal cancer) had substantial regression at all sites (“partial response”), and one patient with metastatic colorectal cancer experienced a total remission with disappearance of all lesions (“complete response”). These responses to MDX-1106 proved to be durable, persisting up to 21+ months post-treatment [48].

A larger on-going phase I trial was initiated to determine the safety and maximum tolerated dose (MTD) of MDX-1106 administered IV twice weekly at doses of 1, 3, and 10 mg/kg. An MTD was not reached, and subsequently, expanded cohorts of up to 16 patients were accrued at all three dose levels for patients with metastatic melanoma and at the 10 mg/kg dose level for patients with metastatic renal cancer, non-small cell lung cancer, colon cancer, and castrate-resistant prostate cancer. At the time of initial analysis, 106 patients who had progressed on standard therapies had been enrolled [52]. An additional update in January 2011 presented data on 126 patients [53]. Similar to the initial single dose trial, treatment with anti-PD-1 was generally well-tolerated. One treatment-related death due to grade 4 pneumonitis and sepsis was reported; other adverse events occurring at low frequency included liver function test elevations, endocrinopathies, and fatigue. This study also found evidence of substantial antitumor activity and durable responses in patients with advanced disease. Among patients with metastatic melanoma, 15 of 46 patients experienced at least a partial response, for an overall response rate of 32.6 percent [54]. In metastatic renal cancer, as of January 2011, seven of 19 (37 percent) patients treated in both MDX-1106 trials had achieved a partial or complete response. Many of the responses to MDX-1106 have been durable, and activity was also observed in patients with non-small cell lung cancer. An additional phase I combination study sponsored by the National Cancer Institute (NCI) of MDX-1106 with ipilimumab for unresectable malignant melanoma has also been initiated. Overall, these trial results are remarkable and provide compelling proof of concept that inhibition of the B7-H1/PD-1 coinhibitory pathway can produce meaningful anti-cancer activity in patients with multiple types of advanced cancer.

Another PD-1 mAb undergoing clinical evaluation is CT-011, a humanized IgG1 mAb specific for PD-1 developed by CureTech Ltd. [55]. A phase I study of 17 patients with advanced hematologic malignancies (including acute myeloid leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma) assessed treatment with one dose of 0.2, 0.6, 1.5, 3.0 or 6.0 mg/kg of CT-011. The agent was well-tolerated, and six patients (33 percent) showed clinical activity from the treatment, including a lymphoma patient experiencing a complete remission lasting 68+ weeks. Additional combination studies of CT-011 with gemcitabine (pancreatic cancer), p53 genetic vaccine (advanced solid cancers), and rituximab (lymphoma) have been initiated.

Fully humanized anti-B7-H1 mAb (MDX-1105) developed by Bristol-Myers Squibb is currently in phase I trials for advanced melanoma, non-small cell lung carcinoma, renal cell carcinoma, and ovarian cancer. Results have not been presented to date.

Enhancing B7-H1 and PD-1 mAb Immunotherapy

The PD-1/B7-H1 molecular pathway is a negative cosignaling pathway that functions as a cellular checkpoint to suppress ongoing inflammatory and immune responses. Therefore, simply blocking this pathway
does not stimulate a de novo immune response. In other words, blockade of this inhibitory pathway lifts the tollbooth gate (i.e., access to cancer cells) and may release the brake on the vehicle (i.e., exhausted effector CD8+ T cells), but it will not accelerate the car through the gate. Pressure on the gas pedal via stimulation such as enhanced TCR signaling and/or costimulatory signaling is also required. Therefore, a combined approach to modulate stimulatory pathways should significantly improve mAb immunotherapeutic outcomes [35,36].

CD137 (4-1BB) is a costimulatory molecule found on T cells that promotes cellular survival and proliferation [35,36]. Several murine transplantable tumor models have now demonstrated that B7-H1/PD-1 pathway blockade in combination with agonist anti-CD137 (4-1BB) mAbs induces significantly enhanced tumor regression when compared to either mAb treatment as a single modality [35,36]. Additionally, a number of other agonist mAbs against costimulatory molecules, such as OX40, CD40, GITR, CD28, and ICOS, may potentially synergize with PD-1/B7-H1 blockade [56,57].

An additional strategy for combination mAb immunotherapy might be termed “multiple brake release therapy” (MBRT), in which several inhibitory molecules are simultaneously targeted for mAb blockade. For example, several studies have investigated PD-1 blockade in combination with blockade of CTLA-4, Tim-3 or Lag-3, all of which are inhibitory CSSMs [58-62]. However, results using MBRT strategy in animal models are less dramatic overall than in combination therapy with costimulatory agonists, as the vehicle will not proceed through the raised tollgate without (stimulatory) fuel for acceleration. Nevertheless, as demonstrated by the anti-PD-1 clinical data, in a significant subset of humans, extensive tumor regression can be achieved by blockade of only coinhibitory signals. Therefore, MBRT may prove to be more effective in patients than in animal models. Consequently, it is possible that a significant number of patients will respond to blockade of a single or multiple coinhibitory signals, while another subset will require both agonist and antagonist mAb cocktails for the best results.

**CONCLUSIONS AND OUTLOOK**

Monoclonal antibody immunotherapeutic abrogation of the PD-1/B7-H1 pathway can profoundly reverse immune inhibition and permit T cell effector function against a broad range of cancers. Questions remain, however, about the mechanisms of action of this molecular axis. Still unclear is the true nature of how T cell exhaustion and deletion occurs and the precise role of PD-1 in the differentiation of this CD8+ T cell subset. The role of PD-1 on CD4+ T cells also remains largely undefined. Questions remain about the effects of PD-1 blockade versus B7-H1 blockade in clinical immunotherapy due to each molecule’s specific expression pattern and function. Moreover, because B7-DC and B7-1 play a significant role in this cosignaling axis and because secondary receptors for B7-H1 and B7-DC have yet to be identified, we are only just beginning to understand the complex interplay of this system. Nevertheless, it is clear that this molecular pathway plays an important role in normal immune modulation and that cancers evade immune activation and anti-tumor immune function by preferentially engaging this cosignaling axis.

Additionally, evidence in murine models suggests that immunotherapy targeting the PD-1/B7-H1 inhibitory pathways in combination with agonist mAbs targeting stimulatory pathways will result in greatly enhanced anti-tumor immune activation. Therefore, as more mAbs move into clinical trials, it is important to keep in mind that even though some immunotherapies may have lackluster performance as single agents, more striking effect may be found in combinatorial therapies.

The human body is in a constant battle for survival against myriad external and internal threats. In the battle against cancer, the normally “good genes” of the B7-H1/PD-1...
molecular axis are manipulated by cancer to its own advantage, much to the detriment of the patient. Targeting this subverted pathway using mAb immunotherapy will allow us to reverse the malignant outcome promoted by good genes gone bad in cancer microenvironments, while maintaining normal immune function in healthy environments. Anti-B7-H1/PD-1 mAb immunotherapies demonstrate significant progress in the war on cancer and should soon become a powerful addition to the oncologist’s toolbox.

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