

Clinical Experiences With Anti-CD137 and Anti-PD1 Therapeutic Antibodies

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Monoclonal antibodies (mAbs) provide a pharmacological platform to block or activate the function of surface receptors. The immune system has evolved receptor-ligand pairs that repress or empower the cellular immune response, which, if tampered with, unleash more potent cellular immunity against tumor antigens. Agonist antibodies directed against CD137 (4-1BB) on the surface of antigen-primed T lymphocytes increase tumor immunity that is curative against some transplantable murine tumors. A fully human IgG4 anti-CD137 antibody is under development with signs of clinical activity and cases of severe liver toxicity that seem to be on-target and dose-dependent effects. Programmed death-1 (PD1) is a surface molecule delivering inhibitory signals important to maintain T-cell functional silence against their cognate antigens. Interference with PD1 or its ligand PD-L1 (B7-H1) increases antitumor immunity. As a result anti-PD1 and anti-PD-L1 human mAbs are under clinical development. Phase I trials with anti-PD1 mAb have yielded encouraging results with durable objective responses and a reasonable safety profile. As new class of drugs in cancer therapy, immunostimulatory mAbs have resulted in redefinition of tumor response criteria and rethinking of the rationale for combining these among each other and with other strategies.

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The tumor microenvironment is the battlefield between immunostimulatory and immunosuppressive forces. Integration of positive and negative signals to lymphocytes is the basis for the manifest immune response against tumor cells. The cytotoxic T-lymphocyte antigen-4 (CTLA-4) is the first example of an indirect immune target against cancer. Mouse and human results show that, by removing the immune-restraining effects due to CTLA-4 binding to B7, it is possible to unleash an effective immune response against neoplastic cells.

Following the promising results of the anti-CTLA-4 anticancer therapy,^{1,2} there is a growing interest about

other receptors or molecules that may be potentially targeted by monoclonal antibodies (mAbs) to enhance antitumor immunity.³ There are two possible types of target: molecules important for checkpoint blockade of the immune cells, and molecules active as immunostimulatory receptors.

This is a review of the current literature regarding two interesting mAbs: the first is directed against CD137 (with a stimulatory agonist or supra-agonist effect that mimics the natural ligand CD137L) and the second is directed to programmed death-1 (PD1) receptor (with an effect similar to anti-CTLA-4 mAb that is exerted by removing the immune brake and by acting as a pharmacological antagonist of this inhibitory receptor).

ANTI-CD137 ANTIBODY

CD137 (4-1BB) is an inducible T-cell surface molecule that belongs to the tumor necrosis factor (TNF) receptor superfamily. Its binding by the natural ligand (CD137L) or by an agonist antibody provides costimulation in a CD28-independent pathway for CD4⁺ and CD8⁺ T cells.^{4,5} Surface expression of CD137 is detectable on activated effector CD8⁺ and CD4⁺ T cells, and also on activated natural killer (NK) cells, NK/T cells, regulatory T cells (Tregs), dendritic cells (DCs), macrophages, neutrophils, and eosinophils.⁶ Recent data indicate that CD137 also is expressed functionally by endothelial cells in tumor capillaries⁷ (Palazon A, et al,

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submitted). CD137 signaling enhances T cell proliferation and Th1 cytokine production and affords protection to CD8⁺ T cells from activation-induced cell death through nuclear factor- κ B (NF- κ B)-mediated activation, and upregulation of the anti-apoptotic Bcl-2 family members Bcl-xL and Bfl-1.⁸ CD137 is not expressed on resting T and B lymphocytes or on activated B lymphocytes.

T cells require two signals for activation: signal 1 is provided by the interaction of the T-cell receptor (TCR) with major histocompatibility complex (MHC) peptide, while signal 2 is provided by costimulatory pathways like B7-CD28, OX40L-OX40, and CD137L-CD137. Activated/mature antigen-presenting cells (APCs) can effectively activate T cells because of their ability to provide both signals. The CD28 costimulatory molecule appears to be the key for the initiation of T cell responses. However, CD137 signals can provide sufficient costimulation even in the absence of the CD28 pathway,⁹ although in the presence of CD28 signals both receptors act synergistically to promote T-cell activation and proliferation.¹⁰

In both murine and human models several authors showed a critical role for costimulation by 4-1BB in the expansion and differentiation of cytotoxic T lymphocytes (CTLs).¹ T-cell ignorance due to engagement of the TCR without enough costimulatory signal is believed to be partially responsible for immune evasion by tumors. Potentiation of costimulatory signals may enhance immunogenicity of tumors. Stimulation of CD137 on DCs induces secretion of interleukin (IL)-6 and IL-12, enhances the DC's ability to stimulate T-cell responses to alloantigens, upregulates MHC class I and costimulatory molecules, and increases the ability of DCs to infiltrate tumors, as well their survival.¹¹ It has been seen that cross-presentation of tumor antigens by DCs is involved in the antitumor effects of anti-CD137 mAb in mice.¹⁰

Animal models showed that the administration of agonistic anti-mouse CD137 mAb cured mice carrying immunogenic tumors (P815 mastocytoma) or even seemingly poorly immunogenic tumors (AG104A fibrosarcoma). These tumor regressions needed the participation of CD8⁺ and CD4⁺ T cells and of NK cells, and were not due to a direct effect of the anti-CD137 mAb, as neither tumor type expressed CD137 on the surface. In other transplantable tumors, NK and CD4⁺ T cells are dispensable for efficacy, while CTLs are absolutely needed. Whether the antitumor effect of CD137 agonists is exerted on other cells beyond CD8⁺ T lymphocytes is a matter of controversy and might be tumor model-dependent.^{12,13}

Stimulation with agonistic CD137 mAb induced a paradoxical suppression of the humoral responses to T-cell antigens in primates and murine models. Earlier studies showed that 2A, an agonistic mAb specific for mouse CD137,¹¹ inhibited the progression of pre-existing autoimmune diseases.^{14,15} It was proposed that an

enhanced CD137 signal on activated autoreactive lymphocytes might push to activation-induced cell death (AICD).¹⁶ Such treatment was shown to reduce anti-CTLA-4-mediated autoimmunity as a side effect.¹⁷ Further studies in autoimmune diseases using a CD137 agonistic mAb (BMS 469492), demonstrated that it inhibits a variety of autoimmune diseases in mice, suggesting that it may reduce the clinical signs of autoimmune response associated with its anticancer activity in humans. Nonetheless, anti-CD137 mAb induced liver infiltrates of highly polyclonal CD8⁺ T lymphocytes in mice.¹⁸

ANTI-CD137 ANTIBODIES: PRECLINICAL DATA

Most studies focused on two anti-CD137 mAb: BMS 469492 that is an anti-murine CD137 mAb, and BMS 663513 that is a fully human IgG4 mAb against human CD137. BMS 469492 was used for studies in murine tumor models, and it does not cross-react with BMS 663513. Both are agonistic mAbs and do not block the interaction of CD137 with its natural ligand, CD137L. It is of note that mAbs that do block CD137L binding show similar functional antitumor activities when compared to nonblocking mAbs. BMS 663513 provides a costimulatory signal to human T cells causing an increase in interferon (IFN)- γ production and increases their survival. In fact, the functional effects of BMS 663513 were demonstrated on human and monkey T cells and peripheral blood mononuclear cells (PBMCs) where IFN- γ production was enhanced compared to controls.³

BMS 469492 was studied as a single agent in the P815 mastocytoma tumor models in mice.¹⁹ Doses of mAbs between 2.5–10 mg/kg administered weekly for 3 weeks demonstrated complete tumor regressions. P815 cells express high levels of MHC class I, while CD137 is expressed below detectable levels. Fifteen of 15 treated mice, rejected the tumor graft when they were rechallenged with live tumor cells about 2 months after the first dose of antibody. These results showed that a memory immune response induced by BMS 469492 protected mice from further tumor growth.¹⁹

Preclinical Data in Mice

The effect of BMS 469492 was further studied with tumor lysate-pulsed dendritic cells (TP-DCs) in the B16-BL6 murine melanoma model. BMS 469492 or TP-DCs alone had no antitumor activity against 3-day established metastases.²⁰ However, the combination produced significant tumor regression in which CD8⁺ and CD4⁺ T cells and NK cells played a role.²⁰ In addition, anti-CD137 mAb was synergistic with other immunostimulatory mAbs,^{21,22} adoptive T-cell therapy,²³ and conventional chemo- and radiotherapy.²⁴ The antitu-

mor effect of BMS 469492 is mediated by the induction of IFN- γ synthesis. In fact, administration of a neutralizing anti-IFN- γ antibody reduced the antitumor effects of BMS 469492. IFN- γ is produced locally in the tumor by recruited T cells and it remains unmeasurable in serum; noteworthy, its exogenous administration did not show antitumor activity in a tumor model.¹⁹

As mentioned earlier, the most exciting feature of CD137 immunostimulation therapy is that it provides strong synergistic effects with many types of therapies at the preclinical level. Among these are chemotherapy, radiotherapy, adoptive T-cell therapy, vaccines, cytokines, and other immunostimulatory mAbs.³ Furthermore, very recent studies reported that anti-CD137 mAbs induced multifocal mononuclear cell infiltrates in the liver of BALB/c and C57BL/6 mice. These infiltrates were composed primarily of CD8⁺ T cells having a diverse TCR V α - β phenotypes. C57BL/6 mice also displayed evidence of dysregulated hematopoiesis in the spleen, lymphocyte trafficking, lymphopenia, thrombocytopenia, and anemia in an anti-CD137 dose-dependent manner. All of these outcomes were T-cell- and anti-CD137-dependent because they did not occur in SCID, nude, Rag^{-/-} (or rat IgG-deficient), or CD137^{-/-} mice. Anti-CD137-treated, spleen cell-reconstituted, but not B-cell-reconstituted, Rag^{-/-} mice had the same outcomes as observed in naive BL/6 mice, and identical results were obtained in anti-CD137-injected 4-1BB ligand^{-/-} mice. CD8⁺ T cells, TNF- α , type I IFNs, and IFN- γ were essential for the induction of various aspects of pathology. These findings suggest that anti-CD137-induced activation leads to the production of proinflammatory cytokines, and they in turn induce many, if not all, of the observed abnormalities found in anti-CD137-treated mice.²⁵ These outcomes have been attributed to enhanced CD8⁺ T-cell survival, induction or suppression of CD4⁺ T-cell help, CD8 regulatory T cells, Th1 cytokine production, enhanced CD8⁺ T-cell and NK cell function, or regulation of antigen (Ag) priming by DCs.²⁶

Anti-CD137 mAb may effectively suppresses T-dependent humoral immunity.²⁷ This observation led to the testing of, and the realization that, anti-CD137 mAbs could suppress and reverse the development of autoimmune diseases, such as experimental autoimmune encephalomyelitis, systemic lupus erythematosus, and collagen-induced arthritis.

Preclinical Data in Monkeys

While studies in mice demonstrated most of the mechanisms of anti-CD137 mAb activity *in vivo*, those in monkeys have been important to test their tolerability. BMS 663513 was studied in cynomolgus monkeys that were immunized by intramuscular injections of both prostate-specific antigen (PSA) and simian immunodeficiency virus (SIV) gag DNA constructs, followed

by BMS 663513. Enzyme-linked immunospot (ELISPOT) analyses of IFN- γ production by monkey PBMCs showed a consistent immune response, with an increased number of antigen-specific IFN- γ -secreting cells when BMS 663513 was added to the vaccine. BMS 663513 increased 1.7-fold the quantity of IFN- γ released, suggesting a possible modulation by anti-CD137 mAb in augmenting the effectiveness of DNA vaccines.³

ANTI-CD137 TOXICOLOGY

Several studies with BMS 469492 in mice and with BMS 663513 in monkeys showed a good tolerability of these two mAbs *in vivo*. The administration of BMS 469492 at doses of 10 or 50 mg/kg twice weekly for 4 weeks to CD-1 mice resulted in skin and liver toxicity. Skin toxicity consisted of alopecia, scabbing, and scaly areas; liver toxicity was characterized by elevation of AST and ALT with local inflammation and increased mRNA expression of CD137, IFN- γ , and IL-12. These toxicities were time- but not dose-dependent. Liver inflammation was not limited to this specific mAb, since repeated doses of mAb 2A to CD137 also increased liver infiltration and induced liver injury in a Fas-independent fashion (Fu Y, et al, unpublished data, August 19, 2010). In fact, Fas-deficient mice have more severe liver injury after antibody treatment, suggesting that liver injury might be associated with either previous activation of autoreactive T cells or with increased liver infiltration. Which cells inside the liver are involved in such toxicity has not been defined yet. The administration of immunosuppressive agents did not significantly reverse the anti-CD137-mediated liver toxicity.

Recent studies by Niu et al²⁸ demonstrated that although the CD137 signaling pathway is another example in which therapeutic manipulation holds promise for its clinical applicability, such manipulation might lead to unexpected adverse events, and to immune system or organ function alterations. Administration of anti-CD137 mAb at 10 or 0.5 mg/kg, given once weekly over a 5-week period, induced a series of adverse events in normal mice that appeared to be confined to the immune and hematopoietic systems. These occurrences may ultimately adversely affect the normal function of several major organs, including liver, lungs, spleen, and bone marrow (BM). CD137 receptor cross-linking, CD8⁺ T cells, and the production of TNF- α , IFN- γ , and type I IFN were found to be essential for the induction of these adverse events. It is unclear whether T cells are the direct targets of anti-CD137 mAb, or whether they are indirectly affected by cytokines, or chemokines produced by other lineages of CD137-expressing cells.

Liver-infiltrating CD8⁺ T cells and TNF- α were essential for the induction of hepatitis, and the absence of either of them prevented the disease in anti-CD137-

injected mice. These activated cells were not likely responding to liver-associated antigens. These observations were inconsistent with an autoimmune involvement, and in favor of cytokine-induced inflammation as the primary cause of liver pathology. In fact, anti-CD137-injected mice recover normal liver function following cessation of treatment. Studies in IFN- γ , TNF- α , and type I IFN receptor (IFNR)-deficient mice concluded that TNF- α and CD8 $^{+}$ T cells are critical to the induction of splenomegaly, lymphadenopathy, hepatomegaly, and multifocal hepatitis, because none of these adverse events developed in their absence. In contrast, IFN- γ and type I IFNs appeared to be essential for T- and B-cell trafficking, and for the 10-fold increase in the number of bone marrow hematopoietic cells, because these events were not observed in type I IFNR $^{-/-}$ mice and IFN- γ $^{-/-}$ mice, and were markedly reduced in anti-CD137-treated wild-type mice injected with neutralizing IFN- γ mAb.

Further studies are important to clarify if all the adverse events seen in mice occur in nonhuman primates and in humans. In fact, previous studies in monkeys showed that the administration of BMS 663513, either at single high doses (10, 30, 100 mg/kg) or at multi-doses of 1, 10, or 100 mg/kg once weekly for 4 weeks, was tolerable. There were no clinical changes, nor skin and liver lesions as previously seen in mice.³

CLINICAL STUDIES WITH ANTI-CD137 ANTIBODIES

Table 1 summarizes the clinical studies using anti-CD137 mAb as an anticancer agent.

The NCT00309023 study was an open-label, ascending, multi-dose phase I-II study conducted in subjects with locally advanced or metastatic solid tumors.²⁹ In the dose-escalation phase of the study, subjects were sequentially assigned to one of six dose-cohorts (0.3, 1, 3, 6, 10, or 15 mg/kg) to receive BMS 663513 as a 60-minute intravenous (IV) infusion once every 3 weeks. In the dose-expansion portion of the study, only subjects with melanoma, renal cell carcinoma (RCC), or ovarian carcinoma were eligible. Patients stratified by tumor types were randomized to receive one of three doses (1, 3, or 10 mg/kg) of BMS 663513. Fifty-four melanoma patients were treated. The reported toxicity was manageable, with fatigue, transaminitis, neutropenia, rash, and diarrhea as the most common adverse events.²⁹ For 106 of the total 115 patients enrolled, the most frequent grade ≥ 2 laboratory abnormalities were increases in ALT (15%) and AST (12%), leukopenia (8%), neutropenia (6%), thrombocytopenia (4%), and hyperbilirubinemia (<1%).²⁹ This study showed clinical activity that justified further development of BMS 663513, both as a single agent and in combination, across a wide range of doses. However, in this first human study an optimal dose and schedule for BMS 663513 was not identified. Clinical activity represented by partial remissions and sustained stable diseases was observed in the 1-, 3-, and 10-mg/kg groups. More toxicity was shown at higher doses (>6 mg/kg). Results of biomarkers studies (activated T-cell markers, RNA expression, and serum biomolecules) indicated that BMS 663513 elicited biological activity with doses as low as 0.3 mg/kg.²⁹

NCT00309023 has provided the rationale for developing a phase II randomized study in previously treated melanoma patients with stage IV disease (NCT00612664).

Table 1. Anti-CD137 Clinical Studies

Status	Study	NTC Identifier	Phase	Condition
Terminated	A study of BMS-663513 administered in combination with chemotherapy to subjects with advanced solid malignancies	NCT00351325	Phase I	Advanced solid malignancies
Terminated	A study of BMS-663513 in combination with chemoradiation in subjects with non small cell lung carcinoma (NSCLC)	NCT00461110	Phase I/ phase II	NSCLC
Terminated	Study of BMS-663513 in patients with advanced cancer	NCT00309023	Phase I	Solid tumors
Withdrawn	Combination of anti-CD137 and ipilimumab in patients with melanoma	NCT00803374	Phase I	Melanoma
Completed	Phase II, second-line melanoma—RAND monotherapy	NCT00612664	Phase II	Melanoma

The primary endpoint was progression-free survival (PFS) at 6 months in each arm. This trial was a randomized, multi-dose, open-label, phase II study of BMS 663513 as second-line monotherapy in metastatic melanoma. There were four arms: arm 1, 0.1 mg/kg every 3 weeks; arm 2, 1 mg/kg every 3 weeks; arm 3, 1 mg/kg every 6 weeks; and arm 4, 5 mg/kg every 3 weeks.³⁰ The study was terminated in May 2009 due to unusually high incidence of grade 4 hepatitis.³¹

ANTI-PD1 ANTIBODY

PD1 (CD279) is an inhibitory co-receptor expressed on antigen-activated T cells, on antigen-specific T cells that are chronically exposed to antigen, and on B cells. It belongs to the CD28 family and has two known ligands: B7-H1/PD-L1 (hereafter B7-H1), the predominant mediator of PD1-dependent immunosuppression, and B7-DC/PD-L2. PD-L1 is expressed on hematopoietic cells and can be upregulated upon their activation. PD-L1 is also found in tissues such as pancreatic islets, heart, endothelium, small intestine, and placenta. Tumor cells that express PD-L1 use this pathway as a mechanism to evade recognition/destruction by the immune system. PD-L2 expression is restricted only to macrophages and DCs and it is also upregulated upon their activation. In murine tumor models, B7-H1 expression confers immune resistance, and interrupting PD1:B7-H1 interactions has antitumor effects. B7-H1 is highly upregulated in many murine and human tumors (either on tumor cells or on nontransformed cells in the tumor microenvironment such as APCs), and its expression is associated with a poor outcome for patients with certain epithelial cancers.³²⁻³⁶ It shows homology to CTLA-4 but provides distinct immune-inhibitory signals. In contrast to early lethality in CTLA-4 knockout mice, PD1 knockouts demonstrate modest late-onset strain- and organ-specific autoimmunity. In fact, PD1 knockout mice develop autoimmune glomerulonephritis and arthritis, which is milder compared to that observed in CTLA-4 knockout mice in which multi-organ autoimmunity is observed within the first few weeks from birth. Suppression of effector T-cell function by PD1 engagement includes deletion/apoptosis, inhibition of proliferation, and production of cytokines such as IL-2 and IFN- γ , and, together with chronic antigen exposure, results in T-cell exhaustion. These data suggest that targeting PD1 may augment tumor-specific T-cell responses. On the basis of these considerations, PD1 blockade has been studied as a possible strategy for cancer immunotherapy.

ANTI-PD1 ANTIBODIES: PRECLINICAL DATA

Some preclinical studies focused on the combination of cellular immunotherapy and anti-PD1 mAb. A murine anti-PD1 mAb (4H2) was developed to study its

effect on murine ligands. This antibody showed ability to inhibit the interaction between mouse PD1 and its ligand PD-L1 or PD-L.³⁷ The variable (V) region sequences of this antibody were determined, and VH and VK sequences were grafted into the murine IgG1Fc region and the murine Cn constant region, respectively, to generate an antibody with reduced binding to mouse Fc receptors. Chinese hamster ovary cell lines that express the chimeric antibody were selected and used for production of the antibody.³⁷ In vivo studies have evaluated the combination of a granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting tumor cell vaccine immunotherapy with this murine anti-PD1 mAb in the poorly immunogenic B16 melanoma and the immunogenic CT26 colon carcinoma tumor models. These two cell lines express low baseline levels of PD1 and PD-L1 both in culture and in vivo. In the 3-day therapy setting, the combination resulted in the survival of 50% and 90% of animals bearing tumors in the B16 and the CT26 tumor models, respectively. Treatment with the anti-PD1 mAb enhanced the effectiveness of the GM-CSF-secreting tumor cell vaccine immunotherapy by significantly increasing the number, potency (as measured by augmented proinflammatory cytokine secretion), and duration of activity of tumor-specific cytolytic T cells. A rapid and persistent infiltration of effector CD8⁺ T cells in the tumor microenvironment was also observed.³⁷ Furthermore, multiple cycles of the combined treatment were required to expand the number of antigen-specific T cells initially primed by the combination therapy. These studies showed that monotherapy with an anti-PD1 antibody did not provide therapeutic benefit to animals bearing poorly immunogenic tumors such as the B16 melanoma but showed some activity in highly immunogenic tumor models such as the CT26 model. However, the addition of the anti-PD1 mAb significantly enhanced antitumor responses induced by the GM-CSF-secreting tumor cells by potentiating tumor-reactive T-cell responses; the combination also produced a prolonged survival of tumor-bearing animals.³⁷

Another important combination of anti-PD1 mAb studied in murine models was with anti-CTLA-4 mAb.³⁸ Previous reports have demonstrated that CTLA-4-blocking antibodies promote T-cell activation and render T-effector cells resistant to Tregs, whereas PD1/PD-L1 blockade resulted in loss of peripheral tolerance.³⁸

Mangsbø et al³⁹ explored single or combined antibody blockade of CTLA-4 and PD1 alone or combined with the toll-like receptor agonists CpG or bacillus Calmette-Guérin for the treatment of murine experimental bladder cancer. In therapeutic studies, tumors were rejected by anti-CTLA-4 mAb, while anti-PD1 mAb suppressed tumor growth. The combination had no additive effect compared with anti-CTLA-4 alone. However, elevated levels of circulating CD107a-expressing CD8⁺ T cells were found in the anti-CTLA-4

plus anti-PD1 group.³⁹ In addition, levels of antinuclear antibodies correlated inversely with tumor size. When CpG or bacillus Calmette-Guérin was combined with anti-CTLA-4, anti-PD1, or anti-PD-L1, it was found that CpG in combination with anti-CTLA-4 or anti-PD1 increased the survival of mice, with anti-PD1 plus CpG being superior to either agent alone. CpG plus anti-CTLA-4 or anti-PD1 increased the numbers of circulating tumor-specific CD107a-expressing CD8⁺ T cells, as well as of activated (CD25⁺FoxP3⁺) CD4⁺ splenocytes.³² Further, the authors investigated the number of Tregs in the tumor area of treated animals, detecting decreased levels after anti-CTLA-4 or anti-PD1 plus CpG therapy. Thus, the combination of CpG with CTLA-4 or PD1 blockade improved long-term survival and led to increased levels of tumor-reactive T cells and reduced numbers of Tregs at the tumor site.³⁹

Pilon-Thomas et al⁴⁰ demonstrated, in vitro and in vivo, the blocking of PD-L1 signaling as a means to enhance combined immunotherapy approaches against melanoma. In vitro blockade of PD-L1 interaction on DCs led to enhanced IFN- γ production and cytotoxicity by antigen-specific T cells. In vivo, the systemic administration of anti-PD-L1 mAb plus melanoma peptide-pulsed DCs resulted in a higher number of melanoma peptide-specific CD8⁺ T cells, but this combination was insufficient to delay the growth of established B16 melanoma. Although the addition of 600 rad of total body irradiation delayed tumor growth, further adoptive transfer of antigen-specific CD8⁺ T cells was needed to achieve tumor regression and long-term survival of treated mice. Lymphopenic mice treated with anti-PD-L1 mAb demonstrated increased activation and persistence of adoptively transferred T cells, including a higher number of CD8⁺ T cells infiltrating the tumor mass.⁴⁰

Other studies explored the role of anti-PD1 in hepatoma and pancreatic carcinoma models.^{41,42} In an experimental murine hepatoma model, the synergistic effects and mechanisms of 4-1BBL and soluble PD1 (sPD1) in tumor therapy were investigated.³⁶ Mice were inoculated intramuscularly (IM) with 5 x 10⁵ H22 tumor cells in the right hind leg to establish the experimental hepatoma and were then randomly divided into five groups (12 mice in each group). The mice in groups A, B, C, and D were injected with normal saline, plasmid pcDNA3.1, plasmid p4-1BBL, and plasmid pPD1A, respectively. The mice in group E, the combinatorial treatment group, were injected with plasmid p4-1BBL and pPD1A together. Either transfection with the 4-1BBL gene alone or with the sPD1 gene alone could inhibit tumor growth to some extent, but a more significant anticancer effect was obtained in the combinatorial treatment group (group E), in which the tumors were completely inhibited in 42% of the mice, compared with zero in the other groups. In addition, the survival rate of mice in group E was 100%, compared with 30% in group B, 65% in group C, and 62% in

group D. The fluorescence-activated cell sorting (FACS) analysis results showed that the expression level of B7-H1 and B7-DC on residual tumor cells in group C (injected with p4-1BBL alone) was higher than that on cells in the other groups. The density of CD8⁺ T cells in the peritumor tissue of group E was significantly increased. This study demonstrated the combinatorial treatment with 4-1BBL and sPD1 could produce a positive synergistic antitumor effect in a murine experimental hepatoma model.⁴¹

In a murine pancreatic cancer model, the therapeutic efficacy of blocking the PD-L1/PD1 pathway was investigated. It was shown that both anti-PD1 and anti-PD-L1 mAbs had significantly inhibited tumor growth, suggesting that the PD-L1/PD1 pathway was critical for growth of pancreatic cancer.³⁶ The results also confirmed that PD-L1 blockade promoted tumor-reactive CD8⁺ T-cell infiltration into the established tumor, thereby resulting in local immune activation as evidenced by the upregulation of several potent effectors including IFN- γ , granzyme B, and perforin. More importantly, data indicated that the combination of gemcitabine with PD-L1 blockade exerted a synergistic antitumor effect on pancreatic cancer.³⁶

The block of PD1 immunosuppression through the engagement of its ligand B7/H1 also has been shown to be crucial in other preclinical studies. B7/H1 is normally expressed by hematopoietic and parenchymal tissue but also constitutively by some tumor cells, in particular, melanoma, kidney, ovary, lung, and colon. The mechanisms of B7-H1/PD1 mediated immunosuppression include apoptosis, anergy, and exhaustion of recently activated effector or memory T cells. Hirano et al⁴² showed that B7-H1 expression in certain mouse tumor models confers resistance to immunotherapy, and suggested that B7-H1 on tumor cells and PD1 on T cells may form a molecular shield (B7-H1/PD1) to prevent destruction by CTLs. The authors also examined the effect B7-H1 expression by tumors on antitumor immunity.⁴² Agonistic mAbs to mouse CD137/4-1BB have been shown to induce regression of established P815 tumors in syngeneic mice in which tumor-specific CD8⁺ CTLs play a central role.⁴² To determine the effect of B7-H1 in this therapy, mice were first inoculated subcutaneously (SC) with cells from mock/P815 or B7-H1/P815. By day 7 mice had developed palpable tumors in the range of 3 to 5 mm in mean diameter and were treated with mAb 2A. As expected, tumor regression was observed after treatment in five of five mice inoculated with mock/P815, whereas all mice treated with control rat IgG progressively developed tumors. The B7-H1/P815 tumor cells grew progressively after treatment by control mAb. Treatment with mAb 2A only temporarily delayed tumor growth; eventually, all of the mice developed large, established tumors. There was no survival benefit from mAb 2A treatment for the B7-H1/P815 tumor-

bearing mice, while those with mock/P815 tumors underwent complete tumor regression after mAb 2A treatment. These results indicated that expression of B7-H1 conferred tumor resistance to CD137 costimulatory therapy.⁴²

All of these preclinical studies showed a critical role of PD1 and its ligand B7/H1 in tumor growth. Blockade may prove promising in combination with other antibodies, vaccines, and chemotherapy.

ANTI-PD1 TOXICOLOGY AND CLINICAL STUDIES

At present only phase I trials that have evaluated anti-PD1 in solid tumors as phase II studies are still under evaluation. Table 2 summarizes the current studies with anti-PD1 mAbs as anticancer agents.

A phase I clinical trial of PD1 blockade with the fully human mAb MDX-1106 (BMS 936558/ONO-538, IgG4) specific for human PD1 was conducted in 39 patients with advanced treatment-refractory solid tumors such as non-small cell lung cancer (NSCLC), hormone-refractory prostate cancer (HRPC), melanoma, colorectal cancer (CRC), and RCC.⁴³ The primary objectives were to characterize the safety and tolerability of a single dose of MDX-1106 in patients with selected malignancies, and to determine the maximum-tolerated dose (MTD) and pharmacokinetics. Secondary objectives included assessing antitumor activity, pharmacodynamics, and immunologic endpoints. Sequential cohorts of

six patients received a 60-minute IV infusion of MDX-1106 at 0.3, 1, 3, or 10 mg/kg and were evaluated for toxicities on a weekly basis for 8 weeks. Dose-limiting toxicity (DLT) was defined as a treatment-related grade <3 adverse event or laboratory abnormality occurring >28 days post-dose. Fifteen additional patients were planned for enrollment at the MTD or at the highest planned dose (10 mg/kg) to confirm safety. Tumor assessment according to Response Evaluation Criteria in Solid Tumors (RECIST 1.0) was performed at 8 and 12 weeks. MDX-1106 was well tolerated: no DLTs were observed after one dose, and a MTD was not defined in this study. Immune-related adverse events (irAEs)⁴² were of special interest because of the presumed mechanism of action of anti-PD1 and prior experience with anti-CTLA-4 mAbs. No grade <3 irAEs occurred in the 28-day period following the first dose of anti-PD1. One patient with metastatic ocular melanoma developed grade 3 inflammatory colitis following five doses (1 mg/kg) administered over 8 months, which responded to corticosteroids and infliximab. This study demonstrated the milder toxicity profile for anti-PD1 mAb as compared to that of anti-CTLA-4 mAb. Furthermore, even though efficacy was not an endpoint, evidence of antitumor activity was seen, with one complete remission and two partial remissions in patients with CRC, melanoma, and RCC; significant tumor regressions were seen in two additional patients with melanoma and NSCLC. The observed clinical activity of MDX-

Table 2. Anti-PD1 Clinical Studies

Status	Study	NCT Identifier	Phase	Condition
Recruiting	A phase Ib study of MDX-1106 in subjects with advanced or recurrent malignancies (MDX1106-03)	NCT00730639	Phase I	mCRPC, RCC, melanoma, NSCLC
Completed	A study of MDX-1106 in patients with selected refractory or relapsed malignancies (MDX1106-01)	NCT00441337	Phase I	Carcinoma, NSCLC, CRC, Melanoma, RCC, mCRPC
Recruiting	ONO-4538 phase I study in patients with advanced malignant solid tumors in Japan	NCT00836888	Phase I	Solid tumor
Recruiting	Dose-escalation study of combination BMS-936558 (MDX-1106) and ipilimumab in subjects with unresectable stage III or stage IV malignant melanoma	NCT01024231	Phase I	Melanoma

Abbreviations: mCRPC, metastatic castration-resistant prostate cancer; RCC, renal cell cancer; CRC, colorectal cancer; NSCLC, non small cell lung cancer.

1106 is most likely exerted through immunologic mechanisms rather than direct tumoricidal effects, since nonhematologic tumors do not express PD1. Importantly, tumor regressions were seen in patients with CRC and NSCLC, suggesting that the capacity of anti-PD1 to enhance antitumor immunity extends beyond the classically immunogenic tumor types of melanoma and RCC.⁴³

A similar phase I trial was conducted on 106 patients with metastatic disease including melanoma, CRC, NSCLC, HRPC, and RCC.⁴⁴ Again, the MTD was not found and the dose of 10 mg/kg was well tolerated with fewer irAEs.

Because of the very good results of phase I trials, phase II and III trials are under evaluation. In particular, it will be interesting to study the combination of anti-PD1 with other agents, such as anti-CTLA-4 mAb, vaccines, and chemotherapy.

Finally, the anti-PD-L1 (B7/H1) mAb MDX-1105 has been developed. This is a fully-human mAb that binds PD-L1 and blocks the binding and the activation of its receptor PD1. At present, it is under evaluation in a phase I clinical trial.⁴⁵

CONCLUSION

Targeted tumor immunotherapy is an exciting field, although in many aspects it will challenge our common wisdom regarding drug development in oncology. The cellular immune response is tightly modulated to control the ability to generate cytotoxicity and inflammation and we are just starting to learn how to unleash or stimulate it within a reasonably safe therapeutic window. Understanding its control offers an opportunity to regulate its intensity. CD137 and PD1 certainly stand out as promising targets for immunostimulatory mAbs in light of their clinical and preclinical records. Their development follows in time behind that one of anti-CTLA-4 mAb that recently showed an overall survival advantage in metastatic melanoma patients in a landmark randomized trial.⁴² In the case of anti-PD1 mAb, the clinical results presented at the annual meeting of the American Society of Clinical Oncology (ASCO) are extremely promising.⁴⁴ It also will be exciting to see if blocking PD-L1 will have at least similar effects in humans and whether expression of PD-L1 on tumor cells correlates with efficacy. In the case of CD137-targeted immunostimulation, finding safer dosing with tolerable liver toxicity is the path forward.

To progress we need to define biomarkers, understand resistance mechanisms, learn how to evaluate and identify responses that would eventually mean survival advantage, and determine how to manage autoimmune complications. Preclinical evidences for synergy with conventional and other immunotherapies are abundant, and stepwise combinations⁴⁶ should lead our way towards much needed efficacy at the bedside.

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