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# Ipilimumab

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*Cancer Immunotherapy*
*Human Anti-CTLA-4 MAb*

BMS-734016
MDX-010

Immunoglobulin $G_1$, anti-(human CTLA-4 [antigen]) (human $\gamma$1-chain), disulfide with human $\kappa$-chain, dimer

CAS: 477202-00-9

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**Abstract**

Investigation into specific immunotherapy for the treatment of cancer has resulted in the identification of more than 50 cancer vaccines that are actively being studied in numerous clinical trials. However, the majority of these vaccines were created using the classic drug-target approach and several large trials have reported disappointing results. An alternative approach involves targeting the immune system as a whole together with its interaction with the tumor. For example, blockade of the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) expressed on activated T-cells can be used alone or in conjunction with a tumor vaccine to potentially enhance antitumor responses. CTLA-4 binds B7 molecules expressed on antigen-bearing cells and prevents their binding to CD28 on T-cells and subsequent enhancement of T-cell receptor (TCR) signaling. Inhibition of CTLA-4 would therefore enable propagation of an ongoing immune response. Ipilimumab (MDX-010, BMS-734016) is a fully human $IgG_1$ anti-CTLA-4 monoclonal antibody (MAb) that is currently undergoing clinical development. This MAb binds to human CTLA-4, preventing the binding of B7 molecules and thus enabling continued CD28-mediated enhancement of TCR signaling. Ipilimumab has shown particular clinical promise, with excellent antitumor activity observed when administered both as monotherapy and in combination with vaccines in patients with melanoma, lymphoma and prostate, ovarian and renal cancers.

## Background

The immune system recognizes and responds to numerous antigens and has evolved a system of regulation to prevent autoimmunity. In the search for genetic and biochemical strategies to identify cancer antigens, investigators have discovered that many tumor cells produce antigens that are self-antigens and therefore are not effectively destroyed by the host immune system. The mechanisms involved in tolerance to self-antigens are numerous. In T-cell immunity, for example, co-stimulatory molecules regulate immunity through enhancement and inhibition of T-cell responses. Signaling from the T-cell receptor (TCR) alone is not sufficient to evoke full immune responses, and a second co-stimulatory molecule is needed to enhance TCR signaling and overcome the threshold for a T-cell response to occur. TCR enhancement primarily occurs via CD28 on T-cells, which is activated by B7 expressed on antigen-bearing cells. Upon activation, T-cells express a second receptor, the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), which also binds B7 molecules. CTLA-4 inhibits T-cell responses, resulting in blockade of the immune response. Studies in CTLA-4 knockout mice have shown that CTLA-4 is essential for maintenance of tolerance. Moreover, CTLA-4 blockade has been shown in numerous studies to result in tumor rejection in mouse models of established tumors. However, transient suppression of CTLA-4 using antibodies in mice may result in enhancement of T-cell responses and can also be accompanied by autoimmune reactions, including encephalomyelitis, colitis and diabetes. Nevertheless, targeting CTLA-4 represents an extremely promising approach to enhance T-cell-mediated immune responses in cancer immunotherapy (1-13).

Investigation into specific immunotherapy for the treatment of cancer has been the focus of many researchers for a number of years and there are currently more than 50 cancer vaccines under active development and more than 400 clinical trials have been initiated. However, the majority of these vaccines were created using the classic drug-target approach and several large

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trials have reported disappointing results. Examination of 440 patients with metastatic cancer treated with 541 cancer vaccines in the Surgery Branch of the National Cancer Institute (NCI) revealed an objective response rate of only 2.6%. In addition, analysis of over 765 patients treated with a variety of cancer vaccines revealed an objective response rate of 3.8%. Thus, it appears that modifications in the current approaches to cancer immunotherapy are needed (14-16).

Researchers have now begun using an alternative approach in cancer immunotherapy in which the immune system is targeted as a whole together with its interaction with the tumor. For example, CTLA-4 blockade can be used alone or in conjunction with a tumor vaccine to potentially enhance antitumor responses. Coupling of anti-CTLA-4 antibodies with antitumor vaccines could direct immune responses toward target antigens and result in effective antitumor responses (17-20).

There are currently two anti-CTLA-4 (CD152) human monoclonal antibodies (MAbs), ipilimumab (MDX-010, BMS-734016) and ticilimumab (CP-675206), undergoing active clinical development. The fully human $IgG_1$ anti-CTLA-4 MAb ipilimumab binds to human CTLA-4 and prevents the binding of B7-1 Ig and B7-2 Ig expressed on antigen-bearing cells. This enables B7 molecules to continue to enhance TCR signaling, thereby propagating an ongoing immune response. Ipilimumab has exhibited particular clinical promise, with excellent results obtained when administered as monotherapy and in combination with anticancer vaccines in patients with melanoma, lymphoma and prostate, ovarian and renal cancer.

## Preclinical Pharmacology

An *in vitro* study using cultures of primary acute myelogenous leukemia (AML) T-cells from patients with newly diagnosed or relapsed AML and induced *ex vivo* to differentiate to dendritic cells (DCs) by sequential modulation of growth factor (SMGF) showed that CTLA-4 ligation significantly downmodulated the immune response. In contrast, treatment of T-cells with ipilimumab during SMGF *ex vivo* priming to suppress CTLA-4 ligation resulted in significant enhancement of total T-cell number, which increased by 2.3-19.6-fold, and in anti-AML activity, such that autologous AML cell lysis increased 1.6-4.7-fold. These effects were observed in 6 of 7 cultures (21).

## Pharmacokinetics and Metabolism

The pharmacokinetics of ipilimumab (3 mg/kg i.v. over 90 min every 3 weeks for up to 6 cycles) were examined in 14 patients with progressive stage IV melanoma (HLA-A*0201$^+$) who were also vaccinated with two vaccines (1 mg s.c. after ipilimumab every 3 weeks) consisting of two modified HLA-A*0201-restricted peptides from the gp100 melanoma-associated antigens gp100:209-217(210M) and gp100:280-288(288V). The mean $C_{max}$ of ipilimumab following the first dose was 72 ± 33 μg/ml and trough levels before the second dose were 12 ± 7 μg/ml. Repeated dosing resulted in moderate accumulation. The mean plasma ipilimumab level at the end of treatment was 99 ± 41 μg/ml, which decreased to 17 ± 10 μg/ml 3 weeks later. There was no correlation detected between plasma levels of the agent or antibody clearance and tumor regression or toxicity (22).

Similar results were obtained in a study in 56 patients with progressive stage IV melanoma treated with ipilimumab 3 mg/kg every 3 weeks or as a single dose of 3 mg/kg followed by 1 mg/kg every 3 weeks for up to 12 cycles, and also vaccinated with gp100:209-217(210M) and gp100:280-288(288V). The mean $C_{max}$ after the first ipilimumab dose was 81 ± 14 μg/ml, with trough levels of 15 ± 5 μg/ml seen 3 weeks later. The $C_{max}$ for subsequent 1 mg/kg doses was 43 ± 10 μg/ml, with a trough level of 7 ± 3 μg/ml (23).

## Safety

A study documented the cutaneous effects of ipilimumab in 6 of 50 patients with advanced melanoma who were treated with the MAb as a single agent (up to 9 mg/kg) every 3 weeks. Skin autoimmune breakthrough events (ABEs) included pruritic, localized erythematous papules/patches and thin plaques without vesiculation erosions. Skin manifestations were generally discrete and rarely involved the face. They developed mainly on the trunk and extensor surface of extremities. One patient presented with scratching-induced Koebner phenomenon. Skin inflammation was detected from 1 to 49 days (mean = 26 days) after a total of 1-3 doses (up to 5 mg/kg). Perivascular CD4-predominant T-cell infiltrates with rare dyskeratotic cells, mild to moderate spongiosis, exocytosis of T-cells (occasional) and mild to moderate eosinophils were detected in the dermis; B-cells and monocytes were rare. Significant increases in peripheral blood eosinophil count were observed in 5 of the 6 patients at the time of dermatitis and during exacerbation. The typical CD8$^+$-rich histology, distribution and maculopapular morphology commonly associated with drug hypersensitivity reactions were not observed. In contrast, patients developed a more focal cutaneous reaction with CD4$^+$ T-cell predominance. Results suggest that ipilimumab-induced suppression of CTLA-4 preferentially leads to activation and/or migration of CD4$^+$ T-cells in the dermis (24).

A case of a 56-year-old woman with stage IV melanoma developing diarrhea after induction therapy with ipilimumab was described. The patient experienced 7-8 episodes of watery, nonbloody diarrhea for 1 week. Abdominal exams and stool studies were negative. However, colonoscopy revealed diffuse ulcerations with nodularity in the ileum and biopsy showed cellular infiltration and crypt abscess formation. The patient was diagnosed with acute ileitis possibly due to autoimmune induction with ipilimumab (25).

Another phase I trial in patients with metastatic hormone-refractory prostate cancer (HRPC) receiving granulocyte-macrophage colony-stimulating factor (GM-CSF;

250 mg/m$^2$/day s.c. on days 1-14 of a 28-day cycle) confirmed the safety of ipilimumab (0.5 mg/kg i.v. on day 1 of each cycle x 4, followed by escalating doses on the same schedule). No dose-limiting toxicity (DLT) was reported for the 8 treated patients at the first ipilimumab dose level. However, 1 case of vertebrobasilar transient ischemic attack (TIA) was associated with the second ipilimumab dose level (1.5 mg/kg x 1 followed by 0.5 mg/kg x 3). No laboratory or clinical autoimmunity, nonspecific activation of CD4$^+$ and CD8$^+$ T-cells or modulation of antigen-specific immunity or CD4$^+$CD25$^+$ regulatory T-cells was observed with treatment (26).

## Clinical Studies

Ipilimumab induced tumor regression and autoimmunity in two clinical studies (see above) conducted in patients with progressive stage IV melanoma (HLA-A*0201$^+$; Karnofsky performance status of 60% or greater; no evidence of autoimmune or immunodeficiency disease). In the first study in 14 patients, 6 patients developed grade 3/4 ABEs including dermatitis, enterocolitis, hepatitis and hypophysitis. Objective tumor responses were observed in 3 patients (2 complete and 1 partial response). In the second study involving 56 patients, a complete response was obtained in 2 patients, which was ongoing at 30 and 31 months. Partial responses were obtained in 5 patients, with durations of 4, 6, 25+, 26+ and 34+ months. The overall objective response rate was 13%. Tumor regression was noted in lung, liver, brain, lymph nodes and s.c. sites. Grade 3/4 ABEs were observed in 14 patients. However, significantly more of these patients achieved a clinical response as compared to the 42 patients who had no evidence of autoimmune toxicity (36% *vs*. 5%). No significant differences in response rate or toxicity were observed between dosing schedules (22, 23). The results from these and several of the following studies are summarized in Table I.

A phase I trial in 17 patients with progressive, unresectable malignant melanoma examined the safety and efficacy of a single ipilimumab dose (3 mg/kg i.v. over 90 min). Plasma levels of the antibody were maintained from 1 to 4 months. Treatment was well tolerated. Only mild adverse events were observed. Mild reversible rash or pruritus was seen in 7 patients and was the only ABE reported. No significant increases in activated peripheral T-cells were observed. Two patients had a partial response which included resolution of 3 soft tissue masses and a reduction in a lung mass of over 50% (27).

A phase I trial in 19 patients with high-risk resected stage III/IV melanoma (HLA-A*0201$^+$) immunized with three tumor antigen epitope peptides from gp100, MART-1 and tyrosinase (1 mg/kg each) examined the safety of ipilimumab (0.3, 1 or 3 mg/kg every 4 weeks for 6 months followed by every 12 weeks for 6 months). Three patients receiving the highest dose and 1 patient treated at 1 mg/kg who seemed to be autoimmune developed grade 3 diarrhea or abdominal pain. The maximum tolerated dose (MTD) of ipilimumab with vaccine on this schedule was concluded to be 1 mg/kg. Evidence of autoimmunity was observed in 8 patients, of whom 3 had disease relapse. Of the 11 patients without autoimmune symptoms, 9 experienced disease relapse. Significant immune responses against gp100 and MART-1 were reported. At 28.5 months of follow-up, 12 patients experienced disease relapse and 3 died. Median time to relapse was 18.3 months (28).

A study in 7 patients with metastatic melanoma (surgically unresectable, grade III/IV) and 2 patients with advanced ovarian carcinoma examined the efficacy of ipilimumab (3 mg/kg i.v. over 90 min). All patients were previously immunized, mostly with DC or irradiated, autologous GM-CSF-secreting tumor cells (GVAX). No serious toxicities associated with ipilimumab were observed, although 5 patients with melanoma developed T-cell reactivity to normal melanocytes. Extensive tumor necrosis was observed in 3 patients with melanoma previously immunized with GVAX. Ipilimumab did not induce tumor necrosis in 4 patients with metastatic melanoma previously immunized with melanosomal antigens. Although tumor biopsies could not be obtained from the 2 patients with ovarian carcinoma previously immunized with GVAX, both displayed reductions or stabilization of blood CA125 levels (29).

Another study in patients with stage IV melanoma (n=10) and renal cell cancer (n=5) who had or had not received peptide immunization demonstrated that the antitumor effects of ipilimumab (3, 5 or 9 mg/kg i.v. over 90 mg/kg every 3 weeks) are due to T-cell activation as opposed to inhibition or depletion of regulatory T-cells. Treatment with ipilimumab had no inhibitory effect on the suppressive activity of CD4$^+$CD25$^+$ cells *in vitro* or *in vivo*. Expression of CD4$^+$CD25$^+$ cells in whole peripheral blood mononuclear cells (PBMCs) or *Foxp3* gene expression in purified populations of CD4$^+$ and CD4$^+$CD25$^+$ cells was also unaffected by treatment. However, treatment with ipilimumab did increase the percentage of CD4$^+$, CD8$^+$, CD4$^+$CD25$^+$ and CD4$^+$CD25$^-$ T-cells in PBMCs expressing HLA-DR (30).

The safety and efficacy of ipilimumab (3 mg/kg/month x 4) alone or in combination with dacarbazine (250 mg/m$^2$ for 5 days/month x 4) were examined in a multicenter, randomized phase II trial conducted in 72 patients with stage IV melanoma. There were 5 grade 3 ABEs reported in both the ipilimumab monotherapy (2 colitis, 2 uveitis and 1 rash) and combination groups (2 grade 3 diarrhea/colitis, 2 grade 3 rash and 1 grade 3 increase in alanine transaminase [ALT]); 1 grade 4 hypersensitivity reaction was also seen in the combination group. All but 1 case of ABE resolved with treatment. One patient receiving combination therapy developed colitis complicated by *Clostridium difficile* and required a colectomy. Two deaths occurred that were possibly related to ipilimumab. Two partial responses (11.9+ and 14+ months) and 4 cases of stable disease were reported in the ipilimumab monotherapy group at week 12 (with follow-up every 3 months until disease progression) and 2 complete responses (12.6+ and 16.3+ months), 4 partial

*Table I: Clinical studies of ipilimumab (from Prous Science Integrity®).*

| Indication | Design | Treatments | n | Conclusions | Ref. |
|---|---|---|---|---|---|
| Cancer, melanoma | Open | Ipilimumab, 3 mg/kg i.v. infusion over 90 min + gp100 antigen 1 vaccine, 1 mg s.c. + gp100 antigen 2 vaccine, 1 mg, s.c. 1x/3 wks | 14 | The administration of ipilimumab induced cancer regression in 21% of patients with metastatic melanoma. However, grade 3/4 autoimmunity was observed in several organ systems in 43% of the patients | 22 |
| Cancer, melanoma | | Ipilimumab, 3 mg/kg i.v. infusion over 90 min 1x/3 wks + Peptide vaccine (n=29)<br>Ipilimumab, 3 mg/kg i.v. infusion over 90 min → 1 mg/kg 1x/3 wks + Peptide vaccine (n=27) | 56 | Ipilimumab combined with peptide vaccine induced tumor regression, which was associated with the induction of autoimmunity in patients with metastatic melanoma | 23 |
| Cancer, melanoma | Open Multicenter | Ipilimumab, 3 mg/kg i.v. over 90 min | 17 | Ipilimumab was well tolerated and could be effective in increasing immune responses in patients with malignant melanoma | 27 |
| Cancer, melanoma | Open | Ipilimumab, 0.3 mg/kg i.v. over 90 min 1x/4 wks x 6 mo (n=7)<br>Ipilimumab, 1.0 mg/kg i.v. over 90 min 1x/4 wks x 6 mo (n=7)<br>Ipilimumab, 3.0 mg/kg i.v. infusion over 90 min 1x/4 wks x 6 mo (n=5) | 19 | Antigen-specific immune responses resulted from vaccination against gp100, MART-1 and tyrosinase peptides in patients treated with ipilimumab, which was well tolerated, with reversible gastrointestinal adverse events at the maximum tolerated dose of 1 mg/kg | 28 |
| Cancer, ovary metastatic, Cancer, melanoma | Open | Ipilimumab, 3 mg/kg i.v. infusion over 90 min | 9 | Ipilimumab induced extensive tumor necrosis in patients with metastatic melanoma or ovarian cancer previously vaccinated with irradiated, autologous granulocyte-macrophage colony-stimulating factor-secreting tumor cells. Ipilimumab did not produce tumor necrosis in patients previously vaccinated with defined melanosomal antigens (dendritic cells engineered to express gp100 and MART-1) | 29 |
| Cancer, melanoma metastatic | Randomized Double-blind Multicenter | Ipilimumab, 3 mg/kg i.v. 1x/mo x 4 mo (n=37)<br>Ipilimumab, 3 mg/kg i.v. 1x/mo + Dacarbazine, 250 mg/m$^2$ o.d. x 5 d 1x/mo x 4 mo (n=35) | 72 | Ipilimumab alone or in combination with dacarbazine induced durable (> 1 year) clinical responses in previously untreated patients with metastatic melanoma. The overall response rate to the ipilimumab/dacarbazine combination was higher compared to ipilimumab monotherapy | 31, 32 |
| Cancer, prostate | Open Multicenter | Ipilimumab, 3 mg/kg i.v. over 90 min | 14 | Ipilimumab was well tolerated and demonstrated inmunological and anti-tumor activity in patients with hormone-refractory prostate carcinoma | 33 |
| Cancer, ovary, Leukemia, acute myeloid | Open | Ipilimumab, 3 mg/kg i.v. | 3 | Antitumor effects were found with a single infusion of ipilimumab in cancer patients previously immunized with GVAX | 36 |
| Cancer, kidney metastatic | Open | Ipilimumab, 3 mg/kg 1x/3 wks (n=20)<br>Ipilimumab, 3 mg/kg → 1 mg/kg 1x/3 wks (n=21) | 41 | Ipilimumab induced tumor regression in patients with metastatic renal cancer | 37 |

responses and 4 cases of stable disease in the combination group. The mean progression-free survival rate for the monotherapy and combination groups was 82 and 99 days, respectively (31, 32).

The efficacy and tolerability of ipilimumab (3 mg/kg i.v. over 90 min) were examined in a multicenter, open-label phase I trial in 14 patients with HRPC. Ipilimumab was well tolerated and plasma levels of the MAb could be detected for 1-4 months. Only mild infusion-related adverse events were reported and 4 patients developed mild, reversible rash or pruritus. One patient developed grade 3 pruritus which resolved with steroid treatment. No other clinical autoimmunity or significant increase in activated peripheral T-cells was observed. A prostate-specif-

ic antigen (PSA) response lasting 3 and 5 months occurred in 2 of the 7 patients who were chemotherapy-naïve and 1 of these patients also had symptomatic improvement (33).

Results from a pilot study demonstrated the efficacy and safety of ipilimumab (3 mg/kg followed by 1.5 mg/kg/month for 3 more months) in 11 patients with advanced malignancies who had tumor progression after previous cancer vaccine therapy. The study included 3 patients with colon cancer previously treated with DC (mutated Ras peptide), 4 patients with non-Hodgkin's lymphoma (2 follicular and 2 mantle cell) treated with patient-specific idiotype vaccines and 4 patients with prostate cancer treated with PSA-expressing recombinant vaccinia virus vectors. Treatment was well tolerated. The majority of toxicities reported were grade 1 and 2 (*e.g.*, skin rash, diarrhea). A case of grade 3 adrenal insufficiency was reported and was possibly related to ipilimumab. No responses were observed in patients with colon or prostate cancer. However, 1 patient with mantle cell lymphoma and 1 patient with follicular lymphoma had tumor regression. In addition, a partial response was seen in a patient with follicular lymphoma. Analysis of tumor biopsies from a responding and a nonresponding patient with follicular lymphoma revealed an increase in T-cell infiltration in the responding patient sample (34).

The tolerability and antitumor efficacy of single-dose ipilimumab (0.1, 0.33, 0.66 and 1 mg/kg i.v. over 90 min) were investigated in a phase I dose-escalating study in 12 patients with relapse of malignancy (2 chronic myelogenous leukemia [CML], 1 chronic lymphocytic leukemia [CLL], 1 AML, 3 Hodgkin's disease, 3 multiple myeloma, 1 renal carcinoma, 1 breast carcinoma) after undergoing allogeneic hematopoietic stem cell transplantation (median time between transplant and ipilimumab = 10.3 months). Donor lymphocyte infusions (DLI) were permitted at 8 weeks after ipilimumab infusion if no graft-*versus*-host disease (GVHD) or malignancy progression was evident. Ipilimumab was well tolerated, with no infusion-related toxicities or clinically significant GVHD reported after single-dose monotherapy with the agent. Four patients received additional DLI. Acute grade 2 GVHD of the skin was seen in 1 patient following DLI and two possible ABEs were reported in 2 patients with AML, including grade 3 polyarthropathy (which resolved with treatment) at 14 weeks after ipilimumab (0.1 mg/kg) and at 6 weeks after DLI, and grade 1 chemical hyperthyroidism with thyroid-stimulating antibody at 6 weeks after ipilimumab (0.66 mg/kg). A partial remission was obtained in a patient with AML (refractory to prior therapies) who received 0.1 mg/kg ipilimumab and molecular remission was detected in a patient with CML maintained off imatinib who received an ipilimumab dose of 0.1 mg/kg. In addition, a patient with previously progressive multiple myeloma who received 0.33 mg/kg ipilimumab had disease stabilization, and regression of malignancy was reported in a patient with CML at an ipilimumab dose of 0.66 mg/kg and following an additional DLI. At a median follow-up of 195 days after ipilimumab infusion, 3 patients had died and 8 were alive with 1 in complete remission and another in partial remission (35).

A phase I study in 48 patients with metastatic melanoma, non-small cell lung cancer (NSCLC), ovarian cancer and AML/myelodysplastic syndrome (MDS) previously immunized with GVAX examined the efficacy of ipilimumab (3 mg/kg by i.v. infusion with possible repeated dosing every 2 months). Two patients with ovarian cancer and 1 patient with AML have been treated. Stable disease was seen in 1 patient with ovarian cancer and a marked reduction in CA125 and tumor regression were observed in the second patient with ovarian cancer after a single dose of the agent. No significant toxicities or autoimmune toxicities were reported (36).

The antitumor efficacy of ipilimumab (group A: 3 mg/kg followed by 1 mg/kg every 3 weeks; group B: 3 mg/kg every 3 weeks) was demonstrated in a phase II study conducted in 41 patients with advanced renal cancer. Significant ABEs, including enteritis (n=9), hypophysitis (n=2) and meningitis (n=1), developed in 3 and 9 patients in groups A and B, respectively. Autoimmune enteritis was resolved with steroid treatment; only 1 patient underwent colectomy for perforation. An objective response was achieved by 1 patient in group A and 5 of the 20 patients in group B had partial responses lasting for 18, 8, 8+, 6+ and 4+ months. All 6 patients who responded were among the 12 patients who developed ABEs and 3 of these 6 patients had previously received IL-12 (37).

Ipilimumab is currently undergoing phase III development for metastatic melanoma. In addition, patients are being recruited for a number of clinical trials to examine the efficacy and tolerability of ipilimumab as monotherapy or in combination with cancer vaccines for the treatment of pancreatic cancer, lymphoma, prostate cancer, colon cancer, ovarian cancer, NSCLC and synovial sarcoma (38-40).

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