



Immunomodulating antibodies in the treatment of metastatic melanoma: The experience with anti-CTLA-4, anti-CD137, and anti-PD1

Ester Simeone & Paolo A. Ascierto

To cite this article: Ester Simeone & Paolo A. Ascierto (2012) Immunomodulating antibodies in the treatment of metastatic melanoma: The experience with anti-CTLA-4, anti-CD137, and anti-PD1, Journal of Immunotoxicology, 9:3, 241-247, DOI: [10.3109/1547691X.2012.678021](https://doi.org/10.3109/1547691X.2012.678021)

To link to this article: <https://doi.org/10.3109/1547691X.2012.678021>



Published online: 23 Apr 2012.



Submit your article to this journal [↗](#)



Article views: 4984



View related articles [↗](#)



Citing articles: 5 View citing articles [↗](#)

ARTICLE

Immunomodulating antibodies in the treatment of metastatic melanoma: The experience with anti-CTLA-4, anti-CD137, and anti-PD1

Ester Simeone and Paolo A. Ascierto

Unit of Medical Oncology and Innovative Therapy, Istituto Nazionale Tumori Fondazione G. Pascale, Naples, Italy

Abstract

Clinical activity of anti-CTLA-4 (cytotoxic T-lymphocyte antigen-4) monoclonal antibodies (mAb) has changed the approaches for the treatment of cancer in terms of patterns of response, duration of response, and adverse event profiles. In fact, antibodies that block the interaction of CTLA-4 with its ligands B7.1 and B7.2 can enhance immune responses, including anti-tumor immunity. Two recent studies using ipilimumab (an anti-CTLA-4 mAb) demonstrated improvements in overall survival in the treatment of advanced melanoma. These studies utilized two different schedules of treatment in different patient categories (first and second line of treatment). However, the results were quite similar despite the different dosage used and the combination with dacarbazine in the first line treatment. Ongoing clinical studies will establish the efficacy of ipilimumab as monotherapy or in combination with other drugs for the treatment of metastatic melanoma and a variety of other cancers. Other antibodies, such as CD137 agonists and PD-1 antagonists, are currently in various stages of pre-clinical and clinical development. 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. 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 anti-tumor immunity. As a result, human mAbs anti-PD1 and anti-PD-L1 are under clinical development. This paper reviews recent studies in the treatment of advanced melanoma with these types of monoclonal antibodies. Ipilimumab can be considered a cornerstone of a new era in melanoma treatment. However, the aim is to optimize the therapy with anti-CTLA-4 antibodies to define the best schedule for next combination regimens (other immunomodulatory antibodies, BRAF/MEK inhibitors, vaccines, etc.) that represent the natural evolution of future melanoma therapy.

Keywords: Melanoma, immunotherapy, anti-CTLA4, ipilimumab, anti-CD137, anti-PD1

Introduction

Monoclonal antibodies targeting immunomodulatory molecules to enhance anti-tumor immunity may represent the next major development in cancer therapy. In fact, re-educating T-lymphocytes to induce an anti-tumor immune response may be beneficial, as it has been shown from the presence of increased T-lymphocytes as predictive of improved clinical outcome (Korman et al., 2006). Unfortunately, tumors can also create an immunosuppressive milieu both locally and systemically that can inhibit or subvert immune responses through

various mechanisms. These include: (1) increased levels of CD4⁺CD25⁺ T-regulatory (T_{reg}) lymphocytes that suppress the function and proliferation of tumor-specific CD4⁺ and CD8⁺ effector T-lymphocytes and natural killer (NK) cells (Korman et al., 2006); (2) induction of myeloid suppressor cells that can suppress T-lymphocyte proliferation and induce T-lymphocyte apoptosis through the metabolism of L-arginine (Krummel and Allison, 1995); (3) local accumulation of tumor-associated macrophages, alternatively activated or M2 macrophages that are capable of not only suppressing T-lymphocyte

Address for Correspondence: Dr Paolo A. Ascierto, Unit of Medical Oncology and Innovative Therapy, Istituto Nazionale Tumori Fondazione 'G. Pascale', Via Mariano Semmola, 80131 Napoli, Italy. Tel: 390815903841. Fax: 390815903841. E-mail: paolo.ascierto@gmail.com

(Received 12 February 2012; revised 07 March 2012; accepted 17 March 2012)

function through interleukin (IL)-10, transforming growth factor (TGF)- β , and arginine metabolism, but that can also enhance tumor growth and invasion (Krummel and Allison, 1995); and (4) up-regulation of the expression of B7-H1 (programmed death ligand-1) that interacts with the co-inhibitory programmed death receptor-1 (PD-1, CD279) on T-lymphocytes, resulting in decreased T-lymphocyte proliferation and cytokine production (Krummel et al., 1996).

A tumor-specific immune response is initiated when antigen-presenting cells (APC) present an antigen in association with major histocompatibility complex (MHC) molecules to T-lymphocytes. T-Lymphocyte activation requires two signals: first, recognition of the antigen complexed with MHC by the T-cell receptor (TCR) and, second, a co-stimulatory signal delivered through B7 proteins (CD80 and CD 86) on the APC surface which interact with CD28 protein on the T-lymphocyte (Figure 1). This process results in T-lymphocyte activation and proliferation. After activation, cytotoxic T-lymphocyte antigen-4 (CTLA-4), another co-stimulatory molecule that also binds B7 (CD80 and CD 86), is then expressed on the surface of T-lymphocytes. Rather than stimulating T-lymphocytes, CTLA-4 provides inhibitory signals to the cells, serving as a negative feedback loop (Korman et al., 2005). Actually, the strategy to potentiate immune response through the inhibition of inhibitory targets like CTLA-4 and PD-1 has reached a relevant role in the treatment of many tumors, but in metastatic melanoma in particular. Table 1 summarizes the pros and contra of the different immunomodulating monoclonal antibodies.

Anti-CTLA-4 antibodies

The mechanism by which the CTLA-4 blockade translates into tumor regression has not been fully elucidated, although there are several hypotheses. The constitutive expression of CTLA-4 on CD25⁺ T_{reg} lymphocytes raises the possibility that CTLA-4 blockade could act by depleting

T_{reg} lymphocytes/blocking their activity, even though there is some evidence to the contrary (Krummel et al., 1996; Korman et al., 2005). Alternatively, a CTLA-4 blockade could act directly on effector CD4⁺ and/or CD8⁺ T-lymphocytes by removing a checkpoint on their proliferation and function. Treatment effects may result from the induction of *de novo* anti-tumor responses or from the activation of endogenous memory responses. By enhancing CD4⁺ T-lymphocyte responses, CTLA-4 inhibition may also induce antibody responses (Korman et al., 2005). Finally, anti-CTLA-4 antibodies could also directly bind to some tumor cells (including melanoma) that constitutively express CTLA-4, thereby perhaps mediating direct cytotoxicity.

Two fully human monoclonal antibodies (mAb) directed against CTLA-4 have been developed. One, ipilimumab (Bristol-Myers Squibb, Princeton, NJ), is currently approved for the treatment of metastatic melanoma but is still in clinical development for other tumors. The other, tremelimumab (CP-675,206; Pfizer, New York, NY), is still under study. While ipilimumab is an IgG₁ antibody and tremelimumab is an IgG₂ antibody, both bind to CTLA-4 with affinities <1 nmol/L.

The dose and regimen of ipilimumab has been explored in several Phase II trials. In particular, patients with metastatic melanoma in a Phase II randomized study received different doses of ipilimumab (0.3 vs 3.0 vs 10 mg/kg) (Wolchok et al., 2010). The results indicated a statistically significant trend of increased response rates with increased dose, suggesting a dose-effect. Overall, the most promising results in terms of best overall response rate (BORR) were obtained with a regimen of 10 mg ipilimumab/kg every 3 weeks for a total of four doses (induction phase) followed by a maintenance period in which ipilimumab was administered every 12 weeks (maintenance phase). Subsequently, the 10 mg ipilimumab/kg dose was utilized in Phase III studies.

In 2010, results from the MDX010-20 clinical trial were published (Hodi et al., 2010). This was the first randomized Phase III trial to have demonstrated a benefit in overall survival (OS) in pre-treated patients with metastatic melanoma. This study showed the superiority of the ipilimumab arm compared to a gp100 vaccine arm: ipilimumab monotherapy (given at 3 mg/kg every 21 days, for four doses) had a median OS of 10.1 months, whereas the gp100 monotherapy OS was only 6.4 months. It should be noted that this clinical trial was activated in 2004, before the data from the dose-ranging Phase II randomized trial were available. The most common adverse events (AE) were immune-related (e.g., cutaneous, gastrointestinal, hepatic, endocrine); specific algorithms were subsequently developed showing that early recognition and correct therapeutic approach with steroid therapy made most of these AE manageable and reversible.

In 2011, results of a second Phase III trial comparing dacarbazine vs dacarbazine + ipilimumab (CA184-024 study) in the treatment of naïve patients with metastatic melanoma were published (Robert et al., 2011). Ipilimumab was administered at a dose of 10 mg/kg

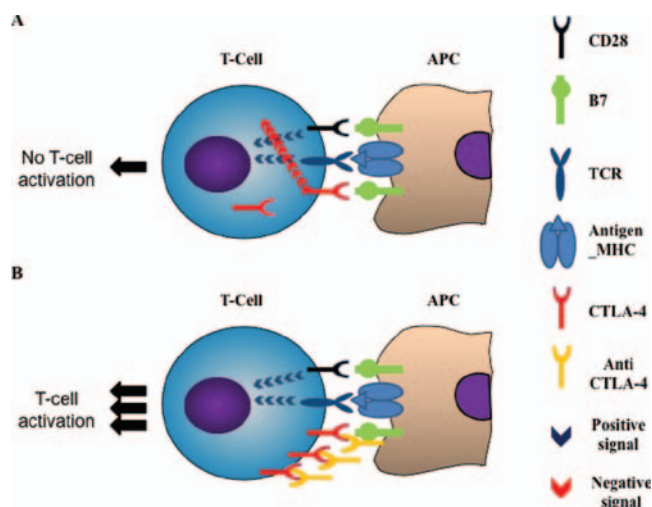


Figure 1. T-Lymphocyte activation requires two signals.

Table 1. Pros and cons about single immunomodulating monoclonal antibodies (mAb).

Agent	Pros	Cons
Ipilimumab (anti-CTLA-4)	Two randomized Phase III trials showed improvement of Overall Survival in melanoma patients as first and second line (Hodi et al., 2010; Robert et al., 2011).	Better schedule and dosage to be defined.
Tremelimumab (anti-CTLA-4)	Treatment schedule with administrations every 90 days with an identical safety profile of ipilimumab.	Negative results in Phase III study compared with standard chemo-therapy (Ribas et al., 2008).
BMS 663513 (anti-CD137)	Possible combination with anti-immune checkpoint blockade mAbs.	Severe toxicity dose related.
MDX-1106 (anti-PD-1)	Effective at lower dosage (i.e., 1 mg/kg). Better safety profile.	Not yet studied in Phase III trial.

every 3 weeks for four doses, followed by maintenance therapy with 10 mg ipilimumab/kg for eligible patients. This study supported (albeit in a manner less than expected) the results of the previous Phase III trial by showing an OS of 11.2 months for patients treated with dacarbazine + ipilimumab and an OS of 9.1 months for patients treated with dacarbazine alone. This study also confirmed the previous toxicity profile with a slight reduction of endocrinopathies and gastrointestinal toxicities. In the dacarbazine + ipilimumab arm, hepatotoxicity appeared to be higher than in the control arm (31.6% vs 2.4% Grade 3–4) and higher than with the prior experience with ipilimumab alone at 3 or 10 mg/kg. In this regard, results from another combination trial with fotemustine could give us more information about efficacy and safety of combination regimens with cytotoxic chemotherapy (Di Giacomo et al., 2011).

Even if they are not directly comparable, given the differences in study design, by looking at the survival curves of the two Phase III trials (Ascierto et al., 2011) they appear to be quite similar. This raises some questions about which are the best dosage (3 vs 10 mg/kg), the best schedule (re-induction vs maintenance), and the combination with chemotherapy. However, there is data from the compassionate use program (CUP) that confirms 3 mg/kg as a sufficient dose to be clinically effective and less toxic (Simeone et al., 2011). In fact, the incidence of severe (i.e., Grade 3–4) AE in the 3 mg/kg schedule was 10–15% (Hodi et al., 2010), while in the ipilimumab combination arm of the CA184-024 study it was 56.3% (41.7% was severe immune-related AE [irAE]) (Robert et al., 2011). It is clear that in the future investigators will need to monitor the incidence of severe irAE, even considering new possible toxicities (e.g., neutropenia, thrombocytopenia, anemia) because the early recognition and treatment is mandatory to reduce the risk in patients (Akhtari et al., 2009; Ahmad et al., 2011). An upcoming randomized trial will directly compare doses of 3 vs 10 mg/kg ipilimumab in patients with advanced melanoma and will likely help to answer the question about efficacy in terms of OS and safety of the two regimens.

Still not yet clarified are the optimal treatment schedule and the role of maintenance or re-induction. For the former, there is still no data to confirm the utility of the

maintenance; in contrast, there is some evidence for the utility of re-induction. In the MDX010-20 study, 21 out of 31 (68%) patients who received re-induction with ipilimumab had a response (complete or partial) or a durable stable disease (SD, at least 6 months) without significant additional toxicities. Furthermore, in the MDX010-08 trial (Hersh et al., 2011) that compared ipilimumab alone (a schedule of 3 mg/kg for four cycles) vs ipilimumab in combination with dacarbazine, patients who progressed on monotherapy could cross-over and receive combination therapy upon progression; however, none of the patients treated with the cross-over had a response. In the ipilimumab alone arm, the BORR was 5.4% and the disease control rate (DCR: complete response + partial response + stable disease) was 21.6%; in the dacarbazine plus ipilimumab combination arm, the BORR was 14.3% and the DCR was 37.1% (Hersh et al., 2011).

Data from completed studies of tremelimumab as a second-line monotherapy for metastatic melanoma (MM) have been published. In a Phase I trial (Ahmad et al., 2011b), 39 patients with solid malignancies (MM, $n = 34$; renal cell, $n = 4$; colon, $n = 1$) were treated with single escalating doses of tremelimumab (0.01–15 mg/kg). Four out of the patients with MM reached objective responses (OR) [two complete responses (3 and 15 mg/kg doses) and two partial responses (PR) (10 and 15 mg/kg doses)], and four additional patients experienced SD. The maximum-tolerated dose was defined as 10 mg tremelimumab/kg. Grade 3 toxicities related to tremelimumab therapy were diarrhea ($n = 3$), dermatitis ($n = 1$), and elevated serum lipase ($n = 1$). Tremelimumab at 15 mg/kg every 90 days (up to four cycles) was tested in a second-line Phase II study (A3671008) in inoperable American Joint Committee on Cancer Stage III/IV melanoma (Ahmad et al., 2011b). The objective response rate (RR) was 7%, median OS was 10.1 months, and 1-year survival rate was 41%. A subsequent Phase III trial (A3671009), in patients with treatment-naïve advanced melanoma, which compared tremelimumab to dacarbazine/temozolomide was discontinued after an interim analysis showed that tremelimumab would not show superiority over standard chemotherapy (Ribas et al., 2008). Median overall survival by intent-to-treat was 11.8 months (95% CI, 10.4–13.9 months) in the tremelimumab arm and 10.7 months (95% CI, 9.3–12.0 months)

in the chemotherapy arm, with a hazard ratio (chemotherapy over tremelimumab) of 1.04 (95% CI, 0.84–1.28).

The failure of tremelimumab may be attributed to the different schedule compared to ipilimumab (the treatment schedule every 90 days); however, this is unlikely since comparison of the response rates (assessed by independent radiology review committees) between the two Phase 2 single arm studies of ipilimumab at 10 mg/kg every 3 weeks and tremelimumab at 15 mg/kg every 90 days demonstrate very similar response and toxicity results (Kirkwood et al., 2010; O'Day et al., 2010). Another possibility is that the restriction of the LDH level to up to twice the upper limit of normality in the tremelimumab Phase 3 trial resulted in an advantage for the control arm that was not present in the ipilimumab Phase 3 trials that did not have this restriction. More likely, the concurrent availability of ipilimumab in several studies that did not exclude prior anti-CTLA4-based therapies (including two expanded access protocols) allowed a significant cross-over to ipilimumab in patients randomized to the control arm of the tremelimumab Phase 3 trial.

Recently, data from the combination of tremelimumab and high doses of interferon (IFN) have been published (Tahini et al., 2011). This was a study about the safety and efficacy of the combination of high dose IFN (HDI) and tremelimumab that enrolled 37 patients with metastatic Stage IV melanoma. One course of therapy consisted of three cycles (one cycle = 28 days). Tremelimumab at 15 mg/kg was administered intravenously at the start of the first cycle. For cycle 1, IFN α -2b was administered intravenously 5 days/week for 4 weeks at 20 MU/m²/day. For cycle 2 onward, the IFN α -2b was administered subcutaneously 3 days/week for 4 weeks at 10 MU/m²/day. The best objective RR (based on Response Evaluation Criteria in Solid Tumors) by intent-to-treat was 24% (90% CI, 13–36%; four complete responses (CR) and five PR that lasted more than 6 months). Fourteen patients (38%) had stable disease (SD) that lasted 1.5–21.0 months. The median progression-free survival was 6.4 months (95% CI, 3.3–12.1 months). The median OS was 21 months (95% CI, 9.5–not reached). Toxicity was acceptable, with Grade 3 and 4 toxicities including neutropenia (six patients; 17%), diarrhea/colitis (four patients; 11%), liver enzyme increase (four patients; 11%), rash (four patients; 11%), fatigue (15 patients; 40%), and anxiety/depression (five patients; 14%). The results of this study were interesting and necessitate further investigation to explore possible combination strategies.

Other immunomodulating antibodies: Anti-CD137 and anti-PD-1

Following the promising results of the anti-CTLA-4 anti-cancer therapy, there is a growing interest about other receptors or molecules that may be potentially targeted by mAb to enhance anti-tumor immunity. These might include against CD137 (with a stimulatory agonist or

supra-agonist effect that mimics the natural ligand CD137L) and PD1 (with an effect like anti-CTLA-4, removing the immune blocking acting as a pharmacological antagonist of this inhibitory receptor).

CD137 (4-1BB) is an inducible T-lymphocyte surface molecule of the tumor necrosis factor (TNF) receptor superfamily. Its binding by the natural ligand (CD137L) or an agonist antibody provides co-stimulation in a CD28 independent way for CD4⁺ and CD8⁺ T-lymphocytes (Wilcox et al., 2002). Surface expression of CD137 is detectable on activated effector CD8⁺ and CD4⁺ cells, and also to activated NK cells, NKT cells, T_{reg} lymphocytes, dendritic cells (DC), macrophages, neutrophils, and eosinophils (Lin et al., 2010). Recent evidence indicates that it is also functionally expressed in tumor capillary endothelial cells (Ascierto et al., 2010a). CD137 signaling enhances T-lymphocyte proliferation and T-helper (T_H)-1 cytokine production, and provides protection to CD8⁺ T-lymphocytes from activation-induced cell death through NF- κ B-mediated activation and up-regulation of the anti-apoptotic B-cell lymphoma 1-2 (Bcl-2) family members Bcl-xL and Bfl-1 (Lee et al., 2002).

Most studies have focused on two anti-CD137 antibodies: BMS 469492—an anti-murine CD137 antibody, and BMS 663513—a fully human IgG₄ against human CD137 (Ascierto et al., 2010a). BMS 469492 was used for studies in murine tumor models, and it does not cross-react with BMS 663513. Both are agonistic mAb and do not block the interaction of CD137 with its natural ligand, CD137L. It is of note that mAb which do block CD137L binding show similar functional anti-tumor activities when compared to non-blocking mAb (Ascierto et al., 2010a). BMS 663513 provides a co-stimulatory signal to human T-lymphocytes, causing an increase in IFN γ production and their survival. In fact, the functional effects of BMS 663513 were demonstrated on human and monkey T-lymphocytes and peripheral blood mononuclear cells (PBMC) by measuring IFN γ production; the results showed that production was enhanced in human and monkey cells as compared to controls that did not see BMS 663513 (Ascierto et al., 2010a).

Another study (NCT00309023) was an open-label, ascending, multi-dose Phase I-II clinical study conducted in subjects with locally advanced or metastatic solid tumors. In the dose escalation portion of the study, subjects were sequentially assigned to one of six dose-cohorts (i.e., 0.3, 1, 3, 6, 10, or 15 mg/kg) to receive BMS 663513 as a 60-min intravenous infusion once every 3 weeks. In the dose expansion part 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 (i.e., 1, 3, or 10 mg BMS 663513/kg). Fifty-four melanoma patients were treated; the reported toxicity was manageable, with fatigue, transaminitis, neutropenia, rash, and diarrhea as the most common adverse events. In a total of 106 patients out of the 115 enrolled, the most frequent Grade $\geq$ 2 abnormalities were: changes in ALT

(15%) and/or AST (12%) activities, leukopenia (8%), neutropenia (6%), thrombocytopenia (4%), and hyperbilirubinemia (< 1%) (Ascierto et al., 2010a).

The NCT00309023 study showed a clinical activity for BMS 663513 that justified its further development 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 the BMS 663513 was not established. Clinical activity represented by partial remissions and sustained stable diseases were observed in the 1, 3, and 10 mg/kg groups. More toxicity was shown at higher doses (> 6 mg/kg). Results of biomarker studies (activated T-lymphocyte markers, RNA expression, and serum biomolecules) indicated that BMS 663513 elicited biologic activity with doses as low as 0.3 mg/kg (Ascierto et al., 2010a). NCT00309023 has provided the rationale for developing a Phase II randomized study (NCT00612664) in previously-treated melanoma patients with advanced disease (Stage IV). The primary end-point was the 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. Unfortunately, the study was discontinued in May 2009 due to an unusually high incidence of Grade 4 hepatitis (Ascierto et al., 2010a). For this reason, the development of BMS 663513 anti-CD137 was stopped. It is likely that a new trial will start in the next few years (NCT01471210) and these studies will deal with safety, tolerability, pharmacokinetics, and immunoregulatory at lower dosage.

Programmed death-1 (PD-1, *aka* CD279) is an inhibitory co-receptor expressed on antigen-activated T-lymphocytes, on antigen-specific T-lymphocytes chronically exposed to antigen, and on B-lymphocytes. It belongs to the CD28 family and it has two known ligands: B7-H1/PD-L1 (referred to hereafter as B7-H1) the predominant mediator of PD-1-dependent immunosuppression, and B7-DC/PD-L2. PD-L1 is expressed on hematopoietic cells and can be up-regulated upon their activation. PD-L1 is also found in cells/tissues such as pancreatic islets, heart, endothelium, small intestine, and placenta. Tumor cells that express PD-L1 use this molecule as part of a mechanism to evade recognition/destruction by the immune system. PD-L2 expression is restricted only to macrophages and DC, and is up-regulated upon activation. In murine tumor models, B7-H1 expression confers immune resistance, and interrupting PD-1:B7-H1 interactions has anti-tumor effects (Sznol et al., 2008). B7-H1 is highly up-regulated in many murine and human tumors (either in tumor cells or non transformed cells in the tumor microenvironment, such as APC); its expression is associated with poor outcome for patients with certain epithelial cancers (Fong and Small, 2008). B7-H1 shows homology with CTLA-4, but provides distinct immune-inhibitory signals. In contrast

to an early lethality seen in CTLA-4 knockout mice, PD-1 knockouts demonstrate modest late-onset strain- and organ-specific autoimmunity. In fact, PD-1 knockout mice develop an autoimmune glomerulonephritis and arthritis that is milder compared to that observed in CTLA-4 knockout mice (who express multi-organ autoimmunity within the first few weeks of birth) (Fong and Small, 2008). Suppression of effector T-lymphocyte function by PD-1 engagement includes deletion/apoptosis, inhibition of proliferation, and production of cytokines such as interleukin (IL)-2 and IFN γ , and, together with chronic antigen exposure, results in T-lymphocyte exhaustion (Fong and Small, 2008). These data suggest that targeting PD-1 may augment tumor-specific T-lymphocyte responses.

A Phase I clinical trial of PD-1 blockade with the fully human mAb MDX-1106 (BMS 936558/ONO-538, IgG $_4$) specific for human PD-1 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 (Sznol et al., 2008). 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 anti-tumor activity, pharmacodynamics, and immunologic end-points. Sequential cohorts of six patients received a 60-min intravenous 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. The dose-limiting toxicity (DLT) was defined as a treatment-related Grade $\geq$ 3 AE or laboratory abnormality occurring $\leq$ 28 days post-dosing. Fifteen additional patients were to be enrolled at the MTD or the highest planned dose (10 mg/kg) to confirm safety. Tumor assessment according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.0) was performed at 8 and 12 weeks. The results showed that MDX-1106 was well-tolerated and no DLT were observed after one dose; an MTD was not defined in this study. Any irAE were of special interest because of the presumed mechanism of action of anti-PD-1 and prior experience with anti-CTLA-4. No Grade $\geq$ 3 irAE occurred in the 28-day period following the first dose of anti-PD-1. One patient with metastatic ocular melanoma developed a Grade 3 inflammatory colitis following five 1 mg/kg doses administered over 8 months; this responded to steroids and infliximab.

This study (Sznol et al., 2008) also demonstrated a milder toxicity profile for anti-PD-1 than for anti-CTLA-4. Furthermore, even if efficacy was not an end-point, evidence of anti-tumor activity was found, 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-1106 is most likely exerted through immunologic mechanisms rather than direct tumoricidal

effects, since non-hematologic tumors do not express PD-1. Importantly, tumor regression was seen in patients with colon and lung cancer, suggesting that the capacity of anti-PD-1 to enhance anti-tumor immunity extends beyond the classically immunogenic tumor types of melanoma and RCC (Sznol et al., 2008). Another similar Phase I trial was conducted on 106 patients with metastatic disease including melanoma, CRC, NSCLC, RCC, and HRPC (Ascierto et al., 2010a). As before, in this study, an MTD was not established and the dose of 10 mg/kg was well tolerated, with a lower incidence of irAE (Ascierto et al., 2010a). Thanks to these very good results of Phase I trials, Phase II and III trials are under evaluation. In particular, it will be very interesting to study the combination of anti-PD-1 with other antibodies (like anti-CTLA-4), vaccines, and chemotherapy (Ascierto et al., 2010a; Ahmad et al., 2011a).

One final note for the Reader: an 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 PD-1. At the moment, it is under evaluation in a Phase I clinical trial.

Perspective

There has been much progress in increasing the understanding of molecular processes involved in the progression of melanoma. For example, it seems clear that the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways are activated through multiple mechanisms and appear to play a major role in melanoma progression. Recently, the FDA and EMA approved vemurafenib (a specific inhibitor of the BRAF protein mutated in V600E) for the treatment of advanced melanoma (in first and second line) for patients harboring the ^{V600E}BRAF mutation (Chapman et al., 2011).

Along these same lines, one should view the synergic actions of some immuno-targeting agents like ipilimumab and anti-PD-1 (Korman et al., 2007). While awaiting the results of such combination treatments in a Phase I study, potential future treatments with these kinds of mAb could also include a combined use of other anti-immuno checkpoint blockade mAb (like anti-CTLA-4, anti-PD-1, and/or anti-PD-L1) in combination with agonistic immunostimulating mAb (such as anti-CD137, anti-OX40, and/or anti-CD40). Another interesting approach could be designing a treatment that utilizes the combination of the targeting molecules (like vemurafenib or MEK inhibitor) with the immunomodulating antibodies. Along these lines, a combination study utilizing both vemurafenib in conjunction with ipilimumab has been initiated. Thus, through the use of novel agonistic immunostimulating mAb and other mAb that target different molecules that have direct impact on different pathways and/or systems (i.e., novel immunotarget agents) involved in the onset/progression of melanoma we and other investigators can change the future history of this insidious disease.

Conclusions

The clinical data so far have revealed some interesting outcomes with this type of antibodies that require specific consideration as clinical development continues. These outcomes include durable OR and SD, variable patterns of response, and a different AE profile, all of which are a function of the mechanism of action of anti-CTLA-4 therapy. In fact ipilimumab showed an effect on OS but doesn't impact on PFS and BORR; Immune-related response criteria (irRC) could help oncologists in evaluating the response. An important challenge for the future will be the identification of the patients who respond to the immunomodulating mAb treatment (biomarkers) (Ascierto et al., 2010a; Ahmad et al., 2011a). The emerging role of other antibodies, in particular anti-PD1, underlines the necessity to improve the knowledge about these for both efficacy and toxicity; the future clinical studies should be oriented to find new biomarkers and possible combination strategies (Ascierto et al., 2010b).

Acknowledgments

This is an article based upon original research presented at the 2nd International Conference on Cancer Immunotherapy and Immunomonitoring (CITIM), Budapest, Hungary, May 2011.

Declaration of interest

Ester Simeone received honoraria from Bristol Myers Squibb; Paolo A. Ascierto participated to Advisory Board from Bristol Myers Squibb, Merck Sharp & Dohme, Roche-Genentech, GSK and received honoraria from Bristol Myers Squibb, Roche Genentech, and Merck Sharp & Dohme.

References

- Ahmad, A., Tarhini, A. A., Moschos, S. J., Tawbi, H. A., Shuai, Y., Gooding, W. E., Sander, C., and Kirkwood, J. M. 2011. Safety and efficacy of combination immunotherapy with IFN α -2b and tremelimumab in patients With Stage IV melanoma. *J. Clin. Oncol.* 30:322-328.
- Ahmad, S., Lewis, M., Corrie, P., and Iddawela, M. 2011a. Ipilimumab-induced thrombocytopenia in a patient with metastatic melanoma. *J. Oncol. Pharm. Pract.* [Epub ahead of print] PubMed PMID:21807763.
- Akhtari, M., Waller, E. K., Jaye, D. L., Lawson, D. H., Ibrahim, R., Papadopoulos, N. E., and Arellano, M. L. 2009. Neutropenia in a patient treated with ipilimumab (anti-CTLA-4 antibody). *J. Immunother.* 32:322-324.
- Ascierto, P. A., Marincola, F. M., and Ribas, A. 2011. Anti-CTLA4 monoclonal antibodies: The past and the future in clinical application. *J. Transl. Med.* 9:196.
- Ascierto, P. A., Simeone, E., Sznol, M., Fu, Y. X., and Melero, I. 2010a. Clinical experiences with anti-CD137 and anti-PD1 therapeutic antibodies. *Semin. Oncol.* 37:508-516.
- Ascierto, P. A., Streicher, H. Z., and Sznol, M. 2010b. Melanoma: A model for testing new agents in combination therapies. *J. Transl. Med.* 8:38.

- Chapman, P. B., Hauschild, A., Robert, C., Haanen, J. B., Ascierto, P., Larkin, J., Dummer, R., Garbe, C., Testori, A., Maio, M., Hogg, D., Lorigan, P., Lebbe, C., Jouary, T., Schadendorf, D., Ribas, A., O'Day, S. J., Sosman, J. A., Kirkwood, J. M., Eggermont, A. M., Dreno, B., Nolop, K., Li, J., Nelson, B., Hou, J., Lee, R. J., Flaherty, K. T., and McArthur, A. G. (BRIM-3 Study Group). 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *New Engl. J. Med.* 364:2507-2516.
- Di Giacomo, A. M., Ascierto, P. A., Fonsatti, E., Pittiglio, E., Queirolo, P., Pilla, L., Ridolfi, R., Santinami, M., Testori, A., Parmiani, G., and Maio, M. 2011. A Phase II study combining ipilimumab and fotemustine in patients with metastatic melanoma: The NIBIT-M1 trial. [2011 ASCO Meeting, abstract #TPS230]. *J. Clin. Oncol.* 29:TPS230.
- Fong, L., and Small, E. J. 2008. Anti-cytotoxic T-lymphocyte antigen-4 antibody: The first in an emerging class of immunomodulatory antibodies for cancer treatment. *J. Clin. Oncol.* 26:5275-5283.
- Hersh, E. M., O'Day, S. J., Powderly, J., Khan, K. D., Pavlick, A. C., Cranmer, L. D., Samlowski, W. E., Nichol, G. M., Yellin, M. J., and Weber, J. S. 2011. A Phase II multi-center study of ipilimumab with or without dacarbazine in chemotherapy-naïve patients with advanced melanoma. *Invest. New Drugs* 29:489-498.
- Hodi, F. S., O'Day, S. J., McDermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J. C., Akerley, W., van den Eertwegh, A. J., Lutzky, J., Lorigan, P., Vaubel, J. M., Linette, G. P., Hogg, D., Ottensmeier, C. H., Lebbé, C., Peschel, C., Quirt, I., Clark, J. I., Wolchok, J. D., Weber, J. S., Tian, J., Yellin, M. J., Nichol, G. M., Hoos, A., and Urban, W. J. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. *New Engl. J. Med.* 363:711-723.
- Kirkwood, J. M., Lorigan, P., Hersey, P., Hauschild, A., Robert, C., McDermott, D., Marshall, M. A., Gomez-Navarro, J., Liang, J. Q., and Bulanhagui, C. A. 2010. Phase II trial of tremelimumab (CP-675,206) in patients with advanced refractory or relapsed melanoma. *Clin. Cancer Res.* 16:1042-1048.
- Korman, A., Chen, B., Wang, C., Wu, L., Cardarelli, P., and Selby, M. 2007. Activity of anti-PD-1 in murine tumor models: Role of 2host" PD-L1 and synergistic effect of anti-PD-1 and anti-CTLA-4. *J. Immunol.* 178:48.
- Korman, A., Yellin, M., and Keler, T. 2005. Tumor immunotherapy: Pre-clinical and clinical activity of anti-CTLA4 antibodies. *Curr. Opin. Invest. Drug.* 6:582-591.
- Korman, A. J., Peggs, K. S., and Allison, J. P. 2006. Checkpoint blockade in cancer immunotherapy. *Adv. Immunol.* 90:297-339.
- Krummel, M. F., and Allison, J. P. 1995. CD28 and CTLA4 have opposing effects on the response of T-cells to stimulation. *J. Exp. Med.* 182:459-465.
- Krummel, M. F., Sullivan, T. J., and Allison, J. P. 1996. Superantigen responses and co-stimulation: CD28 and CTLA4 have opposing effects on T-cell expansion *in vitro* and *in vivo*. *Int. Immunol.* 8:519-523.
- Lee, H. W., Park, S. J., Choi, B. K., Kim, H. H., Nam, K. O., and Kwon, B. S. 2002. 4-1BB promotes the survival of CD8⁺ T-lymphocytes by increasing expression of Bcl-xL and Bfl-1. *J. Immunol.* 169:4882-4888.
- Lin, G. H., Liu, Y., Ambagala, T., Kwon, B. S., Ohashi, P. S., and Watts, T. H. 2010. Evaluating the cellular targets of anti-4-1BB agonist antibody during immunotherapy of a pre-established tumor in mice. *PLoS One* 5:e11003.
- O'Day, S. J., Maio, M., Chiarion-Sileni, V., Gajewski, T. F., Pehamberger, H., Bondarenko, I. N., Queirolo, P., Lundgren, L., Mikhailov, S., Roman, L., Verschraegen, C., Humphrey, R., Ibrahim, R., de Pril, V., Hoos, A., and Wolchok, J. D. 2010. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: A multi-center single-arm Phase II study. *Ann. Oncol.* 21:1712-1717.
- Ribas, A., Hauschild, A., Kefford, R., Punt, C. J., Haanen, J. B., Marmol, M., Garbe, C., Gomez-Navarro, J., Pavlov, D., and Marshall, M. 2008. Phase III, open-label, randomized, comparative study of tremelimumab (CP-675,206) and chemotherapy (temozolomide [TMZ] or dacarbazine [DTIC]) in patients with advanced melanoma. *J. Clin. Oncol.* 26:LBA9011 with advanced melanoma.
- Robert, C., Thomas, L., Bondarenko, I., O'Day, S., Weber, J., Garbe, C., Lebbe, C., Baurain, J. F., Testori, A., Grob, J. J., Davidson, N., Richards, J., Maio, M., Hauschild, A., Miller, W. H., Jr., Gascon, P., Lotem, M., Harmankaya, K., Ibrahim, R., Francis, S., Chen, T. T., Humphrey, R., Hoos, A., and Wolchok, J. D. 2011. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *New Engl. J. Med.* 364:2517-2526.
- Simeone, E., Gentilcore, G., Esposito, A., Curvietto, M., De Rosa, P., De Maio, E., Pistillo, M. P., Queirolo, P., Mozzillo, N., and Ascierto, P. A. 2011. Immunological and biological changes and their correlation with clinical response and survival during ipilimumab in metastatic melanoma Compassionate Use Program. [2011 ESMO Meeting, abstract #9322]. *Eur. J. Cancer* 47(Suppl 1):686.
- Sznol, M., Hodi, F. S., Margolin, K., McDermott, D. F., Ernstoff, M. S., Kirkwood, J. M., Wojtaszek, C., Feltquate, D., and Logan, T. 2008. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients with advanced cancer. *J. Clin. Oncol.* 26:3007.
- Wilcox, R. A., Flies, D. B., Zhu, G., Johnson, A. J., Tamada, K., Chapoval, A. I., Strome, S. E., Pease, L. R., and Chen, L. 2002. Provision of antigen and CD137 signaling breaks immunological ignorance, promoting regression of poorly immunogenic tumors. *J. Clin. Invest.* 109:651-659.
- Wolchok, J. D., Neyns, B., Linette, G., Negrier, S., Lutzky, J., Thomas, L., Waterfield, W., Schadendorf, D., Smylie, M., Guthrie, T. Jr., Grob, J. J., Chesney, J., Chin, K., Chen, K., Hoos, A., O'Day, S. J., and Lebbé, C. 2010. Ipilimumab monotherapy in patients with pretreated advanced melanoma: A randomised, double-blind, multicentre, Phase 2, dose-ranging study. *Lancet Oncol.* 11:155-164.