How anti-PD1 treatments are changing the management of melanoma

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Practice points

- High durable response rates have been seen in patients with unresectable stage III or IV melanoma treated with monoclonal antibodies (mAbs) against the checkpoint molecule PD1 on T cells in large Phase I studies.
- Similar results have been seen in patients treated with mAbs against the ligand for PD1 (PD-L1) on melanoma cells. Phase III studies are in progress.
- The availability of effective treatments may justify more frequent follow-up of patients who are at high risk of relapse after the surgical removal of melanoma.
- Anti-PD1 treatments may render patients operable who were considered inoperable.
- Surgery may have an important role in removing residual metastases in order to achieve complete response.
- High durable response rates and a relative lack of toxicity suggest that treatments aimed at the PD1/PD-L1 axis may soon become the standard of care in the treatment of melanoma.
- Gene signatures from tumors (or blood) are being assessed as biomarkers for the selection of responders to anti-PD1 and to guide combination treatments.
- Current limitations include failure to respond in approximately 30% of patients. Complete responses are only seen in approximately 5–10% patients. Durability has yet to be established, and autoimmune side effects can be limiting.
- Combinations with mAbs against PD-L1, CTLA4 or LAG3 are being tested in clinical trials, as are combinations with BRAF inhibitors.
- Combinations with melanoma vaccines are being planned.
- Combinations with other agents, such as inhibitors of NF- κ B or epigenetic regulators such as HDAC or BET protein inhibitors, are under discussion.
- Effective treatment of brain metastases remains essential for curing patients.

SUMMARY The introduction of immunotherapy based on the blockade of the PD1/PD-L1 checkpoints has been associated with high response rates and durable remissions of disease in patients with metastatic melanoma, to the extent that it is now considered the standard of care for a wide range of patients, irrespective of their *BRAF* or *NRAS* mutation status. In addition, more frequent follow-up of patients who are at high risk of recurrence after surgical treatment appears to be justified, as does neoadjuvant treatments in order to

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render patients treatable by surgery. The limitations of this treatment include failure of some patients to respond, a low rate of complete responses and relapses of the disease during treatment. New initiatives in order to overcome these limitations include the identification of biomarkers for the selection responders and evaluations of treatment combinations that will increase responses and their durability. The latter includes combinations with antibodies against other checkpoints on T cells and cotreatments with inhibitors of resistance pathways in melanoma.

KEYWORDS

- CD8 T cells
- immunotherapy
- melanoma • monoclonal antibodies • PD1/PD-L1
- resistance factors • survival outcomes • TILs

The treatment of melanoma once it has spread beyond locoregional sites has been challenging. Surgical resection of isolated metastases has been and remains an option for a small percentage of patients, but if the number or site of metastases makes this impractical, systemic treatments are needed [1–3]. Prior to 2010, the standard of care was chemotherapy with dacarbazine, which was shown in numerous trials to be as good as or superior to treatment with a number of chemotherapeutic and biological agents [4,5]. In 2010, however, the first of two large, well-controlled trials of immunotherapy with monoclonal antibodies (mAbs) against the CTLA4 checkpoint inhibitor, ipilimumab, was reported [6]. Treatment of (pretreated) patients with monotherapy ipilimumab at 3 mg/kg was shown to increase median overall survival from 6.5 months in the control, gp100 vaccine group to 10.1 months in the ipilimumab group. The 1-year survival rates were 23.5 and 45.6%, respectively, and 13.7 and 23.5%, respectively, at 2 years. These results were followed in 2011 by a report showing that a combination of ipilimumab at 10 mg/kg and dacarbazine was superior to chemotherapy with dacarbazine alone [7]. The median overall survival rates were 11.2 months compared with 9.1 months, respectively. Survival rates at 1 year were 47.3% compared with 36.3%, respectively, and 28.5 versus 17.9%, respectively, at 2 years.

As important as these results were, they have been overshadowed by preliminary results from large Phase I studies of mAbs against the checkpoint inhibitor, PD1, and its ligand PD-L1. As shown in [Figure 1A](#), CTLA4 and PD1 have complementary roles. PD1 appears on T cells that have been stimulated by repeated exposure to antigens in the periphery, in contrast to CTLA4, which is expressed at the initial contact with antigens. When T cells come into contact with the ligand for PD1 (PD-L1) on melanoma cells, their activity is inhibited ([Figure 1B](#)). When the interaction with PD-L1 is blocked, either by mAbs against PD1 or PD-L1, the T cells are able to kill the melanoma cells [8]. Subsequent studies have shown that expression of PD-L1 on

melanoma is induced by IFN- γ from T cells, so that PD-L1 expression represents a protective feedback mechanism in melanoma [9].

The first report on results from treatment with the mAb nivolumab in 2012 (sponsored by Bristol-Myers Squibb [BMS]) indicated that treatment was associated with overall response rates of 28% and median survival times of 24 months [10]. The 1- and 2-year survival rates were 62 and 43%, respectively [11]. Treatment with a second mAb, referred to as MK3475 (sponsored by Merck), produced overall response rates of 38% and higher rates at some dose schedules [12]. Even greater response and survival rates were suggested by preliminary results from the treatment of relatively small patient groups with a combination of nivolumab and ipilimumab [13]. Response rates in patients treated concurrently with 1 mg/kg of nivolumab and 3 mg/kg of ipilimumab were 48%, and the 1-year survival rate in the 17 patients so treated was 81%. These results are now being evaluated in a large, three-arm, Phase III study (CA209-067) comparing ipilimumab 3 mg/kg with nivolumab 1 mg/kg versus ipilimumab 3 mg/kg plus nivolumab 1 mg/kg. A second large Phase III study is comparing MK3475 at a dose level of 10 mg/kg given at 2- or 3-week intervals with ipilimumab at 3 mg/kg. The incidence rates of side effects have been relatively low and manageable, even in studies of the combination of nivolumab and ipilimumab [13]. Concurrent Phase I studies with mAbs against PD-L1 have also shown promise, with nine out of 52 patients showing responses during treatment with the mAb BMS-936559. Progression-free survival (PFS) at 6 months was 42%. There were minimal grade 3–4 side effects [14]. Responses were also seen in nine out of 35 patients treated with another PD-L1 mAb, referred to as MPDL3280A and sponsored by Genentech. PFS at 6 months was 35%. Side effects were also minimal [15].

These results compare well with the results from targeted therapy for the treatment of patients with *BRAF*^{V600}-mutated melanoma, where response rates of approximately 50%

Figure 1. The checkpoint receptors CTLA4 and PD1 have complementary functions, with CTLA4 appearing early in the response and PD1 after repeated stimulation.

were seen with the BRAF inhibitors vemurafenib or dabrafenib alone, with PFS of 5.3 and 5.8 months, respectively. In Phase II studies, the combination of dabrafenib and trametinib had higher response rates of 75.9% and a PFS of 9.4 months, but nevertheless, relapse from these treatments remains a problem and their effects on overall survival are yet to be established (reviewed in [16]).

The rapidity with which these changes have occurred has meant that their full impact on the management of melanoma is yet to be realized and incorporated into treatment plans. We discuss below some of the implications of these treatments and what future developments might be seen.

More effective treatment of metastatic melanoma may justify closer follow-up of patients after surgical treatment of resectable stage II, III or IV melanoma

For the first time in the history of melanoma treatment, there is the promise of systemic treatments that might cure the disease, or at least

result in meaningful prolongation of life should recurrences occur following surgical resection of clinically detectable disease. In view of this, more active follow-up programs may be warranted, such as 6-monthly whole-body CTs for a 3-year period after surgery and 3-monthly visits with hematology and biochemical tests. It is also possible that treatment with anti-PD1 molecules might reduce the risk of recurrent melanoma after surgery for locoregional disease. Although conducting trials in an adjuvant setting seems to be a logical step given the responses in patients with metastatic disease, it is not proven that anti-PD1 molecules will be effective in resected stage II and III disease. In particular, it will be necessary to determine whether micrometastases express PD-L1, as its expression might be a prerequisite for the efficacy of anti-PD1 treatment at this stage of the disease.

Treatment with mAbs against PD1 to increase the effectiveness of surgery

Many patients with localized disease present with locoregional disease that has progressed

to a stage where surgical removal is impractical or hazardous. Anti-PD1 treatments offer the prospect of reducing tumor bulk to the extent that surgical resection may be possible and may also reduce micrometastases at distant sites. Neoadjuvant studies similar to those conducted with ipilimumab by Tarhini and colleagues [17] will also be of great interest. Surgical removal of residual metastases after anti-PD1 treatments may render patients disease free and achieve complete responses. This needs to be tested in a randomized trial.

Will mAbs against PD1 become the new standard of care in metastatic melanoma?

Should the larger randomized studies referred to above confirm the high response rates and durability of mAbs against PD1, as seen in the Phase I studies, it would seem appropriate to make this the treatment of choice for patients with metastatic disease [18]. Exceptions to this might be patients with *BRAF*^{V600} melanoma with rapidly progressive disease, high lactic dehydrogenase (LDH) values and symptoms resulting from the melanoma, such as pain requiring rapid relief.

Much interest is focused on whether patients who respond to anti-PD1 treatments may be identified prior to treatment. Studies on responders to vaccines, such as those targeting MAGE-A3, have identified gene signatures that appear to be predictive of response [19]. Similar gene signatures were identified in responders to other forms of immunotherapy, such as IL-12, and it would not be surprising if they also predict responses to anti-PD1 treatments.

There is some evidence that the expression of PD-L1 on melanoma may be predictive of response [10,20]. Given that PD-L1 may be induced by immune responses, this may be a circular argument and be a component of the immune gene signature. Measurement of PD-L1 as a biomarker has been proven to have a number of practical difficulties. The two major sponsors have different cutoffs for positivity of immunohistochemically stained sections, with BMS taking a level of 5% of tumor cells, while Merck takes 1% as their cutoff. Whether the antibodies used have similar sensitivities is also unknown. The PD-L1 antigen is considered to be labile and its detection requires strict adherence to protocols. Rigorous evaluation of PD-L1 as a biomarker is ongoing. As reviewed elsewhere, it is likely that PD-L1 on

melanoma may also be constitutively induced by oncogenic signals and form a subgroup of melanoma that is separate to those with PD-L1 that is induced by the immune response [8]. The latter may not respond to anti-PD1 treatments unless combined with other treatments that target the oncogenic signals, as discussed elsewhere [21].

Another question is whether the mutation status of melanoma may predict responses to anti-PD1 treatments. *NRAS* mutations and the absence of immune gene signatures were shown to be independent adverse prognostic features [22,23]. Nevertheless, *NRAS*-mutated melanoma was shown to be more sensitive to being killed by lymphokine-activated killer cells [24], and patients with *NRAS*-mutated melanoma showed higher responses and increased PFS rates when treated with high-dose IL-2 [25]. More extensive studies are needed in order to answer whether *NRAS*-mutant melanoma may also respond more favorably to anti-PD1 treatments.

Overcoming the limitations of treatment with anti-PD1 therapy

The Phase I trials have identified two limitations of treatment with anti-PD1 molecules. One is that complete response rates in most of the studies are <10%. Is this because the rate of tumor cell killing is balanced by cell renewal, or is it because the treatment has changed the microenvironment to limit T-cell infiltration? Is the existing T-cell population changed to a regulatory type, or does treatment even increase the number of suppressive macrophages? Is this because of the selection of melanomas that have strong antiapoptotic mechanisms, such as high expression of Bcl-2 or Bcl-A1 [26]?

Another limitation is that some patients (nearly 50%) do not respond or respond but relapse in a short time. The basis for these limitations are as-yet not well documented. It is likely that multiple mechanisms are involved, and systematic studies are needed in order to work through the possibilities. Given that T cells are needed for response, it could be speculated that patients who fail to respond may lack tumor-infiltrating lymphocytes (TILs). Indeed, some authors have classified melanomas broadly into those with or without TILs [27]. The absence of TILs is a problem that is encountered in many forms of immunotherapy [28]. In some instances, metastatic niches may exist that keep

melanoma cells in a dormant state that is not recognized by immune cells [29]. Whether such melanomas are not recognized by the immune system or they suppress immune recognition by the release of cytokines such as IL-10 or TGF- β is not clear [30]. For example, it was reported that endothelin B receptors on the endothelium were responsible for inhibiting T cells from homing to tumors [31].

In patients with TILs but who fail on treatment, it is possible that their melanomas have other checkpoint ligands that act on other checkpoint receptors on T cells, such as LAG3, Tim3 and BTLA4. There is much evidence that the cytokines/chemokines released by melanoma cells shape the microenvironment of the tumor [19,32] and that certain cells, such as myeloid cells, can be immunosuppressive in that environment [33,34]. Alternatively, there is much support for the idea that cytokines from CD8 T cells, such as IFN- γ , can generate negative feedback mechanisms in melanoma cells that result in the production of inhibitory mechanisms, such as indolamine 2,3-dioxygenase, regulatory T cells and the upregulation of ligands for negative checkpoints on T cells, such as PD1 and LAG3 [27,35]. Other possibilities include the loss of MHC class I or II antigens, preventing recognition or the release of immunosuppressive molecules that are impenetrable by lymphocytes, such as galectins, on the surface [36]. It is also possible that the cytokines generated by the immune response may change the tumor into a more resistant form against cytotoxic T lymphocytes (CTLs), as is reported to occur in response to TNF- α [37]. The lack of recognition by TILs may be reflected in the low mitotic rates in TILs or the inability to produce IL-2.

The extent to which inherent resistance factors in melanoma cells may prevent killing by CTLs also needs further exploration. Overexpression of Bcl-2 was found to inhibit granzyme B-induced killing, but not killing by CTL, suggesting that other granzymes were involved. Nevertheless, granzyme B induced a prolonged pressure to die and raised the possibility that BH3 mimetics may have a role along with CTLs [38–40]. The killing of melanoma by CTLs against MART1 on melanoma cells that have been made resistant to melanoma by prolonged coculture could be restored by the treatment of melanoma with inhibitors of NF- κ B [41] or proteasome inhibitors [42], suggesting that

inherent antiapoptotic mechanisms inhibited killing by CTLs.

Combination treatments that build on the results from anti-PD1 treatments

The combination of anti-PD1 treatments with other agents may help to overcome these limitations. Possibilities include combination with other checkpoint inhibitors, such as anti-CTLA4 molecules, which is already being tested in Phase III trials [13]. Other possibilities include combinations of anti-PD1 treatments with anti-PD-L1 treatments, LAG3 and others [8,43]. There is also now a strong case to be made for combining melanoma vaccines with anti-PD1 treatments, as it is highly likely that the ineffectiveness of past vaccine studies may reflect a strong suppression of responses by PD1 on T cells [44]. Given that long-term responses to vaccines are associated with responses to individual specific mutated proteins, combinations with autologous vaccines or epitopes from autologous melanomas are of high interest [45].

The combination of anti-PD1 treatments with signal pathway inhibitors is also of high priority. Combinations with selective BRAF inhibitors similar to those being conducted in combination with ipilimumab will be important in patients with *BRAF*^{V600} mutations, particularly as these inhibitors have been shown to increase immune responses against melanoma [46–50]. However, upregulation of PD-L1 on melanomas failing on BRAF inhibitor treatment may involve other pathways that can be targeted in *BRAF*^{V600} melanomas [51]. Third-generation BRAF inhibitors that do not cause paradoxical activation of the MEK pathway will also be of interest in relation to PD-L1 expression [52]. **Table 1** summarizes ongoing trials with mAbs against PD1 and PD-L1, as well as combinations with other agents.

As discussed elsewhere, another category of inhibitors that has had relatively little attention is the epigenetic regulators that target the epigenetic changes underlying the resistance of melanoma to therapy [53,54]. Initial studies on a class of drugs referred to as BET protein inhibitors have demonstrated potentially interesting combinations with anti-PD1 treatments, particularly in patients with melanomas with high levels of activated NF- κ B and cytokine expression [55].

It is likely that curing patients with melanoma using anti-PD1 treatments will remain

Table 1. Current trials evaluating monoclonal antibodies against PD1 or PD-L1 in the treatment of melanoma.

Trial name	Trial ID	Drug	Sponsor
Phase 1 Biomarker Study of Anti-PD-1 in Advanced Melanoma	NCT01621490	BMS-936558 (anti-PD-1)	BMS
Multiple Class I Peptides and Montanide ISA 51 VG w Escalating Doses of Anti-PD-1 Antibody BMS936558	NCT01176474	BMS-936558	BMS
Multiple Class 1 Peptides and Montanide ISA 51 VG w Escalating Doses of Anti-PD-1 ab	NCT01176461	BMS-936558	BMS
Study of MK-3475 in Participants with Progressive Locality Advanced or Metastatic Carcinoma, Melanoma, or Non-small Cell Lung Carcinoma	NCT01295827	P07990/MK-3475-001	Merck
Study to Evaluate the Safety and Efficacy of two different Dosing Schedules of MK-3475 Compared with Ipilimumab in Participants with Advanced Melanoma	NCT01866319	MK-3475-006	Merck
Study of MK-3475 versus Chemotherapy in Participants with Advanced Melanoma	NCT01704287	MK-3475	Merck
A Phase 1 Study to Evaluate AMP-514	NCT02013804	AMP-514	AstraZeneca/Medimmune
A Phase 1b Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in combination with Vemurafenib (Zelboraf) in Patients with Previously Untreated <i>BRAF</i> ^{V600} -Mutation Positive Metastatic Melanoma	NCT01656642	MPDL3280A	Roche/Genentech
Phase 1 Safety and Tolerability of MEDI4736 in Combination With Dabrafenib and Trametinib or With Trametinib Alone	NCT02027961	MED14736	Medimmune/AstraZeneca
A Phase 1 Study of an Anti-KIR Antibody in Combination with an Anti-PD1 Antibody in Patients with Advanced Solid Tumors	NCT01714739	BMS986015/IPH2102	BMS/AstraZeneca
Study to Assess the Safety, Tolerability, and Pharmacokinetics of AMP-224 in Patients with Advanced Cancer	NCT01352884	AMP-224	Amplimmune/GSK
A Phase 1 Study of MPDL3280A (an Engineered Anti-PDL1 Antibody) in Patients with Locally Advanced or Metastatic Solid Tumors	NCT01375842	MPDL3280A	Roche/Genetech

Ab: Antibody; BMS: Bristol-Myers Squibb; GSK: GlaxoSmithKline; w: With.

elusive unless combined with agents that increase their efficacy against brain metastases. As reviewed elsewhere, new approaches targeting connexins may have therapeutic potential in future studies [56].

Conclusion & future perspective

Anti-PD1 treatments are rapidly becoming the standard of care in treating unresectable stage III and IV melanomas and in changing patient follow-up protocols following surgical resection of locoregional disease. It is likely that such treatments will be used in conjunction with surgery in order to either render patients surgically manageable or to excise residual disease and so achieve complete remissions. An increasing use of gene signatures in the tumor or immune signatures of blood can be expected in order to identify responders. It is also highly likely that such analyses will be used to guide

the use of anti-PD1 treatments in combination either with other checkpoint inhibitors or with signal pathway inhibitors. It would not be unexpected to see a resurgence of vaccine therapy together with anti-PD1 treatments. In order to cure patients who are treated in such a fashion, continued research into effective treatments or the prevention of brain metastases will be required.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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