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Nivolumab in combination with ipilimumab for the treatment of melanoma

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Abstract

Melanoma patients develop resistance to most therapies, including chemo- and targeted-therapy drugs. Single-agent therapies are ineffective due to the heterogeneous nature of tumors comprising several subpopulations. Treatment of melanoma with immune-based therapies such as anti-cytotoxic T-lymphocyte activation-4 and anti-programmed death-1 antibodies has shown modest but long-lasting responses. Unfortunately, only subsets of melanoma patients respond to antibody-based therapies. Heterogeneity in lymphocyte infiltration and low frequency of anti-melanoma-reactive T-cells in tumor lesions are partly responsible for a lack of response to antibody-based therapies. Both antibodies have same biological function but they bind to different ligands at various phases of T-cell activity. Thus, combination therapy of antibodies has shown superior response rates than single-agent therapy. However, toxicity is a cause of concern in these therapies. Future identification of therapy-response biomarkers, mobilization of tumor-reactive T-cell infiltration using cancer vaccines, or non-specific targeted-therapy drugs will minimize toxicity levels and provide long-term remissions in melanoma patients.

Keywords

antibody therapy; anti-CTLA-4; anti-PD-1; immune-checkpoint inhibitors; Melanoma

Melanomas account for ~4% of all dermatological cancers, but 80% of deaths from skin cancer [1,2]. 10-year overall survival rate for advanced melanoma is ~10–15% and in the elderly (age >70), regardless of their disease stage, the survival rate drops dramatically [3]. The incidence of melanoma continues to rise worldwide, and the reasons remain unclear [2,4]. Increased exposures to sun or ultraviolet radiation and frequent use of tanning beds are some of the possible risk factors [2,5]. Despite major strides in molecular medicine, the treatment of metastatic melanoma continues to be a challenge. This is due to the rapid development of resistance to most available chemo-and targeted therapy drugs [4,6,7]. Until very recently, melanomas were considered as a single disease entity and most patients were treated with dacarbazine (DTIC), an alkylating agent, or temozolomide, a second-generation

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drug derivative of DTIC. The objective response rate with these drugs was low (<15%) and it had limited overall survival benefits [7].

Advances in molecular medicine have re-classified melanoma as a highly complex heterogeneous disease comprising of several subpopulations of tumor cells [6,7]. A number of distinct gene mutations and aberrant cell-signaling pathways have been identified in melanomas [2,5,8]. This led to the development of a new generation of targeted therapy drugs such as vemurafenib and dabrafenib targeting mutant BRAF and cobimetinib or trametinib targeting MEK. Melanoma patients treated with targeted therapy drugs show dramatic increase in overall response rate and extended survival [2,6,9]. However, most patients also develop resistance to targeted therapy drugs and present aggressive metastatic tumor lesions on recurrence [6,9].

Recent promising results in the clinics with immune checkpoint reagents such as anti-cytotoxic T-lymphocyte activation-4 (CTLA-4) (ipilimumab) and anti-programmed death (PD)-1 (pembrolizumab, nivolumab) have reenergized the field of tumor immunology with renewed interest in the use of immune-based therapies for treatment of cancer [10]. Unlike dramatic responses seen with targeted therapy drugs, more modest but durable therapy responses are observed with immune checkpoint inhibitors [10]. However, not all patients respond to immune checkpoint inhibitors when anti-CTLA-4 or anti-PD-1 antibodies are used as monotherapy [10–12]. In the following, we review the development of major immune-based therapies in the clinics, briefly summarizing important immune co-stimulatory and immune checkpoint molecules, and highlight recent clinical trial data on single agent use of ipilimumab or pembrolizumab/nivolumab antibodies. Finally, we summarize the current status of clinical trials on combined use of the above immune checkpoint blockade antibodies.

Immune-based therapies

Until very recently, efforts to use immunological agents to treat melanoma patients met with limited success. Early immunological studies relied largely on non-specific and specific activation of the immune system. Some of the nonspecific agents include use of IL-2 or interferon- α (IFN- α) (see reviews [2,13,14]). Both these cytokines modulate melanoma cells to increase tumor-associated antigens and/or HLA class I or II expression to facilitate antigen presentation and promote T-cell activation [2,13,14]. Such agents were largely ineffective due to non-specific activation of T-cells and other immune cell types including dendritic cells, macrophages and natural killer cells, and worked only on a small subset of melanoma patients [14,15]. As both cytokines have a short serum half-life, high dosage was administered that resulted in intolerable toxicity in many patients [13–15]. Administration of slow-release PEGylated cytokines minimized the toxicity issues and was well tolerated in patients [16]. Besides cytokines, other immunological agents such as use of non-specific vaccine (BCG), melanoma-specific whole-cell allogeneic vaccines [Canvaxin and Melacine] or melanoma antigen-specific vaccines (gangliosides, peptides [gp100, MART, MAGE-1/3 and tyrosinase]) were all tried to activate the immune system and most of them failed in clinical trials [17]. Personalized adoptive cell therapy (ACT) using *in vitro* expanded tumor-infiltrating lymphocytes (TILs) has shown mixed responses in melanoma

patients [18]. However, not all major cancer centers are able to adopt the ACT therapy approach due to technical difficulties in generating large number of TILs. As in the case of any other therapy, only a subset of melanoma patients responded to ACT treatment [19]. Melanoma patients in the younger age group fared better in the trials as they could withstand high toxicity issues [20]. Recently, impressive clinical results were obtained with the use of anti-CD19 (B-cell antigen)-directed chimeric antigen receptor (CAR) T-cells in leukemia and lymphoma patients [21]. Efforts are underway to find a suitable CAR T-cell tumor antigen-specific target for melanoma. Many such studies are either in pre-clinical or early clinical trial phases, and the efficacy of CAR-cells needs to be demonstrated in a large cohort of patients.

Low-to-modest clinical responses in immune-based therapies prompted many groups to shift their focus to understand the complex nature of immune regulatory networks. Cancer immunology studies largely benefited from discoveries in viral immunology where it was shown that T-cells that are chronically exposed to antigens are in a state of 'exhaustion' or dysfunction and hence, their inability to clear infection [22,23]. The phenomenon of 'T-cell exhaustion' was also confirmed in many cancer patients that led to the identification of various immune-stimulatory or -regulatory pathways of T-cell activation and downmodulation. This led to the discovery of many biological agents that can be used for modulating co-stimulatory and immune-regulatory molecules to enhance the overall immune responses [24].

Immune regulation

Immune response to antigens is well-regulated either directly or indirectly by cell-to-cell contact or a number of soluble (cytokines or chemokines) factors (see review [17]). T-cell receptor recognition of an antigenic peptide presented on MHC molecules of an antigen presenting cell (APC) provides the primary signal for T-cell activation [24]. For optimal activation, a second signal by interaction of co-stimulatory molecules with its respective ligand on APCs is required [24]. The presence of inhibitory molecules such as CTLA-4 or PD-1 or its ligand PD-L1 can compete or block co-stimulation of T-cells resulting in immune downmodulation [17,24]. Immune checkpoint events are generally safety mechanisms evolved to prevent adverse events of T-cells reacting to self antigens and cause autoimmunity [17]. In cancer patients, due to chronic exposure of T-cells to tumor-associated antigens, upregulation of immune checkpoint molecules is often observed at the site of tumor lesions [17,25]. Recent studies suggest that tumor cells or inflammatory factors present in the tumor microenvironment are responsible for the upregulation of immune checkpoint molecules to facilitate escape of the tumor cells from immune T-cell killing [17,26].

Modulation of immune regulation

The outcome of an immune response can be modulated by altering the intensity of the second signal needed for T-cell activation by use of an agonist antibody against co-stimulatory molecules or by blocking the interaction of inhibitory molecules (CTLA-4 or PD-1) with their respective ligands [24]. Most co-stimulatory molecules belong to the

immunoglobulin superfamily (B7–1/B7–2 [CD80/CD86], CD28) or the TNF receptor superfamily (4–1BB, CD27, CD40) [24]. Thus far, a very cautious approach has been taken with regard to the use of agonistic antibodies in stimulating co-stimulatory molecules that have a potential risk of triggering a cytokine storm and autoimmune attack causing tissue damage. In a Phase I clinical trial, the use of anti-CD28 to boost immune responses was abandoned as six of the eight volunteers developed a massive cytokine storm and severe adverse reactions within an hour of infusion of the agonistic antibody [27]. Readers are referred to excellent reviews available on the pros and cons of targeting co-stimulatory molecules [17,24,28].

Immune checkpoint molecules & their inhibitors

There are a number of immune checkpoint molecules identified and they include CTLA-4, PD-1/PD-L1, lymphocyte-activation gene (LAG)-3, TIGIT and T-cell immunoglobulin (TIM)-3 (see reviews [24,29]). Anti-CTLA-4 (ipilimumab; Bristol-Myers Squibb) was the first immune checkpoint inhibitor to be approved for clinical use. CTLA-4 is expressed on CTL as a late event to regulate the amplitude of T-cell-mediated killing of target cells [30]. There are mixed views on the mechanism of inhibition. CTLA-4 essentially competes with a better binding affinity to B7.1 (CD80)/B7.2 (CD86) molecules, ligands of co-stimulatory molecule (CD28) to downmodulate the T-cell activity [24].

Anti-CTLA-4 therapy

Ipilimumab is a fully engineered human monoclonal antibody (IgG1) that blocks CTLA-4 binding to its ligand B7–1/B7–2 (see BOX 1; [30,31]). High affinity binding of this antibody to B7–1/B7–2 prevents binding of CD28 to its ligand resulting in downmodulation of T-cell activity [31]. Tremelimumab is a human IgG2 monoclonal antibody binding to CTLA-4 and blocking the interaction with B7–1/B7–2 (see BOX 1; [32,33]). Both antibodies were used in clinical trials to treat melanoma patients. Tremelimumab failed to show any beneficial effect when tested in Phase III trials and was subsequently abandoned [33].

In a Phase II trial of melanoma patients, the use of ipilimumab as a single-agent therapy (3 mg/kg or 10 mg/kg body weight, every 3 weeks for 4 cycles followed by maintenance therapy every 3 months) significantly improved the median overall survival (OS) [30,34]. The objective response to singleagent ipilimumab is 10–15% [30]. In Phase III randomized multi-centric trials involving HLA-A2+ melanoma patients (n = 676), the administration of ipilimumab either alone (n = 137) or in combination with experimental gp100 peptide vaccine (n = 403) resulted in a median OS of 10.1 months (for both groups) when compared to gp100 immunized patients (n = 136; median OS 6.4 months) [30]. The addition of gp100 peptide vaccine to ipilimumab did not increase the median OS in patients. This may be due to the timing of gp100 peptide vaccination in relation to ipilimumab injections or low frequency of anti-gp100 reactive T-cells in advanced melanoma patients. About 10–15% of the patients had Grade 3 or 4 immune-related adverse events [35]. Toxicity following ipilimumab was consistently observed in most melanoma patients (~25%) in several trials [10,36]. This is mainly due to the presence of CTLA-4 on many T-cells including those that

are in circulation and residing in normal tissues. Toxicities to ipilimumab are well managed with the administration of corticosteroids.

To minimize adverse immune-related events, efforts are underway to include cytokines or growth factors such as granulocyte-macrophage (GM)-CSF [37,38]. The addition of GM-CSF to ipilimumab resulted in a dramatic decrease in toxicity from 58 to 45% and the gastrointestinal toxicity dropped from 27 to 16% [38]. The combination of IFN- α and ipilimumab improved therapy responses but failed to alter toxicity levels [33]. A trial of bevacizumab plus ipilimumab showed better therapy responses (median OS 25.1%) by enhancing the T-cell infiltration and changes in tumor-stromal vasculature [39].

Ipilimumab has also been used in combination with classical therapy, DTIC or targeted therapy drug, vemurafenib. In a Phase III trial, ipilimumab (10 mg/kg) plus DTIC was compared with DTIC plus placebo [40]. Median OS survival with ipilimumab was 11.2 months compared to 9.1 months in the DTIC plus placebo group. In a Phase I study, the concurrent administration of vemurafenib and ipilimumab resulted in Grade 3 hepatotoxicity with elevated liver enzyme levels [35,41]. The rationale for such trials was that chemo- or targeted therapy drugs could cause tumor cell death and dying cell debris can activate the immune cells.

Anti-PD-1 therapy

Recently, two anti-PD-1 antibodies for treatment of advanced melanoma patients were approved by the US FDA. Pembrolizumab (Merck), formerly known as lambrolizumab, is a high-affinity humanized IgG4 monoclonal antibody and was the first one to be approved by the FDA and more recently, a second anti-PD-1 antibody (nivolumab [Bristol Meyers Squibb]), a fully human monoclonal IgG4 antibody, was approved (see BOX 1; [42]). PD-1 expression on T-cells is restricted to the site of cancer lesions within the inflammatory cytokine environment and thus, a major inhibitor of T-cell in the effector phase [24–26]. Both antibodies, pembrolizumab and nivolumab, can prevent the binding of PD-1 to its ligands PD-L1 or PD-L2, resulting in the disruption of inhibitory mechanisms and restoring T-cell effector phase function [42,43].

Both pembrolizumab and nivolumab were evaluated in many clinical trials. A common observation from these trials was that both antibodies were equally effective in inducing long-term durable responses, which was accompanied by significantly far less toxicity when compared with ipilimumab antibody therapy [33,36,43,44]. The objective response to pembrolizumab or nivolumab is 40–50% and Grade 3 or 4 toxicities are ~10%.

Nivolumab was first evaluated in a Phase I/II study comprising 296 solid tumor patients including melanoma [42]. Patients received nivolumab (1–10 mg/kg) every 2 weeks for 12 cycles [42]. For melanoma, the response rate was ~28% (26/94 patients), which was comparable to other solid tumors such as renal cell and non-small cell lung carcinomas. An extension of this study with multi-dose nivolumab regimen was undertaken in advanced melanoma patients (n = 107) and median OS was 16.8 months, and 1- and 2-year survival rates were 62 and 43%, respectively [45]. A higher 1-year OS was observed in a Phase III

randomized trial with nivolumab (3 mg/kg dose group; 73% OS) when compared to DTIC (42%)-treated patients [46].

Pembrolizumab-treated advance melanoma patients (n = 135) showed responses similar to nivolumab-treated patients when three different dosing strategies (2 mg/kg every 3 weeks or 10 mg/kg every 2 or 3 weeks) were used [47]. Some patients in this trial were ipilimumab non-responders and showed low-grade adverse events similar to nivolumab. Overall response rate with pembrolizumab treatment was 38% that reached as high as 52% with 10 mg/kg dose. Observed responses were durable and median progression-free survival (PFS) was >7 months [47]. Updated report on the Phase I clinical trial of 411 melanoma patients treated across multiple-dose levels indicated a 1-year OS rate of 69% [12,48].

In a Phase III study, advanced melanoma patients (n = 834) were treated with pembrolizumab at a dose of 10 mg/kg every 2 or 3 weeks or ipilimumab at 3 mg/kg every 3 weeks and compared for PFS and OS [49]. The estimated 6-month PFS was similar for patients who received pembrolizumab every 2 and 3 weeks (47.3% and 46.4%, respectively) [50]. PFS was 26.5% in patients who received ipilimumab. Treatment-related adverse events were consistently lower in patients who received pembrolizumab.

Conclusions

Thus far, clinical studies have shown impressive increases in OS in patients treated with immune checkpoint agents [51]. However, not all patients respond to anti-CTLA-4 or anti-PD-1 antibody therapies. Currently, many studies are underway to address the nature of immune non-responsiveness at the tumor site. Poor lymphocytic infiltration of anti-melanoma-specific T-cells at the tumor site is partly responsible for the lack of therapy responses to anti-CTLA-4 or anti-PD-1 antibodies [11,12]. Patients with tumor infiltrating lymphocytes reacting to neo-antigen epitopes have shown better therapy response with anti-CTLA-4 treatment [52]. The administration of bevacizumab (anti-VEGF) antibody in combination with ipilimumab has shown encouraging results with improved median OS [53]. This suggests that combination therapy strategies to improve lymphocyte infiltration will be a key factor in improving OS responses.

Expert commentary

The recent introduction of new generation of targeted therapy drugs and immune-based therapies has revolutionized the field of oncology and cancer immunology. Dramatic therapy responses using targeted therapy drugs and doubling of OS using immune checkpoint blockade antibodies raises hopes for a cure in advanced-stage melanoma patients. Re-classification of melanoma as a highly heterogeneous disease strongly reemphasizes the need to target cancer cells using combination therapy approaches to prevent the growth of resistant clones of tumor subpopulations. For this, one has to use a combination of immune-based and targeted therapies to achieve long-term responses. The choice of using the right combination heavily depends on good biomarker predictions. Potential markers have been identified in single-agent targeted therapy and immune checkpoint antibody trials and one needs to validate them in large cohort of melanoma

patients. Generally, patients whose tumors show PD-L1 expression have better clinical responses with anti-PD-1 antibody therapy [43]. However, *in situ* detection of PD-L1 needs further standardization before it can be used as a predictive biomarker [12,54]. A strong rationale for combining immune checkpoint agents are justified as anti-CTLA-4 and anti-PD-1 antibodies bind to their respective ligands at different phases of T-cell induction and maturation into effector function. In a recent study, analyses of blood and tissue specimens of patients undergoing mono-or combination-therapies of anti-CTLA-4 and anti-PD-1 antibodies revealed that blockade of CTLA-4 induces a proliferative signature in a subset of memory T-cells, whereas PD-1 blockade modulates genes that are involved in T-cell or NK-cell effector functions [55]. Patients receiving combination therapy showed increases in plasma cytokine or chemokine levels when compared to single-agent therapies which may explain enhanced infiltration observed in patients receiving anti-CTLA-4 and anti-PD-1 antibodies [55]. In one study, PD-1 binding to its ligand results in metabolic reprogramming by inhibiting glycolysis and promoting fatty acid oxidation [56]. In contrast, CTLA-4 engagement inhibits glycolysis without altering fatty acid oxidation [56]. Enhanced presence of CD8⁺ T-cells, T-regulatory cells in the lesions, expression of PD-1 and increased levels of IFN- γ may predict therapy responses to anti-PD-1-based therapy [10,57]. Also, a recent study suggesting the presence of T-cell reacting to neo-antigen epitopes is correlated with improved therapy response in anti-CTLA-4 therapy [52]. In light of subtle differences in immune checkpoint blockade mechanisms of anti-CTLA-4 and anti-PD-1 antibodies, a combined therapy approach is justified. Concurrent administration of anti-CTLA-4 and anti-PD-1 antibodies is more beneficial in achieving maximum response rates as indicated in a Phase I trial of stage III or IV melanoma patients [58]. Results from these antibody combination therapies are summarized briefly below.

In a randomized Phase II study of 142 advanced melanoma patients, the combination of ipilimumab and nivolumab showed a high objective response rate (61%) when compared to ipilimumab plus placebo group (11%) [59]. Median PFS was 4.4 months for ipilimumab and this was not reached for the combination therapy group. Complete response was seen in 22% of the patients treated with combined checkpoint inhibitors. A combination therapy trial had acceptable Grade 3 or 4 adverse events that were manageable with immune-suppressive glucocorticoid treatment.

In a Phase III study, 945 untreated melanoma patients with unresectable stage III or IV disease were treated with either nivolumab alone, combination of nivolumab and ipilimumab or ipilimumab alone [43]. Median PFS was 11.5 months for the combined use of checkpoint inhibitors, compared with 2.9 months with ipilimumab or 6.9 months with nivolumab alone [43]. The combination therapy group had a higher overall response (58%) when compared to either nivolumab (44%) or ipilimumab (19%) alone groups. Tumors from patients positive for PD-L1 showed higher (14 months) PFS in the combination therapy group [43,60]. Grade 3 or 4 adverse related events were also higher in the combination therapy group [43]. The above studies do confirm that combination therapies of immune checkpoint antibodies are beneficial in advanced melanoma patients.

Five-year view

Despite the identification of new targeted therapies and immune checkpoint blockade agents, the treatment of melanoma continues to be a challenge. Induction of therapy resistance, lack of infiltrating tumor-specific immune cells and immune non-responsiveness at the tumor site are the major stumbling blocks in the treatment of melanoma. Thus far, most single-agent targeted therapies, including newly approved immune checkpoint inhibitors, have failed to provide long-lasting tumor regressions in melanoma. Nevertheless, doubling of the overall median survival in advanced melanoma patients after treatment with anti-PD-1 antibody is a remarkable achievement. These encouraging observations have led to renewed efforts to find optimal combination agents for use with immune checkpoint antibodies.

Several studies are underway to predict therapy responses to immune checkpoint blockade. Modern imaging techniques to monitor immune T-cell mobilization into tumor lesions will be of immense help in predicting therapy responses. Some of the potential candidates for enhancing the mobilization of T-cells include cancer antigen vaccines for specific activation of tumor-reactive T-cells, BRAF and MEK inhibitors for non-specific activation of immune cells and VEGF inhibitors to enhance leukocytes infiltration into the tumor lesions. The development of additional agents targeting other immune checkpoint molecules such as LAG-3, TIM-3 and TIGIT that are already in early pilot or clinical trials will also provide valuable tools to target melanoma [10]. Some of these molecules are specifically expressed in response to an inflammatory environment and they contribute to T-cell dysfunction.

In the last 5 years, new targeted therapy drugs and immune checkpoint blockade antibodies were approved for the treatment of melanoma patients. An advance in molecular medicine has redefined melanoma as a complex and heterogeneous disease. The clinical community has quickly learned that combination therapy approaches are a must to treat complex diseases such as melanoma. Reducing tumor burden with targeted therapy drugs followed by the activation of immune T-cells by cancer vaccines and treatment with immune checkpoint antibodies are the key steps to achieve long-lasting remissions in melanoma. The future of combination therapy holds great promise as different branches of medicine come together to find a cure for melanoma.

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Box 1**Biological characteristics of anti-CTLA-4 & anti-PD-1 antibodies**

Anti-cytotoxic T-lymphocyte activation-4 (ipilimumab [Bristol Meyers Squibb])

- Engineered human monoclonal antibody (IgG1 isotype)
- Longer half life in plasma
- Capable of antibody-dependent cellular cytotoxicity (ADCC)

Anti-cytotoxic T-lymphocyte activation-4 (tremelimumab [Pfizer])

- Human monoclonal antibody (IgG2 isotype)
- Capable of ADCC

Anti-programmed death-1 (nivolumab [Bristol Meyers Squibb])

- Human monoclonal antibody (IgG4 isotype)
- Poor ADCC and shorter half-life

Anti-programmed death-1 (pembrolizumab [Merck])

- Humanized monoclonal antibody (IgG4 isotype)
- Poor ADCC and shorter half life in plasma

Key issues

- Approval of targeted therapy drugs and immune-based therapies has revolutionized the treatment of advanced melanoma patients.
- Therapy resistance and immune non-responsiveness to immune checkpoint blockade antibodies is a major issue.
- Combination therapy approach of debulking tumor followed by immune checkpoint blockade may effectively overcome immune non-responsiveness to immunological therapies.
- Toxicity to targeted- and immune-based therapies is a major concern with combination therapies.
- Modern imaging techniques to monitor immune cell trafficking into tumor lesions will help in predicting therapy response and reduce toxicity issues when immune-based therapies are used for treatment.
- Mobilization of anti-tumor specific T-cells by cancer vaccines or by non-specific means by use of BRAF and MEK inhibitors will reduce toxicity and provide better OS in patients.
- Enhancement and prolongation of T-cell infiltration into tumor lesions by using VEGF inhibitors.