Review Article

Predictive factors for immunotherapy in melanoma

Cristina Teixidó[1], Maria González-Cao[2], Niki Karachaliou[2], Rafael Rosell[1,2,3,4]

[1]Pangaea Biotech, [2]Dr Rosell Oncology Institute, Quirón Dexeus University Hospital, 08028 Barcelona, Spain; [3]Cancer Biology and Precision Medicine Program, Catalan Institute of Oncology, Hospital Germans Trias i Pujol, 08916 Badalona, Spain; [4]Molecular Oncology Research Foundation (MORe), Barcelona, Spain

Correspondence to: Cristina Teixidó, PhD. Pangaea Biotech, Medical Oncology Service & Laboratory, Quirón Dexeus University Hospital, Sabino Arana 5-19 08028 Barcelona, Spain. Email: cteixido@pangaeabiotech.com.

Abstract: Immunotherapy has emerged as an exciting strategy for cancer treatment. Therapeutic blockade of immune checkpoint regulators favors the ability of T cell responses to increase anti-tumor immunity. The cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1) are two T cell-inhibitory receptors with independent mechanisms of action. Immune checkpoint inhibitors targeting either CTLA-4, PD-1 or its ligand PD-L1 are currently yielding promising results in terms of efficacy in several clinical studies with melanoma patients and are being developed and tested as immunotherapy agents for multiple cancer types. To date, no reliable predictors of activity and efficacy of immunotherapy have yet been identified or validated. Even so, determining which patients derive clinical benefit from immune checkpoint agents remains an important clinical question and efforts to identify predictive markers of response are ongoing. This article reviews the current potential predictive factors for CTLA-4 and PD-1/PD-L1 immune checkpoints inhibitors in melanoma.

Keywords: Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4); immunotherapy; melanoma; programmed cell death-1 (PD-1); PD-L1

Submitted May 19, 2015. Accepted for publication May 21, 2015.
doi: 10.3978/j.issn.2305-5839.2015.05.07
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.05.07

Introduction

Skin cancer is one of the most common cancers worldwide (1,2). Melanoma accounts for less than 5% of all skin cancers but is the most aggressive form and responsible for the greatest number of skin cancer-related deaths at international level (3,4). Whereas most melanomas diagnosed at an early stage can often be treated with surgery alone, a proportion of these cancers may present a loco-regional or a systemic disease recurrence, conferring poor prognosis (5). The global incidence of melanoma is increasing worldwide, with a growing fraction of patients presenting with advanced disease. The clinical benefit from chemotherapy (for example, dacarbazine, temozolamide, fotemustine) in advanced melanoma patients is marginal, with a median duration of response of 7 months and an median overall survival (OS) under 1 year (6,7). The combination of different chemotherapeutic agents has not been demonstrated to affect patient survival, but only in terms of objective response rate (ORR) (8). However, in recent years, the landscape of melanoma treatment has radically changed with the introduction of targeted therapy and immunotherapy.

BRAF codon 600 mutations are detected in between 40% and 60% of melanoma patients, the majorities are the V600E mutation (80% of cases), V600K, V600R, etc. are also found. This genetic alteration represents a predictive biomarker of response to anti-BRAF (vemurafenib, dabrafenib) and anti-MEK kinase inhibitors (trametinib). Significant benefit in term of progression-free survival (PFS), OS and ORR in melanoma patients harboring *BRAF* mutations can be obtained with the use of these targeted agents (9). Almost invariably, however, the disease progresses after several months due to the emergence of acquired resistance (10-12). Melanoma patients negative for BRAF either have other mutations that are not good predictors of responses to specific inhibitors, such as *NRAS* or *CKIT* mutations (13,14), or do not harbor any actionable known molecular alteration treatable with targeted therapy.

Immunotherapy is a novel approach that is beginning to bear fruit and works by manipulating the patient's endogenous immune system (often inhibited and repressed by the presence of a tumor) to react against cancer cells. Unlike anti-BRAF targeted therapy, the effectiveness of immune checkpoint inhibitors is not dependent on specific genetic alterations and may theoretically be applicable to all melanoma patients. However, only temporary and quantitative limited responses to immunotherapy agents have been demonstrated to date, making it a priority to identify those patients most likely to benefit (15,16). Here we review the current available literature regarding activity of immune checkpoint inhibitors in the treatment of advanced melanoma, with a focus on the potential predictive factors of response to anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) antibodies.

Evolution of immunotherapy in melanoma: FDA-approved agents

Based on the presence of anti-tumor immune cells within the tumor tissue, melanoma is considered to be a highly immunogenic disease due to the presence of anti-tumor immune cells within tumor tissue, which are a promising target for immunotherapy. One early milestone was the discovery of interleukin-2 (IL-2) in 1976. IL-2 is a cytokine produced by activated T cells that increases proliferation and activation of cytotoxic T cells, natural killer (NK) cells and monocytes. Immunotherapy with high doses of the immune molecule IL-2 induced long-term, durable, complete responses in a greater number of metastatic melanoma patients (20% of responses, with 5-7% complete responses) than had been previously achieved with dacarbazine, providing the first evidence of activity of immunotherapy in melanoma (17,18). However, to date, no prospective randomized phase III studies showing a survival benefit have been performed with IL-2. Nevertheless, in 1998 the US Food and Drug Administration (FDA) approved IL-2 for treatment of advanced melanoma. Another agent enhancing the immune system is interferon alpha (IFN-α), that showed a statistically significant improvement in both disease-free survival (DFS) and OS in adjuvant treatment of patients with high-risk cutaneous melanoma (19).

The second breakthrough in immune-based therapy was ipilimumab (Yervoy; Bristol-Myers Squibb, New York, US), a monoclonal antibody targeting CTLA-4 (20,21). In March 2011, the FDA approved this targeted agent for treatment of patients with newly diagnosed or previously treated unresectable or metastatic melanoma. This approval was based on a three-arm, multinational, randomized (3:1:1), double blind phase III clinical trial (NCT00094653) conducted in 676 patients with stage III/IV melanoma who experienced disease progression after standard treatment. A total of 403 patients were randomly assigned to ipilimumab plus a glycoprotein 100 (gp100) vaccine, ipilimumab alone, or gp100 alone. Results demonstrated that ipilimumab, with (10 months) or without (10.1 months) a gp100 peptide vaccine, improved OS in patients with previously treated metastatic melanoma compared with gp100 alone (6.4 months). However, only a fraction of patients achieve durable clinical responses that can last a decade and more (22).

Another targetable immune checkpoint is PD-1 and its ligand PD-L1. Antibodies targeting the PD-1/PD-L1 axis have shown promising clinical responses in melanoma. The most advanced antibodies against PD-1 receptor are nivolumab and pembrolizumab. A phase I clinical trial with nivolumab in 296 pretreated patients with solid tumors showed cumulative response rates of 28% among patients with metastatic melanoma (26 of 94) (23). Furthermore, a phase III double-blind trial with nivolumab showed significant improvements in PFS and OS in untreated *BRAF* wild type patients with advanced melanoma as compared with dacarbazine (24). The study showed a significant improvement in ORR (40% *vs.* 13.9%), PFS (5.1 *vs.* 22 months) and 1-year OS (72.95% *vs.* 42.1%) for the group of patients treated with nivolumab compared with those treated with dacarbazine. Nivolumab (Opdivo; Bristol-Myers Squibb) received FDA approval in December 2014 for patients with unresectable or metastatic melanoma and patients with disease progression following ipilimumab and, if patients are *BRAF* V600 mutation positive, a BRAF inhibitor.

A phase I clinical trial testing the activity of the PD-1 inhibitor pembrolizumab in patients with advanced melanoma, including those who progressed on ipilimumab, also showed a high rate of sustained tumor regression (25). In a further study in 173 patients with unresectable or metastatic melanoma with disease progression within 24 weeks of last dose of ipilimumab and, if *BRAF* V600 mutation positive, prior treatment with a BRAF inhibitor, it was demonstrated that patients treated with pembrolizumab experienced an ORR of 26% (26). These findings led to FDA approval of pembrolizumab (Keytruda; Merck Sharp & Dohme Corp, New Jersey, US) in September

2014 for treatment of patients with unresectable or metastatic melanoma who had progressed following ipilimumab and, for those with *BRAF* V600 mutation, following BRAF targeted therapy. To date, more than 100 clinical trials are ongoing testing immune-based therapies for melanoma and trying to determine which therapies can be optimally combined to achieve maximum possible efficacy.

Immune checkpoint therapy and available clinical data

Immune checkpoint therapy has led to important advances in cancer treatment. Rather than cancer cells, it targets molecules involved in regulation of T cells to maintain self-tolerance for removing inhibitory pathways used by tumors to escape immune surveillance. The immune system can both suppress tumor growth by eliminating cancer cells, but can also promote it by selecting for tumor cells able to evade surveillance (27). Cancer cells and tumor-infiltrating lymphocytes (TILs) resist immunoediting phase elimination by up-regulating the expression of inhibitory ligands and receptors that regulate T cell effector functions in a process known as T cell exhaustion (28). The inhibitory receptors on immune cells serve as immune checkpoints to prevent uncontrolled immune pathways. Immune checkpoints are initiated by ligand-receptor interactions and can be effectively blocked by monoclonal antibodies (mAbs), which have been shown to rescue otherwise exhausted antitumor T cells. The two most studied immune checkpoint receptors in cancer immunotherapy are CTLA-4, also known as CD152 and PD-1, also known as CD279; both inhibitory receptors regulate immune responses at different levels and by different mechanisms without overlapping and have distinct patterns of expression (29).

CTLA-4, a gene highly homologous to T cell costimulatory molecule CD28, is an important negative regulator of T cells. This receptor outcompetes CD28 for binding to B7 on antigen presenting cells (APC). Through interaction with its ligands CD80 (B7-1) and CD86 (B7-2), CD28 plays an important role in regulating amount of early activation of naive and memory T cells (30,31). On the other hand, PD-1 binds two B7 family ligands, known as PD-L1 (B7-H1) and PD-L2 (B7-DC) (32,33). In contrast to CTLA-4, interaction of PD-1 with its ligands functions to decrease the ability of already activated T cells in the periphery during the inflammatory response to infection in order to produce an effective immune response and prevent the immune system from rejecting the tumor (34).

Immune checkpoint inhibitors target either T cells or tumor cells to prevent attachment of each to the other so T cells stay activated. This allows activated T cells to infiltrate the tumor and attack cancer cells by their interaction and produce tumor responses, for example in patients with advanced melanoma. Currently, three immune checkpoint agents have been associated with objective clinical responses and have been approved by the FDA for treatment of melanoma (ipilimumab, nivolumab and pembrolizumab). Ipilimumab is the standard of care for patients with advanced melanoma and has achieved considerable improvement in OS for patients with metastatic melanoma when used as monotherapy in phase II and III trials (35,36) and in combination with other therapies (22,37) including cancer vaccines (38).

Another anti-CTLA-4 antibody is tremelimumab, which blocks binding of the APC ligands CD-80 and CD-86 to CTLA-4, allowing them to bind to another T cell surface receptor protein and induce T cell activation. Tremelimumab stimulates the immune system to destroy cancer cells. A phase I dose-escalation study indicated that tremelimumab can safely be administered at doses sufficient to generate antitumor responses in patients with advanced melanoma (39). Although phase I/II clinical studies have induced durable tumor responses in patients with metastatic melanoma (40,41), a phase III trial failed to demonstrate a statistically significant survival advantage in first-line treatment of patients with metastatic melanoma with single-agent tremelimumab over standard chemotherapy (temozolomide or dacarbazine) (42). Nonetheless, tremelimumab has been and is being studied in several clinical trials as single-agent or in combination with other agents in solid tumors. In a small size phase II trial, tremelimumab has shown clinical activity in advanced malignant mesothelioma and was recently approved (April 2015) by the FDA to treat malignant mesothelioma (43).

Multiple mAbs against PD-1 and its ligand (PD-L1) are currently in development and have shown great promise in melanoma and other malignancies. Considering historical data obtained—typical median OS of 6.2 months (95% CI, 5.9-6.5 months), 1-year OS of 25.5% (95% CI, 23.6-27.4%)—with ipilimumab for advanced melanoma (22), the results achieved with anti-PD-1 therapy represent a huge improvement in clinical benefit for these patients. Recently, two very important studies were published in this field. In the first, to identify the most effective immunotherapy treatment, investigators tested ipilimumab monotherapy against pembrolizumab or ipilimumab in combination with nivolumab in metastatic

Table 1 Immunotherapeutic agents in development

Target	Biological function
4-1BB	Stimulatory immune signal
B7-H3	Inhibitory immune signal
B7-H4	Inhibitory immune signal
BTLA	Inhibitory immune signal
CD40	Stimulatory immune signal
CTLA-4	Inhibitory immune signal
GITR	Stimulatory immune signal
ICOS	Stimulatory immune signal
KIR	Inhibitory immune signal
LAG-3	Inhibitory immune signal
OX40	Stimulatory immune signal
PD-1	Inhibitory immune signal
PD-L1	Inhibitory immune signal
TIM-3	Inhibitory immune signal
VISTA	Inhibitory immune signal

BTLA, B and T lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; GITR, glucocorticoid-induced TNFR-related; ICOS, inducible T cell co-stimulator; KIR, killer cell immunoglobulin-like receptor; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death-1; PD-L1, PD-1 ligand; TIM-3, T cell membrane protein 3; VISTA, V-domain immunoglobulin-containing suppressor of T cell activation.

melanoma patients. This phase III study published by Robert *et al.* assigned 834 patients in a 1:1:1 ratio to pembrolizumab every 2 or 3 weeks, or ipilimumab every three weeks. Pembrolizumab prolonged PFS in both pembrolizumab groups (47.3% and 46.4%, respectively) versus ipilimumab (26.5%), and OS was significantly better with pembrolizumab cohorts than ipilimumab (74.1%, 68.4% and 58.2%, respectively). Response rate was also better with pembrolizumab (33.7%, 32.9% and 11.9%, respectively). Drug-associated grades 3 to 5 adverse effects were lower in the pembrolizumab groups than ipilimumab and occurred in 13.3%, 10.1%, and 19.9% of the groups, respectively (44).

The other study, published by Postow and colleagues, assigned untreated patients in a 2:1 ratio to receive ipilimumab combined with either nivolumab or placebo (monotherapy) until disease progression or unacceptable toxic effects. ORR was significantly greater with ipilimumab plus nivolumab (61%) than with ipilimumab monotherapy (11%), with complete responses in 22% of patients who received combined treatment, while patients who received monotherapy had no complete responses (45). Grades 3 to 4 drug-related adverse effects were reported in 54% of patients who received the combination therapy compared with 24% of patients who received ipilimumab monotherapy. ORR in the combination group was even higher than that reported previously by Wolchok and colleagues, which may be explained by the fact that the assigned patients in this trial were untreated (46). The fact that CTLA-4 and PD-1 regulate distinct inhibitory pathways and have non-overlapping mechanisms of action may be another reason for the higher efficacy in the combination cohort versus monotherapy. This was shown by Curran *et al.* in *in vivo* models: combination of CTLA-4 and PD-1 blockade was more than twice as effective as either CTLA-4 or PD-1 alone in driving tumor rejection of B16 melanomas models (47). Similar results for ORR were observed in 33 patients with BRAF-mutated tumors, consistent with the results of a previous phase I study (48). Both of these studies clearly show greater efficacy of anti-PD-1 mAbs for advanced melanoma.

Emerging issues—response evaluation (ircriteria), new antigenic agents

There is a high expectation that these immune checkpoint inhibitors, and others in this class, currently under investigation as melanoma immunotherapy, will also soon be approved for treatment of patients with melanoma and other tumor types (*Table 1*). Pathways that play a role in cancer immunotherapy are becoming increasingly well characterized; the use of new checkpoint inhibitors combinations, such as those combining anti-CTLA-4, anti-PD-1 or anti-LAG-3, including targeted therapies, are being tested to increase PFS and OS. All these options make immunotherapy look like an extremely promising for melanoma. To determine the expected benefit with immunotherapy in an unselected population, several predictive factors have been evaluated and/or are currently under investigation for efficacy and toxicity.

Predictive markers of anti-CTLA-4 therapy

There are currently no confirmed biomarkers predictive of response to anti-CTLA-4 studies. The hypothesis that HLA status could correlate with benefit from ipilimumab was refuted in a retrospective analysis of four advanced melanoma trials which found that ipilimumab's mechanism of action was HLA independent and that similar OS was

observed regardless of HLA subtype (49).

The debate currently rages as to whether the effector T cell or regulatory T cell (Treg) compartment is the primary target of CTLA-4 antibody-mediated blockade. Based on mouse melanoma models, investigators have found that concomitant blockade of both effector T cell and Tregs compartments contributes to the antitumor activity of CTLA-4 inhibitors, suggesting that ipilimumab's primary antitumor effect may be targeting Tregs for destruction within the tumor microenvironment (50). In support of this finding, Hamid *et al.* found no associations between genetic polymorphisms and clinical activity, but did find significant associations between clinical efficacy and high baseline expression of FoxP3, indoleamine 2,3-dioxygenase (IDO) and increase in TILs between baseline and 3 weeks after starting ipilimumab treatment (51).

Whereas a single institution experience conducted in patients treated with ipilimumab demonstrated a significant correlation between survival and absolute lymphocyte count (52), others have shown that nearly all patients treated with ipilimumab had a significant increase in absolute lymphocyte count and that this occurrence was not specifically predictive of OS benefit from ipilimumab (53,54).

A preliminary study in melanoma showed that increase in myeloid-derived suppressor cell (MDSC) number in the peripheral blood by week 24 from pre-treatment baseline was associated with lack of clinical efficacy, suggesting that MDSC may serve both as a predictive and pharmacodynamic marker of treatment outcome (55). However, many IFN-γ-inducible genes and Th1-associated markers showed higher baseline expression in the tumor microenvironment of metastatic melanoma patients who benefited from ipilimumab treatment, suggesting that the genes of this particular type of T cells may serve as early predictors of response rather than predictive markers (56). Moreover, investigators have proposed that increased expression of the inducible co-stimulator (ICOS) molecule after anti-CTLA-4 therapy treatment can be used as a pharmacodynamic biomarker to assess adequate biologic response to treatment (monotherapy or combination) (57,58).

Last year, Snyder *et al.* published what appears to be the first reliable method to predict which melanoma patients will respond to anti-CTLA-4 therapy (ipilimumab and tremelimumab) (59). They discovered a neoantigen peptide landscape that is specifically present in tumors with a strong response to CTLA-4 blockade, and found an association between high mutational load and clinical benefit with CTLA-4 immune checkpoint inhibitors; however, this alone was not sufficient to predict outcome. This suggests that T cells can recognize as non-self (and therefore become more reactive to) tumors that express new antigens (neoepitopes) as a result of high number of non-driver genetic alterations (60,61).

Predictive biomarkers for the PD-1/PD-L1 pathway inhibitors

There is increasing evidence to support the hypothesis that an immune-active tumor microenvironment potentially correlates with improved melanoma patient survival or response to PD-1/PD-L1 pathway inhibitors. Selective infiltrations of CD8 positive T cells have been found to precede melanoma response to anti-PD1 therapy (62). Moreover, Tumeh *et al.* have demonstrated that pre-existing CD8 positive T cell density distinctly located at the invasive tumor margin in melanoma patients was associated with expression of the PD-1/PD-L1 immune inhibitory axis. This was found to be the best predictive parameter of clinical response to PD-1 blocking therapies followed by tumor CD8 positive density, whereas the poorest predictor was CD4 positive T cell density at the tumor and invasive margin (63).

PD-L1 expression is being investigated as a predictive biomarker of response for PD-1/PD-L1 directed therapy (*Table 2*). PD-L1 is expressed in several tumor types, including melanoma, lung, renal, kidney, head and neck and bladder cancer. Preliminary molecular marker studies in melanoma have shown a correlation of PD-L1 expression in pretreatment tumor specimens and objective response to anti-PD-1 therapy (23). However, PD-L1 expression in some studies appears to be associated with better prognosis only in metastatic melanoma lesions, suggesting that its predictive value may not be as clear-cut as initially thought (71). Other issues also add complexity when evaluating different analyses of PD-L1 expression as a predictive factor of response. PD-L1 expression is IFN-γ–inducible and can be present on either the tumor or infiltrating immune cells. Furthermore, there is currently no standardized methodology to measure PD-L1 expression and its evaluation differs between assays.

Taube and colleagues have found a significant correlation between the presence of TILs and PD-L1 expression in the tumor microenvironment. The number, type and location of TILs in primary tumors seem to have prognostic value and its presence may be more important for predicting response than PD-L1 expression alone. However, there is evidence that TILs are necessary but not sufficient for PD-L1 expression in melanoma (71). Patients with better response

Table 2 Correlation of PD-L1 expression by immunohistochemistry and clinical benefit

Reference	Tumor type	Agents (s)	n (PD-L1)	Cell location	ORR (%) PD-L1+	ORR (%) PD-L1-
Topalian *et al*. (23)	Solid	Nivolumab	42	TC	36	0
Robert *et al*. (24)	Melanoma	Nivolumab	418	TC	53	33
Brahmer *et al*. (62)	Solid	Nivolumab	9	TC	75	0
Grosso *et al*. (64)	Melanoma	Nivolumab	38	TC	44	17
Weber *et al*. (65)	Melanoma	Nivolumab	44	TC	67	19
Taube *et al*. (66)	Solid	Nivolumab	41	TC	39	6
				IC	35	11
Hodi *et al*. (67)	Melanoma	Nivolumab	41	TC	44	13
Postow *et al*. (45)	Melanoma	Nivolumab plus ipilimumab	142	TC	58	55
Wolchok *et al*. (46)	Melanoma	Nivolumab plus ipilimumab	56	TC	48	29
Tumeh *et al*. (63)	Melanoma	Pembrolizumab	38	IC	45	–
Hamid *et al*. (68)	Melanoma	MPDL3280A	45	IC	27	20
Herbst *et al*. (69)	Solid	MPDL3280A	175	TC	39	21
				IC	36	16
Segal *et al*. (70)	Solid	MEDI4736	179	–	22	4

ORR, objective response rate; TC, tumor cells; IC, immune cells.

to these therapies are those that express high levels of PD-L1 and have infiltration of T cells within the tumor. Therefore, evaluation of PD-L1 expression by immunohistochemistry (IHC) together with measurement of immune infiltration might be a good predictor of tumor response to anti PD-L1 agents (72). However, there are caveats regarding measuring levels of PD-L1 since its expression is constitutive and its overexpression in response to stimuli can vary according to cell type. In addition, tumors are heterogeneous and the sample used for the assay may not be representative of the whole tumor. For instance, various levels of PD-L1 expression have been found in different metastases and their primary clear cell renal cell carcinomas (73). Moreover, it has been observed that patients with PD-L1 negative tumors can also respond to PD-1 and PD-L1 blockade (46,64,68). For all these reasons, a standardized definition of PD-L1 positivity that links these different assays is needed to evaluate PD-L1 expression as a predictive factor for PD-1 and/or PD-L1 pathway blockade.

Other immune biomarkers have also been assessed. Messina and colleagues found a direct correlation between a 12-chemokine gene expression signature and the presence of lymph nodal structures (immune cells that infiltrate and organize into intratumoral structures which resemble lymph nodes) associated with better OS in melanoma patients, something which may be useful in selecting those patients most suitable for immunotherapy (74).

Finally, tissue studies have demonstrated that tumors with a high somatic mutational frequency (above 10 somatic mutations per megabase of coding DNA), such as melanomas, respond best to PD-1 immune checkpoint inhibitors (75). As commented above, Snyder *et al*. have published similar results with CTLA-4 immunotherapy. The mutational load in melanoma has been found to be associated with clinical benefit but not predictive of response to treatment (59). These mutations may result in the presentation of neoantigens recognizable to the immune system and form a component of a predictive biomarker model of response to checkpoint blockade (76).

Conclusions

Recent advances in immunotherapy have revolutionized the spectrum of treatment options for melanoma patients along with surgery, radiotherapy, chemotherapy and targeted therapy. Immune checkpoint inhibitors are effective cancer treatments and have shown antitumor activity in several clinical studies with melanoma patients. However, not all patients benefit equally and efforts to identify predictive factors of clinical response are ongoing. Blocking the PD-1/PD-L1 pathway with monoclonal antibodies has shown better antitumor responses and safety profile (less toxicity) in clinical studies than has

been seen with prior immunotherapies, such as IL-2 and anti-CTLA-4 agents. Nevertheless, long-term duration of benefit of either anti-PD-1 and/or PD-L1 agents is not as well-known as other treatments options such as the anti-CTLA-4 antibody ipilimumab which has been studied for longer and is better characterized. Evaluation of immunological biomarkers could offer useful prognostic information and facilitate clinical decision-making. It has been observed that characterizing tumors by immune infiltration (intratumoral infiltration), chemokine signature, tumor mutational load and PD-L1 expression, may be key molecular markers to assess the potential for selecting which patients may benefit from which immune checkpoint inhibitor, either in monotherapy or in combination, and may suggest the mechanism of an individual's tumorigenesis. Optimal agents to combine with immune checkpoint mAbs might be those capable of inducing immune infiltration into the tumor microenvironment. Furthermore, PD-L1 expression by IHC is currently the strongest predictive marker of clinical benefit for immune checkpoint therapy but data presented so far do not demonstrate PD-L1 to be a reliable single predictive marker as the epidermal growth factor receptor (EGFR) is for lung cancer or human epidermal growth factor receptor 2 (HER2) for breast cancer. A standardization of PD-L1 IHC is required to explore the relationship between its expression and impact of this on prognosis of melanoma patients treated with PD-1 and/or PD-L1 mAbs. Given that infiltration of TILs is important to obtain an effective antitumor immune response, some categorization of immune infiltration together with PD-L1 expression by IHC or other immunologic assays might help to better predict of tumor response, although the fact that PD-L1 negative patients can also response means clinical application should be approached with caution. Finally, the identification and application of such possible predictive markers for each patient are crucial for the rational development, research and advance of immunotherapy and to guide the optimal choice of immunotherapy treatment.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Teixidó C, González-Cao M, Karachaliou N, Rosell R. Predictive factors for immunotherapy in melanoma. Ann Transl Med 2015;3(15):208. doi: 10.3978/j.issn.2305-5839.2015.05.07

