

Biomarkers associated with checkpoint inhibitors

G. Manson, J. Norwood, A. Marabelle, H. Kohrt, R. Houot

► **To cite this version:**

G. Manson, J. Norwood, A. Marabelle, H. Kohrt, R. Houot. Biomarkers associated with checkpoint inhibitors. *Annals of Oncology*, Elsevier, 2016, 27 (7), pp.1199–1206. 10.1093/annonc/mdw181 . hal-01359396

HAL Id: hal-01359396

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01359396>

Submitted on 7 Oct 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biomarkers Associated with Checkpoint Inhibitors

G. Manson¹, J. Norwood¹, A. Marabelle^{3,4}, H. Kohrt⁵, R. Houot^{1,2}

¹Department of Hematology, CHU Rennes, Rennes, France

²INSERM, U917, Rennes, France

³DITEP, Gustave Roussy Cancer Campus (GRCC), Villejuif, France

⁴INSERM U1015, Gustave Roussy Cancer Institute, Villejuif, France

⁵Department of Medicine, Division of Oncology, Stanford University, Stanford, CA, USA

Corresponding author: Prof. Roch Houot, Department of Hematology, CHU Rennes, 2 rue Henri Le Guilloux, 35033 Rennes Cedex 9, France, Tel : +33 (0)2 99 28 42 26, Fax : +33 (0)2 99 28 41 61, E-mail : roch.houot@chu-rennes.fr

Abstract

Checkpoint Inhibitors (CPI), namely anti-CTLA4 and anti-PD1/PD-L1 antibodies, demonstrated efficacy across multiple types of cancer. However, only subgroups of patients respond to these therapies. Additionally, CPI can induce severe immune-related adverse events (irAE). Biomarkers that predict efficacy and toxicity may help define the patients who may benefit the most from these costly and potentially toxic therapies. In this study, we review the main biomarkers that have been associated with the efficacy (pharmacodynamics and clinical benefit) and the toxicity (irAE) of CPIs in patients.

Key words: Biomarkers, checkpoint inhibitors, cancer, immunotherapy

Key message

Numerous biomarkers may reflect the pharmacodynamics of CPIs and predict their efficacy and/or their toxicity in patients. However, the use of these biomarkers in clinical practice remains ill-defined and will have to be clarified in future studies. Such biomarkers should help to define patients who will benefit most from these costly and potentially toxic drugs.

Introduction

Checkpoint Inhibitors (CPI) are a new class of anti-cancer agents. These antibodies (Ab) target normal immune cells in order to stimulate the anti-tumor response. They work by blocking interactions between inhibitory receptors expressed on T cells and their ligands. In oncology, the two main classes of CPI which are the most advanced in clinical development are the anti-CTLA-4 and anti-PD-1/PD-L1 Abs. Three of these Abs have been approved by the FDA for clinical use (Table 1). CPI can have efficacy across several types of cancer. However, only subgroups of patients respond to these drugs. Additionally, CPI can induce “autoimmune-like” toxicities. These findings raise several questions. What is the impact of CPI on the immune system? Are there biological markers related to their efficacy or toxicity? After a brief review of the mechanisms of action of CPI, we will present the biomarkers that have been associated with pharmacodynamics changes, therapeutic efficacy and toxicity of these treatments in clinic.

CTLA-4 pathway

T-cell activation following the stimulation of its receptor (T cell receptor [TCR]) requires a second signal which results from the interaction between the activating receptor CD28 located on the T cell surface and the CD80 (B7.1) and CD86 (B7.2) molecules expressed by the antigen-presenting cells (APC). Secondly, the activated T cell expresses CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) on its surface, an inhibitory receptor which also recognizes the CD80 and CD86 molecules, but with a much stronger affinity than CD28. The binding of CD80/CD86 to CTLA-4 then inhibits T cells. Anti-CTLA-4 Abs block this interaction and prevent T cell inhibition¹. They could also work by eliminating regulatory T cells (Tregs) that constitutively express this receptor².

PD-1/PD-L1 pathway

PD-1 or “programmed-death 1” is a surface protein which, once bound to its ligand, inhibits the proliferation and function of T cells. PD-1 receptor is expressed by T cells after activation. PD-1 possesses two ligands, PD-L1 and PD-L2. PD-L1 is expressed on the surface of APC (mainly dendritic cells and macrophages) and a broad variety of tissues such as epithelial and endothelial tissues, in response to a stimulation by pro-inflammatory cytokines³. Expression of PD-L1 inhibits the proliferation of activated T cells and is responsible for negative feedback control of inflammation. The other PD-1 ligand, PD-L2, is expressed mainly by APC³. Expression of PD-L1 and/or PD-L2 by tumor cells or cells in the microenvironment allows the tumor to protect itself from the immune system. Blocking monoclonal Abs directed against PD-1 and PD-L1 have been developed in order to prevent PD-1/PD-L1 and PD-1/PD-L2 binding and so to restore the activity of anti-tumor T cells¹.

Biomarkers associated with pharmacodynamics and clinical responses to CPI

The main biological markers associated with pharmacodynamics and/or clinical responses to CPI are listed in Table 2. A multitude of biomarkers has been studied, predominantly involving indices from the patient’s tumor (tumor cells or cells from the microenvironment) or blood (circulating cells or serum). The following section provides a brief summary of each biomarker most advanced in study.

Circulating lymphocytes, neutrophils, eosinophils, and monocytes

Several studies have shown that treatment with anti-CTLA-4 Ab increased the level of circulating lymphocytes⁴. This increase seems to be associated with a better clinical efficacy⁵⁻⁷. More recently, a

study conducted in a 'real world' population of melanoma patients treated with ipilimumab found a correlation between baseline absolute lymphocyte count (ALC) and overall survival (OS was greater in patients with increased ALC at baseline)⁸.

Two studies evaluating an anti-PD-L1 Ab (MPDL3280A) in different cancers showed transient increase of CD8+ HLA-DR+ Ki-67+ cells early during treatment, but there was no correlation with clinical activity^{9,10}.

A study showed that the ratio between neutrophils and lymphocytes (N/L) during treatment with Ipilimumab correlated with clinical efficacy. A N/L ratio below normal limits after 7 and 10 weeks of treatment was significantly associated with a better survival¹¹.

A low level of eosinophils at baseline before treatment was associated with a better overall survival in patients treated with anti-CTLA-4 Ab (Ipilimumab) for a metastatic melanoma¹².

Finally, a high level of CD16-expressing monocytes at baseline was found to be associated with higher response rate and lower Tregs counts in the tumor microenvironment of responding patients¹³.

CD4+ ICOS^{hi} T cells

ICOS is a co-stimulatory receptor expressed on the surface of T cells after activation. In murine models, it has been shown that expression of ICOS is necessary for the efficacy of anti-CTLA-4 Ab. Indeed, mice lacking ICOS or its ligand have a lower response to anti-CTLA-4 Ab than wild-type mice¹⁴.

In patients, it has been described that anti-CTLA-4 Ab increased the number of CD4+ ICOS^{hi} T cells (producing IFN γ) in the blood^{15,16} and in the tumor¹⁶⁻¹⁸. For example, in patients receiving neo-adjuvant therapy with anti-CTLA-4 Ab (Ipilimumab) for bladder cancer before cystoprostatectomy, CD4+ ICOS^{hi} T-Lymphocytes count was significantly increased in prostatic tissue, whether they were healthy or invaded by adenocarcinoma¹⁷. Moreover, a persistent increase of CD4+ ICOS^{hi} T cells in blood after 12 weeks of treatment with anti-CTLA-4 Ab was associated with an improved clinical

outcome (defined as stable disease or objective response) and a better overall survival¹⁸.

Diversity of T cell repertoire

Anti-CTLA-4 therapy may amplify a pre-existing antitumor T cells response and/or generate T cells against new tumor antigens (“epitope spreading”).

A retrospective study including 12 patients harbouring metastatic melanoma evaluated the diversity of the circulating T cell repertoire before treatment with anti-CTLA-4 Ab (Ipilimumab). In this study, response rate was higher in patients presenting with a more diverse T cell repertoire¹⁹.

Cha et al. assessed TCR diversity during CTLA-4 blockade in patients with castration-resistant metastatic prostate cancer and metastatic melanoma, and found that clonotype stability after treatment was associated with a better clinical outcome²⁰.

Kvistborg et al. showed that anti-CTLA-4 therapy broadened the repertoire of melanoma-reactive CD8+ T cells but did not amplify pre-existing T cells. There was no correlation with clinical outcome²¹.

In patients with metastatic melanoma, Tumeh et al found that clinical response to anti-PD1 therapy (pembrolizumab) correlated with i) a more clonal (i.e. more restricted, less diverse) TCR repertoire in pre-treatment tumor samples and ii) an increased clonal expansion of T cells in the tumor after anti-PD1 therapy²².

Immune response against tumor-associated antigens

In patients with melanoma, the presence of Ab targeting NY-ESO-1 (a tumor antigen found in several solid tumors) before treatment and under treatment with Ipilimumab was associated with a better clinical outcome (defined as stable disease or partial/complete response)²³. Among patients seropositive for NY-ESO-1, overall survival was higher in those harbouring CD8 T cells directed against NY-ESO-1²³. Interestingly, Kvistborg et al. demonstrated that anti-CTLA-4 treatment induced new

melanoma-specific CD8 T cells (in blood) but did not significantly expand melanoma-specific T cell responses that were already detected before start of therapy, suggesting that anti-CTLA-4 therapy enhances T cell priming²¹.

Regulatory T cells (Tregs)

Tregs express CTLA-4 constitutively and therefore can be targeted directly by anti-CTLA-4 Abs²⁴. Studies have found a decrease in the number of circulating²⁵ or intratumoral^{16,17} Tregs after Ipilimumab treatment while other studies did not find such depletion^{18,26,27}. Data regarding the correlation between the variation of Tregs and clinical efficacy are likewise contradictory²⁸⁻³⁰.

Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs are an heterogeneous population of cells that exists at basal state and expands under pathological conditions such as infection, inflammation or cancer, and exert suppressive activities, notably on T cells³¹. In certain types of cancer, circulating MDSCs have been found to correlate with tumor stage, tumor volume, and even prognosis³². A recent study by Martens et al. showed a correlation between a low baseline count of MDSCs and better overall survival in melanoma patients treated with anti-CTLA-4³³. Several studies found a decrease in the number of MDSCs following Ipilimumab therapy^{29,34}. In one study, the decrease of certain types of MDSCs under Ipilimumab treatment (given in a neo-adjuvant setting) was associated with higher progression-free survival in patients with melanoma²⁹. Similarly, Kitano et al. showed that a low monocytic MDSCs count at week 6 after Ipilimumab treatment was associated with prolonged overall survival³⁵.

Soluble CD25 (sCD25)

Anti-tumor activity of anti-CTLA-4 Ab seems highly dependent on IL-2, a cytokine involved in the activation of T cells. In murine models, neutralization of IL-2 or blockade of the α and β subunits of its

receptor (CD25 and CD122, respectively) precluded the anti-tumor effect as well as the increase in the ratio of intratumoral effector/regulatory T cells usually found during anti-CTLA-4 Ab therapy³⁶. The soluble form of CD25 (sCD25), capable of capturing IL-2, decreases anti-CTLA-4 Ab efficacy in murine models³⁶. In patients presenting with metastatic melanoma, a high level of sCD25 before treatment with Ipilimumab was associated with treatment resistance³⁶.

Cytokines/chemokines

Treatment with anti-PD-L1 Ab (MPDL3280A) was associated with increased circulating IFN- γ , IL-18 and ITAC (an IFN- γ inducible chemokine which is chemotactic for activated T cells) and decreased IL-6. No correlation was found with clinical efficacy^{9,10}.

Lactate dehydrogenase (LDH)

In some solid tumors and hematologic malignancies, a high level of LDH at baseline is frequently associated with poor prognosis. In advanced melanoma, the site(s) of metastasis and a high level of LDH at baseline are the two most significant prognostic factors³⁷. Increased LDH at baseline seems to be associated with resistance to anti-CTLA-4 Ab therapy in advanced melanoma^{6,8,38}.

Tumor-infiltrating lymphocytes (TIL)

The presence of lymphocytes within the tumor is a favorable prognostic factor in numerous cancers³⁹. The lymphocyte infiltrate also seems to be a predictive factor of response to CPI. In patients with metastatic melanoma treated with anti-CTLA-4 Ab (Ipilimumab), an increase in lymphocyte infiltrate in the tumor between baseline and at three weeks after treatment initiation correlated with clinical response⁴⁰. Moreover, in melanoma patients treated with anti-PD-1 Ab

(Pembrolizumab), response rate was better in patients with high numbers of peri- and intra-tumoral CD8 T cells in their pre-treatment samples²². Analysis of biopsies after treatment with anti-CTLA-4 Ab (Ipilimumab) showed a correlation between a high ratio of intratumoral CD8/regulatory T cells and tumor necrosis^{16,41}. Granzyme B is a cytotoxic granule reflective of CD8 effector function. In the tumor, its expression seems to be increased after anti-PD-1⁴², anti-CTLA-4⁴⁰ or anti-PD-L1 Ab therapy⁹. Sample analysis from patients with metastatic melanoma obtained before and after anti-PD-1 therapy showed a significant increase in granzyme B expressing CD8+ cells in post-dosing biopsies in the responding patients²².

Mutational load

CPI work by amplifying pre-existing anti-tumor responses. Several studies aimed to determine if “immunogenic” tumors, i.e. tumors that can be recognized by the immune system, were more sensitive to CPI. Immunogenicity is associated with the mutational load, in other words with the number of somatic mutations present in the tumor cells⁴³. Indeed, the presence of mutations in the tumor generates neo-antigens (not expressed by normal cells) that are likely to be recognized by the immune system. The more mutations there are, the more the tumor is likely to be immunogenic. A study actually found a significant relationship between cytolytic activity in the tumor (defined by a greater perforin/granzyme B transcript level) and total mutation count in eight tumor types including colorectal and lung cancer⁴⁴. Indeed, clinical studies showed that patients treated with anti-CTLA-4 Ab (Ipilimumab) for melanoma^{45,46} or with anti-PD-1 Ab (Pembrolizumab) for non-small cell lung carcinoma⁴⁷ had better clinical benefit if the mutational load of their tumor was high. Likewise, patients treated with anti-PD-1 Ab (Pembrolizumab) for colorectal cancer had a significantly better response rate and overall survival if they had a mismatch repair deficiency (involved in the repair of DNA replication errors)⁴⁸.

PD-L1 expression within the tumor

Given the mechanism of action of anti-PD-1 and anti-PD-L1 Abs, several studies have tried to determine if the efficacy of these Abs correlated with PD-L1 ligand expression in the tumor. The first studies provided evidence that there was indeed a strong link between PD-L1 expression by tumor cells and the response to anti-PD-1 Ab. Topalian et al. showed that all the responses to Nivolumab were observed in patients whose tumors expressed PD-L1⁴⁹. Likewise, in the KEYNOTE-001 trial, responses to Pembrolizumab correlated with PD-L1 expression by tumor cells⁵⁰. A trial assessing the effect of an anti-PD-L1 (MPDL3280A) on different types of cancer found a correlation between the level of PD-L1 present in the intratumoral immune infiltrate (but not by the tumor cells themselves) and clinical response⁹. However, other studies did not confirm this correlation⁵¹⁻⁵⁵. In a meta-analysis including 1475 patients treated with Nivolumab, Pembrolizumab or MPDL3280A, response rates were significantly higher in PD-L1 positive tumors (34% versus 19.9%)⁵⁶.

These differences regarding the predictive value of PD-L1 may have multiple explanations. First, the analysis of PD-L1 expression within tumors is not standardized. Different staining techniques are available, using different Abs for immunohistochemistry and different levels of positivity. Efforts are currently being made to harmonize the assessment of PD-ligand expression. Also, some studies looked at PD-L1 expression by tumor cells whereas others also included its expression by cells of the microenvironment. These differences could also be explained by the dynamic nature of PD-L1 expression. In fact, PD-L1 is inducible, notably by IFN- γ exposure¹. Therefore, a tumor which does not express PD-L1 at baseline may become PD-L1 positive in an inflammatory background. This could explain why basal expression of PD-L1 does not have a predictive value on response when anti-PD-1 is combined with anti-CTLA-4 Ab in melanoma patients⁵⁷. Inflammation induced by anti-CTLA-4 Abs could indeed induce PD-L1 in tumors formerly negative for this marker⁵³. Lastly, a significant percentage of patients negative for PD-L1 respond to anti-PD-1/PD-L1 therapy.

Indoleamine 2,3-dioxygenase (IDO)

IDO is an enzyme which degrades tryptophan, an essential amino-acid. Its presence lowers tryptophan availability in the tumor microenvironment and increases the concentration of its catabolite. IDO has an immunosuppressive effect by decreasing proliferation, function and survival of T cells⁵⁸. Expression of IDO by some tumors contributes to immune escape. Hamid et al. found a positive correlation between a high expression of IDO at baseline and the clinical activity of Ipilimumab in metastatic melanoma⁴⁰. The presence of IDO could indicate a pre-existing inflammatory/immune response (IDO could be induced by pro-inflammatory cytokines like IFN- γ) which would promote the response to CPI^{59,60}.

Gene expression profile (GEP)

In blood, transcriptome analysis has shown that treatment with an anti-CTLA-4 Ab induces a gene expression profile related to proliferation of T cells, mainly of memory phenotype⁶¹. Anti-PD-1 Abs are responsible for the expression of genes implicated in cytolysis and NK cell function⁶¹. Combination of anti-PD-1 and anti-CTLA-4 Abs induces higher gene expression compared to monotherapy, notably for genes involved in proliferation and coding of cytokines. Some genes can only be induced by the combination of both anti-PD-1 and anti-CTLA-4 Abs⁶¹.

In the tumor, transcriptome analysis performed on biopsies from 45 patients with melanoma before treatment found gene expression profiles (increased expression of immunity-related genes) predictive of response to anti-CTLA-4 Ab (Ipilimumab) therapy⁶². Similarly, GEP performed in melanoma tumors before anti-PD1 treatment (pembrolizumab and nivolumab) revealed a transcriptional signature related to innate anti-PD-1 resistance (IPRES)⁶³. Interestingly, IPRES signatures was not associated with resistance to anti-CTLA-4 suggesting that mechanisms of innate resistance to anti- PD-1 and anti-CTLA-4 may be different.

Melanoma patients responding to MPDL3280A (anti-PD-L1) had an increased expression of IFN- γ and IFN- γ -inducible genes (i.e. IDO1 and CXCL9) on pre-treatment tumours⁹.

Biomarkers associated with toxicity of CPIs

CPI, by stimulating the immune system non-specifically, can trigger side effects called Immune-Related Adverse Events (irAEs). Most frequent irAEs may affect the skin (pruritus, rash, vitiligo), the intestinal tract (colitis), the liver (hepatitis) and the endocrine system. They are usually more pronounced with anti-CTLA-4 Abs than with anti-PD-1 or anti-PD-L1 Abs⁶⁴. Notably, anti-CTLA-4 Abs can cause serious colitis. Some of these irAEs can be life-threatening. IrAEs may require discontinuation of CPI therapy and immunosuppressive treatment. Several studies looked for predictive biomarkers of toxicity. Main results are summarized in Table 3.

Eosinophils

In 156 patients presenting with metastatic melanoma, the circulating eosinophils count before and during treatment with anti-CTLA-4 Ab (Ipilimumab) was associated with irAEs occurrence⁶⁵.

IL-17

In 52 patients treated with anti-CTLA-4 Ab (Ipilimumab) for metastatic melanoma, increase followed by decrease of blood IL-17 levels were respectively associated with the settling and the resolution of colitis⁶⁶.

Gene expression profile (GEP)

Gene expression profiling performed on blood from patients treated with anti-CTLA-4 Ab (Ipilimumab) showed that, out of 10,000 probe sets tested, the increased expression of CD177 and CEACAM1 genes, two markers of neutrophil activation, was associated with digestive toxicity⁶⁷.

Digestive infiltrate by neutrophils

A study of melanoma patients treated with anti-CTLA-4 Ab (Ipilimumab) showed that, on colon biopsies performed just before treatment initiation, lamina propria infiltration by neutrophils as well as other markers of inflammation (cryptic abscess, gland destruction, mucosal erosion) were associated with the occurrence of digestive toxicity⁶⁸.

Lastly, several studies suggest that the occurrence of irAEs could correlate with better efficacy of anti-CTLA-4 Ab (Ipilimumab)^{25,69–71}. However, association between toxicity and efficacy is not entirely clear⁷² and numerous patients respond to CPI therapy without experiencing irAEs.

Conclusions

Numerous biomarkers may reflect the pharmacodynamics of CPIs and predict their efficacy and/or their toxicity in patients. However, the relevance of these biomarkers to clinical practice and their potential for routine application remains ill-defined, and will have to be clarified in larger, prospective studies. A subset of biomarkers seem relevant and accessible enough to be part of our practices (e.g. LDH level, absolute lymphocytes count variation); others look promising but need standardisation of current practices (e.g. measure of PD-L1 expression) and finally, some biomarkers are not yet

accessible to daily practice (e.g. transcriptional analysis, mutational load). In addition, most of the biomarkers described so far have been tested in melanoma patients and therefore require validation in other cancer types and immunotherapeutic agents.

Patients who seem to benefit most from CPIs are those presenting with immunogenic tumors (i.e. with a high mutational load), pre-existing immune response (intratumoral immune infiltrate) and the immune escape ligands being targeted (i.e. PD-L1 for patients treated with anti-PD-1/PD-L1 Abs).

Nevertheless, these biomarkers are not perfect. In the future, better knowledge of the mechanisms of action of CPIs *in vivo* should help us identify other biomarkers in order to define patients who will benefit most from these effective but costly and potentially toxic drugs.

Disclosure

The authors did not receive funding for this study. The authors have declared no conflicts of interest.

References

1. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
2. Selby, M. J. *et al.* Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. *Cancer Immunol. Res.* **1**, 32–42 (2013).
3. McDermott, D. F. & Atkins, M. B. PD-1 as a potential target in cancer therapy. *Cancer Med.* **2**, 662–73 (2013).
4. Wolchok, J. D. *et al.* Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol.* **11**, 155–164 (2010).
5. Ku, G. Y. *et al.* Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting. *Cancer* **116**, 1767–1775 (2010).
6. Delyon, J. *et al.* Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. *Ann. Oncol.* **24**, 1697–703 (2013).
7. D. M. Berman, J. W. J. W. O. H. S. O. S. D. C., Bristol-Myers Squibb, P. N., Memorial Sloan-Kettering Cancer Center, N. Y. N., H. Lee Moffitt Cancer Center & Research Institute, T. F. & Researc, T. A. C. and. Association of peripheral blood absolute lymphocyte count (ALC) and clinical activity in patients (pts) with advanced melanoma treated with ipilimumab. *J Clin Oncol* **27**, (2009).
8. Kelderman, S. *et al.* Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. *Cancer Immunol. Immunother.* **63**, 449–58 (2014).

9. Herbst, R. S. *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563–7 (2014).
10. Powles, T. *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* **515**, 558–562 (2014).
11. Di Giacomo, A. M. *et al.* Long-term survival and immunological parameters in metastatic melanoma patients who responded to ipilimumab 10 mg/kg within an expanded access programme. *Cancer Immunol. Immunother.* **62**, 1021–8 (2013).
12. Schindler, K. *et al.* Pretreatment levels of absolute and relative eosinophil count to improve overall survival (OS) in patients with metastatic melanoma under treatment with ipilimumab, an anti CTLA-4 antibody. in *ASCO Annual Meeting* (2013).
13. Romano, E. *et al.* Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. *Proc. Natl. Acad. Sci.* **112**, 6140–6145 (2015).
14. Fu, T., He, Q. & Sharma, P. The ICOS/ICOSL pathway is required for optimal antitumor responses mediated by anti-CTLA-4 therapy. *Cancer Res.* **71**, 5445–54 (2011).
15. Vonderheide, R. H. *et al.* Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. *Clin. Cancer Res.* **16**, 3485–94 (2010).
16. Liakou, C. I. *et al.* CTLA-4 blockade increases IFN γ -producing CD4+ICOS $^+$ hi cells to shift the ratio of effector to regulatory T cells in cancer patients. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 14987–92 (2008).
17. Chen, H. *et al.* Anti-CTLA-4 therapy results in higher CD4+ICOS $^+$ hi T cell frequency and IFN- levels in both nonmalignant and malignant prostate tissues. *Proc. Natl. Acad. Sci.* **106**, 2729–

- 2734 (2009).
18. Carthon, B. C. *et al.* Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. *Clin. Cancer Res.* **16**, 2861–71 (2010).
 19. Postow, M. A. *et al.* Peripheral T cell receptor diversity is associated with clinical outcomes following ipilimumab treatment in metastatic melanoma. *J. Immunother. cancer* **3**, 23 (2015).
 20. Cha, E. *et al.* Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. *Sci. Transl. Med.* **6**, 238ra70 (2014).
 21. Kvistborg, P. *et al.* Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. *Sci. Transl. Med.* **6**, 254ra128 (2014).
 22. Tumei, P. C. *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
 23. Yuan, J. *et al.* Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 16723–8 (2011).
 24. Sojka, D. K., Huang, Y.-H. & Fowell, D. J. Mechanisms of regulatory T-cell suppression - a diverse arsenal for a moving target. *Immunology* **124**, 13–22 (2008).
 25. Attia, P. Autoimmunity Correlates With Tumor Regression in Patients With Metastatic Melanoma Treated With Anti-Cytotoxic T-Lymphocyte Antigen-4. *J. Clin. Oncol.* **23**, 6043–6053 (2005).
 26. Weber, J. S. *et al.* Ipilimumab Increases Activated T Cells and Enhances Humoral Immunity in Patients With Advanced Melanoma. *J. Immunother.* **35**, 89–97 (2012).
 27. Maker, A. V *et al.* Tumor regression and autoimmunity in patients treated with cytotoxic T

- lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. *Ann. Surg. Oncol.* **12**, 1005–16 (2005).
28. Simeone, E. *et al.* Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. *Cancer Immunol. Immunother.* **63**, 675–683 (2014).
 29. Tarhini, A. A. *et al.* Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. *PLoS One* **9**, e87705 (2014).
 30. Weide, B., Di Giacomo, A. M., Fonsatti, E. & Zitvogel, L. Immunologic Correlates in the Course of Treatment With Immunomodulating Antibodies. *Semin. Oncol.* **42**, 448–458 (2015).
 31. Gabrilovich, D. I. & Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* **9**, 162–74 (2009).
 32. Diaz-Montero, C. M., Finke, J. & Montero, A. J. Myeloid-derived suppressor cells in cancer: therapeutic, predictive, and prognostic implications. *Semin. Oncol.* **41**, 174–84 (2014).
 33. Martens, A. *et al.* Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin. Cancer Res.* (2016).
doi:10.1158/1078-0432.CCR-15-2412
 34. Pico de Coaña, Y. *et al.* Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. *Cancer Immunol. Res.* **1**, 158–62 (2013).
 35. Kitano, S. *et al.* Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. *Cancer Immunol. Res.* **2**, 812–21 (2014).

36. Hannani, D. *et al.* Anticancer immunotherapy by CTLA-4 blockade: obligatory contribution of IL-2 receptors and negative prognostic impact of soluble CD25. *Cell Res.* **25**, 208–224 (2015).
37. Balch, C. M. *et al.* Final version of 2009 AJCC melanoma staging and classification. *J. Clin. Oncol.* **27**, 6199–206 (2009).
38. Wilgenhof, S. *et al.* Single-center experience with ipilimumab in an expanded access program for patients with pretreated advanced melanoma. *J. Immunother.* **36**, 215–22 (2013).
39. Ruffini, E. *et al.* Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. *Ann. Thorac. Surg.* **87**, 365–71; discussion 371–2 (2009).
40. Hamid, O. *et al.* A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. *J. Transl. Med.* **9**, 204 (2011).
41. Hodi, F. S. *et al.* Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 3005–10 (2008).
42. Beavis, P. A. *et al.* CD73: A potential biomarker for anti-PD-1 therapy. *Oncoimmunology* **4**, e1046675 (2015).
43. Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–21 (2013).
44. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity. *Cell* **160**, 48–61 (2015).
45. Snyder, A. *et al.* Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. *N. Engl. J. Med.* 2189–2199 (2014). doi:10.1056/NEJMoa1406498

46. Van Allen, E. M. *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science (80-.)*. **350**, 207–211 (2015).
47. Rizvi, N. A. *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science (80-.)*. **348**, 124–128 (2015).
48. Le, D. T. *et al.* PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
49. Topalian, S. L. *et al.* Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer. *N. Engl. J. Med.* **366**, 2443–2454 (2012).
50. Garon, E. B. *et al.* Pembrolizumab for the treatment of non-small-cell lung cancer. *N. Engl. J. Med.* **372**, 2018–28 (2015).
51. Robert, C. *et al.* Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* **372**, 320–30 (2015).
52. Robert, C. *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.* **372**, 2521–32 (2015).
53. Postow, M. A. *et al.* Peripheral and tumor immune correlates in patients with advanced melanoma treated with nivolumab (anti-PD-1, BMS-936558, ONO-4538) monotherapy or in combination with ipilimumab. *J. Transl. Med.* **12**, O8 (2014).
54. Kefford, R. *et al.* Clinical efficacy and correlation with tumor PD-L1 expression in patients (pts) with melanoma (MEL) treated with the anti-PD-1 monoclonal antibody MK-3475. in *ASCO Annual Meeting J Clin Oncol* 32:5s, 2014 (suppl; abstr 3005[^]) (2014).
55. Daud, A. *et al.* Long-term efficacy of pembrolizumab (pembro; MK-3475) in a pooled analysis of 655 patients (pts) with advanced melanoma (MEL) enrolled in KEYNOTE-001. *ASCO Meet. Abstr.* **33**, 9005 (2015).

56. Carbognin, L. *et al.* Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. *PLoS One* **10**, e0130142 (2015).
57. Larkin, J. *et al.* Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N. Engl. J. Med.* 150531115012002 (2015). doi:10.1056/NEJMoa1504030
58. Mbongue, J. C. *et al.* The Role of Indoleamine 2, 3-Dioxygenase in Immune Suppression and Autoimmunity. *Vaccines* **3**, 703–29 (2015).
59. Jung, I. D. *et al.* Differential regulation of indoleamine 2,3-dioxygenase by lipopolysaccharide and interferon gamma in murine bone marrow derived dendritic cells. *FEBS Lett.* **581**, 1449–56 (2007).
60. Robinson, C. M., Hale, P. T. & Carlin, J. M. The role of IFN-gamma and TNF-alpha-responsive regulatory elements in the synergistic induction of indoleamine dioxygenase. *J. Interferon Cytokine Res.* **25**, 20–30 (2005).
61. Das, R. *et al.* Combination Therapy with Anti-CTLA-4 and Anti-PD-1 Leads to Distinct Immunologic Changes In Vivo. *J. Immunol.* **194**, 950–9 (2014).
62. Ji, R.-R. *et al.* An immune-active tumor microenvironment favors clinical response to ipilimumab. *Cancer Immunol. Immunother.* **61**, 1019–1031 (2012).
63. Hugo, W. *et al.* Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* **165**, 35–44 (2016).
64. Michot, J. M. *et al.* Immune-related adverse events with immune checkpoint blockade: A comprehensive review. *Eur. J. Cancer* **54**, 139–148 (2016).
65. Schindler, K. *et al.* Correlation of absolute and relative eosinophil counts with immune-related

- adverse events in melanoma patients treated with ipilimumab. *ASCO Meet. Abstr.* **32**, (2014).
66. Callahan, M. K. *et al.* Evaluation of serum IL-17 levels during ipilimumab therapy: Correlation with colitis. *ASCO Meet. Abstr.* **29**, (2011).
67. Shahabi, V. *et al.* Gene expression profiling of whole blood in ipilimumab-treated patients for identification of potential biomarkers of immune-related gastrointestinal adverse events. *J. Transl. Med.* **11**, 75 (2013).
68. Berman, D. *et al.* Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. *Cancer Immun.* **10**, 11 (2010).
69. Downey, S. G. *et al.* Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin. Cancer Res.* **13**, 6681–8 (2007).
70. Tarhini, A., Lo, E. & Minor, D. R. Releasing the brake on the immune system: ipilimumab in melanoma and other tumors. *Cancer Biother. Radiopharm.* **25**, 601–13 (2010).
71. Weber, J. Ipilimumab: controversies in its development, utility and autoimmune adverse events. *Cancer Immunol. Immunother.* **58**, 823–30 (2009).
72. Ascierto, P. A. *et al.* Clinical experience with ipilimumab 3 mg/kg: real-world efficacy and safety data from an expanded access programme cohort. *J. Transl. Med.* **12**, 116 (2014).
73. Ribas Antoni, R. C. Association of response to programmed death receptor 1 (PD-1) blockade with pembrolizumab (MK-3475) with an interferon-inflammatory immune gene signature. | 2015 ASCO Annual Meeting | Abstracts | Meeting Library. Available at: <http://meetinglibrary.asco.org/content/151652-156>. (Accessed: 12th October 2015)

Table 1. CPIs FDA-approved for the treatment of cancer

Target	Antibody	Isotype	Company	Indication (date of approval)			
				Melanoma	Non-small cell lung carcinoma	Renal cell carcinoma	
CTLA-4	Ipilimumab	IgG1	BMS	<ul style="list-style-type: none"> Unresectable or metastatic melanoma (2011) Adjuvant treatment of patients with cutaneous melanoma with pathologic involvement of regional lymph nodes of more than 1 mm who have undergone complete resection, including total lymphadenectomy (2015) Patients with BRAF V600 wild-type, unresectable or metastatic melanoma, in combination with nivolumab (2015) 	-	-	
				<ul style="list-style-type: none"> Unresectable or metastatic melanoma with disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor (2014) Initial treatment of patients with unresectable or metastatic melanoma 	<ul style="list-style-type: none"> Metastatic non-small cell lung cancer (NSCLC) whose tumors express programmed death ligand 1 (PD-L1) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy (2015) 	-	
PD-1	Pembrolizumab	IgG4	Merck	<ul style="list-style-type: none"> Unresectable or metastatic melanoma with disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor (2014) Initial treatment of patients with unresectable or metastatic melanoma 	<ul style="list-style-type: none"> Metastatic non-small cell lung cancer (NSCLC) whose tumors express programmed death ligand 1 (PD-L1) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy (2015) 	-	

				(2015)			
	Nivolumab	IgG4	BMS	<ul style="list-style-type: none"> • Unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor (2014) • Patients with BRAF V600 wild-type, unresectable or metastatic melanoma, in combination with ipilimumab (2015) 	<ul style="list-style-type: none"> • Metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab (2015) 		<ul style="list-style-type: none"> • Advanced renal cell carcinoma in patients who have received prior anti-angiogenic therapy (2015)

Table 2. Biomarkers associated with pharmacodynamics and clinical response to CPI

	Biomarker	Ab	Result	
BLOOD	Circulating lymphocytes, neutrophils, eosinophils, and monocytes	Anti-CTLA-4	<ul style="list-style-type: none"> • Increase of circulating lymphocytes count⁴ • Increased lymphocytes count at baseline associated with better overall survival⁸ • Increased lymphocytes count during treatment associated with better clinical efficacy⁵⁻⁷ • Decreased neutrophils/lymphocytes ratio during treatment associated with higher survival¹¹ • Low eosinophil baseline level associated with better survival¹² • High CD16-expressing monocyte level at baseline associated with higher response rate¹³ 	
		Anti-PD-L1	<ul style="list-style-type: none"> • Transient increase of CD8+ HLA-DR+ Ki-67+ lymphocytes^{9,10} 	
	CD4+ ICOS ^{hi} T cells	Anti-CTLA-4	<ul style="list-style-type: none"> • Increase of CD4+ ICOS^{hi} T cell count in blood, tumor and healthy tissues¹⁶⁻¹⁸ • Persistent increase of circulating CD4+ ICOS^{hi} T cell count under treatment associated with better survival¹⁸ 	
	Diversity of T cell repertoire	Anti-CTLA-4	<ul style="list-style-type: none"> • Increase of TCR diversity^{20,21} • Diversity of T cell repertoire associated with higher response rate¹⁹ 	

	Immune response against tumor-antigens	Anti-CTLA-4	<ul style="list-style-type: none"> • Presence of Ab directed against NY-ESO-1 antigen associated with higher clinical benefit in melanoma²³ • Presence of NY-ESO-1 specific T CD8 cells in NY-ESO-1-seropositive patients associated with better survival in melanoma²³
	Tregs	Anti-CTLA-4	<ul style="list-style-type: none"> • Decrease of circulating Tregs count after Ipilimumab treatment in some studies²⁵ • Contradictory data concerning variation of circulating Tregs count and clinical response²⁴⁻³⁰
	MDSCs	Anti-CTLA-4	<ul style="list-style-type: none"> • Decrease of MDSCs count upon Ipilimumab treatment^{29,34} • Low baseline MDSCs count associated with better overall survival³³ • Decrease in several subtypes of MDSCs associated with higher progression-free survival²⁹ • Decrease in monocytic MDSCs during treatment associated with prolonged overall survival³⁵
	Soluble CD25	Anti-CTLA-4	<ul style="list-style-type: none"> • High basal serum level of soluble CD25 associated with treatment resistance³⁶
	Cytokines/chemokines	Anti-PD-L1	<ul style="list-style-type: none"> • Increased level of IFN-γ, IL-18, ITAC and decreased level of IL-6^{9,10}
	LDH	Anti-CTLA-4	<ul style="list-style-type: none"> • High basal serum level of LDH associated with treatment resistance^{6,8,38}
	Gene expression	Anti-CTLA-4	<ul style="list-style-type: none"> • Expression of genes involved in proliferation of T

TUMOR	profile	4	cells, mostly of memory subtype ⁶¹
		Anti-PD-1	<ul style="list-style-type: none"> • Expression of genes involved in cytolysis and NK cells proliferation⁶¹
	Tumor-infiltrating lymphocytes (TIL)	Anti-CTLA-4	<ul style="list-style-type: none"> • Presence of intratumoral lymphocytes associated with better clinical efficacy⁴⁰ • Increased CD4 T cells/Tregs ratio associated with tumor necrosis^{16,41} • Increase of Granzyme B lymphocytes in the tumor after anti-CTLA-4 Ab⁴⁰
		Anti-PD-1	<ul style="list-style-type: none"> • Presence of peri- and intratumoral CD8 T cells associated with higher response rates²² • Increase of granzyme B lymphocytes in the tumor after anti-PD-1⁴² and anti-PD-L1 Ab⁹. • Increase in granzyme B CD8+ T cells after anti-PD1 Ab associated with clinical efficacy in melanoma patients²².
	Diversity of T cell repertoire	Anti-PD-1	<ul style="list-style-type: none"> • More clonal (i.e. more restricted, less diverse) TCR repertoire in pre-treatment tumor samples associated with clinical response²² • Increased clonal expansion of T cells after treatment associated with clinical response²²
	Mutational load	Anti-CTLA-4	<ul style="list-style-type: none"> • High mutational load associated with better efficacy in melanoma^{45,46}
		Anti-PD-1	<ul style="list-style-type: none"> • High mutational load associated with better efficacy in NSCLC⁴⁷

		<ul style="list-style-type: none"> Mismatch-repair deficiency associated with better efficacy in CRC⁴⁸
PD-L1	Anti-PD-1, anti-PD-L1	<ul style="list-style-type: none"> Intratumoral expression of PD-L1 associated with better clinical efficacy^{1,9,49-56}
IDO	Anti-CTLA-4	<ul style="list-style-type: none"> High IDO expression associated with better clinical efficacy⁴⁰
Gene expression profile	Anti-CTLA-4	<ul style="list-style-type: none"> Transcriptomic signature associated with better clinical efficacy in melanoma^{62,73}
	Anti-PD-1	<ul style="list-style-type: none"> Transcriptomic signature associated with innate anti-PD-1 resistance (IPRES) in melanoma and other types of cancer⁶³
	Anti-PD-L1	<ul style="list-style-type: none"> Increased IFN-γ genes expression on pre-treatment tumor biopsies associated with response to anti-PDL1 in melanoma patients⁹

Table 3. Biomarkers associated with CPI toxicity

	Biomarkers	Drugs	Association
BLOOD	Eosinophils	Anti-CTLA-4	<ul style="list-style-type: none"> Increased eosinophils before and after treatment associated with irAEs occurrence⁶⁵
	IL-17	Anti-CTLA-4	<ul style="list-style-type: none"> Increased circulating IL-17 level during treatment associated with digestive toxicity⁶⁶
	Gene expression profile	Anti-CTLA-4	<ul style="list-style-type: none"> Increased expression of CD177 and CEACAM1 genes associated with digestive toxicity⁶⁷
TISSUE	Digestive Neutrophils infiltrate	Anti-CTLA-4	<ul style="list-style-type: none"> Infiltration of lamina propria by neutrophils in colon during treatment associated with digestive toxicity⁶⁸