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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 inhibition[1]. They could also work by eliminating regulatory T cells (Tregs) that constitutively express this receptor[2].

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 cytokines[3]. 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 APC[3]. 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 cells[1].

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 lymphocytes[4]. This increase seems to be associated with a better clinical efficacy[5–7]. 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 survival[11].

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 melanoma[12].

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 patients[13].

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 mice[14].

In patients, it has been described that anti-CTLA-4 Ab increased the number of CD4+ ICOS^{hi} T cells (producing IFNγ) in the blood[15,16] and in the tumor[16–18]. 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 adenocarcinoma[17]. 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 survival[18].

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 repertoire[19].

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 outcome[20].

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 outcome[21].

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 therapy[22].

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-1[23]. 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 priming[21].

Regulatory T cells (Tregs)

Tregs express CTLA-4 constitutively and therefore can be targeted directly by anti-CTLA-4 Abs[24]. Studies have found a decrease in the number of circulating[25] 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 contradictory[28–30].

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 cells[31]. In certain types of cancer, circulating MDSCs have been found to correlate with tumor stage, tumor volume, and even prognosis[32]. 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-4[33]. 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 melanoma[29]. Similarly, Kitano et al. showed that a low monocytic MDSCs count at week 6 after Ipilimumab treatment was associated with prolonged overall survival[35].

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 therapy[36]. The soluble form of CD25 (sCD25), capable of capturing IL-2, decreases anti-CTLA-4 Ab efficacy in murine models[36]. In patients presenting with metastatic melanoma, a high level of sCD25 before treatment with Ipilimumab was associated with treatment resistance[36].

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 factors[37]. 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 cancers[39]. 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 response[40]. 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 samples[22]. 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-1[42], anti-CTLA-4[40] or anti-PD-L1 Ab therapy[9]. 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 patients[22].

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 cells[43]. 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 cancer[44]. 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 carcinoma[47] 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-L1[49]. Likewise, in the KEYNOTE-001 trial, responses to Pembrolizumab correlated with PD-L1 expression by tumor cells[50]. 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 response[9]. However, other studies did not confirm this correlation[51–55]. 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 exposure[1]. 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 patients[57]. Inflammation induced by anti-CTLA-4 Abs could indeed induce PD-L1 in tumors formerly negative for this marker[53]. 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 cells[58]. 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 melanoma[40]. 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 phenotype[61]. Anti-PD-1 Abs are responsible for the expression of genes implicated in cytolysis and NK cell function[61]. 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 Abs[61].

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) therapy[62]. 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 tumours[9].

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 Abs[64]. 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 occurence[65].

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 colitis[66].

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 toxicity[67].

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 toxicity[68].

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 clear[72] 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.

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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	• 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)	-	-
PD-1	Pembrolizumab	IgG4	Merck	• 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	• 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	• 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)	• Metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. Pati ents with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab (2015)	• 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	• Increase of circulating lymphocytes count[4] • Increased lymphocytes count at baseline associated with better overall survival[8] • Increased lymphocytes count during treatment associated with better clinical efficacy[5–7] • Decreased neutrophils/lymphocytes ratio during treatment associated with higher survival [11] • Low eosinophil baseline level associated with better survival[12] • High CD16-expressing monocyte level at baseline associated with higher response rate[13]
		Anti-PD-L1	• Transient increase of CD8+ HLA-DR+ Ki-67+ lymphocytes[9,10]
	CD4+ ICOS^{hi} T cells	Anti-CTLA-4	• Increase of CD4+ ICOS^{hi} T cell count in blood, tumor and healthy tissues[16–18] • Persistent increase of circulating CD4+ ICOS^{hi} T cell count under treatment associated with better survival[18]
	Diversity of T cell repertoire	Anti-CTLA-4	• Increase of TCR diversity[20,21] • Diversity of T cell repertoire associated with higher response rate[19]

	Immune response against tumor-antigens	Anti-CTLA-4	• Presence of Ab directed against NY-ESO-1 antigen associated with higher clinical benefit in melanoma[23] • Presence of NY-ESO-1 specific T CD8 cells in NY-ESO-1-seropositive patients associated with better survival in melanoma[23]
	Tregs	Anti-CTLA-4	• Decrease of circulating Tregs count after Ipilimumab treatment in some studies[25] • Contradictory data concerning variation of circulating Tregs count and clinical response[24–30]
	MDSCs	Anti-CTLA-4	• Decrease of MDSCs count upon Ipilimumab treatment[29,34] • Low baseline MDSCs count associated with better overall survival[33] • Decrease in several subtypes of MDSCs associated with higher progression-free survival[29] • Decrease in monocytic MDSCs during treatment associated with prolonged overall survival[35]
	Soluble CD25	Anti-CTLA-4	• High basal serum level of soluble CD25 associated with treatment resistance[36]
	Cytokines/chemokines	Anti-PD-L1	• Increased level of IFN-γ, IL-18, ITAC and decreased level of IL-6[9,10]
	LDH	Anti-CTLA-4	• High basal serum level of LDH associated with treatment resistance[6,8,38]
	Gene expression	Anti-CTLA-	• Expression of genes involved in proliferation of T

	profile	4	cells, mostly of memory subtype[61]
		Anti-PD-1	• Expression of genes involved in cytolysis and NK cells proliferation[61]
TUMOR	Tumor-infiltrating lymphocytes (TIL)	Anti-CTLA-4	• Presence of intratumoral lymphocytes associated with better clinical efficacy[40] • Increased CD4 T cells/Tregs ratio associated with tumor necrosis[16,41] • Increase of Granzyme B lymphocytes in the tumor after anti-CTLA-4 Ab[40]
		Anti-PD-1	• Presence of peri- and intratumoral CD8 T cells associated with higher response rates[22] • Increase of granzyme B lymphocytes in the tumor after anti-PD-1[42] and anti-PD-L1 Ab[9]. • Increase in granzyme B CD8+ T cells after anti-PD1 Ab associated with clinical efficacy in melanoma patients[22].
	Diversity of T cell repertoire	Anti-PD-1	• More clonal (i.e. more restricted, less diverse) TCR repertoire in pre-treatment tumor samples associated with clinical response[22] • Increased clonal expansion of T cells after treatment associated with clinical response[22]
	Mutational load	Anti-CTLA-4	• High mutational load associated with better efficacy in melanoma[45,46]
		Anti-PD-1	• High mutational load associated with better efficacy in NSCLC[47]

			• Mismatch-repair deficiency associated with better efficacy in CRC[48]
	PD-L1	Anti-PD-1, anti-PD-L1	• Intratumoral expression of PD-L1 associated with better clinical efficacy[1,9,49–56]
	IDO	Anti-CTLA-4	• High IDO expression associated with better clinical efficacy[40]
	Gene expression profile	Anti-CTLA-4	• Transcriptomic signature associated with better clinical efficacy in melanoma[62,73]
		Anti-PD-1	• Transcriptomic signature associated with innate anti-PD-1 resistance (IPRES) in melanoma and other types of cancer[63]
		Anti-PD-L1	• Increased IFN-γ genes expression on pre-treatment tumor biopsies associated with response to anti-PDL1 in melanoma patients[9]

Table 3. Biomarkers associated with CPI toxicity

	Biomarkers	Drugs	Association
BLOOD	Eosinophils	Anti-CTLA-4	• Increased eosinophils before and after treatment associated with irAEs occurence[65]
	IL-17	Anti-CTLA-4	• Increased circulating IL-17 level during treatment associated with digestive toxicity[66]
	Gene expression profile	Anti-CTLA-4	• Increased expression of CD177 and CEACAM1 genes associated with digestive toxicity[67]
TISSUE	Digestive Neutrophils infiltrate	Anti-CTLA-4	• Infiltration of lamina propria by neutrophils in colon during treatment associated with digestive toxicity[68]