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REVIEW ARTICLE

# Treating tumors with immune checkpoint inhibitors: Rationale and limitations

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## ABSTRACT

Immune checkpoints are essential for preventing immunopathology but can also obstruct anti-tumor immune responses. Recent medical advances in blocking these mechanisms have therefore opened promising avenues in the treatment of cancer. Various blocking antibodies targeting the immune checkpoints programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are now approved for human use. This review summarizes the properties of PD-1 and CTLA-4 in physiological and tumor settings, and examines the treatment efficacy, side effects and biomarkers of their inhibitors. Future avenues in the application and development of immune checkpoint inhibitors for the treatment of cancer are also explored.

***Keywords:*** *CTLA-4; PD-1; cancer; immunotherapy; side effects; biomarkers*

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## PD-1 and CTLA-4 are important for immune tolerance

Programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are inhibitory receptors with non-redundant functions. Also known as immune checkpoints, they are important in controlling adaptive immune responses under physiological conditions.

CTLA-4 is primarily expressed by regulatory T cells (Tregs) and may also be upregulated on conventional T cells upon activation[1]. The receptor is normally located in intracellular vesicles and released briefly onto the cell surface during T cell activation[2]. CTLA-4 binds CD80 and CD86 on antigen-presenting cells. This results in negative intracellular signaling, but more importantly prevents CD80 and CD86 from binding to their ligand CD28[1,3]. CTLA-4 binds CD80 and CD86 with much greater affinity, therefore outcompeting the co-stimulatory receptor CD28 during antigen presentation on the same or neighboring T cells[3].

PD-1 can be upregulated in a wide range of immune cell types, including T cells and B cells[4–7]. Most T cells do not constitutively express PD-1 but upregulate the receptor upon activation[8,9]. Unlike CTLA-4, PD-1-mediated inhibitory signaling is primarily intrinsic. The cytoplasmic tail of the receptor contains two amino acid motifs that become phosphorylated upon PD-1 recruitment and initiate intracellular signaling cascades that interfere with CD28- and other TCR-associated signaling[10,11].

PD-1 and CTLA-4 therefore both primarily act on T cells by interfering with the signaling of the costimulatory receptor CD28. CD28 signaling during T cell activation is essential for initiating the T cells' IL-2 production, proliferation and survival. By interfering with this pathway, CTLA-4 and PD-1 lower the responsiveness of T cells during activation[12].

Spatial and temporal differences in the expression of PD-1, CTLA-4 and their ligands mean that both receptors act independently and in a non-redundant manner. CTLA-4 is thought to primarily act during antigen presentation in secondary lymphoid organs, as its ligands CD80 and CD86 are expressed on antigen-presenting cells. PD-1 on the other hand may be more important in peripheral organs, especially during effector phases of immune responses, as its ligands PDL-1 and PDL-2 can be found on a wide range of lymphoid and non-lymphoid cells across the body and are induced by IFN-γ[13–15].

By limiting T cell responses, CTLA-4 and PD-1 are essential for the maintenance of immunological tolerance. CTLA-4 knock-out mice develop T cell proliferative disorders and die at a young age[16,17] and mice deficient in PD-1 develop lupus-like glomerulonephritis and arthritis with age[18,19]. In humans, polymorphisms in the CTLA-4 and PD-1 genes have also been associated with autoimmune conditions[20–22]. However, CTLA-4 and PD-1 may also impair immune responses to pathogens and tumors[23].

## The roles of PD-1 and CTLA-4 in anti-cancer immunity

By inhibiting T cell activation and effector functions, PD-1 and CTLA-4 prevent effective anti-tumor immune responses. T cells that bind strongly to self-antigens are deleted in the thymus. Because tumors are derived from self, tumor-reactive T cells often only bind their cognate antigen with low affinity[24]. These T cells are therefore particularly susceptible to a reduction in the activation threshold caused by PD-1 and CTLA-4.

PD-1 ligands can often be detected in the tumor microenvironment, not just on infiltrating immune cells but also on the tumor cells themselves[25–27]. Tregs may also be recruited to tumor draining lymph nodes or directly into the tumor, suppressing immune responses via a wide range of mechanisms including via CTL-4[28].

Finally, tumor-specific T cells may become additionally susceptible to PD-1- and CTLA-4-mediated signaling by upregulating either or both receptors in a process known as immune exhaustion. During immune exhaustion, T cells exposed to high levels of antigen in the absence of disease resolution upregulate a wide range of inhibitory receptors that limit T cell effector functions, proliferation and survival[29]. Exhausted T cells expressing CTLA-4 and PD-1, among other inhibitory receptors, and with poor functionality have been detected in the tumor microenvironment and circulation in both mice and humans[30–33].

## Blockade of PD-1 and CTLA-4 in tumor patients

Blockade of PD-1 and CTLA-4 can restore anti-tumor immune responses in T cells repressed by these inhibitory receptors. The anti-tumor effect of blocking CTLA-4 *in vivo* was first demonstrated in mice in 1996[34]. Clinical trials in humans followed and yielded promising results in metastatic melanoma and ovarian carcinoma patients, leading in 2011 to the FDA approval of the first immune checkpoint inhibitor, the anti-CTLA-4 antibody Ipilimumab[35,36]. The first anti-PD-1 blocking antibodies Pembrolizumab and Nivolumab were approved in 2014, and other checkpoint inhibitors targeting PD-1 and CTLA-4 have since been approved or are undergoing clinical trials (**Table 1**).

Both CTLA-4 and PD-1 checkpoint inhibitors have resulted in increased patient survival in a number of studies, including those on melanoma, renal cell carcinoma, squamous cell carcinoma and non-small cell lung cancer, when compared to conventional chemotherapies. Recently, a direct comparison between the two checkpoint inhibitors in two separate phase 3 clinical trials found better response and survival rates among patients treated with the anti-PD-1 antibodies, Pembrolizumab and Nivolumab, compared to the anti-CTLA-4 antibody Ipilimumab[37,38]. In advanced stage melanoma patients, objective response rates were 19%, 43.7% and 57.6% and median progression-free survival were 2.9, 6.9, and 11.5 months for anti-CTLA-4, anti-PD-1 and their combined administration, respectively[37]. However, despite these advances,

**Table 1.** PD-1 and CTLA-4 expressing cells, their ligands and blocking antibodies

| | PD-1 | CTLA-4 |
|---|---|---|
| **Expressed on** | T cells, B cells, dendritic cells, monocytes, mast cells, Langerhans cells | T cells |
| **Ligands** | PDL-1, PDL-2 | CD80, CD86 |
| **Antibody therapies** (approved or undergoing clinical trial) | Anti-PD-1: Nivolumab, Pembrolizumab<br><br>Anti-PDL-1: Atezolizumab, Durvalumab, Avelumab | Anti-CTLA-4: Ipilimumab, Tremelimumad |

overall mortality and relapse rates remain high among advanced stage patients treated with immune checkpoint inhibitors.

## Treatment-related adverse events and their management

As PD-1 and CTLA-4 are important both in tumor immune evasion and for maintaining peripheral and central tolerance, indiscriminate blocking of receptor signaling can have important side effects in some patients. Almost all patients treated with either or both immune checkpoint inhibitors develop symptoms. Common side effects include diarrhea, fatigue, pruritus, rash, nausea and hyperthyroidism, all of which are mild in the majority of patients. However, some patients can develop severe adverse effects (grade 3 to 5), including diarrhea, colitis, increased alanine aminotransferase levels, interstitial pneumonia and interstitial nephritis[37–39]. Anti-PD-1 treatment has also been associated with the development of type 1 diabetes mellitus[40], hepatitis[41] and the exacerbation of pre-existing autoimmune conditions such as psoriasis[42,43], among others. It is of note that severe adverse events seem to be more common in melanoma patients treated with anti-CTLA-4 (around 20%) compared to those treated with anti-PD-1 (10%–13%)[37,38].

Severe side effects require careful monitoring and management[37,44]. Guidelines for health care professionals have been set by Bristol-Myers Squibb, the manufacturer of Nivolumab and Ipilimumab, and have been reviewed in detail by Della Vittoria Scarpati and colleagues[45]. In general, high dose oral corticosteroids are recommended for grade 3–4 manifestations and checkpoint inhibitor treatment should be discontinued for grade 4 side effects[45]. Patients with severe diarrhea not responding to high dose corticosteroids may additionally be treated with anti-TNF-α[44,46]. Low-grade skin manifestations may be treated with topical steroids or anti-histamines, and low-grade diarrhea with anti-diarrhea drugs and patient hydration[45]. It should be noted that patients may still respond to immune checkpoint blockade despite the development of adverse events, the administration of corticosteroids or the cessation of treatment[44]. Intriguingly, the development of immune-related adverse events, particularly rash and vitiligo, has even been associated with improved disease outcome in melanoma patients treated with anti-PD-1[47], suggesting that the breaking of tolerance to local (auto-) antigens may also lead to improved anti-tumor immune responses. **Figure 1** shows the visualization of treatment-associated adverse events and their anatomical sites in melanoma patients treated with anti-PD-1 and/or anti-CTLA-4, as reported by Larkin and colleagues[37].

## Predicting the efficacy of anti-PD-1/CTLA-4 treatments

Because of the substantial side effects and treatment cost associated with immune checkpoint inhibitors, there is great interest in identifying biomarkers that will allow the selection of patients who would respond to these treatments.

The most commonly described biomarker for anti-PD-1/PDL-1 treatment efficacy to date is the expression of PDL-1 at the tumor site. Indeed, in various cancers including melanoma, patients that had high PDL-1 expression on tumor cells or infiltrating immune cells were shown to respond better to the treatment than those who did not[37,38,48,49]. However, in a number of other studies, including some involving squamous cell carcinoma of the head and neck, non-small-cell lung cancer, or Merkel cell carcinoma, no significant association was found between treatment efficacy and tumor PDL-1 expression[27,50,51], suggesting that PDL-1 status alone is not sufficient as a predictive biomarker of treatment efficacy.

Various other immune parameters have been associated with improved treatment response (**Table 2**). Th1- and CTLA-4-related gene expressions within the tumor prior to treatment have been linked to subsequent response to anti-PDL-1[47]. In another study, melanoma patients responding successfully to anti-PD-1 treatment were more likely to display high

**Table 2.** Biomarkers that have been associated with patient responses to immune checkpoint inhibitor treatment

| | Anti-PD-1 treatments | Anti-CTLA-4 treatment |
|---|---|---|
| **Pre-treatment** | • PDL-1 expression by tumor and tumor infiltrating immune cells<br>• High tumor mutation rate<br>• Th1-related gene expression<br>• CTLA-4-related gene expression<br>• Increased TGF-β serum levels | • High tumor mutation rate |
| **During/post-treatment** | • Expanded Th9 T cell compartment | • Gut microbiome |

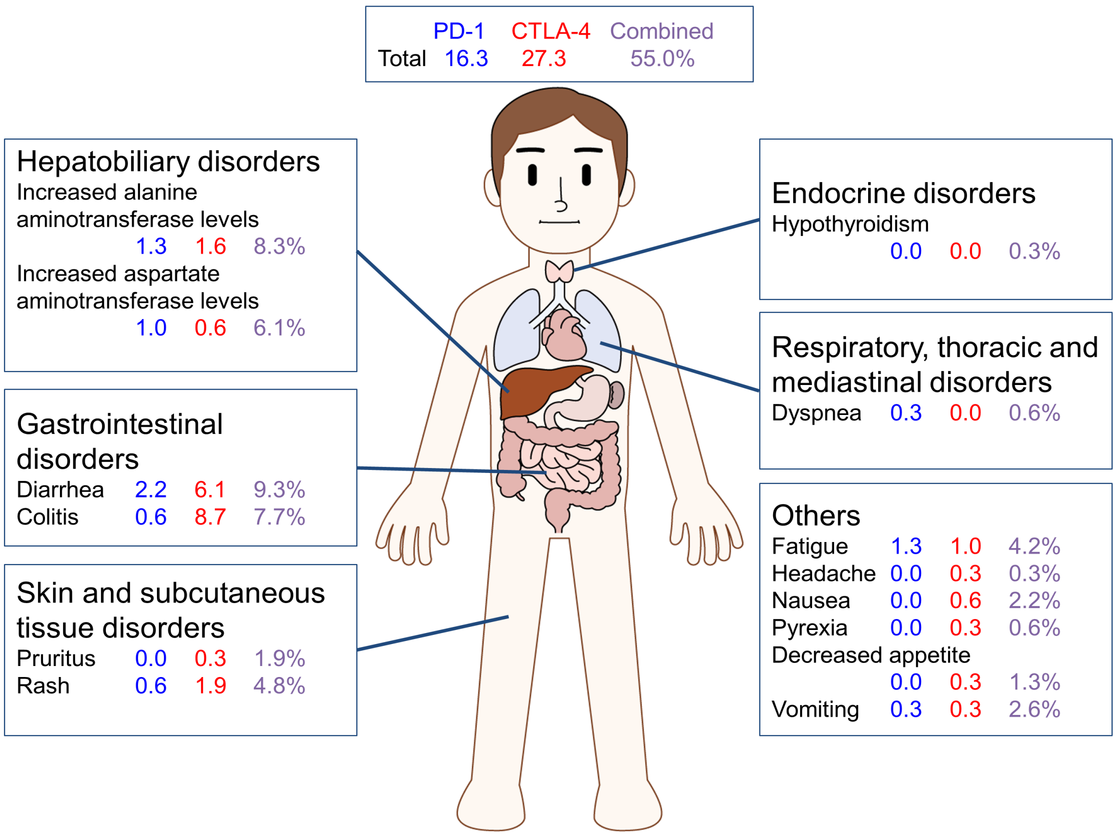


**Figure 1.** An overview of side effects associated with immune checkpoint inhibition. Percentages of grade 3–4 treatment-related adverse events among patients are shown for anti-PD-1 treatment (in blue), anti-CTLA-4 (in red) and combination therapy (in purple). Data presented are based on the study by Larkin *et al*.[37]

TGF-β levels pre-treatment and an expanded IL-9 producing $CD4^+$ T (Th9) cell compartment post-treatment[52]. The authors reported that the addition of PD-1-blocking antibodies increased the IL-4- and TGF-β-mediated induction of Th9 cells *in vitro* and that IL-9 promoted cytotoxic $CD8^+$ T cells *in vitro* and in murine melanoma models. These results suggest that high TGF-β levels in responders prior to treatment may allow increased induction of Th9 cells upon anti-PD-1 treatment, which in turn may improve cytotoxic anti-tumor immune responses[52]. However, it should be noted that the role of Th9 cells in antitumor immunity is still controversial with various studies suggesting that IL-9 can either promote or inhibit anti-tumor immune responses[53–55].

Immune checkpoint inhibitor treatment may also be more effective in tumors that are highly immunogenic due to their high mutation rate. For example, anti-PD-1 antibody administration was more effective in treating colorectal cancers that were mismatch-repair deficient compared to those that were not, and was also particularly effective in non-small cell lung cancers with a higher mutational burden[56,57]. Similarly, the presence of somatic neo-antigens in the tumor cells was associated with the treatment efficacy in melanoma patients treated with anti-CTLA-4[58].

Commensal bacteria may also play a role in influencing the efficacy of immune checkpoint inhibitors. A recent study suggests that anti-CTLA-4 treatment may alter the gut microbiome composition in humans and mice by favoring the growth of *Bacteroides facilis*. In mice, the presence of these bacteria promoted an increase in Th1 polarization and was associated with an improved anti-tumor immune response[59].

Whilst a number of features appear to correlate with the treatment response to anti-CTLA-4 and PD-1, further studies remain necessary to identify more suitable biomarkers that can identify the patients who should (or should not) be treated with immune checkpoint inhibitors. Importantly, tumor tissues are not always available for analysis, and an ideal biomarker would therefore rely on patient samples that are easily accessible, such as blood and stool samples.

## Future avenues for immune checkpoint treatments: Alternative checkpoints and combination therapy

An ideal therapy for cancer should contribute to the complete eradication of tumor cells before they become resistant to the treatment, and be effective in all the patients treated with minimal side effects. Whilst PD-1 and CTLA-4 signaling blockades have greatly improved treatment outcomes, the fact that the majority of patients do not respond, or relapse after treatment, remains a major concern[60].

Although anti-CTLA-4 and anti-PD1/PDL-1 antibodies remain the only immune checkpoint inhibitors clinically approved to date, other similar targets are currently being investigated. Immune checkpoints other than PD-1 and CTLA-4 include the inhibitory receptors TIM-3, LAG-3, and TIGIT (recently reviewed in detail by Anderson *et al.*[61]), and some of these are currently being targeted in phase I and II clinical trials.

The additive effect of PD-1 and CTLA-4 treatment has highlighted that patients might benefit from combination therapies that target non-redundant pathways by combining various immune checkpoint inhibitors[37,38]. Future therapeutic approaches may also combine immune checkpoint inhibitors with other immunological and non-immunological treatments. As the success of immune checkpoint inhibition in humans is related to the existence of anti-tumor immune responses prior to treatment, patients may benefit in particular from therapies that further aim to boost anti-tumor immunity directly[49,56,57]. For example, patients may benefit from cytokine administration in addition to checkpoint inhibition. IL-2 can boost T cell function but prior trials involving IL-2 administration yielded variable results, possibly due to IL-2-induced expansion of Tregs, which in the future may be counteracted with anti-CTLA-4[62–64]. Tumor antigen vaccination may also help, although one previous study did not find any clinical benefit in gp100 vaccination when combined with anti-CTLA-4 and chemotherapy[41]. The combination of checkpoint inhibitors together with the adoptive transfer of *in vitro*-expanded tumor-infiltrating lymphocytes has also yielded promising results in mice[65]. Finally, external factors such as the patients' microbiota may also play an important role in boosting anti-tumor immunity, and recent studies in mice have shown an additive beneficial effect of intestinal *Bifidobacteria* in combination with PDL-1 blockade[66].

## Conclusion

The recent development of immune checkpoint inhibitors targeting CTLA-4 and/or PD-1 has significantly improved disease outcome in a number of cancer patients by boosting anti-tumor immune responses. However, mortality among advanced stage patients and the frequency of treatment-related adverse events remain high with current treatment. The need for predictive markers of treatment efficacy and the development of improved treatment avenues therefore remain as acute as ever.

## Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of their article.

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