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REVIEW ARTICLE

Recent success and limitations of immune checkpoint inhibitors for cancer: a lesson from melanoma

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Abstract

Several researches have been carried over the last few decades to understand of how cancer evades the immune system and thus to identify therapies that could directly act on patient's immune system in the way of restore or induce a response to cancer. As a consequence, "cancer immunotherapy" is conquering predominantly the modern scenario of the fight against cancer. The recent clinical success of immune checkpoint inhibitors (ICIs) has created an entire new class of anti-cancer drugs and restored interest in the field of immuno-oncology, leading to regulatory approvals of several agents for the treatment of a variety of malignancies. The first to be approved in 2011 was the anti-CTLA-4 antibody ipilimumab for the treatment of unresectable or metastatic melanoma. Subsequently, the anti-PD-1s, nivolumab and pembrolizumab, received regulatory approvals for the treatment of melanoma and several other cancers. More recently, three anti-PD-L1 antibodies have received approval: atezolizumab and durvalumab for locally advanced or metastatic urothelial carcinoma and metastatic non-small cell lung cancer (NSCLC) and avelumab for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic Merkel cell carcinoma. This review, starting from the results of melanoma trials, highlights in turn different ICIs and data for different indications in several malignancies are included under each drug class.

Keywords Immuno-oncology · Immune checkpoint inhibitors · Immunotherapy · Combinatorial therapies

Introduction

The concept of cancer immunotherapy can be traced back to 1893 when William Coley first proposed the idea of treating cancer with live bacteria in order to stimulate the immune system. Since that time, interest in immunotherapy has, until recently, been limited because of the low efficacy that was observed with initial efforts and the difficulties in identifying the main mechanisms of immune escape by cancer cells. Nevertheless, over the last few decades, considerable research has increased understanding of how cancer evades the immune system and helped to identify therapies that could act directly on patients' immune systems so as to restore or induce a response to cancer [1]. As a consequence, cancer immunotherapy was defined as 2013's Breakthrough of the Year by *Science* [2]. The recent clinical successes with immune checkpoint inhibitors (ICIs) directed against cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) are the result of deep insights in the field of cancer immunology and immunotherapy, highlighting the mechanisms of cancer immune evasion and manipulating the immune system response to eliminate cancer cells.

The clinical success of ICIs has created an entire new class of anti-cancer drugs and restored interest in the field of immuno-oncology, leading to regulatory approvals of several agents for the treatment of a variety of malignancies. The first to be approved in 2011 was the anti-CTLA-4 antibody ipilimumab for the treatment of unresectable or metastatic melanoma [3]. Subsequently, the anti-PD-1s, nivolumab and pembrolizumab, received regulatory approvals for the treatment of melanoma and several other malignancies. More recently, three anti-PD-L1 antibodies have received approval: atezolizumab and durvalumab for locally advanced or metastatic urothelial carcinoma and metastatic non-small cell lung cancer (NSCLC) and avelumab for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic Merkel cell carcinoma.

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Immune checkpoint inhibitors: from basic science to clinical approval

CTLA-4 and PD-1 are co-inhibitory receptors expressed on the cell surface of T cells which bind to their corresponding ligands (CD80/86 and PD-L1/-L2, respectively) to render T cells anergic [4]. As a consequence, the blocking of these mechanisms can restore an antitumor immune response [Fig. 1]. CTLA-4 (CD152) is a B7/CD28 family member which inhibits T cells, and it is constitutively expressed by Tregs. It can also be upregulated by CD4+ T cells, upon activation, and induces immunosuppression by indirectly reducing signaling through the co-stimulatory receptor CD28. CTLA-4 may also remove CD80 and CD86 from the cell surfaces of APC via trans-endocytosis, reducing the availability of these receptors to other CD28-expressing T cells. The pivotal role of CTLA-4 in immunological tolerance is demonstrated by experiments in mice that lack the CTLA-4 gene in the Forkhead box P3 (FoxP3) + Treg compartment, developing lymphoproliferative disorders. Whereas autoimmune diseases in humans have been associated within the CTLA-4's gene polymorphisms, CTLA-4 signaling is deeply involved in reducing immune responses against infections and cancer cells [5–13].

When autoimmune disease was observed in transgenic mice PD-1−/− T cells [14] and PD-L1 and PD-L2 were discovered as dual ligands for PD-1, both with inhibitory activity on T cells [15, 16], PD-1 was invested in new light and several studies were conducted. PD-1 signaling, like CTLA-4 signaling, can inhibit effector differentiation during the induction phase of a T cell response [17] and develop the suppressive function of inducible Tregs [18]. However, the majority of data highlight an upregulation and maintenance of PD-1 expression on T cells following prolonged antigen exposure, as occurs during chronic viral infection, inflammatory stimuli, and cancer cell proliferation [19–22]. Considering the CTLA-4's role as co-inhibitor of T cell activation, this molecule has become an intriguing target for therapies. In the context of antitumor immunity, pre-clinical and clinical studies have shown that monoclonal antibody directed against CTLA-4 can inhibit Treg-associated immune suppression and promote CD4+ and CD8+ T cell effector function [23, 24].

Anti-CTLA-4

Ipilimumab, a fully human IgG1κ anti-CTLA-4 monoclonal antibody, was the first ICI to be evaluated and approved for the treatment of cancer patients [25, 26]. Ipilimumab promotes T cell-mediated antitumor activity in patients with advanced melanoma by blocking the interaction of CTLA-4 with CD80/CD86 and increasing T cell activation and proliferation with upregulation of antitumor immunity.

Approval of ipilimumab was based on a three-arm phase III study in which 676 pretreated patients received ipilimumab with a peptide vaccine (gp100) or placebo, or gp100 plus placebo [25]. Overall survival (OS) was significantly increased with ipilimumab alone or in combination with the vaccine compared with vaccine alone (10.1 versus 6.4 months). However, there was the risk of immune-related side effects with ipilimumab, occurring in 60% of patients. A subsequent pooled analysis of ipilimumab trials that included 1860 patients reported 3-year survival rates of 22% for all patients, 26% for treatment-naive patients, and 20% for

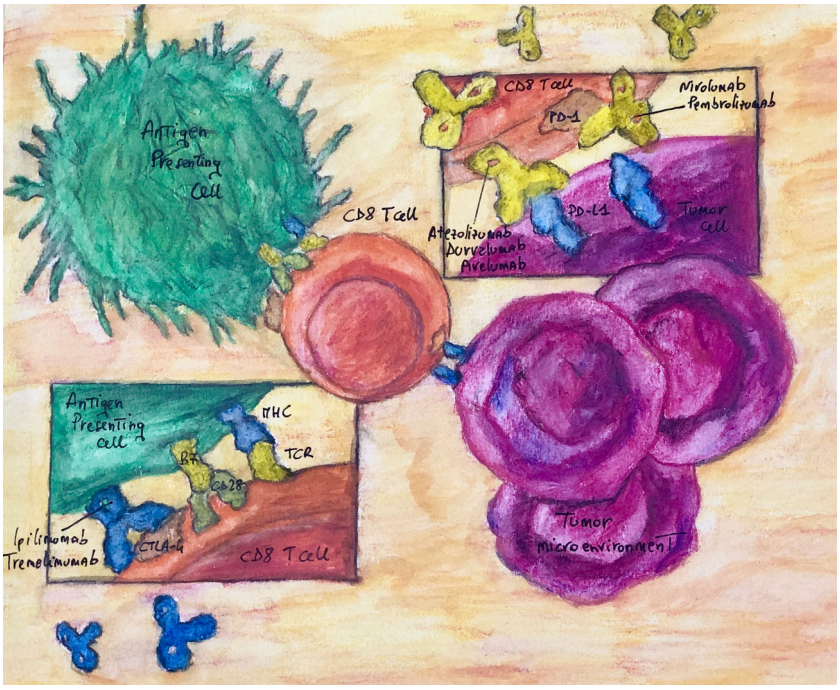

Fig. 1 Mechanisms of action of Immune Checkpoint Inhibitors anti-CTLA4 and anti-PD(L)1: CTLA-4 (a B7/CD28 family member) and PD-1 are co-inhibitory receptors expressed on the cell surface of T cells which bind to their corresponding ligands (CD80/86 and PD-L1/-L2, respectively) to render T cells anergic. The blocking of these mechanisms can restore an antitumor immune response. CTLA-4, cytotoxic T lymphocyte-associated antigen 4; MHC, major histocompatibility complex; PD-1 programmed death 1; PD-L1 programmed death ligand 1; TCR T cell receptor

previously treated patients [27]. When patients enrolled in an expanded access program were also included ($n = 4846$), median OS was 9.5 months (95% CI, 9.0–10.0), with a plateau in the survival curve at 21% that began at approximately 3 years with most patients who were alive at 3 years still alive at 10 years.

A recent phase III study compared ipilimumab 3 mg/kg versus higher dose ipilimumab 10 mg/kg in 727 patients with advanced melanoma [28]. The higher dose treatment was associated with increased median OS (15.7 versus 11.5 months, HR 0.84; $p = 0.04$) but more frequent immune-mediated toxicity, in particular diarrhea, colitis, hepatitis, and hypophysitis.

Ipilimumab was also evaluated in combination with chemotherapy. In a phase III study in 502 patients with previously untreated metastatic melanoma, patients were randomized to receive dacarbazine plus ipilimumab 10 mg/kg every 3 weeks and then every 3 months until progression or dacarbazine plus placebo [29]. The ipilimumab arm demonstrated improved OS compared to dacarbazine alone (11.2 versus 9.1 months). A 3-year survival was 20.8% versus 12.2% (HR = 0.72; $p < 0.001$). The incidence of grade 3/4 adverse events was 56% in the ipilimumab arm and there was evidence of increased liver toxicity. Despite the higher incidence of grade 3/4 adverse events, no toxicity-related deaths occurred. In a subsequent analysis, a 5-year OS rate was 18.2% (95% CI 13.6–23.4) for patients receiving ipilimumab plus dacarbazine compared with 8.8% (95% CI 5.7–12.8) for patients treated with dacarbazine alone ($p = 0.002$) [30].

Although currently approved only for metastatic melanoma and as adjuvant therapy for patients with cutaneous melanoma who received complete resection and total regional lymphadenectomy, ipilimumab has been under evaluation as treatment for several malignancies including renal cell carcinoma, NSCLC, and prostate cancer. A review including many of the completed studies concluded there were modest improvements in survival in particular subsets of cancer patients, despite limited clinical benefit [31].

A fully human IgG2 anti-CTLA-4 monoclonal antibody, tremelimumab, has also being investigated in clinical trials but with no significant survival improvement when used as monotherapy in any of the completed studies.

Anti-PD-1/PD-L1 agents

The discovery of PD-1 in 1992, prior to CTLA-4, was met with limited enthusiasm due to an incomplete comprehension of its function as a co-inhibitory receptor that negatively regulates T cell effector function. However, the anti-PD-1 agents pembrolizumab and nivolumab have since become the standard first-line checkpoint inhibitor treatment having shown superior response rates and improved survival with reduced toxicity compared with ipilimumab.

Pembrolizumab is a humanized IgG4κ-type anti-PD-1 monoclonal antibody. In the randomized phase II KEYNOTE 002 trial that compared pembrolizumab 2 and 10 mg/kg every 3 weeks with investigator choice chemotherapy in patients refractory to ipilimumab, both doses improved PFS and ORR compared with chemotherapy [32] . In the final analysis of this study, improvements in OS versus chemotherapy were non-statistically significant for both pembrolizumab 2 mg/kg (HR 0.86, 95% CI 0.67–1.10, $p = 0.117$) and 10 mg/kg (HR 0.74, 95% CI 0.57–0.96, $p = 0.011$) [33]. Median OS was 13.4 months with pembrolizumab 2 mg/kg (95% CI 11.0–16.4) and 14.7 months (95% CI 11.3–19.5), with pembrolizumab 10 mg/kg compared with 11.0 months (95% CI 8.9–13.8) with chemotherapy, with limited improvement after censoring for crossover. Two-year survival rates were 36% and 38%, versus 30%. PFS, ORR, and duration of response were all improved with pembrolizumab versus chemotherapy, regardless of dose. Pembrolizumab was also been shown to be superior to ipilimumab in the randomized phase III KEYNOTE 006 trial [34]. A total of 834 patients with advanced melanoma received pembrolizumab 10 mg/kg every 2 weeks or 3 weeks or ipilimumab 3 mg/kg every 3 weeks. The estimated 6-month PFS rates were 47.3% for pembrolizumab every 2 weeks, 46.4% for pembrolizumab every 3 weeks, and 26.5% for ipilimumab (HR for disease progression, 0.58; $p < 0.001$ for both pembrolizumab regimens versus ipilimumab). An estimated 12-month OS rates were 74.1%, 68.4%, and 58.2%, respectively (HR for death for pembrolizumab every 2 weeks, 0.63; 95% CI, 0.47–0.83; $p = 0.0005$; HR for pembrolizumab every 3 weeks, 0.69; 95% CI, 0.52–0.90; $p = 0.0036$). Response rates were also superior with pembrolizumab every 2 weeks (33.7%) and every 3 weeks (32.9%), versus ipilimumab (11.9%) ($p < 0.001$ for both comparisons). Treatment-related grade 3–5 adverse events were less frequent with pembrolizumab (13.3% and 10.1%) than with ipilimumab (19.9%). After 4 years of follow-up, pembrolizumab continues to provide durable antitumor activity in treatment-naive and previously treated patients with advanced melanoma, with 86% of patients who are progression-free at 20 months after completing 2 years of pembrolizumab [35].

In addition, based on improved progression-free and overall survival of metastatic NSCLC patients with PD-L1-positive tumors who received pembrolizumab versus either docetaxel- or platinum-based chemotherapy, pembrolizumab obtained accelerated approval in this setting [36, 37]. Accelerated or full approval has also been gained for classical Hodgkin lymphoma, squamous cell carcinoma of the head and neck (SCCHN), urothelial carcinoma, and gastric/gastroesophageal junction adenocarcinoma [38–41].

Nivolumab, a fully human IgG4κ monoclonal antibody, received FDA approval in 2014, as the first anti-PD-1 therapy for cancer. In melanoma, nivolumab had an ORR of 32% and

1-, 2-, 3-, and 4-year OS rates of 63%, 48%, 42%, and 32%, respectively in the phase I CheckMate-003 trial [42]. In a phase III study of the first-line nivolumab versus dacarbazine in 418 patients with advanced melanoma, nivolumab provided superior OS, with 72.9% of patients in the nivolumab arm and 42.1% in the chemotherapy arm alive at 1 year [43]. Median PFS was 5.1 months with nivolumab versus 2.2 months with dacarbazine (HR for death or progression of disease, 0.43; 95% CI, 0.34–0.56; $p < 0.001$) and ORR was 40.0% (95% CI, 33.3–47.0) versus 13.9% (95% CI, 9.5–19.4) ($p < 0.001$). The incidence of grade 3/4 treatment-related adverse events was lower in the nivolumab arm (11.7% versus 17.6%). A study update reported a 2-year OS of 57.7% with nivolumab versus 26.7% with dacarbazine [44]. Moreover, data from a phase III study (CheckMate-037) in patients with metastatic melanoma showed an ORR of 31.7% with nivolumab compared to 10.6% with investigator choice chemotherapy [45]. Grade 3–4 drug-related serious adverse events were observed in 12 (5%) nivolumab-treated patients and nine (9%) patients in the chemotherapy group. No treatment-related deaths occurred.

Compared with ipilimumab, anti-PD-1 therapy offers a significant improvement in terms of ORR, PFS, and OS, as well as reduced toxicity, and consequently these agents have been widely accepted as a potential first-line treatment of patients with advanced melanoma.

More recently, nivolumab was also approved in the adjuvant setting for completely resected stage III/IV melanoma [46]. Going beyond the treatment of melanoma patients, nivolumab has demonstrated survival benefits over traditional therapies in several phase III trials, leading to its approval as either the first-line or second-line therapy for advanced squamous cell lung cancer (SCLC) and nonsquamous NSCLC, advanced RCC, and recurrent SCCHN [47–50]. Moreover, nivolumab was recently granted accelerated approval for the treatment of advanced urothelial carcinoma, advanced hepatocellular carcinoma (HCC), and metastatic DNA mismatch repair–deficient or microsatellite instability-high colorectal cancer, based on significant objective responses rates in patients from Phase II trials [51–53]. Nivolumab has also become the first ICI for treatment of a hematological malignancy, based on the results of two phase I/II trials demonstrating a combined objective response rate of 65% in nivolumab-treated classical Hodgkin lymphoma patients [54, 55].

The anti-PD-L1 agent durvalumab received accelerated approval as the second-line treatment for progressive metastatic urothelial carcinoma, and full approval for treatment of stage III NSCLC not progressed following concurrent chemo-radiotherapy. In the phase III PACIFIC trial, durvalumab achieved 18-month PFS rates of 44.2% versus 27.0% in the placebo arm [56]. Prior to durvalumab, in 2016, atezolizumab, a fully humanized IgG1κ monoclonal antibody, received accelerated approval for the treatment of locally advanced or metastatic urothelial carcinoma and full approval for similar indications of NSCLC, becoming the first anti-PD-L1 immune checkpoint inhibitor anti-PD-L1 to be approved. Indeed, in the case of previously treated NSCLC, in a phase III trial, atezolizumab reached higher OS rates than docetaxel [57], whereas no survival benefit of the second-line atezolizumab versus chemotherapy in advanced urothelial carcinoma patients was obtained in a phase III trial [58]. Another PD-L1 inhibitors, avelumab, a fully human IgG1λ monoclonal antibody, has recently been approved in the USA, the EU, and Japan for the treatment of metastatic Merkel cell carcinoma (MCC), based on significant and durable objective response rates in phase I/II studies [59] It is thus the first therapeutic agent specifically approved for use in this indication, and is approved for use independent of line of treatment due to the confirmed objective responses, early and durable, observed in approximately one-third of patients with chemotherapy-refractory metastatic MCC treated with avelumab. Furthermore, interim results from a separate cohort of patients show an objective response rate for avelumab of > 60% in patients who were chemotherapy-naïve.

Combined CTLA-4 and PD-1 inhibition

Anti-CTLA-4 and anti-PD-1 agents increase antitumor immunity through distinct but complementary mechanisms and pre-clinical models have shown that blocking both receptors, as compared with blockade of either alone, significantly improves antitumor responses [60]. A recent analysis reported that combined PD-1 plus CTLA-4 inhibition resulted in better survival outcomes than either as monotherapy, with the exception of OS in the first-line therapy for which single-agent PD-1 inhibition had similar results as combined PD-1 plus CTLA-4 inhibition.

In a phase I dose escalation study with 53 patients (CheckMate-004), the combination of nivolumab and ipilimumab demonstrated 40% with evidence of clinical activity (conventional, unconfirmed, or immune-related response or stable disease for ≥ 24 weeks observed in 65% of patients with advanced melanoma [61]. At the maximum doses that were associated with an acceptable level of adverse events (nivolumab 1 mg/kg and ipilimumab 3 mg/kg), the ORR was 53%. Grade 3/4 treatment-related adverse events occurred in 53% of patients but toxicity was similar to previous experience with ipilimumab monotherapy. Two-year OS of 79% and 3-year OS of 63%, with median OS not been reached, have been reported [62]. In the subsequent CheckMate-069 phase II study, 142 previously untreated patients with metastatic melanoma were randomized to nivolumab plus ipilimumab or ipilimumab alone [63]. Among patients with BRAF wild-type tumors, confirmed ORR was 61% with ipilimumab plus nivolumab versus 11% with ipilimumab alone ($p < 0.001$); complete responses were

reported in 22% of the combination group compared with no patients in the ipilimumab monotherapy. Median PFS was not reached with the combination therapy and was 4.4 months with ipilimumab alone (HR for disease progression or death, 0.40; 95% CI, 0.23–0.68; $p < 0.001$). Similar results for ORR and PFS were also observed in 33 patients with BRAF-mutant tumors. Grade 3–4 treatment-related adverse events were reported in 54% of patients receiving combination therapy compared with 24% of patients receiving ipilimumab. At a median follow-up of 24.5 months, 2-year OS was 63.8% (95% CI 53.3–7.6) with nivolumab plus ipilimumab and 53.6% (95% CI 38.1–66.8) with ipilimumab alone; median OS had not been reached in either group (hazard ratio 0.74, 95% CI 0.43–1.26; $p = 0.26$) [64].

In the CheckMate-067 phase III trial, nivolumab alone or nivolumab plus ipilimumab was compared with ipilimumab alone in 945 previously untreated patients with metastatic melanoma [65]. At a 9-month median follow-up, median PFS was higher with the combination treatment versus ipilimumab alone (11.5 versus 2.9 months, HR 0.42, 95% CI 0.31–0.57) and with nivolumab monotherapy versus ipilimumab alone (6.9 versus 2.9 months, HR 0.57, 95% CI 0.43–0.76). Although the study was not designed to compare the combination of ipilimumab plus nivolumab with nivolumab monotherapy, the median PFS in the combination arm was superior to that of nivolumab monotherapy (median PFS 11.5 versus 6.9 months, HR 0.74 95% CI 0.60–0.92). Median PFS for patients with positive expression of PD-L1 was 14 months for both the combination and nivolumab monotherapy and 4 months for ipilimumab monotherapy; however, the median PFS for patients who were PD-L1 negative was 11.5, 5.3, and 3 months respectively. ORR was 72%, 58%, and 21% for PD-L1-positive patients and 55%, 44%, and 18%, for PD-L1-negative patients receiving combination, nivolumab alone, or ipilimumab alone, respectively. Treatment-related grade 3/4 adverse events were observed in 16.3% of patients in the nivolumab group, 55.0% in the nivolumab plus ipilimumab group, and 27.3% in the ipilimumab group. After longer term follow-up (minimum of 3 years), median OS had not been reached in the combination group and was 37.6 months in the nivolumab group and 19.9 months in the ipilimumab group (HR for death with nivolumab plus ipilimumab versus ipilimumab, 0.55; $p < 0.001$; HR for death with nivolumab versus ipilimumab, 0.65; $p < 0.001$) [66]. Three-year OS rate was 58% in the combination group and 52% in the nivolumab group, as compared with 34% in the ipilimumab group.

Recently, ipilimumab and nivolumab also showed clinically meaningful intracranial efficacy, with an intracranial clinical benefit rate of 57% (95% CI 47–68) in 94 patients with melanoma who had untreated brain metastases enrolled in a phase II trial with a median follow-up of 14.0 months [67].

Nivolumab in combination with ipilimumab has also been approved for patients with intermediate- and poor-risk advanced renal cell cancer based on significantly higher overall survival and objective response rates with nivolumab plus ipilimumab than with sunitinib [68] and patients with microsatellite instability-high or mismatch repair–deficient metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. Promising results are coming from phase II/III trials in patients with advanced NSCLC and recurrent SCLC [69, 70]. In patients with stage IV or recurrent NSCLC that was not previously treated with chemotherapy, PFS among patients with a high tumor mutational burden was significantly longer with the combination than with chemotherapy [71]. The 1-year progression-free survival rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy, and the median progression-free survival was 7.2 months (95% confidence interval [CI], 5.5 to 13.2) versus 5.5 months (95% CI, 4.4 to 5.8) (hazard ratio for disease progression or death, 0.58; 97.5% CI, 0.41 to 0.81; $p < 0.001$).

In the KEYNOTE 029 study, pembrolizumab 2 mg/kg in combination with lower dose ipilimumab 1 mg/kg had a manageable toxicity profile and provided robust antitumor activity in 153 patients with advanced melanoma [72] ORR was 61% (95% CI 53–69), estimated 1-year PFS was 69% (95% CI 60–75), and estimated 1-year OS was 89% (95% CI 83–93). Grade 3–4 treatment-related adverse events occurred in 45% of patients and grade 3/4 immune-mediated adverse events occurred in 27% of patients. In a recently reported ongoing trial, 22 patients who had progressed immediately prior on an anti-PD-1-based regimen were treated with pembrolizumab 200 mg plus ipilimumab 1 mg/kg every 3 weeks for 4 doses, then pembrolizumab alone [73]. Among the 17 response-evaluable patients, antitumor activity was shown in 8 patients (45%) with 2 complete responses, 6 partial responses. An additional 5 patients had stable disease for a DCR of 76%. Combination strategy therapy with ICIs is under investigation in several malignancies due to the findings that these checkpoint pathways regulate T cell function by distinct mechanisms and at different stages of T cell differentiation.

Combination therapies involving other ICIs, such as durvalumab plus tremelimumab, are also being investigated in several malignancies with manageable toxicity profiles and antitumor activity, in phase I trial for melanoma [74] and NSCLC [81].

Going beyond anti-CTLA-4 and anti-PD(L)-1s

Many patients fail to respond to ICIs due to intrinsic resistance or have an initial response followed by disease progression due to acquired resistance. Mechanisms of treatment resistance are not well understood. However, patients refractory to anti-PD-1 treatment have been recently shown to have

cancer cells less susceptible to T cell-mediated killing via loss of IFN-γ response elements or MHC class I due to acquired mutations in genes encoding for interferon receptor–associated Janus kinase (JAK) 1, JAK2, or β2-microglobulin [75]. Anti-PD-1 or anti-CTLA-4 treatment may also cause upregulation of other inhibitory receptors, as emerged in patients with melanoma or prostate tumor, who exhibited upregulation of the inhibitory receptor V domain Ig suppressor of T cell activation (VISTA) on various tumor-infiltrating immune cells after anti-CTLA-4 treatment [76], as well as in patients affected by lung adenocarcinoma refractory to PD-1 treatment, who showed upregulation of the inhibitory receptor TIM-3 on T cells [77]. Recent pre-clinical study in mice revealed another resistance mechanism to anti-PD-1 therapy, consisting of tumor-associated macrophages (TAM) removing the therapeutic antibody from the surface of the T cells in vivo, thus making them once again susceptible to inhibitory signaling through the receptor. This mechanism could be partially overcome by administration of Fc receptor-blocking agents prior to treatment [78].

Novel combinations involving other inhibitory receptors are being explored to identify treatments that can overcome primary or acquired resistance to anti-PD-1/PD-L1 and/or reduce toxicity compared to combination therapy with anti-CTLA-4 and anti-PD-1. These include the TIM-3, LAG-3, TIGIT, and B and T lymphocyte-associated protein (BTLA) receptors associated with T cell exhaustion as well as VISTA, whose inhibition promoted antitumor immune responses in murine models, and CD96, which has been shown to inhibit NK cell activity in murine cancer models [79–81].

As a potentially synergistic immune pathway to PD-1/PD-L1, anti-lymphocyte-activation gene (LAG)-3 has emerged as an immune checkpoint receptor that regulates T cell function. The anti-LAG-3 therapy BMS-986016 is being investigated in combination with nivolumab. In an ongoing expansion study of 48 heavily pretreated patients with advanced melanoma who were refractory to or relapsed on anti-PD-1/PDL-1 therapy, the ORR was 12.5% [82].

Another regimen combination under investigation is anti-PD-1 plus IDO inhibitor. IDO is an interferon-gamma (IFN-γ)–induced intracellular enzyme that catalyzes the first and rate-limiting step of tryptophan degradation in the kynurenine pathway [83]. In tumors, depletion of tryptophan and production of kynurenine and other metabolites lead the local tumor microenvironment to an immunosuppressive state that helps tumor cells evade immuno-surveillance. Epacadostat is an oral inhibitor of the IDO-1 enzyme [84] that is being evaluated in combination with both pembrolizumab and nivolumab. In an open-label phase I/II study in multiple tumor types (ECHO-202/KEYNOTE-037), epacadostat plus pembrolizumab showed promising antitumor activity in patients with advanced melanoma [85]. In 54 evaluable patients with melanoma, the ORR was 56% and the disease control rate (DCR) was 71%. Median PFS was 12.4 months, and 18-month PFS was 49%. Among treatment-naïve patients with advanced disease, ORR was 58% and the DCR was 74%. Epacadostat plus pembrolizumab showed a favorable safety profile, with an incidence of related grade 3/4 toxicity of 20%. This combination is being further evaluated versus pembrolizumab monotherapy in a phase III study of 706 patients with advanced melanoma (ECHO-301/KEYNOTE-252). Similarly, in the open-label phase I/II ECHO-204 study of patients with advanced solid tumors, epacadostat plus nivolumab was generally well tolerated and showed promising activity [86]. In 40 patients with advanced melanoma untreated with immune-therapies, except for ipilimumab, as a first-line therapy, ORR was 63% and the DCR was 88%. Response was observed regardless of PD-L1 expression. Toxicity was manageable, although treatment-related grade 3 rash and treatment-related adverse events (TRAEs) leading to discontinuation were increased with a higher dose of twice daily epacadostat (300 vs. 100 mg). However, the pivotal phase III ECHO-301/KEYNOTE-252 trial of epacadostat in combination with pembrolizumab which enrolled over 700 patients with unresectable or metastatic melanoma did not meet the primary endpoint of improved PFS compared to pembrolizumab monotherapy and the study was prematurely stopped [87]. Other phase III trials of epacadostat in combination with pembrolizumab or nivolumab were also subsequently halted.

Another IDO-1 inhibitor, BMS-986205, has also been tested in combination with nivolumab. In a phase I/IIA trial in patients with cervical, bladder, or other advanced cancers (CA017-003), BMS-986205 plus nivolumab showed an antitumor activity and had a favorable safety profile in 289 heavily pretreated patients, with grade 3/4 TRAEs in 11% of patients and no treatment-related deaths [88]. Phase III development of BMS-986205 has also been revised in light of the failure of epacadostat, with studies in combination with nivolumab in patients with metastatic melanoma, NSCLC, and head and neck cancer having been stopped, although other studies are ongoing.

Another combination therapy to be under investigation is anti-PD1 plus NKTR-314, which is a CD122-biased immune-stimulatory cytokine that selectively binds to the IL-2 receptor-β. Biased signaling preferentially activates and expands effector T cells and NK cells over regulatory T cells and increases proliferation of TILs and PD-1 expression on effector T cells in the tumor microenvironment. In the ongoing phase, I/II PIVOT-02 NKTR-214, in combination with nivolumab, showed encouraging antitumor activity with notable ORR in PD-L1-negative patients (42% melanoma, 53% RCC, 60% urothelial), with a low rate of grade 3 TRAEs including immune-mediated adverse events, supporting the evaluation of NKTR-214 plus nivolumab in registrational trials. Moreover, robust translational data confirm rationale for

activation of the immune system in the tumor microenvironment with a conversion of PD-L1-negative tumors to PD-L1 positive on treatment, and an ongoing enrollment in PIVOT-02 is continuing for additional tumor types in immune-therapy-naïve and refractory settings [89].

Macrophages may also interfere with antitumor immunity or even directly restrict therapeutic antibodies [90], thus their depletion through a colony-stimulating factor-1 receptor (CSF-1R) inhibitor is being explored in clinical trials in association with anti-PD-1s after having shown efficacy in a glioblastoma mouse model [91]. Because ICIs mostly work by removing inhibitory stimuli on the immune system rather than directly activating the immune response, combination therapies that include immuno-stimulatory substances may also be suitable for patients. The combination of anti-CTLA-4 with cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) in mouse melanoma models, in fact, resulted in increasing tumor rejection [78, 92]. Since the genetically modified herpes simplex virus talimogene laherparepvec is able to replicate in tumor cells and to release GM-CSF, attracting immune cells into the tumor environment been evaluated in recent clinical trials in combination with either CTLA-4 or PD-1 in advanced-stage melanoma patients, obtaining increased treatment response rates compared to the ICIs alone [93–96, 123–126].

A pivotal role in this direction of immuno-stimulation may be that of the gut microbiome, with administration of intestinal *Bifidobacteria* alone associated with reduced tumor growth in a murine B16 melanoma model by promoting dendritic cell-mediated CD8+ T cell responses. In addition, administration of *B. fragilis* to sterile mice treated with anti-CTLA-4 resulted in reduced tumor growth [97]. Moreover, clinical studies associated the presence of fecal *A. muciniphila* with a favorable outcome in anti-PD-1 treatment [98]. These findings suggest that patients may improve their response to ICIs treatment with an appropriate management of their intestinal flora, although ongoing and further studies are waited to confirm clinical efficacy.

Conclusion

Although ICIs have raised the life expectancy for cancer patients, toxicity and mortality still represent a challenge for researchers and clinicians. Further studies in the huge field of immuno-oncology are needed in order to maximize patient outcomes. We envisage that knowledge about baseline antitumor immunity could provide new insight for the selection of patients who are most likely to respond, leading to the use of ICIs with other complementary drugs to help those patients who are, instead, less likely to respond to the current regimens.

Authors' contributions Ottaviano Margaret wrote the manuscript; she acted as corresponding author;

De Placido Sabino provided to data interpretation and reference checking;

Ascierto Paolo Antonio provided to conceptualization of work and its realization; he also checked the references.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this review formal consent is not required.

Conflict of interest Ottaviano Margaret and De Placido Sabino have not potential conflicts of interest.

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