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Comprehensive Review of PD1/L1 Inhibition in Metastatic Solid Tumors: Safety, Efficacy and Resistance

Dae Won Kim[1], Marc Uemura[2] and Adi Diab[2]

[1]Department of Medical Oncology, Moffitt Cancer Center

[2]Department of Melanoma Medical Oncology, The University of Texas M.D. Anderson Cancer Center

Corresponding author: Adi Diab, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030; Tel: 713-745-7336; Fax: 713-745-1046; E-mail: adiab@mdanderson.org

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Abstract

Since pembrolizumab (Keytruda®) was approved for advanced melanoma in September 2014, multiple PD-1 blockade agents have been explored in other malignancies. Emerging clinical data has demonstrated durable clinical activity and safety of PD-1/L1 blockade agents in diverse cancers including melanoma, non-small cell lung cancer, renal cell carcinoma, urothelial cancer, Hodgkin lymphoma, and head and neck cancer. Thus, PD-1/L1 blockade agents have led to a paradigm shift in cancer therapy. In this review, current indications of PD-1 blockade agents in advanced solid malignancies will be discussed.

Keywords: Cancer Immunotherapy; Pembrolizumab; Nivolumab; Atezolizumab

Introduction

Rapid advances in tumor immunology have improved our understanding of key regulators that mediate T cell responses, leading to the development of new immunotherapeutic approaches targeting various immune checkpoints such as cytotoxic T lymphocyte associated protein 4 (CTLA-4) and programmed death-1 (PD-1). PD-1 is a negative immune regulator which plays an essential role in suppressing antitumor immunity in the local tumor environment. PD-1 is expressed on the surface of activated T cells and has two ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). Antigen presenting cells (APCs) and tumor cells broadly express PD-L1 on their surface, and the expression of PD-L1 is upregulated by interferon which is predominately produced by effector T cells. The ligation of PD-1 and PD-L1 inhibits T cell proliferation and activation, and ultimately can induce apoptosis of antigen-specific T cells to prevent collateral tissue damage and autoimmune disease. Tumor cells hijack the PD-1/PD-L1 pathway to inhibit antitumor immunity, and various cancer cells have been reported to upregulate PD-L1 to escape immune surveillance. Several different PD-1/PD-L1 antibodies have been extensively studied in a wide spectrum of malignancies. These efforts are rapidly translating into remarkable clinical successes as PD-1/PD-L1 blocking agents, which currently include atezolizumab (Tecentriq®), nivolumab (Opdivo®) and pembrolizumab, have been FDA-approved for multiple malignancies, including head and neck squamous cell carcinoma, melanoma, non-small cell lung cancer, renal cell carcinoma, and urothelial cancer. In this review, we will discuss the current indications for PD-1 and PD-L1 blockade agents in solid tumors with summarized data from clinical studies.

Clinical Indications of PD-1 Blockade Agents in Solid Tumors

Melanoma

Melanoma is highly immunogenic tumor. In the last 20 years, immunotherapeutic approaches such as cytokine therapy, cancer vaccines, immune checkpoint inhibition and adaptive T cell transfer therapy have been extensively studied in metastatic melanoma. Although the remarkable success of ipilimumab (Yervoy®, a CTLA-4 blocking agent) and targeted therapy for BRAF mutations has revolutionized the metastatic melanoma treatment, an unmet need remains for new therapeutic approaches that induce higher rates of durable clinical response. Extensive research efforts in tumor immunology have identified key immune regulatory molecules including PD-1/PD-L1, targeting of which has demonstrated significant clinical activity in melanoma.

Pembrolizumab

Pembrolizumab, a humanized anti-PD-1 IgG4 monoclonal antibody, was the first PD-1 blockading agent approved by the U.S. Food and Drug Administration (FDA) in September 2014. Initially, pembrolizumab was approved only for patients with metastatic melanoma who demonstrated disease progression following ipilimumab and a BRAF inhibitor if the patient harbored a BRAFV600 mutation. This approval was based on a phase 1 study including 173 patients with ipilimumab-refractory

advanced melanoma [1]. In the study, the patients received pembrolizumab at 2 mg/kg or 10 mg/kg every 3 weeks until disease progression or intolerable toxicity. The overall response rate (ORR) for both doses was 26%, and the treatment was well-tolerated with only 12% of patients experiencing grade 3-4 drug related adverse events. PD-L1 expression in the tumor microenvironment was evaluated in the enrolled patients, and increased expression of PD-L1 was associated with longer progression free survival (PFS) (hazard ratio (HR) 0.76, P<0.001) and overall survival (OS) (HR 0.76, P<0.001) [2].

In a subsequent phase 2 study, 540 patients with refractory metastatic melanoma were randomized to pembrolizumab 2 mg/kg or 10 mg/kg every 3 weeks or to investigator-choice chemotherapy (1:1:1) [3]. With a median follow-up of 10 months, median PFS was significantly improved in both pembrolizumab groups; in the 2 mg/kg group PFS was 5.4 mos. (HR 0.57, 95% confidence interval (CI) 0.45-0.73; P<0.0001) and in the 10 mg/kg group PFS was 5.8 mos. (HR 0.5, 95% CI 0.39-0.64; P<0.0001) compared with the chemotherapy group (3.6 mos.).

The clinical efficacy of pembrolizumab was verified in a phase 3 studies which included 834 patients with advanced, refractory melanoma who had received no more than one prior line of systemic therapy [4]. Patients were randomized to pembrolizumab 10 mg/kg every 2 weeks or 3 weeks vs. ipilimumab 3 mg/kg every 3 weeks in a 1:1:1 ratio. OS was significantly improved in the pembrolizumab every 2 weeks (median OS: not reached (NR), HR 0.63, 95% CI 0.47-0.83; P<0.001) and 3 weeks (median OS: NR, HR 0.69, 95% CI 0.52-0.9; P=0.004) arms compared with ipilimumab (median OS: 16.0 mos.). Overall response rates were 33.7% for pembrolizumab every 2 weeks (P<0.001 vs ipilimumab), 32.9% for every 3 weeks (P<0.001) and 11.9% for ipilimumab. Severe treatment related toxicities (>grade 3) were significantly lower in the pembrolizumab groups (13.3% and 10.1%) than in the ipilimumab group (19.9%). Based on these results, in December 2015, the FDA expanded the treatment indications for pembrolizumab to include first line of therapy for patients with advanced melanoma.

Nivolumab

Nivolumab is another human anti-PD-1 IgG4 monoclonal antibody approved for advanced melanoma. Similar to pembrolizumab, nivolumab was FDA-approved for patients with refractory unresectable or metastatic melanoma in December 2014. Thereafter, this indication was extended to include frontline therapy in January 2016. In a phase 1/2 dose escalation cohort expansion study, 107 patients with metastatic, refractory melanoma received nivolumab at doses from 0.1 to 10 mg/kg every 2 weeks [5,6]. Median OS was 16.8 mos. with ORR of 31%. Interestingly, clinical responses were durable with 2 years of the median response duration. Tumor PD-L1 expression was evaluated in the study: None of the 17 patients with PD-L1–negative tumors had an objective response while 9 patients (36%) with PD-L1 expressing tumors had an objective response [6].

A subsequent phase 3 study confirmed nivolumab's clinical activity [7]. In the study, 272 patients with ipilimumab-refractory metastatic melanoma were randomly allocated to nivolumab 3 mg/kg every 3 weeks or investigator's choice chemotherapy (dacarbazine or paclitaxel). With 167 evaluable patients, confirmed ORR was 31.7% in the nivolumab group and 10.6% in the chemotherapy group. Grade 3-4 drug related toxicities were reported in 5% of nivolumab-treated patients and 9% of chemotherapy-treated patients.

Nivolumab's clinical efficacy and safety were subsequently verified in patients with treatment-naïve, advanced melanoma. In a phase 3 study of previously untreated patients with advanced melanoma, nivolumab demonstrated improved OS rate at 1 year (72.9% vs. 42.1%, HR 0.42, 95% CI 0.25-0.73; P<0.001), prolonged PFS (5.1 months vs. 2.2 months HR 0.43, 95% CI 0.34-0.56; P<0.001) and improved ORR (40% vs. 13.9%; P<0.001) compared with chemotherapy (dacarbazine) [8]. Additionally, nivolumab also demonstrated superior clinical benefits than ipilimumab in a phase 3 study of treatment-naïve metastatic melanoma patients. In particular, PFS was significantly longer (6.9 mos. vs. 2.9 mos., HR 0.57, 95% CI 0.43-0.76; P<0.001) than ipilimumab, similar to that seen for pembrolizumab in a similar patient population [9].

Nivolumab plus ipilimumab

Several preclinical studies have demonstrated that combined CTLA-4 and PD-1 blockade was more clinically effective than either alone [10,11] and this strategy has been evaluated in numerous clinical studies. A phase 1 study of concurrent ipilimumab and nivolumab found that the maximum-tolerated dose of concurrent therapy was 3 mg/kg of ipilimumab and 1 mg/kg of nivolumab [12]. The regimen was clinically active with an ORR of 40%, and tumor reduction of >80% was observed in most of the responding patients. However, 53% of patients developed grade 3 or 4 adverse events, and 21% of patients discontinued therapy due to treatment-related toxicities. A subsequent randomized phase 2 study comparing ipilimumab with or without nivolumab showed that the combination resulted in higher response rates (61% vs. 11%, P<0.001), with complete responses in 22% of patients, and improved PFS (HR 0.40; P<0.001) in treatment naïve patients with BRAFV600 wild-type melanoma [13]. However, an OS benefit was not observed (HR 0.74, 95% CI 0.43-1.26; P=0.26) [14], which may be attributed to the trial design in which 62% of patients in the ipilimumab alone arm crossed over to nivolumab at the time of progression. Again, combination therapy was more toxic with 54% of patients experiencing grade 3 or 4 toxicities compared with 24% of patients receiving ipilimumab alone. Based on this study, the combination regimen was FDA-approved for patients with BRAFV600 wild-type advanced melanoma in September 2015.

A subsequent randomized, three-arm, phase 3 study comparing nivolumab monotherapy, ipilimumab monotherapy, and concurrent nivolumab with ipilimumab confirmed the statistically superior clinical outcomes of the combination compared to ipilimumab alone (median PFS: 11.5 mos. vs. 2.9 mos., HR 0.42; P<0.001) in untreated patients with unresectable

stage III or stage IV melanoma regardless of BRAF mutation status [9]. Based on this study, the clinical indication for combination ipilimumab plus nivolumab was extended to include patients with unresectable stage III or stage IV melanoma regardless of BRAF mutational status. Notably, in patients with PD-L1 expressing tumors, the median PFS was 14.0 mos. in both the ipilimumab plus nivolumab combination group and the nivolumab monotherapy group while median PFS was significantly prolonged with the combination group compared with nivolumab alone in patients with PD-L1 negative tumor, suggesting a possible predictive marker of PD-L1 for more aggressive therapy. However, updated data demonstrated superior outcome of the combination to nivolumab alone regardless of PD-L1 expression in tumor [15].

Non-small cell lung cancer (NSCLC)

In contrast to melanoma, due to the non-immunogenic nature of advanced lung cancer and their sensitivity to contemporary chemotherapy agents, immunotherapeutic approaches have historically not been well-studied in lung cancer. However, recent data have demonstrated that lung cancers, and especially squamous lung cancers which are associated with tobacco use, intrinsically express highest mutational burdens [16]. We know now that these mutations generate neoantigens which can be recognized by effector T cells and induce antitumor immune responses. While immunotherapeutic approaches with cancer vaccines failed to show clinic efficacy in lung cancer, recent studies with PD-1/PD-L1 blocking agents have demonstrated promising results.

Nivolumab

Nivolumab was the first immunotherapy agent approved for the treatment of advanced lung cancer. The safety and efficacy of nivolumab were confirmed in both squamous and non-squamous cell NSCLC. In a phase 3 trial, 272 patients with metastatic squamous cell NSCLC who had disease progression during or after first line chemotherapy were randomly assigned to nivolumab or docetaxel [17]. Nivolumab treatment was associated with improved OS (median OS: 9.2 mos. vs. 6.0; HR 0.59, P<0.001), PFS (median PFS: 3.5 mos. vs. 2.8; HR 0.62, P<0.001) and ORR (20% vs. 9%, P=0.008) compared with docetaxel in patients with advanced, previously- treated squamous cell NSCLC. The expression of PD-L1 was not prognostic or predictive. Grade 3-4 treatment related toxicities were observed in 7% of patients in the nivolumab group compared with 55% in the docetaxel group.

In another phase 3 trial, 582 patients with advanced non-squamous cell NSCLC that had progressed during or after platinum-based doublet chemotherapy were treated with nivolumab or docetaxel [18]. Nivolumab was associated with longer median OS (12.2 mos. vs. 9.4; HR 0.72; 95% CI: 0.59-0.89, P=0.002) and higher ORR (19% vs. 12%; P=0.02) with lower incidence of grade 3-4 toxicities (10% vs. 54%) than docetaxel. Additionally, nivolumab treatment was associated with longer OS, PFS and higher ORR in PD-L1 expressing tumors (≥ 1%) while OS and PFS were similar between the two arms in patients with PD-L1 negative tumors (<1%). Based on the results from these two studies, nivolumab has been FDA-approved for patients with advanced NSCLC (both squamous and non-squamous cell carcinomas) whose disease progressed during or after platinum-based chemotherapy regardless of PD-L1 expression. Most recently, in a randomized phase 3 trial of nivolumab verses physician choice platinum based doublet chemotherapy nivolumab failed to show superior PFS as first line therapy in stage IV/recurrent NSCLC patients with ≥ 5% PD-L1 tumor expression [19].

Currently, several clinical studies of nivolumab plus ipilimumab, or nivolumab plus platinum-based chemotherapy as first-line treatment are underway for advanced NSCLC

Pembrolizumab

In contrast to nivolumab, pembrolizumab has been approved for previously treated patients with advanced NSCLC whose tumors express PD-L1 (>1%). In the large phase 1 study which led to FDA approval, 495 patients with advanced NSCLC regardless of prior treatment received pembrolizumab 2 mg/kg or 10 mg/kg every 3 weeks or 10 mg/kg every 2 weeks until disease progression [20]. Treatment was well-tolerated with grade 3 or higher adverse events of 9.5%. The ORR was 18% in the 394 previously-treated patients and 24.8% in the 101 previously untreated patients. Median PFS and OS were 3.0 and 9.3 mos., respectively, in the previously treated patients and 6.0 and 16.2 mos., respectively in the previously untreated patients. Importantly, pembrolizumab efficacy correlated with PD-L1 expression. The ORR and median PFS were 45.2% and 6.4 months in the patients with tumors expressing PD-L1 ≥ 50%, 16.5% and 4.1 months with PD-L1=1-49% and 10.7% and 4.0 months with PD-L1<1%.

In a subsequent randomized phase 2/3 study, 1034 patients with previously treated with NSCLC with PD-L1 expression at least 1% were randomized to pembrolizumab 2mg/kg, pembrolizumab 10 mg/kg or docetaxel every 3 weeks [21]. OS was significantly prolonged for pembrolizumab 2 mg/kg and 10 mg/kg compared with docetaxel (10.4 mos. and 12.7 mos. vs. 8.5 months [HR 0.71, 95% CI 0.58-0.88 and HR 0.61, 95% CI 0.49-0.75]), while PFS was similar in all groups. Pembrolizumab treatment was much more effective than docetaxel in patients whose tumors expressed PD-L1 of >50% with median OS of 14.9 mos. with pembrolizumab 2 mg/kg, 17.3 mos. with pembrolizumab 10mg/kg and 8.2 mos. with docetaxel (HR 0.54; 95% CI: 0.38-0.77, P=0.0002 and HR 0.50; 95% CI: 0.36-0.70, P<0.0001).

Based on this remarkable efficacy in tumors with PD-L1 expression ≥ 50%, pembrolizumab has been evaluated as frontline treatment in patients with metastatic NSCLC whose tumors have ≥ 50% PD-L1 [22]. In the phase 3 study, 305 patients were randomized to pembrolizumab (200mg every 3 weeks) or platinum-based chemotherapy. Pembrolizumab was associated with prolonged PFS (10.3 mos. vs. 6.0 mos., HR 0.50; 95% CI: 0.37-0.68, P<0.001) and improved OS at 6 months (80.2% vs. 72.4%, HR 0.60; 95% CI: 0.41-0.89, P=0.005) compared with chemotherapy. The ORR was also higher in the pembrolizumab group (44.8% vs. 27.8%) with fewer high grade [3-5] adverse events (26.6% vs. 53.3%) than in the

chemotherapy group. These results led to FDA-approval in October 2016 of pembrolizumab as frontline therapy for patients with metastatic NSCLC whose tumors have ≥ 50% PD-L1 without EGFR or ALK genomic tumor aberrations.

Atezolizumab

Atezolizumab, a humanized anti-PD-L1 IgG1 monoclonal antibody was the first anti-PD-L1 antibody approved for previously treated metastatic NSCLC. The approval was based on a survival benefit demonstrated with atezolizumab in two randomized studies. In a phase 2 trial of 287 patients with previously treated advanced NSCLC, patients were randomly assigned to atezolizumab or docetaxel [23]. The median OS for atezolizumab was significantly longer than docetaxel (12.6 mos. vs. 9.7 mos., HR 0.73; 95% CI 0.53-0.99, P=0.04). While the ORR was similar (17% vs 15%) in both groups, the objective responses of atezolizumab were durable with a median duration of 14.3 mos. compared with 7.2 mos. for docetaxel. PD-L1 expression on tumors or tumor infiltrating immune cells correlated with improvement in survival with atezolizumab. Furthermore, atezolizumab showed favorable toxicity profiles compared with docetaxel (grade 3-4 toxicities: 11% vs. 39%). In a subsequent phase 3 study of atezolizumab in patients with previously treated advanced NSCLC, atezolizumab also demonstrated favorable toxicity profiles and improved OS (13.8 mos vs. 9.6 mos., HR 0.73, P=0.0003) regardless of PD-L1 expression in comparison to docetaxel [24]. The survival benefit was more prominent in patients with high PD-L1 expression on tumors or tumor infiltrating immune cells. Results from these trials led to FDA-approval of atezolizumab in October 2016 for the treatment of patients with advanced NSCLC whose disease progressed on platinum-containing chemotherapy and on EGFR/ALK directed therapies if such mutations were present.

Renal cell carcinoma (RCC)

Renal cell carcinoma with clear cell histology has been considered a highly immunogenic tumor since spontaneous regression of primary and metastatic tumors have been observed [25,26], and high dose interleukin-2 has demonstrated durable responses in select patients [27]. Therefore, checkpoint inhibitors have been extensively studied in metastatic RCC with clear cell type. In this review, we will focus only on the clear cell histologic subtype.

Nivolumab

Several early clinical studies of nivolumab demonstrated promising antitumor activity and acceptable tolerability in patients with previously treated metastatic RCC6. These provided the rationale for the phase 3 trial of nivolumab in patients with advanced RCC who received one or two prior anti-angiogenic therapies [28]. In this study, a total of 821 patients received either nivolumab or everolimus. As compared to the everolimus treated patients, the nivolumab group demonstrated improved OS (25.0 mos. vs. 19.6 mos., HR 0.73, P=0.002) and higher ORR (25% vs. 5%, P<0.001) with fewer grade 3 or 4 adverse events. Moreover, the clinical benefit from nivolumab was observed regardless of PD-L1 expression.

VEGF is one of the critical mediators of immune suppression. VEGF regulates antitumor immunity by inhibiting dendritic and effector T cell function, [29] while enhancing immunosuppressive regulatory T cells [30] and myeloid derived suppressor cells [31]. Clinical data demonstrate that elevated VEGF levels are associated with poor clinical response to immunotherapy such as high dose IL-2 [32] and ipilimumab [33]. Therefore, a clinically relevant combinatorial strategy with increased therapeutic potential may include and VEGF and PD-1 inhibitor. Currently, several clinical trials investigating this strategy are underway.

Urothelial cancer

The clinical efficacy of immunotherapy with intravesical Bacillus Calmette-Guerin (BCG) has been shown in localized, non-muscle invasive urothelial cancer. However, no systemic immunotherapy has shown antitumor activity in metastatic bladder cancer until recently. Although platinum-based chemotherapy remains the standard of care for treatment naïve metastatic urothelial cancer with response rates ranging from 30-56% [34], most patients develop disease progression after frontline chemotherapy. Unfortunately, second line treatments, which historically involved other chemotherapy agents, have minimal clinical benefit. Thus, in this setting, there is a significant unmet need for effective, well-tolerated approaches in refractory urothelial cancer. Recently, a large phase I trial demonstrated promising results of atezolizumab in metastatic urothelial cancer and it led to further evaluation of blockade of PD-1/PD-L1 in metastatic urothelial cancer [35].

Atezolizumab

Atezolizumab is the first, FDA-approved immunotherapy agent for patients with metastatic urothelial cancer. It is approved following platinum-based chemotherapy or within 12 months of receiving platinum-based chemotherapy in either before or after surgical resection. This approval was based on durable activity and tolerability demonstrated in a phase 2 trial which investigated 310 patients with chemotherapy refractory, metastatic urothelial cancer [36]. In this trial, the ORR was 15% and 38 patients (84%) had ongoing responses at a median follow-up of 11.7 months. Although the objective responses correlated with PD-L1 expression on tumor infiltrating immune cells, 8% of objective responses were observed even in patients with no PD-L1 expression. Therapy was well-tolerated with grade 3-4 immune mediated toxicities occurring in only 5% of patients.

Head and neck squamous cell carcinoma (SCC)

Cytotoxic chemotherapy is the standard therapeutic option for patients with recurrent or metastatic head and neck SCC. However, the prognosis of these patients, even with therapy, is dismal with a median survival of 6-10 months [37]. Given the low survival rates and significant toxicities associated with standard cytotoxic chemotherapy regimens, more effective agents in this setting are also needed. Recently, studies have shown that dysregulation and evasion of the immune system is closely associated with the development and progression of

head and neck cancers [38]. Recently several immunotherapeutic approaches, including checkpoint blockade have been evaluated these patients.

Pembrolizumab

The FDA approved pembrolizumab for patients with recurrent or metastatic head and neck SCC with disease progression on or after platinum-based chemotherapy based on early non-randomized data from the KEYNOTE-012 trial [39-41]. In this study, 174 patients received pembrolizumab 10 mg/kg every 2 weeks or 200mg every 3 weeks. Objective responses were observed in 28 patients (16%); among all responders, 23 patients (82%) had responses of 6 months or longer. Patients with positive PD-L1 expression had higher ORR (22% vs. 4%; P=0.021) than PD-L1 negative tumors. Safety data were similar to those reported with pembrolizumab in melanoma and NSCLC. However, contrast to melanoma and NSCLC, the recommended dose is 200mg every 3 weeks for head and neck SCC instead of 2 mg/kg every 3 weeks.

Nivolumab

More recently, nivolumab was also studied in a phase 3 trial in 361 patients with recurrent SCC of the head and neck whose disease had progressed on platinum-based chemotherapy [42] In this study, patients received either nivolumab 3 mg/kg every 2 weeks or standard, single agent systemic therapy which included either methotrexate, docetaxel or cetuximab. The median OS (7.5 vs. 5.1 mos; HR 0.70; 97.73% CI, 0.51-0.96; p=0.01) and 1-year survival rate (36.0% vs. 16.6%) favored the nivolumab group; however, the median PFS was no statistically significant (2.0 mos. for nivolumab vs. 2.3 mos. for chemotherapy, HR=0.89; 95% CI, 0.70-1.13; p=0.32). Like previous trials, nivolumab was well-tolerated with grade 3 or 4 treatment-related adverse events 13.1% in the nivolumab group vs. 35.1% in the standard therapy group. Because of this data, nivolumab is also now FDA-approved for recurrent or metastatic head and neck SCC with disease progression on platinum-based chemotherapy.

PD-L1 Expression in Tumor Microenvironment as a Biomarker

While several clinical studies confirmed the remarkable clinical activity of PD-1/PDL-1 blockading agents, including atezolizmuab, nivolumab and pembrolizumab, in patients with numerous malignancies, there are limitations to their efficacy. For example, more than 50% of patients did not have clinical benefit from PD-1/PD-L1 inhibitors, and some of initially responding patients eventually developed disease progression. Various biomarkers and tumor characteristics used to predict clinical responses have been evaluated for appropriate selection of patients that are likely to benefit from PD-1/PD-L-1 blockade. Several biomarkers have been suggested, including pre-existing CD8+ T cells in the tumor microenvironment [43], high tumor mutational loads [44], neoantigen heterogeneity [45], high relative eosinophil count [46], high relative lymphocyte count [46], low LDH [46], absence of metastasis other than involvement of the soft tissue or lung [46], and PD-L1 expression in the tumor microenvironment [43]. Among these markers, PD-L1 expression in the tumor microenvironment has been extensively studied. As described above, PD-L1 expression in pre-treatment tumor samples correlated with improved clinical outcome to PD-1/PD-L1 blockading agents in head and neck squamous cell carcinoma [41], melanoma [2,6], NSCLC [18,20] and urothelial cancer [35,36].

Currently, PD-L1 expression is routinely used in patients with metastatic NSCLC as a biomarker for pembrolizumab treatment, both in the frontline and refractory setting. However, using PD-L1 as an absolute predictive biomarker remains problematic for a number of reasons. 1) A significant number of patients with PD-L1 negative tumors respond to PD-1/PD-L1 blocking agents [17,28]. The specific mechanism by which this happens, assuming PD-L1 is the most relevant biomarker is not known. 2) A significant discordance of PD-L1 expression has been reported between primary tumors and metastatic lesions in several malignancies including melanoma [47], NSCLC [48] and RCC [49]. How this discordance affects treatment outcome if PD-L1 is the main biomarker is not clear. 3) There are at least 12 different anti-PD-L1 antibodies and several different staining techniques for determination of PD-L1 expression which have different sensitivity [48]. Choosing the correct methodology for PD-L1 expression in the context of each tests' sensitivity and use with specific PD-1/PD-L1 blocking agent is challenging and not yet defined .4) The cut-off value of PD-L1 staining positivity has not been elucidated. In most of the immunotherapy trials presented, patients with wide ranges of PD-L1 expression (ie: >1%; 1-49%, and >50%) had positive response to PD-1/PD-L1 blockade. Thus, in order to overcome these challenges and accurately identify patients who will benefit from PD-1/PD-L1 blocking agents, further studies improving our understanding of the tumor microenvironment are needed.

Anti-PD-1/PD-L1 Treatment in Preexisting Autoimmune Disease or Organ Transplantation

Considering the 3-8% prevalence of autoimmune disease in the US and the number of organ transplants performed each year, it is not uncommon for cancer patients to present with a concurrent autoimmune condition or previous organ transplant [50]. Complicating this issue, epidemiological studies have shown that patients with autoimmune disease or organ transplantation have an increased risk of diverse cancers [51,52]. To date, the use of immune checkpoint therapy has been limited in these patients, due to concerns of potentially exacerbating their pre-existing autoimmune disease or potentiating graft rejection.

Several case reports have demonstrated the efficacy and safety of PD-1/PD-L1 blocking agents in patients with concurrent autoimmune disease. Notably, our group recently published on a patient with advanced melanoma and Crohn's disease who we treated with tocilizumab (anti-IL-6 antibody) to prevent autoimmune exacerbation and pembrolizumab [53]. Despite this, the efficacy and safety of checkpoint inhibitors are largely

unknown in this patient population as they are routinely excluded from immunotherapy clinical trials. A recent retrospective study suggested that anti-PD-1 therapy may be safely and effectively administered to cancer patients who have pre-existing autoimmunity with close monitoring for autoimmune flares [54]. In this study of 52 patients which demonstrated an ORR of 33%, 15 (29%) had active autoimmune symptoms and 16 (31%) were on systemic immunosuppression at anti-PD-1 initiation. 22 patients (38%) experienced exacerbation of their pre-existing autoimmune disease at a median of 1.3 months after anti-PD-1/PD-L1 initiation including 7/13 with rheumatoid arthritis, 3/3 with polymyalgia rheumatic, 2/2 with Sjogren's disease, 1/2 with scleroderma, 2/2 with immune thrombocytopenic purpura, 3/8 with psoriasis and 1/4 with Graves' disease. Interestingly, no flare was observed in 5 patients with neurological disorders and 6 patients with gastrointestinal autoimmunity. Among the 22 flares, 3 patients (6%) had grade 3 flares, and 2 patients discontinued treatment. Immune related toxicities other than flares were observed in 18 patients (29%) including 5 grade 3 toxicities, and 3 of them discontinued anti-PD-1.

In regard to checkpoint inhibitor use in patients with organ transplantation, two cases of allograft rejection were reported after anti-PD-1 therapy in patients with kidney transplantation [55,56]. In addition, two cases of successful administration of ipilimumab in patients with kidney transplantation have also been reported [57]. The paucity of data available in this unique population, further studies is needed to delineate the role of PD-1/PD-L1 blocking agents in patients with pre-existing autoimmune disease or organ transplantation.

Anti-PD-1 in Patients with Metastatic Brain Disease

Metastatic brain disease commonly develops in patients with melanoma, NSCLC and RCC. Although advances in local therapy such as stereotactic radiosurgery have improved local control of brain metastases, managing these patients remains challenging as many systemic agents have poor central nervous systemic (CNS) response. Historically, standard chemotherapy was considered ineffective for brain metastases due to the presence of the blood-brain barrier (BBB). However, recent data have demonstrated antitumor activity of nivolumab and pembrolizumab in the CNS. In a non-randomized, phase 2 trial, the safety and activity of pembrolizumab was evaluated in patients with untreated brain metastasis from melanoma or NSCLC who did not have associated neurological symptoms and did not require systemic steroid therapy [58]. Positive tumor PD-L1 expression was required in the NSCLC arm while it was not necessary in the melanoma arm. Among 36 patients (18 melanoma and 18 NSCLC) studied, the ORR of brain metastases was 22% and 33%, respectively. Toxicity profiles were similar with previous reports.

With the advent of improved target localization and radiation delivery techniques, radiation therapy such as stereotactic radiosurgery and whole brain radiation has been the cornerstone of treatment for brain metastases. Preclinical data suggest that radiation therapy may potentiate antitumor immunity through a variety of mechanisms. These include upregulation of MHC class I [59] and PD-L1 [60] on cancer cells, induction of immunological tumor cell death through T cell infiltration [61], activation of dendritic cells [62] and secretion of multiple inflammatory cytokines and chemokines such as TNFα, IFN-γ and CXCL16 [61]. Because of this, the combination of radiation therapy and checkpoint inhibition has been evaluated in several recent trials. For example, safety and efficacy were reported in melanoma patients receiving nivolumab and stereotactic radiation for the treatment of brain metastasis [63]. In this retrospective study, 26 patients with metastatic brain disease were treated with stereotactic radiation before, during and after nivolumab. The treatment was well-tolerated with a median OS after nivolumab of 12 months, with 12-month local and distant brain metastasis control rates of 85% and 53%.

Anti-PD-1 Refractory Disease

To improve the clinical outcome in patients with anti-PD-1/PD-L1 resistant tumors, innate and acquired resistance mechanisms of PD-1 inhibitors have been extensively studied. So far, several resistance mechanisms have been suggested including: 1) Constitutive activation of WNT/β-catenin signaling pathway leading to lack of T cell infiltration [64]; 2) loss of PTEN increasing expression of immunosuppressive cytokines and decreasing T cell infiltration [65]; 3) expression of indoleamine 2,3-dioxygenase (IDO) which suppresses effector T cells and activates regulatory T cells [66]; 4) upregulation of genes involving mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis and wound healing [67]; 5) loss of function mutations in the genes encoding Janus kinase 1 (JAK1) or Janus kinase 2 (JAK2) resulting in insensitivity to the anti-proliferative effects of interferon γ on cancer cells [68]; and [6] mutations in the gene encoding beta 2 microglobulin leading to loss of expression of major histocompatibility complex (MHC) class I [68].

With improved understanding of these resistance mechanisms, several combination approaches employing PD-1/PD-L1 blocking agents with other therapeutic modalities are undergoing evaluation to overcome this resistance and improve clinical outcomes. Such modalities include targeting other immunosuppressive molecules such as CTLA-4, LAG-3, TIM-3 and IDO. Other potential targets include combinations with CD137 or OX-40 agonists, TLR agonist, oncolytic virotherapy, cancer vaccines and adoptive T cell therapies.

Conclusion

The efficacy and safety of PD-1/PD-L1 blocking agents have been validated across a wide spectrum of cancer types, and PD-1/PD-L1 blockade has changed the landscape of cancer therapy. In addition to current anti-PD-1/PD-L1 antibody FDA-approvals for metastatic melanoma, NSCLC, RCC, urothelial cancer and head and neck SCC, approvals for additional cancer types are anticipated the near future. Despite the remarkable success of PD-1/PD-L1 blockade, however, durable clinical responses are still limited in subgroups of patients, and

numerous studies are ongoing to try and improve clinical outcomes.

Conflicts of Interest

No conflicts of interest to disclose

References

1. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, et al. (2014) Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 384: 1109-1117.
2. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, et al. (2016) Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma. J Clin Oncol 34: 4102-4109.
3. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, et al. (2015) Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 16: 908-918.
4. Robert C, Schachter J, Long GV, Arance A, Grob JJ, et al. (2015) Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 372: 2521-2532.
5. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, et al. (2014) Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 32: 1020-1030.
6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454.
7. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, et al. (2015) Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol 16: 375-384.
8. Robert C, Long GV, Brady B, Dutriaux C, Maio M, et al. (2015) Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 372: 320-330.
9. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey L, et al. (2015) Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 373: 23-34.
10. Curran MA, Montalvo W, Yagita H, Allison JP (2010) PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 107: 4275-4280.
11. Duraiswamy J, Kaluza KM, Freeman GJ, Coukos G (2013) Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res 73: 3591-3603.
12. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, et al. (2013) Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369:122-133.
13. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, et al. (2015) Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 372: 2006-2017.
14. Hodi FS, Chesney J, Pavlick AC, Robert C, Grossmann KF, et al. (2016) Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol.
15. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, et al. (2016) Updated results from a phase III trial of nivolumab (NIVO) combined with ipilimumab (IPI) in treatment-naive patients (pts) with advanced melanoma (MEL) (CheckMate 067). 2016 ASCO Annual Meeting.
16. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, et al. (2013) Signatures of mutational processes in human cancer. Nature 500: 415-421.
17. Brahmer J, Reckamp KL, Baas P, Crinò C, Eberhardt WE, et al. (2015) Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 373: 123-135.
18. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, et al. (2015) Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 373: 1627-1639.
19. Socinski M, Creelan B, Horn L, Reck M, Paz-Ares L, et al. (2016) NSCLC, metastaticCheckMate 026: A phase 3 trial of nivolumab vs investigator's choice (IC) of platinum-based doublet chemotherapy (PT-DC) as first-line therapy for stage iv/recurrent programmed death ligand 1 (PD-L1)−positive NSCLC, ESMO 2016.
20. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, et al. (2015) Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 372: 2018-2028.
21. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, et al. (2016) Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 387: 1540-1550.
22. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, et al. (2016) Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med.
23. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, et al. (2016) Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 387: 1837-1846.
24. Barlesi F, Park K, Ciardiello F, von Pawel J, Gadgeel S, et al. (2016) Primary analysis from OAK, a randomized phase III study comparing atezolizumab with docetaxel in 2L/3L NSCLC, European Society For Medical Oncology.
25. de Riese W, Goldenberg K, Allhoff E, Stief C, Schlick R, et al. (1991) Metastatic renal cell carcinoma (RCC): spontaneous regression, long-term survival and late recurrence. Int Urol Nephrol 23: 13-25.
26. Edwards MJ, Anderson JA, Angel JR, Harty JI (1996) Spontaneous regression of primary and metastatic renal cell carcinoma. J Urol 155: 1385.
27. Klapper JA, Downey SG, Smith FO, Yang JC, Hughes MS, et al. (2008) High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma : a retrospective analysis of response and survival in patients treated in the surgery branch at the National Cancer Institute between 1986 and 2006. Cancer 113: 293-301.
28. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, et al. (2015) Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 373: 1803-1813.
29. Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, et al. (1996) Production of vascular endothelial growth factor by

human tumors inhibits the functional maturation of dendritic cells. Nat Med 2: 1096-1103.

30. Terme M, Pernot S, Marcheteau E, Sandoval F, Benhamouda N, et al. (2013) VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 73: 539-549.

31. Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, et al. (1998) Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood 92: 4150-4166.

32. Sabatino M, Kim-Schulze S, Panelli MC, Stroncek D, Wang E, et al. (2009) Serum vascular endothelial growth factor and fibronectin predict clinical response to high-dose interleukin-2 therapy. J Clin Oncol 27: 2645-2652.

33. Yuan J, Zhou J, Dong Z, Tandon S, Kuk D, et al. (2014) Pretreatment serum VEGF is associated with clinical response and overall survival in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res 2: 127-132.

34. Sternberg CN, Donat SM, Bellmunt J, Millikan RE, Stadler W, et al. (2007) Chemotherapy for bladder cancer: treatment guidelines for neoadjuvant chemotherapy, bladder preservation, adjuvant chemotherapy, and metastatic cancer. Urology 69: 62-79.

35. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, et al. (2014) MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 515: 558-562.

36. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, et al. (2016) Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 387: 1909-1920.

37. Baxi S, Fury M, Ganly I, Rao S, Pfister DG (2012) Ten years of progress in head and neck cancers. J Natl Compr Canc Netw 10: 806-810.

38. Ferris RL (2015) Immunology and Immunotherapy of Head and Neck Cancer. J Clin Oncol 33: 3293-3304

39. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, et al. (2016) Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. Lancet Oncol 17: 956-965.

40. Mehra R, Seiwert TY, Mahipal A, Weiss J, Berger R, et al. (2016) Efficacy and safety of pembrolizumab in recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC): Pooled analyses after long-term follow-up in KEYNOTE-012, 2016 ASCO Annual Meeting.

41. Chow LQ, Haddad R, Gupta S, Mahipal A, Mehra R, et al. (2016) Antitumor Activity of Pembrolizumab in Biomarker-Unselected Patients With Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma: Results From the Phase Ib KEYNOTE-012 Expansion Cohort. J Clin Oncol.

42. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas D, et al. (2016) Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med 375: 1856-1867.

43. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, et al. (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515: 568-571.

44. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, et al. (2015) Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 348: 124-128.

45. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, et al. (2016) Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 351: 1463-1469.

46. Weide B, Martens A, Hassel JC, Berking C, Postow MA, et al. (2016) Baseline Biomarkers for Outcome of Melanoma Patients Treated with Pembrolizumab. Clin Cancer Res.

47. Madore J, Vilain RE, Menzies AM, Kakavand H, Wilmott JS, et al. (2015) PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res 28: 245-253.

48. McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorf D, Pelekanou V, et al. (2016) Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. JAMA Oncol 2: 46-54.

49. Callea M, Albiges L, Gupta M, Cheng SC, Genega EM, et al. (2015) Differential Expression of PD-L1 between Primary and Metastatic Sites in Clear-Cell Renal Cell Carcinoma. Cancer Immunol Res 3: 1158-1164.

50. Jacobson DL, Gange SJ, Rose NR, Graham NM (1997) Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol 84: 223-243.

51. Engels EA, Pfeiffer RM, Fraumeni JF Jr, Kasiske BL, Israni AK, et al. (2011) Spectrum of cancer risk among US solid organ transplant recipients. JAMA 306: 1891-1901.

52. Franks AL, Slansky JE (2012) Multiple associations between a broad spectrum of autoimmune diseases, chronic inflammatory diseases and cancer. Anticancer Res 32: 1119-1136.

53. Uemura M, Trinh VA, Haymaker C, Jackson N, Kim DW, et al. (2016) Selective inhibition of autoimmune exacerbation while preserving the anti-tumor clinical benefit using IL-6 blockade in a patient with advanced melanoma and Crohn's disease: a case report. J Hematol Oncol 9: 81.

54. Menzies AM, Johnson DB, Ramanujam S, Atkinson VG, Wong AN, et al. (2016) Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders (AD) or major toxicity with ipilimumab (IPI). 2016 ASCO Annual Meeting. Chicago, IL, USA.

55. Lipson EJ, Bagnasco SM, Moore J Jr, Jang S, Patel MJ, et al. (2016) Tumor Regression and Allograft Rejection after Administration of Anti-PD-1. N Engl J Med 374: 896-898.

56. Alhamad T, Venkatachalam K, Linette GP, Brennan DC (2016) Checkpoint Inhibitors in Kidney Transplant Recipients and the Potential Risk of Rejection. Am J Transplant 16:1332-1333.

57. Lipson EJ, Bodell MA, Kraus ES, Sharfman WH (2014) Successful administration of ipilimumab to two kidney transplantation patients with metastatic melanoma. J Clin Oncol 32: 69-71.

58. Goldberg SB, Gettinger SN, Mahajan A, Chiang AC, Herbst RS, et al. (2016) Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. Lancet Oncol 17: 976-983.

59. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, et al. (2006) Radiation modulates the peptide repertoire, enhances

MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med 203: 1259-1271.

60. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, et al. (2015) Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 520:373-377.

61. Frey B, Rubner Y, Kulzer L, Werthmöller N, Weiss EM et al. (2014) Antitumor immune responses induced by ionizing irradiation and further immune stimulation. Cancer Immunol Immunother 63: 29-36.

62. Diab A, Jenq RR, Rizzuto GA, Cohen AD, Huggins DW, et al. (2013) Enhanced responses to tumor immunization following total body irradiation are time-dependent. PLoS One 8: e82496.

63. Ahmed KA, Stallworth DG, Kim Y, Johnstone PA, Harrison LB, et al. (2016) Clinical outcomes of melanoma brain metastases treated with stereotactic radiation and anti-PD-1 therapy. Ann Oncol 27: 434-441.

64. Spranger S, Bao R, Gajewski TF (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 523: 231-235.

65. Peng W, Chen JQ, Liu C, Malu S, Creasy C (2016) Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. Cancer Discov 6: 202-216.

66. Holmgaard RB, Zamarin D, Munn DH, Wolchok JD, Allison JP (2013) Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J Exp Med 210: 1389-1402.

67. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, et al. (2016) Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell 165: 35-44.

68. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, et al. (2016) Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med 375: 819-829.