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Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance

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Abstract

Immune checkpoint inhibitors (ICIs) have made an indelible mark in the field of cancer immunotherapy. Starting with the approval of anti-cytotoxic T lymphocyte-associated protein 4 (anti-CTLA-4) for advanced-stage melanoma in 2011, ICIs—which now also include antibodies against programmed cell death 1 (PD-1) and its ligand (PD-L1)—quickly gained US Food and Drug Administration approval for the treatment of a wide array of cancer types, demonstrating unprecedented extension of patient survival. However, despite the success of ICIs, resistance to these agents restricts the number of patients able to achieve durable responses, and immune-related adverse events complicate treatment. Thus, a better understanding of the requirements for an effective and safe antitumor immune response following ICI therapy is needed. Studies of both tumoral and systemic changes in the immune system following ICI therapy have yielded insight into the basis for both efficacy and resistance. Ultimately, by building on these insights, researchers should be able to combine ICIs with other agents, or design new immunotherapies, to achieve broader and more durable efficacy as well as greater safety. Here, we review the history and clinical utility of ICIs, the mechanisms of resistance to therapy, and local and systemic immune cell changes associated with outcome.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have revolutionized the field of tumor therapy. Whereas chemotherapy and radiotherapy remain the mainstay of treatment for most cancer types, ICIs are now first-line therapies for various solid and liquid tumors. ICIs work by releasing the inhibitory brakes of T cells, resulting in robust activation of the immune system and productive antitumor immune responses. Even though T cells have been the linchpins of ICI therapy, ICIs work not only by invigorating T cells but also by activating other cells of the innate and adaptive arms, all of which function in concert to orchestrate an effective response against tumors. ICI-driven changes in the immune response are observed both within tumors and in peripheral organs, such as the draining lymph nodes and peripheral blood. In fact, peripheral immune responses are critical in achieving favorable clinical outcomes. To date, ICIs targeting three different molecules have been approved by the US Food and Drug Administration (FDA) for use in humans. An antibody against cytotoxic T lymphocyte-associated protein 4 (CTLA-4) called ipilimumab was the first to receive approval for the treatment of metastatic melanoma. The second class of ICIs consists of antibodies that block the inhibitory receptor, programmed cell death 1 (PD-1), on T cells, which interacts with its ligands, PD-L1 and PD-L2, to thwart active T cell responses. Currently, two anti-PD-1 antibodies, pembrolizumab and nivolumab, are FDA approved. These drugs, like ipilimumab, were first approved for the treatment of advanced-stage melanomas but have since been approved to treat various cancer types. Last, antibodies against PD-L1 form the third group of FDA-approved ICIs. Three anti-PD-L1 antibodies—atezolizumab, durvalumab, and avelumab—are used primarily for the treatment of urothelial carcinoma, non-small-cell lung cancer (NSCLC), and Merkel cell carcinoma. Over the years, the clinical use of anti-PD-1 and anti-PD-L1 antibodies has greatly exceeded that of anti-CTLA-4 due to a combination of greater clinical efficacy and superior tolerability.

Despite the fact that many patients experience dramatic tumor regression in response to ICIs, most patients and cancers do not respond to these therapies. Of the patients who initially respond, some develop resistance at a later time and experience tumor relapse; such patients are described as having acquired resistance. Patients who never experience a therapeutic response are described as having primary resistance. Although we do not yet fully understand the mechanisms responsible for either form of resistance, dissecting them should not only pave the way for predicting responsive and nonresponsive patients but ultimately could reveal the means for converting nonresponders to responders. Perhaps the most prominent predictive markers of effective ICI therapy are the compositions of immune cells within tumors and in the periphery in patients prior to and after therapy. In this article, we review (*a*) the biology and clinical use of FDA-approved ICIs, (*b*) the mechanisms of primary and acquired resistance to these therapies, and (*c*) the tumoral and systemic immune cell changes at baseline and following ICI therapy and their association with clinical efficacy.

IMMUNE CHECKPOINT INHIBITORS

The last decade has witnessed unprecedented advances in cancer immunotherapy, and by far the most widely used immunotherapeutic agents are blocking antibodies targeted to immune inhibitory receptors such as CTLA-4, PD-1, and PD-L1. Although antibodies against these molecules are already FDA-approved therapies for various cancer types, copious antibodies and small molecules targeting other immune checkpoints such as LAG3, TIGIT, TIM3, B7H3, CD39, CD73, adenosine A2A receptor, and CD47 are in clinical development. In this section, we focus mainly on the roles of CTLA-4, PD-1, and PD-L1 in inhibiting the immune response and the roles of antibodies that block these molecules in cancer immunotherapy.

CTLA-4

The immune system functions in measured steps to first mount and subsequently dampen an active immune response. CTLA-4, an inhibitory receptor expressed primarily by T cells, functions to dampen T cell activity and is upregulated upon T cell activation (1–3). When a naive T cell encounters its cognate antigen, in order to become activated, it requires not only stimulation through its T cell receptor (TCR) but also a second costimulation signal. The second signal is transmitted upon engagement of CD28 on T cells with B7.1/CD80 or B7.2/CD86 on antigen-presenting cells (APCs) (4–6). Once TCR signaling is induced, intracellularly stored CTLA-4 molecules traffic to the cell surface, where they can bind and compete for the same ligands as CD28, but with greater affinity, thereby dampening costimulation (7, 8). Inhibitory signals then drive the T cell to a resting state. CTLA-4 inhibits T cell activity through both T cell–intrinsic and T cell–extrinsic mechanisms. Intrinsically, signaling through the receptor results in the recruitment of phosphatases; inhibition of transcription factors associated with T cell activity, such as NF-κB, NFAT, and AP-1; and activation of ubiquitin ligases (9–15). Extrinsically, CTLA-4 can compete with CD28 for ligand binding, thus lowering the effective level of costimulatory signals received by T cells (16). Apart from its increased expression on the surface of activated T cells, CTLA-4 is also constitutively expressed on the surface of regulatory T cells (Tregs), which play an important role in suppressing immune responses. Studies have demonstrated that CTLA-4 controls the function and generation of Tregs (17–22). Given the importance of CTLA-4 in controlling T cell responses, it is not surprising that *Ctla4*$^{-/-}$ mice develop a severe autoinflammatory condition marked by widespread T cell activation and proliferation and infiltration of these polyclonal T cells into myriad tissues, resulting in premature death (23–25). With knowledge of the potent immunosuppressive functions of CTLA-4, scientists turned to CTLA-4 blockade as a potential means of boosting antitumor immunity.

The first evidence that inhibiting CTLA-4 activity can impart in vivo antitumor effects emerged in 1996 (26). Mice implanted with colon carcinoma or fibrosarcoma cells either were cured of their tumors or demonstrated drastically reduced tumor burden when injected with anti-CTLA-4 antibodies (26). Another compelling facet of the study was that the mice not only rejected the initial tumors but also developed long-lasting protective immunity and were protected from rechallenge with the same tumor (26). The efficacy of anti-CTLA-4 antibodies in other transplantable mouse tumor models—prostatic and ovarian carcinoma—was subsequently established (27, 28). Even though the anti-CTLA-4 antibody proved effective against various cancer types in mice, it failed to impact the growth of poorly immunogenic murine B16 melanoma and SM1 mammary carcinoma (29). However, the antibody was highly effective against these tumors when used in combination with a cell-based granulocyte-macrophage colony-stimulating factor (GM-CSF) vaccine (30, 31). These and other studies demonstrating the efficacy of an anti-CTLA-4 antibody in curing tumors in mice eventually led to exploration of the utility of anti-CTLA-4 in treating human tumors. In 2010, results from a phase III clinical trial in patients with metastatic melanoma proved to be a breakthrough in cancer immunotherapy (32). This study demonstrated that the anti-CTLA-4 antibody ipilimumab extended the median overall survival of patients by more than 3 months compared with a melanoma-specific peptide vaccine. Not only was it the only new treatment that had shown increased survival of patients with advanced-stage melanoma, but it also demonstrated that an ICI alone was responsible for these potent antitumor effects. Critically, analogous to what was observed in preclinical studies, the 20% of patients whose tumors were completely cleared maintained their response for the duration of the study, with a median follow-up time of approximately 30 months. Thus, in 2011, the FDA approved the antibody for treating late-stage melanoma patients. Although as a monotherapy ipilimumab

is not highly effective in other tumor types, it is currently being tested in combination with other treatment modalities, such as chemotherapy, radiotherapy, and immunotherapies, in clinical trials for many cancer types. Thus, ipilimumab has proven to be an effective means of boosting the antitumor response, and its success in the clinic paved the way for the development of other ICIs.

PD-1 and PD-L1

PD-1 was first identified in 1992 as a protein involved in regulating programmed T cell death (33). Over the years, knowledge of its expression pattern, structure, and function has been well established. Although PD-1 is expressed on the surface of most activated immune cells, such as macrophages, dendritic cells (DCs), Langerhans cells, B cells, and T cells, its expression is most highly upregulated on exhausted T cells (34, 35). Similar to CTLA-4, engagement of PD-1 with its ligands, PD-L1 and PD-L2, leads to suppression of T cell immune responses (36, 37). Both PD-L1 and PD-L2 are expressed on hematopoietic and nonhematopoietic cells, including APCs and cancer cells (36, 37). Engagement of PD-1 with its ligands leads to phosphorylation of its cytoplasmic immunoreceptors. Specifically, phosphorylation of the immunoreceptor tyrosine-based switch motif (ITSM) recruits the Src homology region 2 domain-containing phosphatase-2 (SHP-2) (38). SHP-2 mediates the inhibitory functions of PD-1 by repressing the activity of several intracellular molecules involved in propagating signals downstream of the TCR (38). In B cells, recruitment of SHP-2 dephosphorylates effector molecules, including Syk and PI3K, and in T cells, signaling through PD-1 dephosphorylates ZAP70 and CD3ζ , both of which are involved in transducing signals downstream of TCR engagement (38, 39). These dephosphorylation events directly blunt the inflammatory response of the affected cells. Alternatively, SHP-2 can also inhibit the Ras/MEK/ERK pathway, which controls cell proliferation, growth, and survival, contributing to restrained proliferation following activation (40). Because PD-1 is involved in curtailing immune responses, it is not surprising that PD-1 knockout mice are characterized by systemic autoimmune inflammation resulting in arthritis, nephritis, and myocarditis (41–43). However, unlike mice lacking CTLA-4, the absence of PD-1 in mice does not lead to premature death, as these autoimmune complications manifest much later in life. The exact reason for this difference is not fully understood, but it is clear that PD-1 and CTLA-4 play biologically nonredundant roles in suppressing developing immune responses. For example, CTLA-4 is expressed early during T cell activation, whereas PD-1 is expressed largely at later stages of T cell activation. Additionally, in contrast to the PD-1 ligands, which are expressed on various cell types, CTLA-4 ligands are expressed mostly by APCs in secondary lymphoid organs. Last, the mechanisms by which these signaling axes inhibit T cell responses are also distinct, indicating nonoverlapping roles in T cell suppression.

Numerous preclinical studies carried out in the early 2000s revealed the utility of targeting the PD-1/PD-L1 axis to boost antitumor immunity (44–49). A seminal paper in the field demonstrated that interaction between PD-L1 on tumor cells and PD-1 on cytotoxic T cells resulted in accelerated tumor growth and that treatment with an anti-PD-L1 antibody or tumor implantation in PD-1 knockout mice resulted in reduced tumor growth (44). Subsequent human studies have consistently shown that PD-L1 and PD-L2 expression not only increases in cancerous tissues but is also linked to poor disease prognosis in many cancer types (50–56). These data, from both preclinical and clinical studies, paved the way for the initiation of clinical trials to test the efficacy of anti-PD-1 and anti-PD-L1 antibodies in treating patients with cancer. In a phase I clinical trial of patients with advanced-stage, ipilimumab-refractory melanoma, 26% of participants treated with pembrolizumab, an anti-PD-1 antibody, demonstrated either a complete or partial response (57). In the subsequent phase III clinical trial, more than 30% of patients with advanced melanoma

achieved an objective response following pembrolizumab monotherapy compared with 12% of patients treated with ipilimumab alone (58). In a separate phase III clinical trial, the antitumor activity of nivolumab, a different anti-PD-1 antibody, was tested in patients with unresectable stage III or stage IV melanoma who had received no prior treatments (59). Randomized patient groups were treated with a combination of nivolumab and ipilimumab, with nivolumab alone, or with ipilimumab alone. At the end of the trial, progression-free survival was highest in patients who received the combination therapy, followed by patients treated with nivolumab alone, and was lowest in patients treated with ipilimumab alone (59). This stratification in progression-free survival hinted at the possibility of combining ICIs to achieve maximum antitumor efficacy. Taken together, these breakthrough clinical trials propelled FDA approval of the anti-PD-1 antibodies, pembrolizumab and nivolumab, for the treatment of melanoma. The subsequent demonstration that pembrolizumab can induce sustained tumor regression in a substantial proportion of patients with NSCLC was another landmark (60), as NSCLC is one of the most common cancer types and was, prior to the arrival of ICIs, thought to be a poorly immunogenic tumor (61). The phase III trial enrolled patients with metastatic NSCLC and required tumoral PD-L1 expression as an entry criterion. In this trial, the patients treated with pembrolizumab demonstrated markedly increased response rates, progression-free survival, and overall survival compared with patients treated with chemotherapy, leading to FDA approval of pembrolizumab as a first-line treatment. The potency of anti-PD-1 in the clinic was further proved by its recent, first-in-class FDA approval as a tissue-agnostic therapy for any unresectable or metastatic tumors exhibiting microsatellite instability or deficient mismatch repair (62). Today, nivolumab and pembrolizumab are approved for the treatment of myriad cancer types, including both solid and liquid tumors (**Table 1**).

Apart from ICIs that target PD-1, antibodies against one of its ligands, PD-L1, expressed by both APCs and tumor cells, are also effective antitumor agents. This is not surprising given that PD-L1 blockade inhibits not only the PD-1/PD-L1 axis but also PD-L1/CD80 *cis* interactions on DCs, freeing more CD80 molecules to enhance T cell priming (63). In recent years, three anti-PD-L1 antibodies, atezolizumab, durvalumab, and avelumab, have been approved by the FDA to treat several cancer types (64). Although all three antibodies block the interaction of PD-L1 with PD-1 and CD80, atezolizumab and durvalumab contain a modification in the fragment crystallizable (Fc) region that eliminates antibody-dependent cellular cytotoxicity to prevent depletion of T cells that express PD-L1 (64). On the other hand, avelumab contains the native Fc region, which is capable of engaging Fc-γ receptors on natural killer (NK) cells to induce antibody-dependent cellular cytotoxicity (64). Atezolizumab was initially approved by the FDA for the treatment of localized and metastatic urothelial carcinoma on the basis of results of a phase II clinical trial that showed overall response rates of 10% in patients whose disease had progressed after platinum-based chemotherapy treatment (65). These results propelled scientists to examine the antitumor efficacy of anti-PD-L1 antibodies in combination with various chemotherapeutic regimens, and to date, atezolizumab, in combination with chemotherapy, has been approved by the FDA for the treatment of squamous and nonsquamous NSCLC, small cell lung cancer, and PD-L1-positive triple-negative breast cancer (66–69). Apart from atezolizumab, durvalumab and avelumab are also approved for use in patients with urothelial carcinoma (70–72), and avelumab is approved for use in patients with Merkel cell carcinoma (73). Not surprisingly, novel combination therapies of anti-PD-L1 antibodies with other immunotherapies and chemotherapies form the basis of many ongoing clinical trials.

As shown in **Table 1**, ICIs are approved for various cancer types, with anti-PD-1 antibodies having received the greatest number of approvals. Yet what has become apparent over the course of their use in patients is that the clinical success of checkpoint therapy has not come without drawbacks, as (*a*) only a minority of patients treated with ICIs respond to therapy; (*b*) some patients

Table 1 List of all FDA-approved ICIs with their current indications

Therapy	Approved indication	Additional notes
Anti-CTLA-4		
Ipilimumab	Metastatic melanoma	It can be used as adjuvant therapy to prevent disease relapse after surgical resection of primary tumor and lymph nodes containing metastatic disease.
Anti-CTLA-4 in combination with anti-PD-1		
Ipilimumab and nivolumab	Metastatic RCC	NA
	Metastatic colorectal cancer	They are approved for MSI-H and dMMR tumors that have failed chemotherapy treatments with fluoropyrimidines, oxaliplatin, and irinotecan.
	HCC	They are used in patients who have failed treatment with sorafenib.
Anti-PD-1		
Nivolumab	Metastatic melanoma	It can used as adjuvant therapy to prevent disease relapse after surgical resection of primary tumor and lymph nodes containing metastatic disease. It can be used alone or in combination with ipilimumab.
	Late-stage NSCLC	It is used in patients whose tumors have either spread or grown and in patients for whom platinum-based chemotherapy drugs have failed.
	Late-stage SCLC	It is used in patients whose tumors have either spread or grown and in patients for whom two types of chemotherapy drugs, with at least one being platinum based, have failed.
	Metastatic RCC	It can be used in patients whose cancers have grown or spread and in patients for whom other first-line cancer drugs have failed.
	Hodgkin's lymphoma	It is used in patients who have received an autologous stem cell transplant treatment along with brentuximab vedotin before or after the transplant. Alternatively, it is used in patients who have received at least three different types of treatments, including a stem cell transplant.
	HNSCC	It is used in patients with relapsed disease or in patients whose cancers have spread. These patients must have failed platinum-based chemotherapy drugs.
	Urothelial carcinoma	It is used in patients whose tumors have either grown or spread. These patients must also have failed platinum-based chemotherapy drugs.
	Metastatic colorectal cancer	It is approved for MSI-H and dMMR tumors that have failed chemotherapy treatments with fluoropyrimidines, oxaliplatin, and irinotecan. It can be used alone or in combination with ipilimumab.
	HCC	It is used in patients who have failed treatment with sorafenib. It can be used alone or in combination with ipilimumab.
Pembrolizumab	Metastatic melanoma	It can be used as adjuvant therapy to prevent disease relapse after surgical resection of primary tumor and lymph nodes containing metastatic disease.
	Metastatic nonsquamous NSCLC	It is used in combination with pemetrexed and platinum-based chemotherapy as a first-line therapy in patients with no EGFR or ALK genomic tumor aberrations.

(*Continued*)

Table 1 (*Continued*)

Therapy	Approved indication	Additional notes
	Metastatic squamous NSCLC	It is a first-line therapy used in combination with carboplatin and either paclitaxel or protein-bound paclitaxel.
	NSCLC	It is an approved single agent therapy in patients whose tumors express PD-L1, have no EGFR or ALK genomic tumor aberrations, and harbor tumors that cannot be surgically removed or treated with chemoradiation.
	Metastatic NSCLC	It is an approved single agent therapy in patients whose tumors express PD-L1 and whose disease has progressed with platinum-based chemotherapy. FDA-approved therapy for EGFR or ALK genomic tumor aberrations should have failed patients with these aberrations.
	Metastatic SCLC	It is approved for use in patients who fail platinum-based chemotherapy and at least one other standard-of-care therapy.
	Metastatic HNSCC	In combination with platinum- and fluorouracil-based chemotherapies, it is a first-line therapy for patients with metastatic disease or unresectable tumors. It is also used as a single agent, first-line therapy for patients whose tumors have PD-L1 expression. These patients must also have metastatic disease or unresectable tumors. It can be used as a single agent therapy in patients with metastatic disease for whom platinum-based chemotherapy has failed.
	Hodgkin's lymphoma	It can be used in patients who have relapsed after treatment with three or more prior lines of therapy.
	Primary mediastinal large B cell lymphoma	It can be used in patients who have relapsed upon treatment with two prior lines of therapy.
	Metastatic urothelial carcinoma	It is used in patients who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1. It is also used in patients who are not eligible for any platinum-based chemotherapy regardless of PD-L1 status. It is used in patients who have failed platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.
	Non-muscle invasive bladder cancer	It is used in patients with BCG-unresponsive, high-risk tumors with carcinoma in situ with or without papillary tumors who are ineligible for or have elected not to undergo cystectomy.
	Solid tumors	It is used in patients with metastatic MSI-H or dMMR solid tumors for whom prior line therapies have failed or in patients who do not have an alternative option.
	Metastatic gastric and gastroesophageal junction carcinoma	It is used in patients whose tumors express PD-L1 and for whom two or more prior lines of therapy with fluoropyrimidine- and platinum-containing chemotherapy and, if appropriate, HER2/neu-targeted therapy have failed.
	Recurrent locally advanced or metastatic squamous cell carcinoma of the esophagus	It is used in patients whose tumors express PD-L1 and for whom one or more prior lines of therapy have failed.

(*Continued*)

Table 1 (*Continued*)

Therapy	Approved indication	Additional notes
	Recurrent or metastatic cervical cancer	It is used in patients for whom chemotherapy treatments have failed and whose tumors express PD-L1.
	HCC	It is used in patients for whom sorafenib treatment has failed.
	Recurrent locally advanced or metastatic Merkel cell carcinoma	NA
	Metastatic RCC	It is used in combination with axitinib as a first-line therapy.
Anti-PD-L1		
Atezolizumab	Locally advanced or metastatic urothelial carcinoma	It is used in patients who are not eligible for cisplatin-containing chemotherapy and whose tumors express PD-L1. It is also used in patients who are not eligible for any platinum-based chemotherapy regardless of PD-L1 status. It is used in patients for whom platinum-based chemotherapy has failed or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.
	Metastatic nonsquamous NSCLC	It is used in combination with bevacizumab, paclitaxel, and carboplatin as a first-line therapy in patients with no EGFR or ALK genomic tumor aberrations. It is used in combination with protein-bound paclitaxel and carboplatin as a first-line therapy in patients with no EGFR or ALK genomic tumor aberrations. It is an approved single agent therapy in patients whose disease has progressed with platinum-based chemotherapy. FDA-approved therapy for EGFR or ALK genomic tumor aberrations should have failed patients with these aberrations.
	Metastatic triple-negative breast cancer	It is used in combination with protein-bound paclitaxel in patients whose tumors express PD-L1.
	ES-SCLC	It is used in combination with carboplatin and etoposide as a first-line therapy.
Durvalumab	Locally advanced or metastatic urothelial carcinoma	It is used in patients for whom platinum-based chemotherapy has failed or whose disease had progressed within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.
	NSCLC	It is used in patients with unresectable tumors and whose disease has not progressed following concurrent platinum-based chemotherapy and radiotherapy.
	ES-SCLC	It is used in combination with etoposide and either carboplatin or cisplatin as a first-line treatment.
Avelumab	Metastatic Merkel cell carcinoma	NA
	Locally advanced or metastatic urothelial carcinoma	It is used in patients for whom platinum-based chemotherapy has failed.
	Locally advanced or metastatic RCC	It is used in combination with axitinib as a first-line therapy.

Abbreviations: ALK, anaplastic large-cell lymphoma kinase; BCG, Bacillus Calmette–Guérin; CTLA-4, cytotoxic T lymphocyte-associated protein 4; dMMR, mismatch repair deficient; EGFR, epidermal growth factor receptor; ES-SCLC, extensive-stage small cell lung cancer; FDA, US Food and Drug Administration; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitor; MSI-H, microsatellite instability-high; NA, not applicable; NSCLC, non-small-cell lung cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; RCC, renal cell carcinoma; SCLC, small cell lung cancer.

who initially respond to therapy later develop resistance to these immune modulators; and (*c*) a large proportion of ICI-treated patients develop immune-related adverse events (irAEs), some of which can be life-threatening. We discuss these ICI-associated issues in the next sections.

RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS

Although ICI therapies have improved patient outcomes across numerous cancer types, only a minority of patients treated with ICIs achieve a durable response. Even among patients with melanoma, which has one of the highest rates of response to ICI, 60–70% of patients do not experience an objective response to anti-PD-1 therapy; of those that do, 20–30% demonstrate eventual tumor relapse and progression (74, 75). Understanding why most patients do not respond to or fail to sustain their response to ICI therapy has been a topic of intense study, with mechanisms of resistance generally categorized into two types: primary and acquired. Primary resistance refers to those cases in which no initial response to checkpoint therapy was observed; acquired resistance encompasses those cases in which patients initially responded to an ICI but later became refractory. Here, we review some of the mechanisms that contribute to both primary and acquired resistance.

Primary Resistance

Response rates to ICI treatment vary widely among different cancers, ranging from greater than 80% in patients with refractory Hodgkin's lymphoma to little or no response in patients with mismatch repair–proficient colorectal cancer (76, 77); many tumor indications fall within a response rate range of 20% to 40% (78). Thus, primary resistance, or nonresponse, to ICIs clearly remains a critical issue. One recent cross-sectional study using publicly reported cancer statistics and response rates to ICI therapy presented a sobering estimate of its benefit in the clinic: Of all the patients who met the eligibility criteria for ICI therapy in 2018, only an estimated 12.5% would have benefitted (79). Thus, a greater understanding of the multiple factors that can contribute to primary resistance is necessary to increase the proportion of patients who stand to benefit from this form of cancer immunotherapy.

One of the most commonly used biomarkers for predicting response to ICIs is the intensity of PD-L1 expression on tumor cells, which is positively correlated with response to ICIs in several cancer settings (74, 75, 80). Expression of PD-L1 by the tumor is important not only because it ensures, at the most basic level, that targetable PD-1 receptor–ligand interactions exist in the tumor microenvironment but also because PD-L1 expression correlates with parameters associated with immune activation in the tumor, including activated $CD8^+$ T cell responses as well as antigen presentation (75). Thus, it stands to reason that tumors with absent or low expression of PD-L1 are more likely to exhibit resistance to anti-PD-1 and anti-PD-L1 therapies, and this assertion is supported by response rates of only up to 10% in patients with PD-L1-low melanoma and patients with NSCLC treated with anti-PD-1 compared with 40–50% in patients with high PD-L1 expression (74, 80). However, PD-L1-negative tumors can still occasionally respond to anti-PD-1 therapy, and high PD-L1 expression, although correlative, is by no means sufficient for generating a response following anti-PD-1 therapy, indicating that other factors are involved in determining response and nonresponse to ICIs (80–82).

Another well-recognized biomarker that predicts response or nonresponse to ICIs is the mutational burden of the tumor, with higher tumor mutational loads strongly associated with response to both anti-CTLA-4 and anti-PD-1 across multiple cancer indications (83–86). The underlying cause of the high mutational burden is not important for determining whether a tumor will respond to ICIs, as melanoma and NSCLC, which acquire mutations through

UV- and carcinogen-induced DNA damage, respectively, respond just as well to ICIs as do tumors that acquire mutations through genomic instability or deficient mismatch repair (74, 80, 87). The common thread that unites these types of tumors and that potentiates their response to ICIs is their high neoantigen load, which leads to increased visibility of the tumor to the immune system and the development of a more potent antitumor T cell response following ICI treatment. Given this contribution of mutational burden to ICI efficacy, tumors with low mutational burdens, such as prostate and pancreatic cancers, might be expected to respond poorly to ICIs, and this is indeed the case (88). The importance of mutational load is even evident within subtypes of the same cancer; in colorectal cancer, anti-PD-1 therapy was effective in patients with tumors exhibiting microsatellite instability or deficient mismatch repair, which demonstrated a 40% objective response rate, and not in patients with tumors with intact mismatch repair, which exhibited a 0% response rate (77). Thus, as a general observation, tumor types exhibiting low tumor mutational burdens are commonly associated with resistance or nonresponsiveness to ICIs. Nonetheless, exceptions do exist. Metastatic renal cell carcinoma and polyomavirus-positive Merkel cell carcinoma, which exhibit low tumor mutational burdens, often respond to anti-PD-1 and anti-PD-L1 therapies, respectively (87, 89). Notwithstanding these exceptions, low mutational load, and therefore low availability of neoantigens, is a major contributor to primary resistance to ICIs.

Beyond low mutational burden, primary resistance to checkpoint therapies can also occur if critical signaling pathways are disrupted in the tumor. Interferon-γ (IFN-γ) is an important cytokine for initiating and maintaining a potent antitumor response not only through promoting $CD8^+$ cytotoxic T cell activity and skewing toward a Th1 response but also through exerting antiproliferative, proapoptotic effects and inducing major histocompatibility complex class I (MHC I) upregulation in tumor cells (90, 91). In the clinic, a strong IFN-γ signature has been identified as a useful predictor and indicator of response in anti-PD-1-treated patients across various cancer indications (75, 92, 93). Given the importance of IFN-γ in antitumor immunity, tumor-intrinsic mutations that disrupt signaling in the IFN-γ pathway have been identified to confer resistance to ICIs. Tumors with mutations in genes such as *JAK1/2* and *IFNGR1/2* were retrospectively identified in patients who did not respond to either anti-CTLA-4 or anti-PD-1, suggesting that tumor-intrinsic IFN-γ signaling is critical for the efficacy of checkpoint blockade (94, 95). In addition to genes modulating IFN-γ signaling, MHC molecules are crucial for modulating and participating in the developing immune response following ICI therapy. For example, *MEX3B*, a gene responsible for downregulating MHC I expression in tumors, was expressed at higher levels in nonresponders to anti-PD-1 than in responders (96). In another study, MHC I downregulation via transcriptional repression was associated with primary resistance to anti-CTLA-4 therapy but not to anti-PD-1 therapy, and the response to anti-PD-1 was more dependent on MHC II expression linked to an IFN-γ-regulated module of genes (97). Thus, mutations in critical antitumor pathways, such as in IFN-γ and antigen presentation, can be a source of primary resistance to ICIs.

Although PD-L1 expression and tumor mutational status have been established and utilized as biomarkers of ICI response, other factors that impact the response have begun to emerge. One such factor involves the microbiome (98). For example, in preclinical models, the therapeutic response to anti-CTLA-4 therapy was found to be dependent on *Bacteroides* species, as germ-free mice or antibiotic-treated mice did not respond to anti-CTLA-4, but response was rescued through gavage of *Bacteroides fragilis* (99). In addition, fecal transplants from patients who benefitted clinically from anti-CTLA-4 therapy favored the growth of *B. fragilis*, linking this species with improved response. Similar findings for anti-PD-1 therapy have been reported, although different microbes have been implicated (100, 101). This idea is further supported by reports of decreased overall survival and progression-free survival in ICI-treated patients who received

antibiotics (101–103). Thus, a patient's overall microflora makeup may contribute to primary resistance, as well as response, to ICIs.

A final mechanism contributing to primary resistance involves the epigenetic properties of tumors. Several studies have demonstrated that mutations in genes involved in the SWI-SNF chromatin remodeling complex may sensitize human tumors to ICIs and that treatment with an epigenetic modulator can potentiate the ICI-mediated antitumor response through increased production of chemokines responsible for recruiting $CD8^+$ T cells (104–107). However, whether the epigenetic landscape of the tumor cells or T cell exhaustion is contributing to resistance is not well understood, leaving much room for further understanding of how epigenetics can lead to nonresponse to ICIs (108, 109).

Acquired Resistance

Unlike primary resistance to ICIs, acquired resistance, also known as secondary resistance, is not as well understood. One mechanism purporting to contribute to the development of acquired resistance is evolution or selection of tumors that acquire mutations in critical pathways involved in the checkpoint blockade response. Many of the same pathways implicated in primary resistance are also implicated here, as these pathways are integral for both developing and maintaining an effective antitumor response. One of these critical pathways, as highlighted above, is the IFN-γ response pathway. Whole-exome sequencing of baseline and relapsed tumors in a small cohort of patients with melanoma exhibiting acquired resistance to pembrolizumab revealed that tumor outgrowth occurred in two patients harboring loss-of-function mutations in *JAK1* and *JAK2* (110). These relapsed tumors showed loss of response to IFN-γ and thus resistance to its cytostatic effects. In the same study, tumor cells in another patient demonstrating relapse contained a frameshift deletion of β_2-microglobulin (*B2M*), disrupting MHC I trafficking to the cell surface and thereby rendering the tumor cells invisible to cytotoxic $CD8^+$ T cells (110). Similar disruptions in MHC I presentation in patients with lung cancer were also reported, implicating dysregulated antigen presentation as a potent mediator of acquired resistance (111). In one case report of a patient with metastatic uterine leiomyosarcoma treated with anti-PD-1, despite demonstrating a robust response to ICI, one metastatic nodule remained insensitive to the immunotherapy (112). Analysis of the genome and proteome of this tumor revealed a mutation in *PTEN* and lower expression of several neoantigens. Although there is no proof that these features were responsible for the resistance, loss of neoantigen expression and consequent evasion from cytotoxic T cell attack likely were major factors. A similar mechanism of acquired resistance to anti-PD-1 and combination anti-PD-1/anti-CTLA-4 in a larger cohort of patients with NSCLC was described (113). Here, whole-exome sequencing of tumors before and after therapy also revealed a loss of putative neoantigens in resistant tumors, lending further support to the hypothesis that loss of neoantigens, whether a result of genetic events or clonal selection, can lead to ICI resistance.

Although other potential mechanisms of acquired resistance have been proposed and examined in preclinical studies, it is clear that the difference in our understanding of primary resistance and of acquired resistance is considerable. Though the two types of resistance may share pathways in common, as described above for mutations in the IFN-γ pathway, it is conceivable that tumors that initially respond to ICIs may utilize immune evasion strategies different from those utilized by tumors that are resistant at the outset. Although our understanding of acquired resistance is far too undeveloped to determine whether this is the case, this gap in knowledge needs to be remedied, as better understanding of resistance, either primary or acquired, will be necessary to identify the best path forward for treating patients. **Figure 1** depicts our current understanding of the mechanisms of resistance.

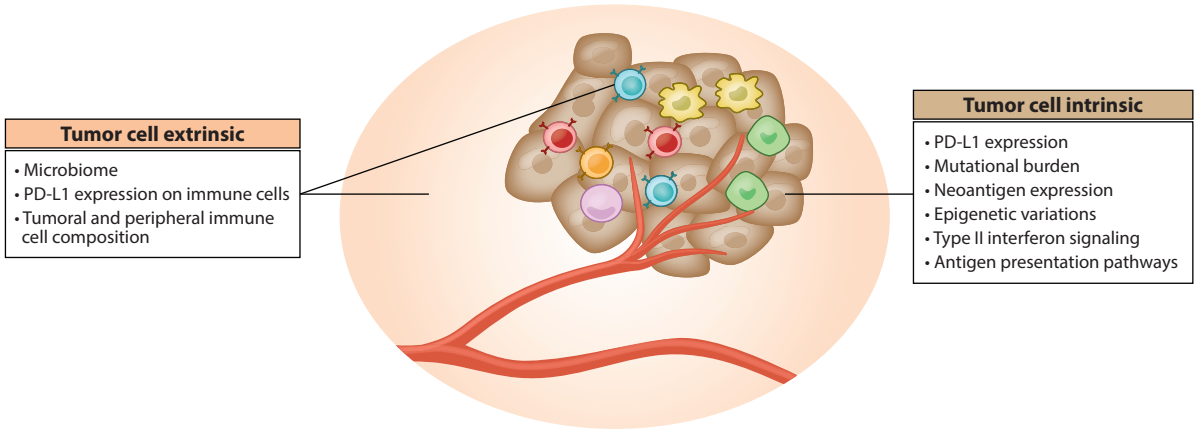

Figure 1

Tumor-intrinsic and tumor-extrinsic mechanisms of resistance to ICI therapy. Some patients who receive ICIs do not respond to treatment (primary resistance), while others respond to initial therapy but eventually relapse (acquired resistance). Although significant effort has been devoted to understanding the mechanisms of primary resistance, studies of acquired resistance have begun only recently. Abbreviations: ICI, immune checkpoint inhibitor; PD-L1, programmed cell death ligand 1.

IMMUNE-RELATED ADVERSE EVENTS

Despite the significant benefit ICIs have brought to patients, overactivation of the immune system to boost antitumor immunity can act as a double-edged sword, leading to the development of irAEs that can range from mild rash to severe colitis to life-threatening myocarditis (114, 115). Newly developed autoimmune disease, such as type I diabetes in patients with no prior history of the disease, following checkpoint therapy has also been reported to occur, demonstrating the potential adverse consequences of inhibiting the body's tolerance pathways (115, 116). irAEs are common among those receiving ICIs, as 30–60% of patients experience irAEs of any grade and approximately 10–20% experience more severe grade 3 or 4 irAEs (114, 115, 117). The frequency and severity of irAEs differ between different immune checkpoint modalities, with anti-PD-1 typically exhibiting a more favorable safety profile compared with anti-CTLA-4. In patients with untreated melanoma, treatment with anti-CTLA-4 led to grade 3 or 4 irAEs in 27.3% of patients and treatment with anti-PD-1 led to such severe irAEs in only 16.3% of patients (59). Combination treatment of advanced melanoma with both anti-CTLA-4 and anti-PD-1 led to a marked increase in both the incidence and the severity of irAEs, with 55% of those patients exhibiting high-grade irAEs (59). Aside from differences in irAE frequency and severity, anti-CTLA-4 and anti-PD-1 therapy also results in irAEs that differ in organ presentation, with anti-CTLA-4 resulting in more hypophysitis and more severe colitis cases and anti-PD-1 resulting in more pneumonitis, thyroiditis, and nephritis cases (114, 118). These differences may be the result of the different mechanisms of T cell inhibition by CTLA-4 and PD-1, as the former is understood to function more proximally to T cell activation and the latter is thought to function at later stages in the periphery (119).

Depending on the severity of the irAE in a given patient, cessation of ICI therapy and administration of immunosuppressive or immunomodulatory agents, such as glucocorticoids, may follow. Given this, one question that has arisen in the field is whether the immunosuppression needed to control irAE presentation can undermine the antitumor effects of checkpoint therapy. In general, discontinuation of ICI therapy and subsequent treatment with immunosuppressive agents do not appear to negatively impact patient outcomes (120, 121). However, there are exceptions, indicating

that better understanding of the interplay between the developing antitumor response following ICI therapy and the use of immunosuppressive agents is needed (117, 122, 123). Another important question is whether irAE presentation correlates with ICI efficacy. Several studies have shown an association between development of vitiligo and improved objective response rates and overall survival in patients with melanoma (124–126). This association may be stronger than those with other irAE manifestations due to the autoimmune reaction targeting shared antigens between healthy melanocytes and the melanoma cells, allowing the occurrence of vitiligo to serve as a surrogate measure of the developing antitumor response. Whether irAEs broadly correlate with improved response to ICIs, however, is more controversial. Studies claiming improved outcomes associated with irAEs can be found alongside studies reporting no apparent correlation, regardless of the type of ICI used (120, 127–130). Thus, further granularity with regard to the association of irAE manifestations (i.e., target organ, severity, and timing) with clinical response in particular cancers may prove more useful in dissecting the true prognostic implications of irAE development in patients receiving ICIs.

LOCAL AND SYSTEMIC IMMUNE RESPONSES TO IMMUNE CHECKPOINT INHIBITORS

Systemic administration of ICIs impacts not just T cells but also other immune cells throughout the body. Among preclinical studies, perhaps the first to characterize changes in the systemic immune response following immunotherapy was from Spitzer et al. (131). In this report, the authors demonstrated that, upon immunotherapy administration in mammary tumor-bearing mice, effective therapy was characterized by marked changes in immune cells not only in the tumor but also in peripheral organs such as the draining lymph nodes, blood, and bone marrow (131). These changes throughout the host were critical to the antitumor response, as administration of fingolimod, a small molecule that inhibits egress of lymphocytes from the lymph nodes, abrogated the effect of the immunotherapy. The tumor rejection phase was characterized by proliferating immune cells in the draining lymph nodes but not in the tumor, suggesting that the immune response in the periphery is important for maintaining and activating immune cells participating in the antitumor response. The authors also discovered that a subset of peripheral effector-memory-like $CD4^+$ T cells was associated with effective immunotherapy and could initiate an antitumor response upon adoptive transfer to untreated tumor-bearing mice (131). Altogether, this study brought to light that the systemic immune response following immunotherapy was critical in determining effective versus ineffective immunotherapy and that a particular $CD4^+$ T cell subset was an important participant in effective therapy.

In accord with the concept set forth by Spitzer et al. (131), numerous studies of ICI-treated patients have highlighted the importance of immune cells both at the tumor site and in the periphery. These studies examined tumoral and systemic immune cell milieus at baseline and after ICI treatment and measured the degree of association with clinical parameters, such as therapeutic response, to ascertain the contribution of different immune cell populations and phenotypes to ICI efficacy. Importantly, unlike preclinical models in which any tissue can be examined in detail at different time points and individual immune cell types can be evaluated for their contributions to antitumor immunity, these sorts of invasive studies cannot be carried out in patients. Nonetheless, examination of blood and tumors from patients has revealed several biomarkers that correlate with clinical outcome. Here, we describe the findings surrounding these studies.

A paradigm in the field of tumor immunology is that lymphocytes, especially $CD8^+$ T cells and NK cells, are critical for antitumor immunity. Thus, the increased presence of these lymphocytes, at baseline or after ICI therapy, would be expected to predict a favorable response to therapy.

Indeed, several studies have demonstrated that increased lymphocyte counts in the blood are associated not only with favorable treatment outcome but also with increased overall survival in patients with melanoma treated with ICIs (132–135). Examination of the lymphocytes that correlated with such beneficial outcomes has repeatedly implicated effector and memory $CD8^+$ T cell and NK cell populations (136–141). For example, the frequency of $CD8^+$ effector memory cells in the peripheral blood of patients with advanced melanoma 9 weeks after anti-CTLA-4 treatment correlated with clinical benefit and overall survival (142). Using time-of-flight mass cytometry, Subrahmanyam et al. (137) performed phenotypic and functional characterizations of immune cells in the peripheral blood of patients with melanoma at baseline to show that anti-CTLA-4 and anti-PD-1 treatments are associated with distinct predictive biomarkers. Whereas $CD45RA^+$ naive $CD8^+$ T cells negatively correlated with response with ipilimumab treatment, $CD45RA^-CD8^+$ T cells and $CD8^+$ T effector memory cells ($CD45RA^-CCR7^-$) were more abundant in responsive patients (137). By contrast, nonresponders to PD-1 treatment exhibited lower levels of CD69 and lower production of MIP-1β by NK cells (137). Similarly, another study demonstrated that a high frequency of $CD45RA^-CCR7^-CD27^+CD28^+$ effector memory $CD8^+$ cells at baseline in the blood of patients with late-stage melanoma correlated with higher and more durable clinical response rates following ICI therapy (143). Further, decreased expression of PD-1 on these $CD8^+$ T cells during therapy was a hallmark of better treatment outcome (143). Like observations regarding memory $CD8^+$ T cells in the peripheral blood, Sade-Feldman et al. (144) reported that intratumoral memory-like $CD8^+$ T cells were also more strongly associated with response to ICI therapy. This study reported that these cells uniquely expressed the transcription factor TCF7, which marks a critical subset of "stem-like" intratumoral $CD8^+$ T cells responsible for mediating antitumor immunity in mice (145).

Apart from memory $CD8^+$ T cells, several studies have tried to dissect how expression of PD-1 on $CD8^+$ T cells changes with ICI therapy and the predictive value of PD-1-expressing $CD8^+$ T cells in ICI therapy. Perhaps unsurprisingly, the presence of $CD8^+PD\text{-}1^{hi}$ T cells in the tumors of patients with melanoma and patients with NSCLC prior to anti-PD-1 administration was predictive of treatment response and correlated with overall survival (146, 147). Aside from the presence of these T cells at baseline being a favorable predictive marker of anti-PD-1 therapy, proliferation of circulating $CD8^+PD\text{-}1^{hi}CTLA\text{-}4^+$ T cells, within 3–4 weeks of ICI treatment, was also associated with responders in patients with melanoma and patients with NSCLC (148, 149). Further, in a study of patients with NSCLC, the favorable correlation between $CD8^+PD\text{-}1^{hi}$ T cells and response to treatment was attributed not only to their tumor reactivity but also to their secretion of the chemokine CXCL13, which contributed to the recruitment of other immune cells, such as B cells and T follicular helper cells, that may participate in antitumor responses (147). The location and functional status of $CD8^+$ T cells within tumors have also been correlated with ICI therapy outcome. For example, a study of patients with melanoma showed that the presence of $CD8^+$ T cells at the tumor invasive margins at baseline correlated with ICI response and that intratumoral $CD8^+$ T cell proliferation during treatment was associated with tumor regression (150). Overall, these studies support the concept that increased abundance of $CD8^+$ T cells and $CD8^+$ T cells with memory-like phenotypes in peripheral and tumor tissues prior to or after initiation of therapy is correlated with a favorable response to ICIs.

Even though $CD8^+$ T cells have been traditionally viewed as the primary effector cells responsible for antitumor immunity, several clinical studies of patients treated with ICIs have highlighted a potential role for $CD4^+$ T cells in antitumor immunity. An early study of patients with melanoma treated with ipilimumab showed that 8–14 weeks after the first dose, a heightened frequency of $CD4^+$ T cells in blood correlated with increased overall survival (135). However, no further characterization of the $CD4^+$ T cells or their cell surface phenotype was carried out. More

recently, Kagamu et al. (151) found that increased frequencies of $CD62L^{low}CD4^+$ T cells in the circulation of patients with NSCLC before treatment with anti-PD-1 were predictive of greater progression-free survival following ICI treatment. Additionally, another study revealed that a higher frequency of memory $CD45RA^-CD4^+$ T cells in the blood of patients with melanoma at baseline was associated with a favorable response to ipilimumab, whereas an increased presence of naive $CD45RA^+CD4^+$ T cells correlated with nonresponders (137). Along similar lines, single-cell RNA-sequencing analysis performed on tumor biopsies from patients with melanoma revealed that at baseline, responders had a higher frequency of memory $CD4^+$ T cells, as defined by expression of CCR7, CD28, and CD62L (144). Similar to $CD8^+$ T cells, lack of PD-1 downregulation on $CD4^+PD\text{-}1^{hi}$ T cells following treatment with anti-PD-1 resulted in a higher risk of death in patients with advanced melanoma (152). Given these findings, it is clear that the frequency of $CD4^+$ T cell populations prior to therapy and the dynamic changes they undergo during therapy can serve as important predictive biomarkers distinguishing ICI responders from nonresponders.

Since a large portion of $CD4^+$ T cells in tumors are Tregs, which actively curb antitumor immune responses, one can posit that these cells may function to dampen the effects of ICIs and therefore would be associated with a worse response following ICI treatment. However, while decreases in Tregs in the peripheral blood and tumors of patients with metastatic melanoma treated with ICIs have been associated with ICI response and increased patient survival, Balatoni et al. (153) demonstrated that patients with melanoma who exhibited a higher frequency of Tregs in lymph node metastases responded better to ipilimumab compared with patients who had fewer Tregs (133). Additionally, a separate study found that an abundance of Tregs in the blood of patients with melanoma at baseline correlated with a favorable outcome following ipilimumab treatment (135). Woods et al. (154) also reported that an increased proportion of circulating Tregs in patients with melanoma after treatment with anti-PD-1 therapy correlated with a favorable response. As has been the case in many preclinical studies, a higher ratio of effector $CD8^+$ T cells to Tregs in the tumors of previously vaccinated patients with melanoma correlated favorably with tumor necrosis (155). Last, Liakou et al. (156) showed that in patients with bladder cancer, CTLA-4 blockade led to increased inducible T cell costimulator (ICOS) expression on IFN-γ producing $CD4^+$ T cells. The increased abundance of $ICOS^+CD4^+$ T cells resulted in an increased ratio of these cells to Tregs (156) both in the tumor and in peripheral blood. Although the authors did not correlate these changes with clinical outcome, this study nonetheless points to the possibility that ICI therapy brings about similar tumoral and systemic changes in immune cells, suggesting that circulating immune cells may provide a window into changes in immune cells in the tumor. Overall, it is difficult to conclude whether an increased frequency of Tregs prior to or following therapy is favorable for patients treated with ICIs. Examining the ratios of effector T cells to Tregs, rather than the absolute Treg numbers, may be a more fruitful approach to deciphering the prognostic utility of monitoring changes in this population.

For any T cell to become activated and acquire effector functions, its TCR must recognize its cognate antigen presented on an MHC molecule. The antigen specificity of a T cell is determined by its TCR, and because each cell expresses multiple copies of only a single TCR, T cells are highly specific in their response. This is important for our discussion, as numerous studies have attempted to determine whether TCR richness or diversity of V-D-J arrangements can predict the response to ICI therapy. Whereas some studies have found that high TCR diversity in peripheral blood and tumors prior to ICI therapy is predictive of effective treatment, others have pointed to ICI therapy–associated increases in TCR clonality, that is, enrichment of T cells with the same TCRs, as a sign of effective therapy (150, 157–161). Contrary to these studies, in patients with metastatic

pancreatic cancer, longer survival has been associated with lower baseline circulating clonality and a high number of expanded clones following anti-CTLA-4 treatment but not anti-PD-1 treatment (162). In this study, no TCR parameters were found to be associated with clinical outcomes in patients treated with anti-PD-1. In another study, patients with castration-resistant prostate cancer or advanced melanoma who maintained their oligoclonal circulating baseline TCRs after receiving anti-CTLA-4 responded better to therapy than those in which more of those clonotypes were lost (163). In patients with basal cell carcinoma and patients with squamous cell carcinoma treated with anti-PD-1, TCR clones present in the tumor posttreatment consisted of a significant number of clones that were not present in the tumors prior to ICI therapy (164). This finding suggests that, contrary to the concept of in situ reinvigoration of exhausted $CD8^+$ T cells that was widely considered to be the mechanism of ICI therapy, an effective ICI response may actually depend on an influx of novel, less exhausted T cells into the tumor site. Further investigation by Yost et al. (164) revealed that many of the new posttreatment clonotypes infiltrating the tumor could be identified in the blood, implicating the importance of T cell activation in the periphery in the developing antitumor response. The authors did not draw any correlations between TCR repertoire and clinical outcome, but the results certainly alter the view of how an ICI may exert its antitumor effects. These findings are also supported by a study performed by Wu et al. (165) that similarly suggested a peripheral origin for intratumoral $CD8^+$ clonotypes, on the basis of the presence of expanded clones infiltrating the tumor as well as normal adjacent tissue and blood. Overall, most of these studies point to increased TCR diversity, whether at baseline or after therapy initiation, as a favorable prognostic marker for ICI therapy. This is perhaps not surprising, as generating T cell responses to various tumor epitopes may help minimize tumor selection and eventual tumor escape. These studies also put forth the idea that assessing T cell diversity or clonality in the blood may be useful for understanding the T cell profile in the tumor, as Yost et al. and Wu et al. implicate the periphery as the source of newly infiltrated T cells in the tumor. If such blood analysis of the TCR repertoire is to be widely used for predicting clinical outcome, more studies would need to be done to determine the predictive value of such testing.

Apart from T cells, macrophages and myeloid-derived suppressor cells (MDSCs) have also been extensively studied in the context of ICI therapies. In preclinical models, both of these innate immune cell types aid tumor growth and metastasis through their secretion of factors that suppress antitumor immune responses, especially those of $CD8^+$ T cells and NK cells (166, 167). In accord with these findings, numerous studies of patients with cancer have indeed found that these myeloid cell subsets, whether in the tumor or in peripheral tissues, were negatively correlated with effective ICI therapy (134, 161, 168–171). In one study of patients with advanced melanoma, analysis of the tumor immune landscape using deconvolution of bulk RNA-sequencing data found that patients who responded to anti-PD-1 demonstrated lower tumoral macrophage infiltration (161). Similarly, a decrease in the abundance of tumor-infiltrating macrophages in patients with melanoma treated with anti-CTLA-4 and anti-PD-1 antibodies distinguished responders from nonresponders (172). In other studies, both a low baseline frequency of MDSCs and a low frequency of MDSCs in the blood of patients with melanoma following ipilimumab treatment were associated with favorable therapy outcome (134, 169–171). Additionally, renal cell carcinomas that exhibited a high tumoral myeloid signature were associated with significantly lower progression-free survival following anti-PD-L1 treatment, supporting the detrimental effects of some myeloid populations on ICI efficacy (173). By contrast, a recent study revealed that an increased frequency of circulating monocytes at baseline, unlike macrophages and MDSCs in tumors and other tissues, predicts superior ICI responses (174). Thus, when Krieg et al. (174) analyzed peripheral blood from patients with advanced melanoma at baseline using both mass and flow cytometry, $CD14^+CD16^-HLA\text{-}DR^{hi}$ monocytes were a strong predictor

of progression-free and overall survival after treatment with anti-PD-1 antibody. In contrast, Martens et al. (135) showed that a low absolute monocyte count in circulation is associated with better ipilimumab therapy outcome in patients with melanoma. Altogether, these results suggest that, whereas the negative role for tissue macrophages and MDSCs in ICI treatment is well established, our understanding of the role of circulating monocytes remains blurred.

What is clear from these studies is that, similar to the analysis of immunotherapy in tumor-bearing mice by Spitzer et al. (131), ICI treatment of patients induces significant immune changes both in the periphery and in the tumor site. The mechanisms by which the developing system-wide antitumor response is orchestrated or thwarted following ICI therapy are only beginning to be clarified, but additional studies that examine the association of different immune cell populations with positive or negative patient outcomes should yield further insight into the roles of these cells in the developing antitumor response. Furthermore, it may be important to clarify the differential

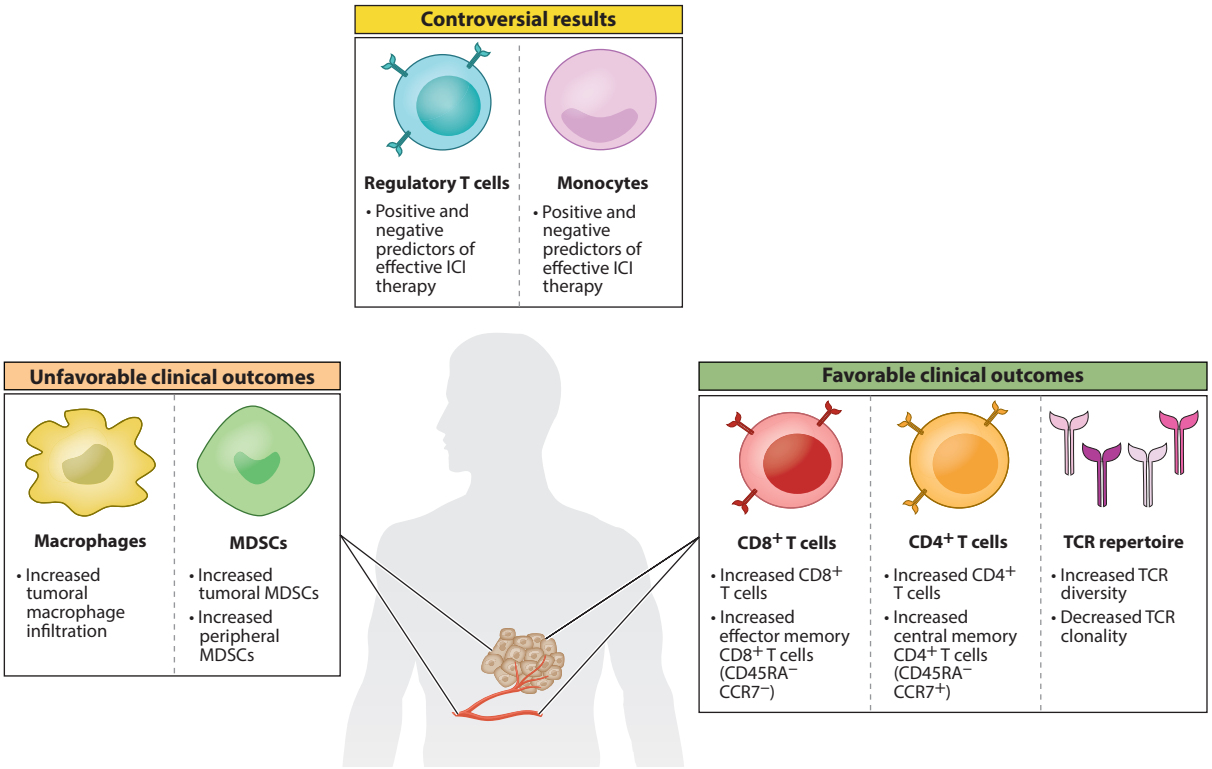

Figure 2

Immune cell populations in tumors and peripheral blood that correlate with favorable and unfavorable clinical outcomes following ICI therapy. Most human studies that have examined immune cell abundance, phenotype, and function in the context of ICI therapy have been undertaken for the purpose of biomarker discovery and have focused on peripheral blood and tumors in patients with melanoma, NSCLC, or both. These studies have revealed that high frequencies of memory $CD4^+$ and $CD8^+$ T cells prior to or after ICI therapy correlate with favorable responses to treatment. However, the mechanisms by which these T cells promote a favorable therapy outcome are unclear. Additionally, a diverse TCR repertoire is also linked to effective therapy. In contrast to T cells, high frequencies of macrophages and MDSCs, both within tumors and in peripheral blood, prior to or after ICI treatment are generally associated with unfavorable clinical outcomes. This is not surprising given the largely immunosuppressive roles of these cells in tumor immunity. Last, contradictory data exist on the role of other immune cell types, especially Tregs and monocytes, in the response to ICIs. Abbreviations: ICI, immune checkpoint inhibitor; MDSC, myeloid-derived suppressor cell; NSCLC, non-small-cell lung cancer; TCR, T cell receptor; Treg, regulatory T cell.

immune responses elicited by anti-CTLA and anti-PD-1 therapies. One study, for example, has suggested that anti-CTLA-4 induces mainly proliferation of $ICOS^+CD4^+$ effector T cells and that anti-PD-1 is associated primarily with reinvigoration $CD8^+PD\text{-}1^{hi}$ T cells (175). Given the extensive interactions between immune cells that are required to generate an effective immune response, it is unlikely that quantifying a single cell population or parameter will accurately predict response or nonresponse to ICI treatment, and a more holistic approach encompassing multiple cell types will be necessary. In **Figure 2**, we have summarized the immune cell types and their correlation to ICI therapy outcome.

CONCLUDING REMARKS

ICI treatment has revolutionized the field of cancer immunotherapy. Here, we reviewed the origins and development of the most prominent ICI agents, as well as the challenges they present in the clinic, such as resistance to therapy and irAEs. We further described data suggesting the importance of the tumoral and systemic immune responses elicited by ICIs and how investigating changes in these immune responses or particular immune cell populations can be leveraged to try to better understand what characterizes an effective response.

It is also important to acknowledge that, although ICIs are the most widely used, they are not the only FDA-approved immunotherapies. A DC vaccine for the treatment of advanced prostate cancer and chimeric antigen receptor T cells for the treatment of several hematological malignancies are among other immunotherapies that have also received FDA approval (176, 177). However, the role of systemic immunity is not well understood in these modalities. To achieve a more comprehensive understanding of how the immune system initiates and maintains an effective antitumor response, researchers need to additionally investigate the systemic effects of other immunotherapies. In the end, these efforts directed at understanding both resistance to ICIs and the systemic response defining efficacious therapy all converge on two goals: to broaden the number of patients who can benefit from existing immunotherapies and to help guide and inform future therapies.

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LITERATURE CITED

1. Valk E, Rudd CE, Schneider H. 2008. CTLA-4 trafficking and surface expression. *Trends Immunol.* 29(6):272–79
2. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, et al. 1994. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1(5):405–13
3. Krummel MF, Allison JP. 1995. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J. Exp. Med.* 182(2):459–65
4. Linsley PS, Clark EA, Ledbetter JA. 1990. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *PNAS* 87(13):5031–35

5. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. 1991. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med.* 173(3):721–30
6. Azuma M, Ito D, Yagita H, Okumura K, Phillips JH, et al. 1993. B70 antigen is a second ligand for CTLA-4 and CD28. *Nature* 366(6450):76–79
7. Valk E, Leung R, Kang H, Kaneko K, Rudd CE, Schneider H. 2006. T cell receptor-interacting molecule acts as a chaperone to modulate surface expression of the CTLA-4 coreceptor. *Immunity* 25(5):807–21
8. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. 1991. CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* 174(3):561–69
9. Chuang E, Fisher TS, Morgan RW, Robbins MD, Duerr JM, et al. 2000. The CD28 and CTLA-4 receptors associate with the serine/threonine phosphatase PP2A. *Immunity* 13(3):313–22
10. Chuang E, Lee K-M, Robbins MD, Duerr JM, Alegre M-L, et al. 1999. Regulation of cytotoxic T lymphocyte-associated molecule-4 by Src kinases. *J. Immunol.* 162(3):1270–77
11. Lee K-M, Chuang E, Griffin M, Khattri R, Hong DK, et al. 1998. Molecular basis of T cell inactivation by CTLA-4. *Science* 282(5397):2263–66
12. Marengère LEM, Waterhouse P, Duncan GS, Mittrücker H-W, Feng G-S, Mak TW. 1996. Regulation of T cell receptor signaling by tyrosine phosphatase SYP association with CTLA-4. *Science* 272(5265):1170–73
13. Fraser JH, Rincón M, McCoy KD, Le Gros G. 1999. CTLA4 ligation attenuates AP-1, NFAT and NF-κB activity in activated T cells. *Eur. J. Immunol.* 29(3):838–44
14. Olsson C, Riebeck K, Dohlsten M, Michaëlsson E. 1999. CTLA-4 ligation suppresses CD28-induced NF-κB and AP-1 activity in mouse T cell blasts. *J. Biol. Chem.* 274(20):14400–5
15. Hoff H, Kolar P, Ambach A, Radbruch A, Brunner-Weinzierl MC. 2010. CTLA-4 (CD152) inhibits T cell function by activating the ubiquitin ligase Itch. *Mol. Immunol.* 47(10):1875–81
16. Masteller EL, Chuang E, Mullen AC, Reiner SL, Thompson CB. 2000. Structural analysis of CTLA-4 function in vivo. *J. Immunol.* 164(10):5319–27
17. Read S, Malmström V, Powrie F. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192(2):295–302
18. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, et al. 2000. Immunologic self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* 192(2):303–10
19. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, et al. 2006. Blockade of CTLA-4 on $CD4^+$ $CD25^+$ regulatory T cells abrogates their function in vivo. *J. Immunol.* 177(7):4376–83
20. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, et al. 2008. CTLA-4 control over $Foxp3^+$ regulatory T cell function. *Science* 322(5899):271–75
21. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, et al. 2009. $CD4^+$ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J. Exp. Med.* 206(2):421–34
22. Zheng SG, Wang JH, Stohl W, Kim KS, Gray JD, Horwitz DA. 2006. TGF-β requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive $CD4^+CD25^+$ regulatory cells. *J. Immunol.* 176(6):3321–29
23. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3(5):541–47
24. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, et al. 1995. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270(5238):985–88
25. Chambers CA, Sullivan TJ, Allison JP. 1997. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of $CD4^+$ T cells. *Immunity* 7(6):885–95
26. Leach DR, Krummel MF, Allison JP. 1996. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 271(5256):1734–36
27. Kwon ED, Hurwitz AA, Foster BA, Madias C, Feldhaus AL, et al. 1997. Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. *PNAS* 94(15):8099–103

28. Yang YF, Zou JP, Mu J, Wijesuriya R, Ono S, et al. 1997. Enhanced induction of antitumor T-cell responses by cytotoxic T lymphocyte-associated molecule-4 blockade: The effect is manifested only at the restricted tumor-bearing stages. *Cancer Res.* 57(18):4036–41
29. Hurwitz AA, Townsend SE, Yu TF, Wallin JA, Allison JP. 1998. Enhancement of the anti-tumor immune response using a combination of interferon-γ and B7 expression in an experimental mammary carcinoma. *Int. J. Cancer* 77(1):107–13
30. van Elsas A, Hurwitz AA, Allison JP. 1999. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J. Exp. Med.* 190(3):355–66
31. Hurwitz AA, Yu TF, Leach DR, Allison JP. 1998. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. *PNAS* 95(17):10067–71
32. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, et al. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8):711–23
33. Ishida Y, Agata Y, Shibahara K, Honjo T. 1992. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* 11(11):3887–95
34. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, et al. 2009. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 114(8):1537–44
35. Salmaninejad A, Valilou SF, Shabgah AG, Aslani S, Alimardani M, et al. 2019. PD-1/PD-L1 pathway: basic biology and role in cancer immunotherapy. *J. Cell Physiol.* 234(10):16824–37
36. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, et al. 2000. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192(7):1027–34
37. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, et al. 2001. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat. Immunol.* 2(3):261–68
38. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. 2001. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting Src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *PNAS* 98(24):13866–71
39. Sheppard K-A, Fitz LJ, Lee JM, Benander C, George JA, et al. 2004. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3ζ signalosome and downstream signaling to PKCθ. *FEBS Lett.* 574(1–3):37–41
40. Patsoukis N, Brown J, Petkova V, Liu F, Li L, Boussiotis VA. 2012. Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. *Sci. Signal.* 5(230):ra46
41. Nishimura H, Minato N, Nakano T, Honjo T. 1998. Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. *Int. Immunol.* 10(10):1563–72
42. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. 1999. Development of lupus-like autoimmune diseases by disruption of the *PD-1* gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11(2):141–51
43. Nishimura H. 2001. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 291(5502):319–22
44. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. 2002. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *PNAS* 99(19):12293–97
45. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, et al. 2005. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res.* 65(3):1089–96
46. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, et al. 2003. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat. Med.* 9(5):562–67
47. He Y-F, Zhang G-M, Wang X-H, Zhang H, Yuan Y, et al. 2004. Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine. *J. Immunol.* 173(8):4919–28

48. Blank C, Brown I, Peterson AC, Spiotto M, Iwai Y, et al. 2004. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8$^+$ T cells. *Cancer Res.* 64(3):1140–45
49. Iwai Y. 2004. PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. *Int. Immunol.* 17(2):133–44
50. Thompson RH, Gillett MD, Cheville JC, Lohse CM, Dong H, et al. 2004. Costimulatory B7-H1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. *PNAS* 101(49):17174–79
51. Thompson RH, Kuntz SM, Leibovich BC, Dong H, Lohse CM, et al. 2006. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer Res.* 66(7):3381–85
52. Ohigashi Y, Sho M, Yamada Y, Tsurui Y, Hamada K, et al. 2005. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clin. Cancer Res.* 11(8):2947–53
53. Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. 2007. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol. Immunother.* 56(8):1173–82
54. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, et al. 2007. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8$^+$ T lymphocytes are prognostic factors of human ovarian cancer. *PNAS* 104(9):3360–65
55. Nomi T, Sho M, Akahori T, Hamada K, Kubo A, et al. 2007. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin. Cancer Res.* 13(7):2151–57
56. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, et al. 2007. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin. Cancer Res.* 13(6):1757–61
57. 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(9948):1109–17
58. Robert C, Schachter J, Long GV, Arance A, Grob JJ, et al. 2015. Pembrolizumab versus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 372(26):2521–32
59. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, et al. 2015. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* 373(1):23–34
60. Reck M, Rodríguez-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.* 375(19):1823–33
61. Remark R, Becker C, Gomez JE, Damotte D, Dieu-Nosjean M-C, et al. 2015. The non-small cell lung cancer immune contexture. A major determinant of tumor characteristics and patient outcome. *Am. J. Respir. Crit. Care Med.* 191(4):377–90
62. Marcus L, Lemery SJ, Keegan P, Pazdur R. 2019. FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin. Cancer Res.* 25(13):3753–58
63. Mayoux M, Roller A, Pulko V, Sammicheli S, Chen S, et al. 2020. Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Sci. Transl. Med.* 12(534):eaav7431
64. Akinleye A, Rasool Z. 2019. Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. *J. Hematol. Oncol.* 12(1):92
65. 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(10031):1909–20
66. West H, McCleod M, Hussein M, Morabito A, Rittmeyer A, et al. 2019. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 20(7):924–37
67. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, et al. 2018. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N. Engl. J. Med.* 378(24):2288–301

68. Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, et al. 2018. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N. Engl. J. Med.* 379(23):2220–29
69. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, et al. 2018. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N. Engl. J. Med.* 379(22):2108–21
70. Powles T, O'Donnell PH, Massard C, Arkenau H-T, Friedlander TW, et al. 2017. Efficacy and safety of durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. *JAMA Oncol.* 3(9):e172411
71. Apolo AB, Infante JR, Balmanoukian A, Patel MR, Wang D, et al. 2017. Avelumab, an anti-programmed death-ligand 1 antibody, in patients with refractory metastatic urothelial carcinoma: results from a multicenter, phase Ib study. *J. Clin. Oncol.* 35(19):2117–24
72. Patel MR, Ellerton J, Infante JR, Agrawal M, Gordon M, et al. 2018. Avelumab in metastatic urothelial carcinoma after platinum failure (JAVELIN Solid Tumor): pooled results from two expansion cohorts of an open-label, phase 1 trial. *Lancet Oncol.* 19(1):51–64
73. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, et al. 2016. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol.* 17(10):1374–85
74. 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(21):2018–28
75. Ott PA, Bang Y-J, Piha-Paul SA, Razak ARA, Bennouna J, et al. 2019. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J. Clin. Oncol.* 37(4):318–27
76. Nayak L, Iwamoto FM, LaCasce A, Mukundan S, Roemer MGM, et al. 2017. PD-1 blockade with nivolumab in relapsed/refractory primary central nervous system and testicular lymphoma. *Blood* 129(23):3071–73
77. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, et al. 2015. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* 372(26):2509–20
78. Ribas A, Wolchok JD. 2018. Cancer immunotherapy using checkpoint blockade. *Science* 359(6382):1350–55
79. Haslam A, Prasad V. 2019. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. *JAMA Netw. Open* 2(5):e192535
80. Daud AI, Wolchok JD, Robert C, Hwu W-J, 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(34):4102–9
81. Stenehjem DD, Tran D, Nkrumah MA, Gupta S. 2018. PD1/PDL1 inhibitors for the treatment of advanced urothelial bladder cancer. *OncoTargets Ther.* 11:5973–89
82. Yearley JH, Gibson C, Yu N, Moon C, Murphy E, et al. 2017. PD-L2 expression in human tumors: relevance to anti-PD-1 therapy in cancer. *Clin. Cancer Res.* 23(12):3158–67
83. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, et al. 2017. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol. Cancer Ther.* 16(11):2598–608
84. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, et al. 2018. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 362(6411):eaar3593
85. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, et al. 2015. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350(6257):207–11
86. 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(6230):124–28
87. Boyiadzis MM, Kirkwood JM, Marshall JL, Pritchard CC, Azad NS, Gulley JL. 2018. Significance and implications of FDA approval of pembrolizumab for biomarker-defined disease. *J. Immunother. Cancer* 6(1):35
88. Maleki Vareki S. 2018. High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. *J. Immunother. Cancer* 6(1):157

89. Kaufman HL, Russell JS, Hamid O, Bhatia S, Terheyden P, et al. 2018. Updated efficacy of avelumab in patients with previously treated metastatic Merkel cell carcinoma after ≥1 year of follow-up: JAVELIN Merkel 200, a phase 2 clinical trial. *J. Immunother. Cancer* 6(1):7
90. Ni L, Lu J. 2018. Interferon gamma in cancer immunotherapy. *Cancer Med.* 7(9):4509–16
91. Garris CS, Arlauckas SP, Kohler RH, Trefny MP, Garren S, et al. 2018. Successful anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-γ and IL-12. *Immunity* 49(6):1148–61.e7
92. Karachaliou N, Gonzalez-Cao M, Crespo G, Drozdowskyj A, Aldeguer E, et al. 2018. Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. *Ther. Adv. Med. Oncol.* 10:1758834017749748
93. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, et al. 2017. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J. Clin. Investig.* 127(8):2930–40
94. Gao J, Shi LZ, Zhao H, Chen J, Xiong L, et al. 2016. Loss of IFN-γ pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. *Cell* 167(2):397–404.e9
95. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, et al. 2017. Primary resistance to PD-1 blockade mediated by *JAK1/2* mutations. *Cancer Discov.* 7(2):188–201
96. Huang L, Malu S, McKenzie JA, Andrews MC, Talukder AH, et al. 2018. The RNA-binding protein MEX3B mediates resistance to cancer immunotherapy by downregulating HLA-A expression. *Clin. Cancer Res.* 24(14):3366–76
97. Rodig SJ, Gusenleitner D, Jackson DG, Gjini E, Giobbie-Hurder A, et al. 2018. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Sci. Transl. Med.* 10(450):eaar3342
98. Nowicki TS, Hu-Lieskovan S, Ribas A. 2018. Mechanisms of resistance to PD-1 and PD-L1 blockade. *Cancer J.* 24(1):47–53
99. Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, et al. 2015. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 350(6264):1079–84
100. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, et al. 2018. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359(6371):97–103
101. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, et al. 2018. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 359(6371):91–97
102. Tinsley N, Zhou C, Tan G, Rack S, Lorigan P, et al. 2020. Cumulative antibiotic use significantly decreases efficacy of checkpoint inhibitors in patients with advanced cancer. *Oncologist* 25(1):55–63
103. Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, et al. 2018. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann. Oncol.* 29(6):1437–44
104. Shen J, Ju Z, Zhao W, Wang L, Peng Y, et al. 2018. ARID1A deficiency promotes mutability and potentiates therapeutic antitumor immunity unleashed by immune checkpoint blockade. *Nat. Med.* 24(5):556–62
105. Pan D, Kobayashi A, Jiang P, Ferrari de Andrade L, Tay RE, et al. 2018. A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. *Science* 359(6377):770–75
106. Keenan TE, Burke KP, Van Allen EM. 2019. Genomic correlates of response to immune checkpoint blockade. *Nat. Med.* 25(3):389–402
107. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, et al. 2015. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature* 527(7577):249–53
108. Marwitz S, Scheufele S, Perner S, Reck M, Ammerpohl O, Goldmann T. 2017. Epigenetic modifications of the immune-checkpoint genes *CTLA4* and *PDCD1* in non-small cell lung cancer results in increased expression. *Clin. Epigenet.* 9:51
109. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, et al. 2016. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 354(6316):1160–65
110. 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(9):819–29

111. Gettinger S, Choi J, Hastings K, Truini A, Datar I, et al. 2017. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. *Cancer Discov*. 7(12):1420–35
112. George S, Miao D, Demetri GD, Adeegbe D, Rodig SJ, et al. 2017. Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blockade therapy in metastatic uterine leiomyosarcoma. *Immunity* 46(2):197–204
113. Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, et al. 2017. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. *Cancer Discov*. 7(3):264–76
114. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, et al. 2019. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. *Nat. Rev. Clin. Oncol.* 16(9):563–80
115. Bajwa R, Cheema A, Khan T, Amirpour A, Paul A, et al. 2019. Adverse effects of immune checkpoint inhibitors (programmed death-1 inhibitors and cytotoxic T-lymphocyte-associated protein-4 inhibitors): results of a retrospective study. *J. Clin. Med. Res.* 11(4):225–36
116. Chae YK, Chiec L, Mohindra N, Gentzler R, Patel J, Giles F. 2017. A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. *Cancer Immunol. Immunother.* 66(1):25–32
117. Das S, Johnson DB. 2019. Immune-related adverse events and anti-tumor efficacy of immune checkpoint inhibitors. *J. Immunother. Cancer* 7(1):306
118. Postow MA, Sidlow R, Hellmann MD. 2018. Immune-related adverse events associated with immune checkpoint blockade. *N. Engl. J. Med.* 378(2):158–68
119. Buchbinder EI, Desai A. 2016. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *Am. J. Clin. Oncol.* 39(1):98–106
120. Horvat TZ, Adel NG, Dang T-O, Momtaz P, Postow MA, et al. 2015. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. *J. Clin. Oncol.* 33(28):3193–98
121. Weber JS, Hodi FS, Wolchok JD, Topalian SL, Schadendorf D, et al. 2017. Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma. *J. Clin. Oncol.* 35(7):785–92
122. Esfahani K, Miller WH. 2017. Reversal of autoimmune toxicity and loss of tumor response by interleukin-17 blockade. *N. Engl. J. Med.* 376(20):1989–91
123. Faje AT, Lawrence D, Flaherty K, Freedman C, Fadden R, et al. 2018. High-dose glucocorticoids for the treatment of ipilimumab-induced hypophysitis is associated with reduced survival in patients with melanoma. *Cancer* 124(18):3706–14
124. Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. 2016. Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. *Clin. Cancer Res.* 22(4):886–94
125. Teulings H-E, Limpens J, Jansen SN, Zwinderman AH, Reitsma JB, et al. 2015. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J. Clin. Oncol.* 33(7):773–81
126. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, et al. 2016. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. *JAMA Dermatol.* 152(1):45–51
127. Sanlorenzo M, Vujic I, Daud A, Algazi A, Gubens M, et al. 2015. Pembrolizumab cutaneous adverse events and their association with disease progression. *JAMA Dermatol.* 151(11):1206–12
128. Downey SG, Klapper JA, Smith FO, Yang JC, Sherry RM, et al. 2007. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin. Cancer Res.* 13(22 Part 1):6681–88
129. Weber JS, O'Day S, Urba W, Powderly J, Nichol G, et al. 2008. Phase I/II study of ipilimumab for patients with metastatic melanoma. *J. Clin. Oncol.* 26(36):5950–56
130. Grimaldi A, Simeone E, Festino L, Giannarelli D, Palla M, et al. 2015. Correlation between immune-related adverse events and response to pembrolizumab in advanced melanoma patients. *J. Immunother. Cancer* 3(Suppl. 2):P186

131. Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, et al. 2017. Systemic immunity is required for effective cancer immunotherapy. *Cell* 168(3):487–502.e15
132. De Angulo G, Yuen C, Palla SL, Anderson PM, Zweidler-McKay PA. 2008. Absolute lymphocyte count is a novel prognostic indicator in ALL and AML: implications for risk stratification and future studies. *Cancer* 112(2):407–15
133. Simeone E, Gentilcore G, Giannarelli D, Grimaldi AM, Caracò C, et al. 2014. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. *Cancer Immunol. Immunother.* 63(7):675–83
134. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, et al. 2016. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin. Cancer Res.* 22(12):2908–18
135. Martens A, Wistuba-Hamprecht K, Yuan J, Postow MA, Wong P, et al. 2016. Increases in absolute lymphocytes and circulating $CD4^+$ and $CD8^+$ T cells are associated with positive clinical outcome of melanoma patients treated with ipilimumab. *Clin. Cancer Res.* 22(19):4848–58
136. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, et al. 2005. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N. Engl. J. Med.* 353(25):2654–66
137. Subrahmanyam PB, Dong Z, Gusenleitner D, Giobbie-Hurder A, Severgnini M, et al. 2018. Distinct predictive biomarker candidates for response to anti-CTLA-4 and anti-PD-1 immunotherapy in melanoma patients. *J. Immunother. Cancer* 6(1):18
138. Felix J, Lambert J, Roelens M, Maubec E, Guermouche H, et al. 2016. Ipilimumab reshapes T cell memory subsets in melanoma patients with clinical response. *Oncoimmunology* 5(7):1136045
139. Pirozyan MR, McGuire HM, Emran AA, Tseng H-Y, Tiffen JC, et al. 2020. Pretreatment innate cell populations and CD4 T cells in blood are associated with response to immune checkpoint blockade in melanoma patients. *Front. Immunol.* 11:372
140. Ribas A, Shin DS, Zaretsky J, Frederiksen J, Cornish A, et al. 2016. PD-1 blockade expands intratumoral memory T cells. *Cancer Immunol. Res.* 4(3):194–203
141. Gide TN, Quek C, Menzies AM, Tasker AT, Shang P, et al. 2019. Distinct immune cell populations define response to anti-PD-1 monotherapy and anti-PD-1/anti-CTLA-4 combined therapy. *Cancer Cell* 35(2):238–55.e6
142. de Coaña YP, Wolodarski M, Poschke I, Yoshimoto Y, Yang Y, et al. 2017. Ipilimumab treatment decreases monocytic MDSCs and increases CD8 effector memory T cells in long-term survivors with advanced melanoma. *Oncotarget* 8(13):21539–53
143. Wistuba-Hamprecht K, Martens A, Heubach F, Romano E, Geukes Foppen M, et al. 2017. Peripheral CD8 effector-memory type 1 T-cells correlate with outcome in ipilimumab-treated stage IV melanoma patients. *Eur. J. Cancer* 73:61–70
144. Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, et al. 2018. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 175(4):998–1013.e20
145. Siddiqui I, Schaeuble K, Chennupati V, Fuertes Marraco SA, Calderon-Copete S, et al. 2019. Intratumoral $Tcf1^+PD\text{-}1^+CD8^+$ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity* 50(1):195–211.e10
146. Daud AI, Loo K, Pauli ML, Sanchez-Rodriguez R, Sandoval PM, et al. 2016. Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. *J. Clin. Investig.* 126(9):3447–52
147. Thommen DS, Koelzer VH, Herzig P, Roller A, Trefny M, et al. 2018. A transcriptionally and functionally distinct $PD\text{-}1^+$ $CD8^+$ T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade. *Nat. Med.* 24(7):994–1004
148. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, et al. 2017. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* 545(7652):60–65
149. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, et al. 2017. Proliferation of PD-1+ CD8 T cells in peripheral blood after PD-1–targeted therapy in lung cancer patients. *PNAS* 114(19):4993–98
150. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, et al. 2014. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 515(7528):568–71
151. Kagamu H, Kitano S, Yamaguchi O, Yoshimura K, Horimoto K, et al. 2020. $CD4^+$ T-cell immunity in the peripheral blood correlates with response to anti-PD-1 therapy. *Cancer Immunol. Res.* 8(3):334–44

152. Zappasodi R, Budhu S, Hellmann MD, Postow MA, Senbabaoglu Y, et al. 2018. Non-conventional inhibitory $CD4^+Foxp3^-PD\text{-}1^{hi}$ T cells as a biomarker of immune checkpoint blockade activity. *Cancer Cell* 33(6):1017–32.e7. Erratum. 2018. *Cancer Cell* 34(4):691
153. Balatoni T, Mohos A, Papp E, Sebestyén T, Liszkay G, et al. 2018. Tumor-infiltrating immune cells as potential biomarkers predicting response to treatment and survival in patients with metastatic melanoma receiving ipilimumab therapy. *Cancer Immunol. Immunother.* 67(1):141–51
154. Woods DM, Ramakrishnan R, Laino AS, Berglund A, Walton K, et al. 2018. Decreased suppression and increased phosphorylated STAT3 in regulatory T cells are associated with benefit from adjuvant PD-1 blockade in resected metastatic melanoma. *Clin. Cancer Res.* 24(24):6236–47
155. Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, et al. 2008. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. *PNAS* 105(8):3005–10
156. Liakou CI, Kamat A, Tang DN, Chen H, Sun J, et al. 2008. CTLA-4 blockade increases IFNγ-producing $CD4^+ICOS^{hi}$ cells to shift the ratio of effector to regulatory T cells in cancer patients. *PNAS* 105(39):14987–92
157. Roh W, Chen P-L, Reuben A, Spencer CN, Prieto PA, et al. 2017. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. *Sci. Transl. Med.* 9(379):eaah3560
158. Arakawa A, Vollmer S, Tietze J, Galinski A, Heppt MV, et al. 2019. Clonality of $CD4^+$ blood T cells predicts longer survival with CTLA4 or PD-1 checkpoint inhibition in advanced melanoma. *Front. Immunol.* 10:1336
159. Postow MA, Manuel M, Wong P, Yuan J, Dong Z, et al. 2015. Peripheral T cell receptor diversity is associated with clinical outcomes following ipilimumab treatment in metastatic melanoma. *J. Immunother. Cancer* 3:23
160. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, et al. 2018. Neoadjuvant PD-1 blockade in resectable lung cancer. *N. Engl. J. Med.* 378(21):1976–86
161. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, et al. 2017. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 171(4):934–49.e16
162. Hopkins AC, Yarchoan M, Durham JN, Yusko EC, Rytlewski JA, et al. 2018. T cell receptor repertoire features associated with survival in immunotherapy-treated pancreatic ductal adenocarcinoma. *JCI Insight* 3(13):e122092
163. Cha E, Klinger M, Hou Y, Cummings C, Ribas A, et al. 2014. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. *Sci. Transl. Med.* 6(238):238ra70
164. Yost KE, Satpathy AT, Wells DK, Qi Y, Wang C, et al. 2019. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat. Med.* 25(8):1251–59
165. Wu TD, Madireddi S, de Almeida PE, Banchereau R, Chen Y-JJ, et al. 2020. Peripheral T cell expansion predicts tumour infiltration and clinical response. *Nature* 579(7798):274–78
166. Ostrand-Rosenberg S, Fenselau C. 2018. Myeloid-derived suppressor cells: immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J. Immunol.* 200(2):422–31
167. Noy R, Pollard JW. 2014. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 41(1):49–61
168. Neubert NJ, Schmittnaegel M, Bordry N, Nassiri S, Wald N, et al. 2018. T cell-induced CSF1 promotes melanoma resistance to PD1 blockade. *Sci. Transl. Med.* 10(436):eaan3311
169. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, et al. 2014. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunol. Immunother.* 63(3):247–57
170. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, et al. 2015. Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. *Clin. Cancer Res.* 21(24):5453–59
171. Sade-Feldman M, Kanterman J, Klieger Y, Ish-Shalom E, Olga M, et al. 2016. Clinical significance of circulating $CD33^+CD11b^+HLA\text{-}DR^-$ myeloid cells in patients with stage IV melanoma treated with ipilimumab. *Clin. Cancer Res.* 22(23):5661–72

172. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, et al. 2017. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep*. 18(1):248–62
173. McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, et al. 2018. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. *Nat. Med.* 24(6):749–57
174. Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, et al. 2018. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nat. Med.* 24(2):144–53
175. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang N-AAS, et al. 2017. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. *Cell* 170(6):1120–33.e17
176. Hollingsworth RE, Jansen K. 2019. Turning the corner on therapeutic cancer vaccines. *NPJ Vaccines* 4(1):7
177. Majzner RG, Mackall CL. 2019. Clinical lessons learned from the first leg of the CAR T cell journey. *Nat. Med.* 25(9):1341–55