Mechanistic and pharmacologic insights on immune checkpoint inhibitors

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Abbreviations: CTLA-4: cytotoxic T lymphocyte antigen-4, IFN: interferon, PD-1: programmed death-1, irAE(s): immune related adverse event(s)

ABSTRACT

The concept of augmenting the immune system to eradicate cancer dates back at least a century. A major resurgence in cancer immunotherapy has occurred over the past decade since the identification and targeting of negative regulators with antibody therapies to augment the anti-tumor immune response. Unprecedented responses across a broad array of cancer types elevated this class of therapies to the forefront of cancer treatment. The most successful drugs to date target the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) pathways. The immune biology of these pathways was illuminated through thoughtful pre-clinical experiments over the past 20 years. The characterization of these negative immune regulators, also known as immune checkpoints, subsequently led to the successful clinical development four drugs in six different cancer types to date, and progress continues. Despite these successes, significant challenges remain including the development of predictive biomarkers, recognition and management of immune related toxicities, and elucidating and reversing mechanisms of primary and secondary resistance. Ongoing work is expected to build upon recent accomplishments and allow more patients to benefit from this class of therapies.

INTRODUCTION

The notion of employing the immune system to target cancer was conceived well over a century ago when Dr. William Coley of the New York Hospital successfully treated sarcoma patients by the injection of bacteria to invoke an immune response [1]. In 1893 he reported a remarkable case series of 38 patients, 15 who were treated intentionally with bacterial injection and 23 who had incidentally developed bacterial infections (erysipelas). He reported cures in 12/38 patients, however two patients died due to the inoculation of the bacteria. As a result of those deaths, immunotherapy fell out of favor for many decades, especially given the advent of radiation and chemotherapy. At the turn of the 21st century, the discovery of negative regulators of anti-tumor immunity, or immune checkpoints, re-invigorated the field of cancer immunotherapy. In 2011 the first therapy targeting negative immune regulation was approved by regulatory agencies for the treatment of metastatic melanoma. This drug, ipilimumab, was an antibody designed to target cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), also known as CD152. Subsequently, programmed cell death-1 (PD-1) pathway was characterized and found to have inhibitory effects on antitumor immunity. Targeting PD-1 or its ligand PD-L1 with antibodies has led to even more successes and is potentially the most broadly effective cancer therapeutic strategy to date.

Despite the clinical triumphs of modern immune checkpoint blockade, there still remains uncertainty around the precise mechanisms of action at play in the tumors of patients. The concept of “releasing the breaks” on the immune response is generally straightforward, but the specific immune cell populations and sites of action require complex analysis. In this article we explore the pharmacologic aspects of immune

checkpoint therapies and reveal mechanistic insights gained over the past several decades of immunology research. Given the demonstrated clinical success of targeting CTLA-4 and PD-1/PD-L1, we focus primarily on those targets.

CANCER CIRCUMVENTS AN ADAPTIVE IMMUNE RESPONSE

In order to explore potential mechanisms of action of immune checkpoint blockade, it is important to first review the knowledge that is understood on components of effective antitumor immunity. Malignancy develops and progresses due to its circumvention of one or more elements of the immune response. The hallmark of oncogenesis is abnormal genetic changes occurring in tumor cells, including mutations, chromosomal alterations, epigenetic modifications, gene expression changes, splice variants, and other disruptions that drive cellular proliferation and growth. The cornerstone of adaptive immunity is the recognition of neoantigens, or abnormal peptides generated from non-synonymous mutations, by the immune system [2]. Within a tumor microenvironment, this requires uptake of peptide fragments by specialized antigen presenting cells (APCs) driven by Type I interferons, which cross-present them to T cells in the tumor draining lymph nodes [3, 4].

Engagement of the neoantigen:major histocompatibility (MHC) complex and the T cell receptor alone is insufficient to activate tumor-antigen specific T cells. Additional costimulation must occur through CD28, which is active upon binding of B7-1 (CD80) or B7-2 (CD86) on the APC (Figure 1) [5]. If an appropriate ratio of T cell activating-to-inhibitory signal is present, a T cell will increase metabolism, proliferate, and eventually traffic back through the circulation to the tumor where it can engage and destroy tumor

cells through enumeration of perforin and granzyme. Negative regulatory pathways have been identified at essentially all of the aforementioned steps. Growth and metastasis of neoplastic cells depends on circumventing antigen presentation, T cell activation, recruitment of immune cells to the tumor microenvironment, and/or cytolytic activity of T cells. This is often done by exploitation of negative regulatory pathways, such as CTLA-4 and PD-1, at each stage of the immune response (Figure 1).

MECHANISM OF ACTION OF IMMUNE CHECKPOINT TARGETS

The efficacy of CTLA-4 and PD-1/PD-L1 immune checkpoint inhibitors has been demonstrated in preclinical models and numerous human clinical trials, ultimately leading to regulatory approval. Important questions still remain regarding the precise mechanism of action that results in tumor regression for both targets. In this section we review current the understanding of immunobiology in this context, which has expanded rapidly in the past decade.

CTLA-4

Activation of T lymphocytes requires a costimulatory signal from CD80 or CD86 binding CD28. In 1987 a new member of the immunoglobulin superfamily analogous to CD28 was discovered on lymphocytes and it was called cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), also known as CD152. It contains 4 exons (a signal peptide sequence, an IgV-like ligand-binding domain, a transmembrane region, and a cytoplasmic domain) and is highly conserved across human and mouse species [6]. Early studies found that its expression was restricted to activated T cells and suggested that it stimulated T lymphocyte proliferation, similar to CD28 [7, 8]. As CTLA-4 was further

experimentally examined, data began to suggest that it was, in fact, a negative regulator of immune function [9-11]. Binding of CTLA-4 did not induce IL-2 production as observed with CD28, and CTLA-4 knockout mice were generated and found to develop uncontrolled inflammation involving the pancreas, heart, liver, and lungs, causing death within the first month of life [12]. CTLA-4 was also noted to bind CD80 and CD86 with a much higher affinity than CD28 [13]. This competitive binding alone likely accounts for a substantial proportion of its inhibitory effect. However, the binding itself leads to downstream effects via tyrosine phosphatases that inhibit cellular proliferation and production of pro-cytotoxic signaling molecules such as IL-2 [10]. A blocking antibody was developed soon after and tested in the preclinical setting. Inhibition of CTLA-4 was found to induce tumor rejection and protection from secondary challenge in a transplantable murine colon carcinoma model [14]. This study demonstrated proof-of-concept that disinhibiting the immune response could be used as a cancer therapeutic.

Despite validation of this concept, some controversy has emerged over the dominant mechanism of action for the efficacy of anti-CTLA-4 antibodies. Emerging pre-clinical data points to additional physiologic effects of CTLA-4 that play an important role in immune inhibition. The action of CTLA-4 blockade has been shown to be dependent on CD8⁺ cytotoxic lymphocytes, but also CD4⁺ regulatory T cells (Tregs) [15]. Tregs have emerged as important negative regulators of immune response, and CTLA-4 is constitutively expressed in this lymphocyte subset [16]. Expression of CTLA-4 is driven by the transcription factor Forkhead box P3 (FOX-P3), which is the *sine qua non* of Tregs. There is emerging data that binding of CTLA-4 also induces other modes of immune inhibition such as indoleamine-2,3-dioxygenase (IDO) [17].

PD-1 and PD-L1

In 1992 Tasuku Honjo and colleagues reported the discovery of a novel member of the immunoglobulin gene superfamily [18]. In an effort to understand the molecular mechanism behind programmed cell death, 2B4.11 (a murine T cell hybridoma cell line) and LyD9 (a murine hematopoietic progenitor cell line) were used to isolate cDNA that was common to both upon stimulation that resulted in cell death. Hence the isolated nucleotide sequence was deemed programmed cell death-1, or PD-1. Conserved across human and mouse, this gene is part of the CD28/CTLA-4 subfamily and has five exons encoding a signal peptide sequence, an IgV-like ligand-binding domain, a stalk/transmembrane region, a cytoplasmic domain encoding an immunoreceptor tyrosine-based inhibitory motif (ITIM), and a cytoplasmic domain encoding an immunoreceptor tyrosine-based switch motif (ITSM) [19]. Subsequent studies revealed that PD-1, or CD 279, exists as a monomer on the cell surface of activated T cells and is induced by TCR signaling, γ -chain cytokines (IL-2, IL-7, IL-15, IL-21), and type I interferons [20, 21]. Unlike CTLA-4, PD-1 is also expressed on activated B cells, NK cells, antigen presenting cells, and myeloid cells. Two ligands for PD-1 have been identified, PD-L1 (CD274) and PD-L2 (PDCD1LG2). Both are B7-homologs found on chromosome 9 only 42 kb apart [22]. Both ligands are expressed in response to γ -chain cytokines, Type I interferons, and interferon- γ . GM-CSF and IL-4 can also induce expression of PD-L2. Expression of PD-L1 occurs not only on immune cells, but also epithelial cells including cancer cells. Binding of ligands to PD-1 inhibits T cell activation, and thus dampens the immune response.

THERAPEUTIC SUCCESSES AND CHALLENGES OF IMMUNE CHECKPOINT BLOCKADE

Once CTLA-4 and PD-1/PD-L1 were determined to be negative regulators of anti-tumor immunity, clinical exploration of antibodies targeting those pathways ensued. Between 2011 and 2017 four immune checkpoint inhibitors were approved for six different diseases (Table 1). Below we review the successes and difficulties encountered during the clinical development of these drugs. Numerous other immune checkpoint inhibitors are currently under development and expected to be approved in the coming years.

Ipilimumab

The first clinically successful immune checkpoint inhibitor was ipilimumab, a fully human monoclonal IgG1 antibody targeting CTLA-4. This drug was first approved for the treatment of metastatic melanoma in 2011. Therapeutic success with this drug demonstrated proof of concept that targeting negative immune regulation could lead to tumor regression in patients, ushering in a new era in cancer immunotherapy. The first clinical study in this drug was published in 2003 and included 14 patients with metastatic melanoma [23]. In that study, ipilimumab (MDX-010) at 3 mg/kg was administered intravenously every 3 weeks in combination with subcutaneous vaccination with two modified HLA-A*0201-restricted peptides, gp100:209-217(210M) and gp100:280-288(288V). The mean peak plasma concentration of the antibody after the first dose was 72 +/- 33 µg/mL with a trough before the second dose of 12 +/- 7 µg/mL. There was cumulative levels observed with a mean post-therapy value after all cycles of 99 +/- 41 µg/mL. The weeks after completion of therapy this value declined to 17 +/- 10 µg/mL. In

that study, there was no observed correlation between concentration and efficacy or toxicity.

Patients were heavily pretreated including many with prior immunotherapy such as IFN- α , IL-2, or peptide vaccines. In spite of the pretreatment, objective responses were observed in three patients, including two complete responses. Even in this small study, incredible insight was gained into the clinical management of immune checkpoint inhibitors, most of which remains relevant to current clinical research in immune checkpoint modulation. For instance, pseudoprogression, or radiologic progression likely resulting from immune cell infiltration rather than tumor growth, was reported in one patient who had a subsequent complete response. This concept is now well understood to clinicians using immune checkpoint inhibitors. One responding patient had a brain metastasis, thus indicating that immune checkpoint inhibition could impact CNS as well as visceral metastases. Autoimmune toxicities were observed and characterized in great detail. High-grade toxicities included dermatitis, enterocolitis, hepatitis, and hypophysitis. It important to highlight these observations noted in this early study, which informed much of what is known today about the clinical use of immune checkpoint blockade.

Ipilimumab then progressed to two large phase III studies in metastatic melanoma. In one study, patients with progression on prior therapy were randomized to three treatment arms including doublets of ipilimumab (3 mg/kg intravenous infusion every 3 weeks), a gp100 peptide vaccine, and/or placebo [24]. Overall survival was better in the ipilimumab group (10.0 months, 95% confidence interval, 8.5-11.5) versus the group receiving gp100 vaccine alone (6.4 months, 95% CI, 5.5 - 8.7). No difference was

observed between the ipilimumab groups receiving placebo or gp100 vaccine. The second study compared, in the first line setting, administration of decarbazine 850 mg/m² plus either placebo or ipilimumab at 10 mg/kg given every 3 weeks [25]. Again an improvement in overall survival was observed with the rate at three years being 20.8% versus 12.2%. These studies led to approval of ipilimumab in 2011 for metastatic melanoma. One of the most important benefits of immune checkpoint blockade over other therapies is the observed durability of responses. Long-term pooled follow up data from ipilimumab-treated patients suggests that the survival rate plateaus at around 20% at the 3 year-mark and extends to at least 10 years [26].

Despite the major breakthroughs gained from the discovery and development of ipilimumab, significant challenges remain. The precise optimal dosing strategy remains to be determined. The higher dose (10 mg/kg) used in the phase III decarbazine +/- ipilimumab trial resulted in a greater incidence of high-grade toxicities. Subsequent studies have established that efficacy is also dose dependent when comparing 0.3 mg/kg, 3 mg/kg, and 10 mg/kg [27]. Results from additional studies comparing 3 versus 10 mg/kg remain pending (NCT 01515189). Management of toxicities is an important challenge as some patients can have severe, and even-life threatening, immune-related adverse events (iRAEs). As experience has grown using ipilimumab, strategies to mitigate risk have been developed including corticosteroid administration. One of the most important factors is early recognition by clinicians. If acted upon quickly, the majority of iRAEs are reversible.

Pembrolizumab

Successful targeting of CTLA-4 as a cancer therapeutic led to the pursuit and discovery of numerous other negative regulators of immune function. The PD-1 pathway was next proven to be a viable target in cancer immunotherapy. Pembrolizumab is a humanized, monoclonal IgG4- κ antibody against PD-1. It was first approved in 2014 after results were published from a randomized clinical trial in metastatic melanoma patients. In this study, there were three nonrandomized cohorts with the first receiving pembrolizumab at a dose of 10 mg/kg every 2 weeks, and the other 2 cohorts receiving pembrolizumab every 3 weeks at either 2 mg/kg or 10 mg/kg [28]. Peak and trough serum concentrations were lower by 5-fold in patients receiving 2 mg/kg versus 10 mg/kg every 3 weeks. The trough concentration was 20% higher in patients receiving 10 mg/kg every 2 weeks versus 10 mg/kg every 3 weeks. The half-life was estimated to be 14-21 days.

The response rate for the 135 treated patients was 38%. As with ipilimumab, durability of responses was demonstrated with 81% of responding patients still receiving treatment after 11 months. Prior use of ipilimumab did not show any association with response. The efficacy of pembrolizumab in ipilimumab-refractory melanoma was confirmed in a larger 540-patient study that showed 6-month progression free survival improved to 38% with pembrolizumab versus 16% with investigator's choice chemotherapy [29].

A later expanded analysis of pembrolizumab from 655 patients from multiple studies confirmed durability of response with 74% lasting more than 12 months [30]. Response rate was 33% and median overall survival was 23 months. Compared to ipilimumab, fewer high-grade adverse events were observed with pembrolizumab. Grade

3-4 toxicities were reported in only 14% of patients, with the most common being fatigue (2%), rash (1%), diarrhea (1%), asthenia (15), and dyspnea (1%). When any grade toxicity was considered, fatigue, pruritus, and rash were the most common toxicities. Overall pembrolizumab was very well tolerated with minimal toxicities in the majority of patients.

In addition to the improved side-effect profile, pembrolizumab was also demonstrated to be more broadly effective against a wider variety of cancers compared with anti-CTLA-4 therapy. A 495-patient study in advanced non-small cell lung cancer (NSCLC) showed an overall response rate of 19.4% and median overall survival of 12 months [29]. Importantly, this study also assessed the PD-L1 expression by an immunohistochemical assay that ultimately defined the population of patients most likely to respond. PD-L1 positivity was defined as the percentage of cells (neoplastic and mononuclear inflammatory cells) that showed membranous staining. In a population of patients with baseline tumor expression of >50%, the response rate was 45.2%. Median overall survival was not reached in this group. In 2015, pembrolizumab gained approval for metastatic non-small cell lung cancer with >50% PD-L1 expression after progression on platinum-based chemotherapy. The demonstration of clinical efficacy in second-line NSCLC led to the exploration of pembrolizumab in the first line setting for patients with tumors having >50% PD-L1 expression [31]. In this randomized phase III study, pembrolizumab resulted in a longer progression free survival than investigator's choice chemotherapy (10.3 versus 6.0 months). Pembrolizumab was then approved in the first line setting.

Advanced head and neck squamous cancer patients with disease progression on or after platinum-containing chemotherapy were treated with pembrolizumab and the overall response rate was 16%, with 82% of responses lasting 6 months or longer [32]. This study led to its approval for head and neck squamous cancer in 2016. Encouraging data have been reported for several other indications including urothelial bladder cancer and Merkel cell carcinoma, for which there has never been an approved therapy [33, 34].

Nivolumab

Nivolumab is a fully human IgG4 anti-PD-1 antibody that was developed at the same time as pembrolizumab. A phase I study included cohorts of multiple tumor types and activity was seen in metastatic melanoma, renal cancer, and non-small cell lung cancers [35]. In melanoma the response rate was 28% with most responses lasting >1 year. Non-small cell lung cancer and renal cancer had response rates of 18% and 27%, respectively. An expansion cohort in melanoma was then reported that showed an objective response rate of 31% and a median overall survival of 16.8 months (95% CI, 12.5 to 31.6 months) [36]. Nivolumab was approved for metastatic melanoma in 2014, the same year as pembrolizumab's approval.

In renal cancer, it was studied head to head versus everolimus in the second line setting. It showed a longer median overall survival (25.0 versus 19.6 months) and the hazard ratio for death of 0.73 (98.5% CI, 0.57 to 0.93; P=0.002) indicated superiority according to prespecified criteria. There were fewer grade 3 to 4 adverse events in the nivolumab group (19% versus 37%). These data resulted in the 2015 approval of nivolumab for metastatic renal cancer in the second-line setting.

Nivolumab was tested further in previously treated squamous non-small cell lung cancer in comparison to docetaxel, a standard second line therapy [37]. Nivolumab was found to have a 42% 1 year overall survival rate compared with 24% for docetaxel. The median overall survival was 9.2 months for nivolumab compared to 7.3 months for docetaxel, with 41% lower risk of death for nivolumab ($p < 0.001$). In non-squamous, non-small cell lung cancer a randomized study with 582 patients also showed a longer overall survival with nivolumab versus docetaxel (12.2 versus 9.4 months). Median progression-free survival was not different between groups (2.3 months for nivolumab versus 4.2 months for docetaxel), yet progression free survival at 1 year was higher for nivolumab (19% versus 8%). This discrepancy highlights the challenges with immune checkpoint therapy studies, since the duration of response is often not captured in other clinical endpoints such as median progression free survival data. As with pembrolizumab, nivolumab was studied in the first-line setting after efficacy was demonstrated in the second line. A randomized phase III study between nivolumab versus physician's choice combination chemotherapy failed to demonstrate any difference in progression free survival. Unlike the first-line pembrolizumab trial for NSCLC, this trial enrolled patients with just 5% or greater PD-L1 expression. It is likely that the failure of this trial to achieve its endpoint was related to patient selection. Nivolumab has since gained approval in advanced or metastatic urothelial carcinoma and head and neck squamous carcinoma.

Atezolizumab

The fourth approved immune checkpoint inhibitor was atezolizumab, which came on the market first for the treatment of urothelial bladder cancer. This was the first

approved drug for advanced bladder cancer in over 20 years [38]. It is a human IgG1 antibody targeting PD-L1, in contrast to PD-1. Early studies in advanced urothelial cancer showed an objective response rate 43% in patients with PD-L1 positive tumors (defined by expression of PD-L1 by tumor infiltrating immune cells by immunohistochemistry) [39]. The complete response rate of 7% was notable, and the duration of response was greater than 6 months in almost all responders, consistent with the durability observed with other immune checkpoint inhibitors. Atezolizumab subsequently gained approval in non-small cell lung cancer based on improved overall survival versus docetaxel (12.6 versus 9.7 months) [40].

Limitations to checkpoint inhibitors: Resistance, toxicities, and biomarkers

One of the most important limitations to immune checkpoint inhibitors is their modest overall response rate. While the depth and durability of responses have been life-changing for many cancer patients, most patients will continue to have disease progression despite treatment with anti-CTLA-4 and anti-PD-1 targeted immunotherapy. Patients with initial responses to immune checkpoint inhibitors also frequently develop progression after a period of time. As our understanding of tumor immunology continues to grow tremendously, the importance of elucidating potential mechanisms of primary and secondary resistance is has become paramount.

Pre-clinical data dating back to 2010 suggested that the combination of both anti-CTLA-4 and anti-PD-1 therapies has a higher response rate than either agent alone [41, 42]. Subsequent clinical trial data has confirmed that this strategy overcomes primary resistance in at least a subset of patients. In 2015 a double blind study was published in

which 143 metastatic melanoma patients were randomized 2:1 to ipilimumab (3 mg/kg) combined with nivolumab (1 mg/kg) or placebo every three weeks in the first-line setting [43]. For BRAF-wild type patients, the objective response rate was 61% in the nivolumab group versus 11% in the placebo group. The median overall survival was not reached in the nivolumab group and only 4.4 months with placebo. The resulting hazard ratio for death was 0.4 (95% CI 0.23-0.68, $P < 0.001$). In another study, three arms were used to compare the combination versus either agent alone [44]. Median progression-free survival was longest in the combination arm (11.5 months, 95% CI, 8.9 to 16.7). PD-L1 expression by immunohistochemistry was used as an exploratory biomarker. For patients with PD-L1-positive tumors the median progression-free survival was 14.0 months in both nivolumab or the combination group. In contrast, patients with PD-L1-negative tumors seemed to benefit from the addition of ipilimumab, as PFS 11.2 months for the combination versus 5.3 months with nivolumab alone. [95% CI, 8.0 to not reached] vs. 5.3 months [95% CI, 2.8 to 7.1]). In 2015 the combination was approved for use in metastatic melanoma. Further studies are investigating this combination in other cancer types.

One of the touted benefits of immune checkpoint blockade is its impressive tolerability in comparison to traditional cytotoxic therapies, but the magnitude of this benefit diminishes when combining therapies [43, 44]. In both combination studies noted above, patients receiving anti-CTLA-4 with anti-PD-1 therapy had much higher rates of grade 3-4 drug related adverse events (54-55%) versus the monotherapy groups (16-27%). While generally these toxicities are typically reversible and treatable, they are serious and clinicians need to remain vigilant in order to expeditiously identify and treat

severe immune related adverse events (irAEs). Toxicities most commonly observed with immune checkpoint inhibition are quite distinct from those related to cytotoxic chemotherapy or molecular targeted therapies. Common symptoms of irAEs include rash, diarrhea, shortness of breath, and cough, which reflect underlying dermatitis, colitis, pneumonitis, and hypothyroidism [45-48]. Furthermore, any other new or unusual symptoms must be considered to be drug-related until proven otherwise. Even more rare autoimmune manifestations such as inflammatory arthritis, sicca syndrome, myositis, vasculitis, lupus nephritis, and immune thrombocytopenia (ITP) are now being reported with the widespread use of these drugs [49]. Even irreversible autoimmune diabetes has been reported after PD-1 targeted therapy [50, 51]. Nonetheless, most irAEs are treatable with early cessation of the drug and/or administration of corticosteroids, and the incidence of fatal irAEs is <1% [48].

Given that a significant number of patients do not benefit from immune checkpoint inhibitors, much exploratory research has been aimed at identifying predictive biomarkers. PD-L1 expression predicts a higher response to anti-PD-1 therapy rate in most cancers where activity has been observed, however, its negative predictive value is low. In some studies, up to 33% of PD-L1-negative patients responded to therapy [52]. Heterogeneity of tumor sampling, variable assay methodology, and differences in cells assessed (immune infiltrating cell versus tumor cell PD-L1 expression) likely explain much of the difficulty in using this biomarker. PD-L1 expression is associated with the presence of tumor-infiltrating T lymphocytes. It has become evident though several studies that T cell infiltration itself is associated with a higher response rate to immunotherapies compared with patients with tumors that are devoid of immune cells

[42, 53, 54]. This phenotype has been referred to as the T cell-inflamed tumor microenvironment (Figure 2), and can be identified by immunohistochemistry or gene expression profiling. Ongoing research is aimed at understanding the mechanisms behind development of the non-T cell-inflamed, or immune resistant, phenotype. Additionally, mutational burden has been linked with improved response to immune checkpoint blockade and this characteristic appears to be independent of the T cell inflamed phenotype [55]. Yet neoantigen burden is not consistent as a predictive biomarker on its own, since there are observed responses in some patients with relatively low tumor mutational burden and vice versa. Elucidating the complex interplay between neoantigens and pre-existing adaptive immunity will require prospective trials designed with clearly defined assay characteristics and endpoints, in order to increase the scientific yield in assessing predictive biomarkers [56].

CONCLUSIONS AND FUTURE DIRECTIONS

Cancer therapy has undergone a sea change with the development and widespread adoption of immune checkpoint therapy. The remarkable complete responses observed in some patients with diseases previously considered rapidly fatal cannot be understated. Looking forward, the growth of successful immunotherapeutic strategies will be dependent on further developing a deeper understanding of the immune biology of resistant tumors so that a wider group of patients benefit.

There are many hypotheses regarding why some tumors do not respond to immune checkpoint inhibitors. Broadly speaking, resistance mechanisms can be categorized as tumor-intrinsic, environmental, or genetic. Tumor-intrinsic molecular

alterations that may lead to resistance have been identified in human samples, such as β -catenin, FGFR3, PTEN, and JAK 1/2 [55, 57-59]. Exploration into the mechanisms at play and/or their use as predictive biomarkers is ongoing. Interesting developments into environmental factors governing resistance include commensal gut bacteria that have been now linked with resistance to immune checkpoint blockade [60, 61]. Finally, it is possibly that genetic factors influencing immune response may impact antitumor immune responses, although a potential pharmacogenomic association has not yet been explored in depth.

Owing to the successes of anti-CTLA-4 and anti-PD-1 immunotherapy, numerous other immune checkpoints are now under investigation as monotherapy or in combination, including lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin-3 (TIM-3), and indoleamine-2,3-dioxygenase (IDO) [62-64]. Furthermore, combining immunotherapy with chemotherapy, radiotherapy, or molecularly targeted therapy is also being explored across nearly all tumor types. Given the thousands of potential combination studies possible, clinical trials need to be selectively designed with a sound preclinical and scientific rationale, which necessitates collaboration between basic, translational, and clinical scientists. With these recent breakthroughs in cancer immunity, and more anticipated, patients now have more hope than ever before.

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Figures/Tables:

Table 1. Approved cancer therapies targeting negative immune regulation

Figure 1. Binding of the T cell receptor to the peptide:MHC complex alone is not sufficient to activate T cells. Costimulation is necessary from the binding of B7-1/B7-2 to CD28. Inhibitory receptors such as PD-1 and CTLA-4 have been discovered, which blunt costimulation, prevent T cell activation, and result in T cell anergy and/or apoptosis.

Figure 2. Depiction of the non- T cell-inflamed versus T cell-inflamed tumor microenvironment. T cell-inflamed tumors are characterized by the presence of tumor-infiltrating lymphocytes, dendritic cells, chemokines, and type I IFN. The infiltration of tumor-antigen specific T cells results in the development of adaptive immune resistance via negative immune regulatory pathways, such as PD-L1 and other molecules not depicted, which suppress the anti-tumor immune response. Thus, blocking negative regulatory pathways is more likely to lead to T cell activation and regression of tumors. Non-T cell inflamed tumors lack immune infiltrating cells and are unlikely to respond to immune checkpoint blockade, as there is no adaptive resistance present to reverse.

Figure 1

Figure 2

Non T cell-inflamed

T cell-inflamed

Table 1. Approved cancer therapies targeting negative immune regulation

Target	Drug	Approval year	Indication	Dose/schedule
CTLA-4	Ipilimumab	2011	Melanoma (metastatic/unresectable)	3 mg/kg IV every 3 weeks for a maximum of 4 doses
		2015	Melanoma (adjuvant)	10 mg/kg IV every 3 weeks for 4 doses, then every 12 weeks up to 3 years
PD-1	Pembrolizumab	2014	Melanoma (metastatic/unresectable)	2 mg/kg IV every 3 weeks
		2015	Non-small cell lung cancer (first-line if PD-L1>50%)	
		2015	Non-small cell lung cancer (post-platinum chemotherapy if PD-L1>1%)	200 mg IV every 3 weeks
		2016	Head and neck cancer	
	Nivolumab	2014	Melanoma (metastatic/unresectable)	
		2015	Non-small cell lung cancer (post-platinum chemotherapy)	240 mg IV every 2 weeks
		2015	Renal cell carcinoma	
		2016	Hodgkin Lymphoma	3 mg/kg IV every 2 weeks
		2016	Head and neck cancer	
		2017	Urothelial cancer	240 mg IV every 2 weeks
PD-L1	Atezolizumab	2016	Urothelial cancer, lung cancer	
		2016	Non-small cell lung cancer (post-platinum chemotherapy)	1200 mg IV every 3 weeks
CTLA-4 + PD-1	Ipilimumab + Nivolumab	2015	Melanoma (metastatic/unresectable)	Nivolumab 1 mg/kg IV with ipilimumab 3 mg/kg IV every 3 weeks for 4 doses, then nivolumab 240 mg IV every 2 weeks

Blocking immune checkpoints