Review Article

Importance of immunopharmacogenomics in cancer treatment: Patient selection and monitoring for immune checkpoint antibodies

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In the last 5 years, immune checkpoint antibodies have become established as anticancer agents for various types of cancer. These antibody drugs, namely cytotoxic T-lymphocyte-associated antigen, programmed death-1, and programmed death ligand-1 antibodies, have revealed relatively high response rates, the ability to induce durable responses, and clinical efficacy in malignancies not previously thought to be susceptible to immune-based strategies. However, because of its unique mechanisms of activating the host immune system against cancer as well as expensive cost, immune checkpoint blockade faces novel challenges in selecting appropriate patient populations, monitoring clinical responses, and predicting immune adverse events. The development of objective criteria for selecting patient populations that are likely to have benefit from these therapies has been vigorously investigated but still remains unclear. In this review, we describe immune checkpoint inhibition-specific challenges with patient selection and monitoring, and focus on approaches to remedy these challenges. We also discuss applications of the emerging field of immunopharmacogenomics for guiding selection and monitoring for anti-immune checkpoint treatment.

Introduction and principles of immune checkpoint blockade

Immunotherapies such as immune checkpoint antibodies have revolutionized cancer treatment. Rather than directly targeting cancer cells, immune checkpoint antibodies target proteins that inhibit the host's natural immune response towards cancer cells and then strongly activate the host immune response to eliminate cancer cells. At present, the three major target molecules for such blockade are cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed death-1 (PD-1), and programmed death ligand-1 (PD-L1). Both CTLA-4 and PD-1/PD-L1, which act through distinct mechanisms, are key regulators responsible for maintaining homeostasis during the T cell-mediated immune response. To date, ipilimumab (anti-CTLA-4 antibody), nivolumab and pembrolizumab (anti-PD-1 antibodies) are approved for advanced melanoma, and nivolumab and pembrolizumab are also approved for advanced non-small-cell lung cancer (NSCLC).[1–3]

Application of antibodies against these immune checkpoint molecules in cancer therapy was first proposed in the 1990s.[4,5] A type I transmembrane protein, CTLA-4 (also called CD152) is expressed on the surface of regulatory T cells and regulates the amplitude of the T cell response. Antigen-presenting cells (APCs) display antigens on MHC to the T cell receptor (TCR) of T lymphocytes. Effective T cell responses require costimulatory signals transmitted through the engagement of CD28 on the surface of T cells with CD80 (also known as B7.1) and CD86 (B7.2) on APCs (Fig. 1).[5] CTLA-4 competes with CD28 for binding with CD80 and CD86, resulting in the inhibition of TCR signaling, suppression of effector T cell activation, and attenuation of the T cell-mediated immune response.[6] Because of these functions, CTLA-4 plays indispensable roles in the prevention of autoimmunity and the perpetuation of self-tolerance.[4] Anti-CTLA-4 antibody was the first immune checkpoint antibody shown to have antitumor potential, as shown in a landmark study in mice in 1996.[4–6] Clinical trials later proved that CTLA-4 blockade could also be applied to have potent antitumor abilities in humans.[7,8]

Programmed death-1 is also a negative regulator of the T cell immune response that physiologically functions to avoid collateral damage caused by an overactive T cell response in peripheral tissues (Fig. 2). PD-1 is expressed on activated T cells as well as B cells and natural killer cells, and engages with B7 family ligand partners, either PD-L1 (also known as B7-H1 and CD274) or PD-L2 (B7-DC and CD273), found in peripheral tissue cells, APCs, and tumor cells.[9] Binding of
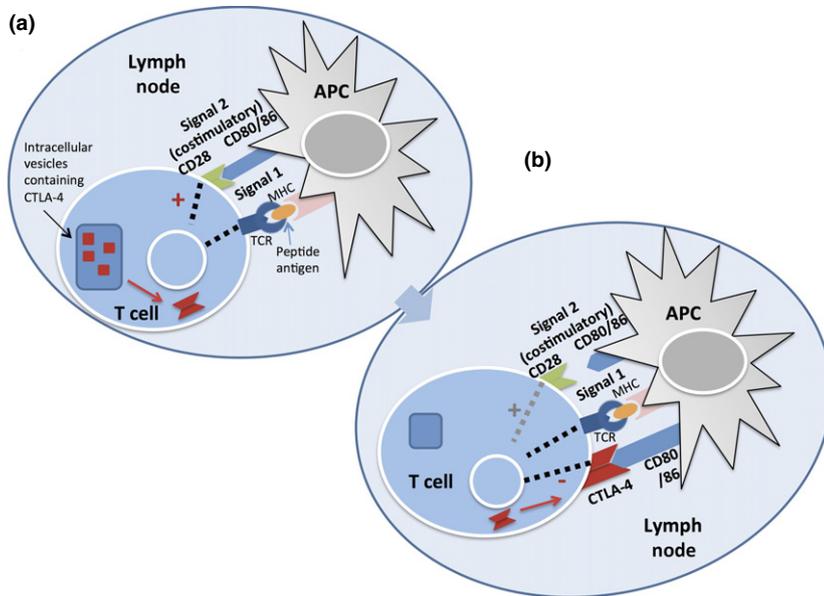
(a)

(b)

Fig. 1. (a) T cell activation requires both signal 1, mediated by antigen presentation on MHC by dendritic cells to the T-cell receptor (TCR), and costimulatory signals from CD80/86 engagement with CD28 on the surface of T cells. This initial activation sends signals to release cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) from intracellular vesicles. (b) Downregulation of T cell activity can occur distinctly through two mechanisms. CTLA-4 is upregulated on the surface of T cells in response to initial activation, and outcompetes binding to CD80/86. In the periphery, tumor cells can present programmed death ligand-1 to programmed death-1 on the surface of T cells and also induce downregulation of T cell activity. APC, antigen-presenting cell; MHC, major histocompatibility complex.
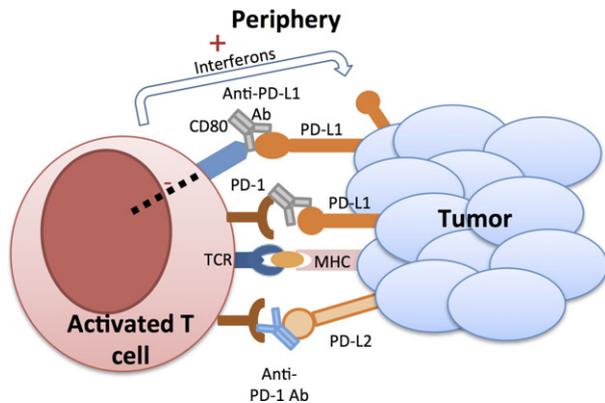

Fig. 2. Schematic of the actions of anti-programmed death-1/programmed death ligand-1 (PD-1/PD-L1) antibodies. In the periphery, PD-1 can be inducibly expressed on the surface of T cells, as well as B cells and monocytes. T cell activation releases interferons that cause the upregulation of PD-L1 and PD-L2 on the surface of tumors (as well as on T cells, B cells, and antigen-presenting cells, not shown here). Binding between PD-1 and PD-L1 causes downregulation of T cell activity and is intended to limit overly aggressive immune response in the periphery, but is capitalized by tumor cells to limit the antitumor response of the adaptive immune system. PD-L1 on tumor cells also binds to CD80 on T cells, further initiating downregulation of activated T cells. Anti-PD-1 antibodies disrupt the PD-1/PD-L1 interaction, as well as PD-1/PD-L2 and PD-L1/CD80 interaction. TCR, T-cell receptor; ab, antibody; MHC, major histocompatibility complex.

selves from cytotoxic T cell attack by increasing expression of PD-L1 on their surface, which anergizes activated T cells. Studies in mice showed that blockade of the PD-1/PD-L1 interaction could be used as a promising anticancer strategy.[13] Of note, the mechanisms to block PD-1 or PD-L1 are not equivalent because blockade of PD-L1 leaves the PD-1/PD-L2 interaction intact, which may maintain T cell anergy.

The U.S. FDA approved ipilimumab, a fully humanized monoclonal IgG1 antibody against CTLA-4, in 2011 for treatment of advanced melanoma. At the time, ipilimumab was the first anti-immune checkpoint agent to show survival benefit in patients with metastatic melanoma.[14] Pembrolizumab, a PD-1 antibody, was granted accelerated approval in 2014 by the FDA in advanced melanoma for patients previously treated with ipilimumab or BRAF inhibitors based on two studies.[15,16] Two anti-PD-1 antibodies, pembrolizumab and nivolumab, showed superior overall survival in ipilimumab-refractory melanoma compared to chemotherapy, conclusively establishing these antibodies as standard of care after ipilimumab in advanced melanoma.[17,18]

Progress in clinical trials: optimizing the regimen

After the approval of these three agents in advanced melanoma and NSCLC, these drugs have been tested in various cancer types. In some trials, the immune checkpoint blockade antibodies are combined with each other or other systemic therapies. For example, in both advanced and untreated melanoma, compelling evidence is emerging that PD-1 blockade may be more efficacious than CTLA-4 blockade. The first high-profile phase III trial to compare pembrolizumab with ipilimumab showed improved progression-free and overall survival rates in a pembrolizumab-treated group, compared with ipilimumab alone, with lower incidence of drug-related grade 3–5 adverse events.[19] Combination trials published in 2015 have indicated that the combination of nivolumab and ipilimumab also has superior progression-free survival over ipilimumab monotherapy,[20–22] strongly suggesting PD-1 blockade may be superior to CTLA-4 blockade in melanoma.

Combining the immune checkpoint antibodies with other therapies, including systemic chemotherapies and molecular

PD-L1/2 to PD-1 inhibits kinase signal pathways involved in T cell activation. Chronic antigen exposure, caused by chronic viral infection or cancer, was first shown to induce high expression of PD-1 (considered to represent a state of T cell exhaustion or anergy) which can be reversed upon the blockade of the PD-1/PD-L1 interaction.[10]

Similar to CTLA-4, cancer cells capitalize on the inhibitory role of the PD-1/PD-L1 interaction to evade host T cell immune attack on cancer cells.[11] Quantitative analysis of 150 melanoma specimens revealed that PD-L1 expression in tumor cells is tightly colocalized with tumor-infiltrating lymphocytes in cancer tissues and is also upregulated geographically in areas of high γ-interferon production.[12] Therefore, cancer cells protect them-

targeted therapies, has also been explored, although such combinations are often affected by higher incidences of adverse events.[23,24] For example, the combination of ipilimumab and dacarbazine has better survival than dacarbazine alone for untreated melanoma patients, but 56% of patients treated with the combination treatment experienced grade 3–4 treatment-related adverse events.[25]

In addition to melanoma and NSCLC, immune checkpoint blockade is gaining traction in other cancer types, including refractory non-Hodgkin's lymphoma, metastatic bladder cancer, intensively treated renal cell carcinoma, and colorectal cancers with mismatch repair deficiencies (Table 1).[26–29] With over 130 active clinical trials registered in the USA, it is beyond the scope of this review to highlight all the cancer types and combinations of immune checkpoint blockade therapies that are underway. Of note, while the immune checkpoint antibodies have shown positive results in a large number of clinical trials, at least one phase III trial using ipilimumab in metastatic castrate-resistant prostate cancer failed to demonstrate a positive result.[30]

Immunotherapy-specific challenges in clinical evaluation

One of the most exciting characteristics of the checkpoint inhibitors is that the clinical responses observed have been remarkably durable even after cessation of treatment.[31] In a pooled analysis of 1861 patients treated with ipilimumab, 22% survived for at least 3 years, with the Kaplan–Meier survival curve achieving a plateau that extended from 3 to 10 years after treatment.[32] Similar durability has been seen with anti-PD-1 inhibitors. In two major NSCLC trials, 28% and 27% of patients survived at least 18 months.[33] In melanoma, 43% of patients survived at least 2 years.[34] Hence, patient selection and monitoring for immunotherapy, if established, has the potential to offer unprecedented durable responses in other refractory stages of disease.

The first immune checkpoint blockade-specific challenge in patient selection is how to define clinical efficacy with immune checkpoint inhibitors. In general, tumor responses to immunotherapies have shown tendencies to deviate from the widely used Response Evaluation Criteria in Solid Tumors (RECIST), meaning that a subset of patients who ultimately survive on therapy do not meet criteria for objective response during the trial period. The primary biological response of immune checkpoint blockade is blockage of immune suppressive molecules, resulting in activation of intratumoral infiltrated T lymphocytes and increase in cytokine production. Due to the inflammation caused by these secreted cytokines, tumor sizes may initially enlarge (termed pseudo-progression)[31] on radiographic assessment early after the initiation of treatment, before causing tumor shrinkage.[35,36] Accordingly, a delayed separation of survival curves from control arms has been observed in multiple trials. For example, in follow-up reports of the earliest CTLA-4 blockade trials, the average time among all patients to achieve complete response was 30 months, a period before which clinical trials often conclude.[37] Therefore, immune checkpoint blockade therapy requires innovative strategies for monitoring and evaluating patient response based on the mode of action of the drugs.

Patient selection: predictive biomarkers

In the era of precision medicine, it is critically important to address how to select the patients that are likely to derive benefit from immune checkpoint blockade therapy.[38,39] Investigation into identifying factors involved in the immune checkpoint pathways in the tumor immune microenvironment has been extensively carried out and several molecules have been proposed to predict clinical response.

Intratumoral PD-L1 expression for patient selection. A number of studies investigating the safety and efficacy of PD-1/PD-L1 antibodies reasonably hypothesized that tumor cell expression levels of PD-L1 would predict response to both therapies. An early phase I trial suggested that tumors with positive PD-L1 staining by immunohistochemistry showed better response to anti-PD-1 agents than PD-L1-negative tumors, with an objective response rate of 36% versus 0%.[40] A meta-analysis of 1475 patients concluded that PD-L1-positive tumors also have an improved response rate compared to PD-L1-negative tumors (34% vs 19.9%).[41] However, no clear threshold for positivity of PD-L1 has been defined. Although many trials applied 5% staining in tumor cells as positive, a phase I study of patients with NSCLC treated with pembrolizumab applied a cut-off of 50% positive staining in tumor cells and obtained an objective response rate of 45.2% with a median overall survival of 26 months in PD-L1-positive patients. Although the authors concluded that the PD-L1 positivity in >50% of tumor cells is a promising biomarker, tumors with as few as 1% of tumors cells staining positive for PD-L1 still showed a median overall survival of >8 months.[42] In addition, multiple trials have shown no correlation or inconclusive correlation between the clinical response and PD-L1 status in cancer tissues (Table 2). Therefore, the mechanism of how PD-L1-negative patients respond to anti-PD-1 treatment still needs to be clarified. Of interest, pembrolizumab was recently approved specifically for use in NSCLC for PD-L1-positive tumors as defined by a commercial immunohistochemical diagnostic assay.[3]

While many studies define PD-L1 positivity according to tumor cell expression, PD-L1 expression on immune cells, including macrophages, dendritic cells, and T lymphocytes, has also been investigated. One study of anti-PD-L1 antibody in multiple tumor types reported significant association between PD-L1 expression in tumor-infiltrating immune cells and clinical response ($P < 0.007$).[43] However, a different study of 41 patients comprised of five solid-tumor types treated with nivolumab showed no association between PD-L1 expression in immune cells with objective response.[12]

The variability of the results listed above is generated by a number of factors, including the complexity and dynamic nature of the PD-1/PD-L1 interaction, subjectivity of PD-L1 staining, variations in techniques and assays used, tumor heterogeneity, and use of archival tissues that may not reflect the status of PD-1/PD-L1 interaction at time of treatment.[44] Because of the variability of results across trials and the inability to exclude patients with negative PD-L1 expression as poor responders, there is still no solid evidence for applying PD-L1 expression level on tumor or immune cells as a sole criterion for patient selection.[45]

Intratumoral and tumor microenvironment correlate as predictors. Intensive efforts have also highlighted specific factors in the tumor microenvironment as potential predictive biomarkers because of their influence on drug action. Herbst et al.[43] analyzed pretreatment and on-treatment tumor biopsies from patients treated with MPDL3280A across seven solid malignancies and found an association between high CTLA-4 expression in pretreatment tissue samples and good therapeutic response. Other investigators have similarly shown that high intratumoral baseline expression of FoxP3 and indoleamine

Table 1. Major clinical trials with immune checkpoint blockade

Therapy	Author, year, journal	Cancer type	Phase (no. of patients)	Findings, median PFS in months unless otherwise stated
Pembrolizumab versus ipilimumab	Robert, 2015, NEJM[19] (KEYNOTE-006)	Advanced melanoma	Phase 3 (834)	5.5 (pembrolizumab every 2 weeks) versus 4.1 (pembrolizumab every 3 weeks) versus 2.8 (ipilimumab)
Nivolumab with ipilimumab versus nivolumab or ipilimumab monotherapy	Larkin, 2015, NEJM[20]	Untreated melanoma	Phase 3 (945)	2.9 (ipilimumab) versus 6.9 (nivolumab) versus 11.5 (combination)
Nivolumab plus ipilimumab versus ipilimumab monotherapy	Postow, 2015, NEJM[21]	Untreated melanoma	Phase 2 (142)	Not reached (combination) versus 4.4 (ipilimumab) (BRAF-WT tumors)
Nivolumab plus ipilimumab, concurrently and sequentially	Wolchok, 2013, NEJM[22]	Advanced melanoma	Phase 1 (86)	ORR 40% (21/52) (concurrent) versus 20% (6/30) (sequential)
Pembrolizumab	Garon, 2015, NEJM[42] KEYNOTE-001	NSCLC	Phase I (495)	3.7 (all pts); 3.0 (previously untreated pts); 6.0 (previously untreated); PD-L1 positive expression: PFS 6.3
Pembrolizumab	Le, 2015, NEJM[27]	Mismatch repair-deficient cancers	Phase 2 (41)	Immune-related PFS 78% (7/9) (mismatch repair deficient) versus 11% (2/18) (mismatch proficient) colorectal cancer
Pembrolizumab	Ribas, 2015, Lancet Oncology[17] KEYNOTE-002	Ipilimumab-refractory melanoma	Phase 2 (540)	PFS at 6 months: 34% (2 mg/kg) versus 38% (10 mg/kg) versus 16% (ICC)
Lambrolizumab	Hamid, 2013, NEJM[15]	Advanced melanoma	Phase 1 (135)	>7.0 (all patients)
Pembrolizumab	Robert, 2014, Lancet[16]	Ipilimumab-refractory melanoma	Phase 1 (173)	5.5 (pembrolizumab 2 mg/kg) versus 3.5 (10 mg/kg) Immune-related response criteria: PFS 7.8 (2 mg/kg)versus 8.8 (10 mg/kg)
Ipilimumab	Hodi, 2010, NEJM[14]	Advanced melaonma	Phase 3 (676)	Median OS 10.0 (ipilimumab + gp100) versus 6.4 (gp100 alone)
Ipilimumab	Robert, 2011, NEJM[25]	Untreated melanoma	Phase 3 (502)	OS 11.2 (dacarbazine + ipilimumab) versus 9.1 (dacarbazine + placebo)
Nivolumab	Weber, 2015, Lancet Oncology[18] Checkmate 037	Ipilimumab or BRAF inhibitor (BRAF mutated)-refractory melanoma	Phase 3 (272)	4.7 (nivolumab) versus 4.2 (ICC)
Nivolumab	Motzer, 2015, JCO[28]	Clear-cell, previously treated renal cell carcinoma	Phase 2 (168)	2.7 (0.3 mg/kg) versus 4.0 (2 mg/kg) versus 4.2 (10 mg/kg)
Nivolumab	Robert, 2015, NEJM[19]	Untreated melanoma without BRAF mutation	Phase 3 (418)	5.1 (nivolumab) versus 2.2 (dacarbazine)
Nivolumab	Rizvi, 2015, Lancet Oncology[57] Checkmate-063	Advanced refractory NSCLC	Phase 2 (117)	PFS 1.9; OS 8.2
Nivolumab	Brahmer, 2015, NEJM[58]	Advanced squamous cell NSCLC	Phase 3 (272) chemo	3.5 (nivolumab) versus 2.8 (docetaxel) OS 9.2 (nivolumab) versus 6.0 (docetaxel)
Nivolumab	Topalian, 2012, NEJM[40]	Multiple solid tumors	Phase 1 (296)	Objective responses noted across varying doses in NSCLC, melanoma, and renal cell cancer; none in colorectal or prostate cancer
Nivolumab	Ansell, 2015, NEJM[26]	Relapsed or refractory Hodgkin's Lymphoma	Phase I (23)	PFS at 6 months, 86%
MPDL3280A (anti-PDL1)	Powles, 2014, Nature[39]	Metastatic bladder cancer	Phase 1 (68)	ORR at 6 weeks, 43% among PD-L1 positive tumors and 11% for negative tumors
MPDL3289A (anti-PDL1)	Herbst, 2014, Nature[43]	Multiple advanced cancers	Phase 1 (277)	Objective responses (complete or partial) in all tumor types tested
BMS-936559 (anti-PDL1)	Brahmer, 2012, NEJM[59]	Advanced cancers	Phase 1 (207)	Objective responses seen in melanoma, NSCLC, renal cell cancer, ovarian cancer (none in colorectal or pancreatic)

A representative, though not comprehensive, list of high-profile clinical trials with immune checkpoint blockade is detailed. The therapy, authors and journal information, phase, and major findings are provided. BRAF-WT: B-raf wild-type; ICC: investigator's choice chemotherapy; JCO, Journal of Clinical Oncology; NEJM, New England Journal of Medicine; NSCLC, non-small-cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Table 2. Programmed death ligand-1 (PD-L1) status as predictive biomarker

Therapy	Cancer	Author, year, journal	Antibody and PD-L1+ definition	% Tumors PD-L1-positive	PD-L1-positive	PD-L1-negative	Conclusion
Pembro and ipi	Advanced Melanoma	Robert, 2015, NEJM	Merck 223C 1% tumor cells	>80	PFS and overall response not stated between the two groups		Insufficient sample size (too few PD-L1-negative tumors) to draw conclusions
Nivo and ipi in combination versus monotherapy	Untreated melanoma	Larkin, 2015, NEJM	Dako 28-8 clone (BMS assay) >5% tumor cells positive	23.6	PFS, months Nivo 14 Combo 14 Ipi 3.9	5.3 11.2 2.8	For PD-L1- patients, combo treatment may be most beneficial
Nivo and ipi in combo versus ipi monotherapy	Untreated melanoma	Postow, 2015, NEJM	Dako 28-8 (BMS) >5% tumor cells	30	ORR, % Combo 58 Ipi 18	55 4	In combo therapy, PD-L1 status not prognostic, but may be beneficial in pts receiving ipi alone
Concurrent versus sequential combo treatment with nivo and ipi	Advanced melanoma	Wolchok, 2013, NEJM	Dako 28-8 (BMS) >5% tumor cells	38	ORR, % Concurrent 46 Seq 50	41 7	Objective responses seen regardless of PD-L1 status
Pembro	NSCLC	Garon, 2015, NEJM	Merck 22C3 >50% membrane staining	>50%: 23.3 1–49%: 37.5 <1%: 39.2	PFS, months 6.3	4.0	PD-L1 staining >50% may be a valuable biomarker, but PD-L1 neg pts still derive benefit
Nivolumab	Advanced melanoma	Weber, 2015, Lancet Oncology	Dako 28-8 (BMS) >5% tumor cells	~50%	ORR, % Nivo-treated 43.6	20.3	PD-L1 appeared to be associated with response, but small sample sizes
Nivolumab	Previously treated clear-cell renal-cell carcinoma	Motzer, 2015, JCO	Dako 28-8 (BMS) >5% tumor cells	27	PFS, months/ORR, % Nivo-treated 4.9/31	2.0/28	PD-L1 status was associated with response, but not conclusively since PD-L1 negative patients also responded
Nivolumab	Melanoma	Robert, 2015, NEJM	Dako 28-8 (BMS) >5% tumor cells	35.4	ORR, % Nivo-treated 52.7	33.1	PD-L1 status was not as important as nivolumab's superiority over dacarbazine

Table 2 (Continued)

Therapy	Cancer	Author, year, journal	Antibody and PD-L1+ definition	% Tumors PD-L1-positive	PD-L1-positive	PD-L1-negative	Conclusion
Nivolumab	NSCLC	Brahmer, 2015, NEJM	Dako 28-8 (BMS) >1, 5, and 10% of tumor cells		ORR, % Nivo-treated 17	17	PD-L1 was neither predictive nor prognostic
Nivolumab	Advanced cancers	Topalian, 2012, NEJM	5H1 >5% tumor cells	59%	ORR, %: Nivo-treated 36	0	PD-L1 may be a prognostic marker
Nivolumab	Non-Hodgkin's lymphoma	Ansell, 2015, NEJM	10 patient samples underwent PDL1 and PDL2 copy number analysis		All had 3–15 copy number gains in PDL1 and PDL2		Pathway activation of PDL1/2 copy number gain prognostic for response
MPDL3280A	Metastatic bladder cancer	Powles, 2014, Nature	>5% of tumor or tumor-infiltrating immune cells	27% on immune cells 4% on both immune and tumor cells	ORR, % Immune cell staining 43	11	Immune cell PD-L1 staining may be prognostic for MPDL3280A response
MPDL3280A	Advanced cancers	Herbst, 2014, Nature	Ventana clone SP142) >5% of tumor or tumor-infiltrating immune cells	12–36% (immune cell expression) depending on tumor type; 1–24% (tumor cell) depending on tumor type	Correlation between immune cell IHC staining ($P = 0.007$ all patients) No correlation found with tumor cell staining		Immune cell PD-L1 staining may be prognostic for MPDL3280A response

Summary of a subset of clinical trials that have examined the correlation between PD-L1 immunohistochemistry (IHC) on either tumor or immune cells with clinical response. BMS, Bristol-Myers Squibb; combo, combination; ipi, ipilimumab; JCO, Journal of Clinical Oncology; NEJM, New England Journal of Medicine; nivo, nivolumab; NSCLC, non-small cell lung cancer; ORR, objective response rate; pts, patients; pembro, pembrolizumab; PFS, progression-free survival; Seq, sequential.

2,3-dioxygenase, a metabolic enzyme that inhibits the immune responses through depletion of amino acids, is associated with clinical activity of ipilimumab.[46]

Anti-PD-1/PD-L1 therapies may have improved prognosis in patients whose tumors have pre-existing coalitions of cytotoxic T lymphocytes that are in an anergic state.[31] Serial on-treatment biopsies from 46 patients with melanoma treated with pembrolizumab revealed that patients who responded well to pembrolizumab had higher densities of CD8$^+$ T cells at the invasive tumor margin and center and that the densities of CD8$^+$ T cells in close proximity to PD-1$^+$/PD-L1$^+$ tumor cells increased after treatment.[47]

However, while it is not clinically practical to obtain serial biopsy samples to monitor patient immune response, pretreatment biopsy samples may be acceptable surrogates for capturing a signature of the balance between active and suppressive immune elements. Quantitative and standardized means of assessing the balance between active and suppressive immune factors are critically important for the development of validated and robust criteria for selecting and monitoring patients for immune checkpoint inhibitors.

Mutational landscape of tumors as predictors of neoantigens. Advances in sequencing technology in the clinical setting have also ushered in new strategies for identifying biomarkers to immune checkpoint blockade treatment.[48] Non-synonymous somatic mutations are considered to be the basis for generation of cancer-specific neoantigens, which are likely to be recognized by and induce clonal expansion of certain T cells. This hypothesis was indirectly supported by findings that mutations in DNA-damage repair genes increases somatic mutation burden, and are associated with longer recurrence-free survival in surgically resected muscle-invasive bladder cancer patients.[49] A high somatic mutation burden should theoretically increase the probability of generating neoantigens that can be presented with HLA molecules on the surface of cancer cells, recognized by CD8$^+$ T cells, and can induce clonal expansion of cytotoxic T cells. Indeed, in tumors treated with pembrolizumab, the overall mutational burden correlated with response to therapy; interestingly, the absolute burden of predicted neoantigens seemed to be a better predictor.[50]

Bioinformatics approaches provide useful tools for predicting neoantigens from whole exome and transcriptome sequencing data. For example, in a study of mice injected with the d42m1-T3 sarcoma cell line, mutations occurring in the *Lama4* and *Alg8* genes were successfully identified as d42m1-T3-specific neoepitopes that stimulated a CD8$^+$ T cell response.[51] In these methods, prediction of binding to individual HLA molecules is essential for identifying possible neoantigens. Although the total number of somatic missense mutations correlated with long-term response to ipilimumab, a signature of preserved tetrapeptides in neoepitope polymers was a more accurate predictor of clinical response in melanoma.[52]

Avenues for future direction: immunopharmacogenomics

The work carried out thus far in patient selection and monitoring in immune checkpoint therapy has underlined the importance of deeply understanding both the immune and genetic landscape of tumors in order to predict clinical response. The next step will be integrating the knowledge gained from these studies and applying it to modulating and improving clinical response. We have proposed a new study field, termed immunopharmacogenomics, which links the pharmacological response to cancer genomics with immunogenomics using massively parallel next-generation sequencing of the TCR repertoire. Immunopharmacogenomics has shown promise in both serving as a pharmacodynamics marker of immunotherapeutic activity and potentially modulating the clinical response. The TCR sequencing of tumor-infiltrating lymphocytes (TILs) from pretreatment biopsy samples, with comparison of on-treatment or post-treatment biopsy samples, can provide critical information about the changes in TIL repertoire during immune checkpoint inhibitor therapy. For example, deep sequencing of TCR repertoires from serial tumor tissue biopsies on treatment showed a 10-fold clonal expansion in cancer tissues in responders, but less or no expansion of clonal T cells in non-responsive patients.[47] While serial tissue biopsies are difficult to obtain, peripheral blood samples collected from patients on anti-CTLA antibody therapy showed an increase in TCR diversity for most patients on therapy, suggesting that TCR sequencing can be a tool for pharmacodynamics monitoring.[53] Deep sequencing of the TCR, both within the tumor and in the peripheral blood, can therefore provide direct quantification of the clonality and specificity of T cells.[38]

In addition, identifying TCR sequences that are expanded in tumors of patients treated with immune checkpoint blockade has the potential for new therapeutic interventions such as production of genetically engineered T cells targeting cancer cells. Particularly, there is significant interest and progress in identifying T cell clones that recognize neoantigens generated by somatic missense mutations in cancer cells.[48] The oligoclonal expansion of these T cells, which recognize neoantigens, may be potential immune responses against cancer. T-cell receptor deep sequencing has already been used to identify oligoclonal expansion of CD8$^+$-PD-1$^+$ TILs in melanoma tumors that are specific for mutated antigens.[54] Therefore, immunopharmacogenomics may both offer insight into patient selection and monitoring on immune checkpoint blockade as well as offer avenues to enhance the clinical response.[55,56] Tissue and blood samples, collected from patients on immune checkpoint antibody therapy, are needed to further validate this work.

Conclusions

Although the immune checkpoint inhibitors are already successes as anticancer agents, we are still far from knowing which patients may benefit from the use of immune checkpoint monotherapies or from knowing at what point to alter the direction of treatment. Immunopharmacogenomics may have a strong foothold in addressing lingering questions about predictive biomarkers for immunotherapy.

In summary, the class of immune checkpoint inhibitors has already changed how we think of anticancer strategies. In chess, the point of victory is called checkmate, stemming originally from the Russian phrase, "shakh mat" or "death to the king." In the balance between natural immunity and cancer tissues, immune checkpoint inhibitors, by unleashing the body's armament of self-defense already poised for action, may have the potential to, at last, bring death to cancer. There remains much work to do, however, to bring that potential to its full realization.

Disclosure Statement

The authors have no conflict of interest.

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