Review Article

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

Noura Choudhury¹ and Yusuke Nakamura²

¹Pritzker School of Medicine, University of Chicago, Chicago; ²Section of Hematology/Oncology and Center for Personalized Therapeutics, Department of Medicine, University of Chicago, Chicago, Illinois, USA

Key words
Biomarkers, checkpoint inhibitors, immunopharmacogenomics, immunotherapy, T-cell receptor

Correspondence
Yusuke Nakamura, 5841 South Maryland Avenue, MC 2115, Chicago, IL 60637, USA.
Tel: +1-773-834-1405; Fax: +1-773-702-0963; E-mail: ynakamura@medicine.bsd.uchicago.edu

Funding Information
No sources of funding were declared for this study.

Received October 26, 2015; Revised December 11, 2015; Accepted December 14, 2015
Cancer Sci 107 (2016) 107–115
doi: 10.1111/cas.12862

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
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 themselves 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, 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
targeted therapies, has also been explored, although such combinations are often affected by higher incidences of adverse events. 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.

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, intensely treated renal cell carcinoma, and colorectal cancers with mismatch repair deficiencies (Table 1). 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.

Immuno...
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.

| Therapy | Author, year, journal | Cancer type | Phase (no. of patients) | Findings, median PFS in months unless otherwise stated |
|---------|------------------------|-------------|-------------------------|--------------------------------------------------------|
| Pembrolizumab versus ipilimumab | Robert, 2015, NEJM(190) (KEYNOTE-006) | Advanced melanoma | Phase 3 (834) | 5.5 (pembrolizumab every 2 weeks) versus 4.1 (pembrolizumab every 3 weeks) versus 2.8 (ipilimumab) |
| Pembrolizumab | Garon, 2015, NEJM(442) 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(277) | 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) |
| Pembrolizumab | Hamid, 2013, NEJM(15) | Advanced melanoma | Phase 1 (135) | Not reached (combination) versus 4.4 (ipilimumab) (BRAF-WT tumors) |
| Pembrolizumab | Robert, 2014, Lancet(16) | Ipilimumab-refractory melanoma | Phase 1 (173) | 5.5 (pembrolizumab 2 mg/kg) versus 3.5 (10 mg/kg) Immunerelated response criteria: PFS 7.8 (2 mg/kg) versus 8.8 (10 mg/kg) |
| Pembrolizumab | Hodi, 2010, NEJM(14) | Advanced melanoma | Phase 3 (676) | Median OS 10.0 (ipilimumab + gp100) versus 6.4 (gp100 alone) |
| Pembrolizumab | 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(190) | 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) | 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) |
### 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 |
| 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 5.3 Combo 14 11.2 Ipi 3.9 2.8 |
| 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 41 Seq 50 7 |
| 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 |
| Nivolumab Advanced melanoma | Weber, 2015, Oncology | Dako 28-8 (BMS) >5% tumor cells | ~50% | ORR, % Nivo-treated 43.6 20.3 |
| 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 |
| Nivolumab Melanoma | Robert, 2015, NEJM | Dako 28-8 (BMS) >5% tumor cells | 35.4 | ORR, % Nivo-treated 52.7 33.1 |

Note: Insufficient sample size (too few PD-L1-negative tumors) to draw conclusions. For PD-L1+ patients, combo treatment may be most beneficial. In combo therapy, PD-L1 status not prognostic, but may be beneficial in pts receiving ipi alone. Objective responses seen regardless of PD-L1 status. PD-L1 staining >50% may be a valuable biomarker, but PD-L1 neg pts still derive benefit. PD-L1 appeared to be associated with response, but small sample sizes. PD-L1 status was associated with response, but not conclusively since PD-L1 negative patients also responded. PD-L1 status was not as important as nivolumab’s superiority over dacarbazine.
| 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                                                                          |
| Nivolumab        | Advanced cancers              | Topalian, 2012, NEJM  | 5H1 >5% tumor cells            | 59%                    | ORR, %:        | Nivo-treated  | 36               | 0                                                                           |
| 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                                                                          |
| 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; pembrolizumab; PFS, progression-free survival; Seq, sequential.
2,3-dioxygenase, a metabolic enzyme that inhibits the immune response through depletion of amino acids, is associated with clinical activity of ipilimumab.\(^{(40)}\)

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 of 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 increase 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 *Lamc4* 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.\(^{(54)}\) 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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