

Clinical pharmacologic aspects of immune checkpoint inhibitors in cancer therapy

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During the past two years, three immune checkpoint inhibitors, ipilimumab, nivolumab and pembrolizumab, have been approved and revolutionized cancer immunotherapy. Translational and clinical pharmacology of these agents have contributed in identifying patients who will receive benefit, dose effect relationship and surrogate endpoints of clinical benefit. In addition, population pharmacokinetics/pharmacodynamics have facilitated scientific clinical development, which has led to accelerated approval of these agents. This paradigm may show how early phase studies may allow identification of subgroup of patients who can benefit and subsequent approval of drugs based on smaller patient population. This may speed the access of effective treatment for patients with life-threatening diseases.

Introduction

Coley had reported the shrinkage of sarcoma after the bacterial infection erysipelas in the late 19th century.[1] The low rate of response portrayed two important issues: the potential of harnessing the immune system against cancer and the importance of selecting the right patients. These issues are persistent despite recent breakthroughs of cancer immunotherapy. In addition, many clinical pharmacology issues have risen regarding optimum dose and response evaluation. This review is a tutorial on the development of recently approved immune checkpoint inhibitors focused on clinical pharmacology and translational research.

T lymphocytes are pivotal in immunologic surveillance a process critical for the identification and elimination of tumor cells. [2] T cell receptor (TCR) is the key molecule that is related to the recognition of tumor cells.[3] After the TCR binds with the major histocompatibility complex (MHC) on the cell surface of tumor cells, various other ligand-receptors interactions come into place, which are co-stimulatory and co-inhibitory. The latter are often called "immune checkpoints" and include cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death-1 (PD-1), T-cell immunoglobulin domain and mucin domain 3 (TIM3), and lymphocyte-activation gene 3 (LAG3).

During the past two years, the three drugs, ipilimumab, nivolumab and pembrolizumab, have revolutionized cancer immunotherapy. These immune checkpoint inhibitors block the co-inhibitory signals related to tumors, which subsequently leads to an immune response against tumors.[4]

Pathology of Cancer Immunotherapy

Pathology has been a cornerstone in identifying patients who might respond to immune checkpoint inhibitors. Pathologic evaluation reveals patients' characteristics that suggest "immune targetable lesion" that include inflamed tumor environment, gene mutation load and molecular markers related with immune escape mechanisms. Inflamed tumor environment are comprised of T cells which are tumor-infiltrating lymphocytes (TILs) recruited into the tumor. TILs are seen in various entities including melanoma, breast cancer and colorectal cancer and the quantity of TILs at diagnosis is often associated with prognosis.[5] Further stratification is being tried to characterize lymphocytes and spatial distribution of immune infiltration (immune contexture).[6,7] For example, Mlecnik et al have further characterized the type and density of the TIL by immunostaining with CD8 and CD45RO and divided the location of the response into center of the tumor versus the invasive margin in colorectal cancer that generated an immune score. [8] The clinical significance of the score suggested that the state of the local adaptive immune response was related to improved clinical outcome. Further data from large clinical studies demonstrate that immune cell infiltrating tumors consists of many

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subpopulations. CD8(+) cytotoxic T lymphocytes, Th1 and Th17 CD4(+) T cells, natural killer cells, dendritic cells and M1 macrophages, are often indicators of good prognosis.[9-11] On the other hand, high levels of immune cells involved in co-inhibitory mechanism including intratumoral CD4(+)CD25(+) FOXP3(+) regulatory T cells, Th2 CD4(+) T cells, myeloid-derived suppressor cells, M2 macrophages and neutrophils have frequently been associated with a poor prognosis. In addition, gene expression signatures are being used as immunoprofiling tools for antigens expressed on tumor cells and surface receptors on immune cells[12] The prognostic and predictive value of immune score and immunoprofiling are being evaluated to guide cancer immunotherapy.[13]

This relationship has been used to identify patients responsive to immune checkpoint inhibitors. For decades, lymphocytic reaction to colorectal cancer have been associated with longer survival in several large studies with mismatch-repair deficient/micro-satellite instability high (MSI-high) patients, and has been considered an indicator of a host immune response to tumor cells that lead to improved survival.[14] The lymphocytic reaction seen in MSI-high patients has been translated into high response to anti-PD-1 therapy in colorectal cancer.[15] In addition, MSI-high patients with non-colorectal cancers including uterus, stomach, biliary tract, and small intestine also showed a response rate of about 70%. This implied that MSI-high was compelling predictive marker of response to immune checkpoint inhibitors. This led to the hypothesis that MSI-high would lead to high mutagenicity and the formation of neoantigenicity, which would generate an immune response that the checkpoint inhibitors would unleash. Similar findings are being expected in patients with missense mutations of the proofreading domain of DNA polymerase (POLE) in other tumors.[16]

Repertoires of neoantigens have been suggested to be biomarkers for immune checkpoint inhibitors based on the initial therapeutic benefits in melanoma and non-small cell lung cancer which have high mutational load. While the value of mutational load in predicting response to immune checkpoint inhibitors is being evaluated, there have also been results that the mutational load did not correlate with the density of T-cell inflammation in metastatic melanoma patients.[17,18] In addition, despite similar rates in mutation, colorectal cancer patients have lower response compared to non-colorectal cancer patients with mismatch-repair deficiency.[15] Therefore, it maybe only one of the factors related to T cell inflammation and response to immune checkpoint inhibitors.

Presently, the ligand of PD-1 (PD-L1) expression in pre-treatment tumor biopsies is a candidate biomarker for clinical response to anti-PD-1 therapy.[4] While PD-L1 is generally not detectable in normal tissues besides the tonsil, inflammatory cytokines can upregulate its expression in various cell types, including tumors.[19] This indicates that tumors can upregulate PD-L1 in response to local cytokines and evade local effector T cell, which suggests that immunosurveillance may exist even in

advanced cancers. PD-L1 can also be expressed constitutively on cancer cells through oncogenic signaling pathways.[20,21] PD-L1 expression has been observed in various solid human malignancies, including melanoma, breast, lung, kidney cancer as well as Hodgkin disease, and is a major factor in evaluating responses to anti-PD-1/PD-L1 therapies.[22] Teng et al had recently reported a tumor categorization based on T-cell infiltration and PD-L1 expression.[23] However, the value of PD-L1 expression as a biomarker is challenged by many findings. Various antibodies to PD-L1 show different immunohistochemistry staining according to specific condition and some lack specificity.[24] In addition, expression of PD-L1 is dynamic and is influenced by cytokines such as interferon- γ . Recent studies suggest that PD-L1 expression by tumor cells can also be influenced by cytotoxic agents and targeted therapies.[25] Thus, the timing of the biopsy to determine PD-L1 expression may be critical. For example, following anti-PD-1 therapy, biopsied specimens of regressing lesions were densely infiltrated by CD8(+) cytotoxic T lymphocytes.[26]

An immune-active tumor microenvironment appears to favor clinical response to anti-CTLA-4 therapy with ipilimumab. Patients with high pretreatment expression levels of immune-related genes were more likely to respond favorably to ipilimumab.[27] Furthermore, ipilimumab appears to induce two major changes in tumors from patients who exhibited clinical activity: increased expression of genes involved in immune response and decreased expression of genes for melanoma-specific antigens and genes involved in cell proliferation. Many IFN- γ -inducible genes and Th1-associated markers showed increased expression after ipilimumab treatment in T cells, which suggests accumulation of these cells at tumor sites and play an important role in mediating the antitumor activity of ipilimumab.

Optimal Dosage of Immune Checkpoint Inhibitors

Ipilimumab

Early trials reported single dose of 3 mg/kg.[28,29] Phan et al chose a dose of 3 mg/kg with a vaccine derived from the melanoma antigen gp100. In their first trial, they treated 14 patients of which eight experienced grade 3/4 toxicities including severe colitis, rash and hypophysitis. Subsequently, a dose ranging trial ranging from 2.8 to 20 mg/kg followed by a phase II extension of the trial in which 23 stage IV melanoma patients received 10 mg/kg of ipilimumab four times every 3 weeks was performed.[30] These results led to a one-three-arm randomized study, 217 previously treated melanoma patients received ipilimumab at either 0.3, 3 or 10 mg/kg every 3 weeks for four cycles followed by maintenance therapy every 3 months.[31] A clear dose-response for objective response rates was observed: 11.1% for 10 mg/kg, 4.2% for 3 mg/kg, and 0% for 0.3 mg/kg. Grade 3/4 immune-related adverse events also occurred in a dose proportional fashion: 36.0% for 10 mg/kg, 10.9% for 3 mg/

kg, and 0.0% for 0.3 mg/kg. The data of this trial was analyzed using a population pharmacokinetic method, which showed a two-compartment model with zero-order intravenous infusion and first-order elimination. Model-based simulation to assess achievement of the target trough concentration of 20 µg/mL before the last dose in the induction phase indicated that the target concentration would be achieved in about 95% of patients in the 10 mg/kg group, but only in 30% and 0% of patients in the 3 mg/kg and 0.3 mg/kg groups, respectively. Therefore, the final dose and balance between efficacy and safety remains a question. The 3 and 10 mg/kg doses have been compared in a phase III trial that completed accrual in 2010 (ClinicalTrials.gov registry number NCT01515189). The primary endpoint of the trial is overall survival; results are pending.

Pharmacokinetic characteristics of ipilimumab are similar to those of other monoclonal antibodies (Table 1). Ipilimumab is a humanized immunoglobulin (Ig)G1 monoclonal antibody that follows a first-order, two-compartment model.[32] The biphasic elimination comprises of a rapid distribution phase ($t_{1/2, \alpha}$ 27.4 hours) and a slow elimination phase ($t_{1/2, \beta}$ 14.7 days). This is comparable to endogenous IgG which shows relatively small volume of distribution (3-9 L), low clearance (8-12 mL/hour), and long biological half-life (20-25 days).[33] Model-predicted trough concentration after the first ipilimumab dose exceeded the target trough concentration of 3 µg/mL in about 99% and 100% of patients at the 3 and 10 mg/kg doses, respectively. The cut-off for maximal binding for CD80 and CD86 has been reported as target concentrations of 20 µg/mL and 3 µg/mL, respectively.[34] Recent data have also shown that the activity of ipilimumab may also involve selective depletion of regulatory T cells within tumor lesions, which suggests that target concentrations may not reflect the clinical endpoints sufficiently.[35] The results from the phase III trial (ClinicalTrials.gov registry number NCT01515189) will help us fully understand the mechanism of action and dose-effect relationship for ipilimumab.

Nivolumab and Pembrolizumab

Both nivolumab and pembrolizumab are recently approved IgG4 anti-PD-1 agents, which have many common features both in efficacy and safety; therefore, many aspects of the clinical development overlap in dose-response and efficacy evaluation, unlike ipilimumab.

Both agents were intensively evaluated in early phase trials. Nivolumab was first evaluated in a phase I/II study in 296 patients with a variety of heavily pretreated malignancies including melanoma, non-small cell lung cancer, prostate cancer, renal cell cancer and colorectal cancer.[36] Patients received nivolumab at doses of 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg of body weight every 2 weeks for up to 12 cycles until disease progression or a complete response occurred. No maximum-tolerated dose was defined and only 14% of the patient experienced grade 3/4 drug-related adverse events. The median time to the peak concentration of nivolumab was 1 to 4 hours after the start of infusion. The pharmacokinetics of the nivolumab was linear, with a dose-proportional increase in the peak concentration and the area under the curve (Table 1). The pharmacodynamic relation was relatively flat to dose; median PD-1-receptor occupancy by nivolumab was 64 to 70% according to dose levels of 0.1 to 10.0 mg/kg every 2 weeks. The spectrum, frequency, and severity of immune-related adverse events (irAEs) were similar across the dose levels tested. Further, phase III trials have selected 3.0 mg/kg every 2 weeks for melanoma, non-small cell lung cancer and renal cell carcinoma.[37-39]

Pembrolizumab was evaluated in 135 patients with advanced melanoma being treated with three separate dosing strategies: 10 mg/kg of body weight every 2 or 3 weeks or 2 mg/kg every 3 weeks.[26] Response rates across all dose levels were 38%, with patients on the highest dose of pembrolizumab showing a response rate of 52%. Responses were durable, and the median progression-free survival was longer than 7 months. A subsequent prospective, randomised analysis was performed using both 2 and 10 mg/kg given every 3 weeks to patients with ipilimumab-refractory advanced melanoma. The response rate was similar (21% vs 25%) at both doses and the safety profile was similar.[40] Pharmacokinetic analysis from the prior trials showed that pembrolizumab had relatively low clearance and limited volume of distribution (Table 1). Pooled population analysis showed low variability in its central volume of distribution (CV of 13.5%) and further predicted that dosage regimens 200 mg every 3 weeks and 2 mg/kg every 3 weeks were equivalent in exposure.[41] Presently, a flat dose of 200 mg every 3 weeks has become the standard dosage regimen for many studies.

Table 1. Pharmacokinetic characteristics of approved immune checkpoint inhibitors*

	Immunoglobulin subtype	Systemic Clearance	Volume of distribution at steady state	Terminal half-life
Ipilimumab	IgG1	15.3 mL/h (38.5%)	7.21 L (10.5%)	14.7 days (30.1%)
Nivolumab	IgG4	9.5 mL/h (49.7%)	8.0 L (30.4%)	26.7 days (101%)
Pembrolizumab	IgG4	8.3 mL/h (28%)	7.7 L (14%)	26 days (24%)

*Mean (percent coefficient of variation) based on FDA package insert values.

Population modeling has also helped evaluate the response of immunotherapy. Modeling of tumor size over time after dosing of pembrolizumab has identified baseline tumor sizes, number of target lesions and number of lymph node lesions as important covariates in the response to treatment.[42] These associations may suggest that anti-PD-1 agents exert direct effects on tumors. Recently, a mouse model has shown that PD-1 signaling stimulated tumor growth and antibody-mediated PD-1 blockade suppressed tumor growth even in immunocompromised or PD-1-deficient mice.[43] Subsequent studies are needed to validate this, but the successful development of a mixed-effects model that captures tumor size reduction in cancer patients may be useful for further decisions to find the optimal dosage with various partner agents.

Safety of Immune Checkpoint Inhibitors

Immune checkpoint inhibitors are related to a specific spectrum of side effects (irAEs), which consist of colitis, diarrhea, hypophysitis, hepatitis, nephritis, rash and vitiligo; these events are autoimmune or autoinflammatory. Despite this common mechanism, the pattern and severity of the checkpoint inhibitors differ according to target.

Ipilimumab

Non-clinical studies have suggested the role of CTLA-4 in autoimmunity. CTLA-4-deficient mice developed lethal lymphoproliferative disease with infiltration of visceral organs by activated T cell blasts.[44]

In animal models, blockade of CTLA-4 led to intensification of T cell-associated vitiligo and enhanced antitumor immunity.[45,46] However, ipilimumab did not cause any notable clinical or pathological toxicity at the dose range from 3 to 30 mg/kg at extensive evaluation in cynomolgus monkeys.[29]

At a meta-analysis, overall incidence of all-grade irAEs and grade 3/4 irAEs were 72 % and 24 %, respectively.[47] The risk ratio of ipilimumab at 10 mg/kg compared with 3 mg/kg was over three times the overall incidence level of grade 3/4 irAEs. Enterocolitis is one of the most common and severe irAEs, which have been associated with 5% mortality in a pooled analysis.[48] Systemic steroids for irAEs were effective in most cases and did not seem to affect efficacy.[49] Unfortunately, prophylactic steroids and other preventive strategies to avoid irAEs have not shown clinical benefit.[50] The response rate was also significantly higher among ipilimumab-treated patients who developed irAEs. Among those melanoma patients experiencing enterocolitis, the response rate was 36% as compared with 11% for patients with metastatic melanoma who did not develop enterocolitis, which supports the exposure effect relation for both efficacy and toxicity.[48]

Nivolumab and Pembrolizumab

Adverse events are similar in nivolumab and pembrolizumab including fatigue, rash, pruritus and diarrhea with grade 3/4

irAE occurring in 10–14% of patients.[36] Compared to ipilimumab, irAEs are significantly less frequent with the anti-PD-1 antibodies. Although rare with less than 1% incidence, deaths due to pneumonitis have been reported. Although the irAEs are less than ipilimumab, a dose-response relationship has been shown in pembrolizumab.[26] The highest incidence of overall treatment-related adverse events was seen among the patients receiving 10 mg/kg of pembrolizumab every 2 weeks, as compared with the patients receiving 10 mg/kg every 3 weeks and those receiving 2 mg/kg every 3 weeks (23%, vs. 4% and 9%, respectively). Treatment-related pneumonitis was reported in 4% of the patients; none of the cases were grade 3 or 4. Steroids are also the main treatment for irAEs associated with anti-PD-1 antibodies and presently show no deleterious effects on clinical outcome. In a pooled analysis from nivolumab melanoma clinical trials, the objective response rate in patients treated with immunosuppression was 28.8%, and the response rate in patients who did not receive immunosuppressants to treat a side effect was 32.3%.[51]

Endpoints of Immune Checkpoint Inhibitors

Clinical endpoints are hard to define as immunotherapy differs from prior agents in many aspects. Traditionally, tumor shrinkage that improves cancer symptoms has been objectively assessed with Response Evaluation Criteria in Solid Tumors (RECIST).[52] While, high response rates with cytotoxic chemotherapy have not always been translated into overall survival benefit, low response rates with certain immunotherapy have been translated into a benefit in overall survival.[53,54] Progression-free survival and overall survival have been well-recognized endpoints for many therapies. However, in the case of immunotherapy, this becomes quite challenging because of two aspects: durability and timing of clinical effects. In survival studies, the interval between time of randomization and death or disease progression is compared. These studies are designed based on exponential distribution assumption that hazards affects compared groups by the same ratio at all times, i.e., proportional hazards. This implies that the clinical effect of the experimental arm over the control is observed from the beginning and the survival curves will eventually drop down to zero survival probability. However, with recent develops in immunotherapy, 20–30% of the patients reach long-term survival; zero survival probability is not reached in the experimental arm. In addition, these differences appear in relatively later time points, so that the hazard ratio changes over time. Developing adequate methods to evaluate endpoints of immunotherapy remains challenging.

Conclusion

Cancer immunotherapy is opening a new paradigm and evolving very quickly. This paradigm challenges translational and clinical pharmacology in many aspects including identifying patients who will receive benefit, dose-effect relationship and sur-

rogate endpoints of clinical benefit. Methods including population pharmacokinetics/pharmacodynamics have enhanced the speed of clinical development of these agents. However, it is still challenging to validate clinical endpoints that have true clinical implications. To evaluate the mechanism of action, more studies should take advantage of highly interventional small clinical trials focused on the translational study of tumor biopsies. These early phase studies may allow identification of molecularly- or clinically-defined subgroup of patients who can benefit and the sample sizes of subsequent studies designed after identification of the subgroups would be smaller than those of conventional designs.

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Conflict of interest

Hun Jung is an employee of and holds stock options in MSD.

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