



REVIEW ARTICLE

## Pharmacometrics and systems pharmacology of immune checkpoint inhibitor nivolumab in cancer translational medicine

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**Abstract:** Nivolumab, a fully human immunoglobulin G4 (IgG4) monoclonal antibody (mAb) that targets the programmed cell death-1 (PD-1) inhibitory receptor expressed on lymphocytes and dendritic cells, has been approved for metastatic melanoma, advanced squamous non-small cell lung cancer (NSCLC) and metastatic renal cell carcinoma. In this review, systems pharmacology and pharmacometrics of this immunopharmaceutical are discussed. Mechanistic actions of T-cell biology with respect to both “priming phase” (anti-cytotoxic T lymphocyte-associated antigen 4 (anti-CTLA-4) mAb; ipilimumab) and “effector phase” (anti-PD-1 mAb; nivolumab) are discussed, respectively. Key pharmacometric variables in anticancer efficacy of nivolumab such as target engagement, metabolism, pharmacology systems and clearance are elucidated with an emphasis on current knowledge from pre-clinical as well as phase 1, 2 and 3 clinical trials, including the data presented at the American Society of Clinical Oncology (ASCO) 2015 and European Cancer Congress 2015. Nivolumab biomarkers, safety, and synergistic combination immunotherapies are delineated. Nivolumab, administered via intravenous infusion, has an acceptable safety profile and good efficacy. Indeed, the way forward to leverage maximum benefits for the cancer patient may be to synergize anti-PD-1 blockade with complementary targets in immune checkpoint pathways or other oncogenic signal transduction pathways. The encouraging results with nivolumab lend credence to the promise of immune checkpoint blockade as a therapeutic strategy that has come of age in clinical oncology. Of necessity, the burden of “financial toxicity” on cancer patients and families must be factored in considering nivolumab therapy. The problem of ligand PD-L1 being a weak biomarker in clinical practice is discussed. Appropriate patient selection methods including immunopharmacogenomics may be used to identify those patients who are most likely to benefit from anti-PD-1 therapy. Taken together, the potential success of nivolumab strengthens the case for accelerated development of immunopharmaceuticals and basic drug discovery in oncology, and potentially non-oncology indications, by stakeholders such as clinical oncologists, pharmacists and scientists in academia and the pharmaceutical industry in a concerted fashion.

**Keywords:** Nivolumab; PD-1; cancer; biomarkers; pharmacometrics; systems pharmacology; pharmacokinetics; T-cell; translational medicine

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### Introduction

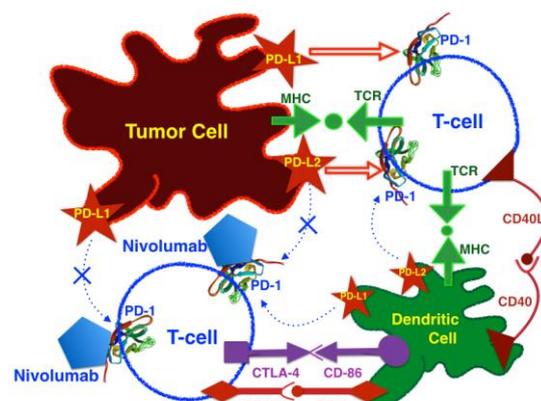
Novel personalized cancer treatment paradigms that target the tumor stroma in solid cancers have recently high-

lighted the emergence of engineered monoclonal antibodies as their essential cornerstones<sup>[1]</sup>. Immunomodulatory mAbs directly interact with soluble, or most often, cellular immune components to exert their therapeutic

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effects and, thus, differ from their tumor-targeting counterparts<sup>[2]</sup>. In response to membrane-bound and soluble ligands, inhibitory receptors on immune cells either abort or mitigate the intensity of immune response in a variety of ways including halting proliferation, raising the activation threshold, inhibiting/deviating differentiation of effector function, or by favoring apoptosis; collectively such inhibitory mechanisms which are evolutionarily-selected are known as checkpoints<sup>[3]</sup>. Sznol and Longo noted that cancer cells deploy many subterfuges to limit detection by host immune defenses, and, amongst these, the one that may be most exploitable in activation of a counterattack is immune checkpoint activation<sup>[4]</sup>. Indeed, immuno-oncology agents that target checkpoints within the cascade of immune regulatory molecules are presently fast becoming the hotbed of immuno-oncology<sup>[5]</sup>. US FDA-approved antibody ipilimumab (Yervoy<sup>®</sup>) targets cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) but is limited by the toxicity and low responses<sup>[6]</sup>. Massari et al.<sup>[7]</sup> observed that the interaction between programmed cell death-1 (PD-1) [structure elucidated in Cheng et al.<sup>[8]</sup> and RCSB Protein Data Bank<sup>[9,10]</sup> (PDB) available at [www.rcsb.org](http://www.rcsb.org) bearing PDB ID: 2M2D, see *Figure 1*], an inducible inhibitory receptor expressed on lymphocytes and dendritic cells, and PD-L1 ligand, expressed by tumor cells, results in a decrease of the T-cell response, thus, the inhibition of the PD-1/PD-L1 axis by targeted antibodies increases the T-cell proliferation and cytotoxicity and is a promising mechanism to stimulate the antitumor activity of the immune system so as to improve therapeutic outcomes in cancer patients. The mAb nivolumab (Opdivo<sup>®</sup>) that blocks PD-1 (CD279), resulting in anti-inhibition of tumor-specific immune responses, has been recently approved for use by the US FDA in the therapy of both metastatic melanoma and renal cell carcinoma as well as for advanced (metastatic) non-small cell lung cancer (NSCLC)<sup>[11]</sup>. Swanson and Sinha<sup>[12]</sup> observed that combined use of ipilimumab and nivolumab, with the latter being a fully humanized IgG4 antibody against PD-1<sup>[13]</sup>, may have a more pronounced effect than monotherapy with pre-clinical data suggesting beneficial effects in head and neck malignancies as well. Anti-PD-1 and anti-PD-L1 antibodies have demonstrated long-term responses coupled with minimal side-effects in patients suffering from triple-negative breast cancer, melanoma, chemotherapy-refractory Hodgkin's disease, and also kidney, lung and bladder cancers<sup>[14]</sup>. In this review, the current pre-clinical and clinical data on anti-PD-1 nivolumab with an emphasis on systems pharmacology and pharmacometric evaluation of this mAb are deline-

ated in order to appreciate the value of immune checkpoint blockade as a novel tool in the oncotherapeutic arsenal for advanced cancers.



*Figure 1* Systems Pharmacology of immune checkpoint inhibitor nivolumab. Immune checkpoint PD-1 (programmed death-1) receptor is expressed on the surface of T-cells whereas ligands PD-L1 and PD-L2 are expressed on the surface of tumor cells. As activated effectors traffic back into peripheral tissues, they come under the influence of PD-1/PD-L1- and PD-1/PD-L2-mediated signaling which is known as the “effector phase”. PD-L1 and PD-L2 bind to PD-1 and attenuate the protective immune response. Upon intravenous infusion to patients with melanoma or non-small cell lung cancer or renal cell carcinoma, nivolumab binds to PD-1 rendering this receptor unavailable for binding to the ligands, thus inhibiting the attenuation of immune response, enabling tumor cell kill and therapeutic success. CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) competes with stimulatory receptor CD28 for binding to its ligands (CD80/CD86) leading to inhibition of T-cell activation and expansion predominantly during initial activation by dendritic cells and other antigen-presenting cells (APCs) which is referred to as the “priming phase”. Costimulatory and coinhibitory ligand-receptor interactions between a T-cell and a dendritic cell or tumor cell in the tumor microenvironment are shown (Note: The structure of PD-1 was obtained from RCSB Protein Data Bank (PDB ID 2M2D) as cited in the text of the manuscript. The exact extracellular binding domain of PD-1 has not been elucidated in this schematic representation. The dendritic cell interactions are adapted from Ott et al<sup>[19]</sup>).

### Nivolumab mechanism of action, pharmacokinetic/pharmacodynamic (PK/PD) metrics, biomarkers, and disposition

Nivolumab enhances the human body's ability to recognize cancer by helping the immune system to mobilize lymphocytes which have been inactivated by melanoma cells, and has been shown to greatly improve survival in malignant melanoma when compared to conventional

chemotherapy<sup>[15]</sup>. PD-1 is expressed by activated T-cells and downregulates T-cell effector functions upon binding to its ligands, PD-L1 and PD-L2, on tumor cells in the tumor microenvironment<sup>[16]</sup>. T-cell activation is regulated by a balance of inhibitory and stimulatory signals that work in concert to co-ordinate the response to a threat faced by the immune system<sup>[17]</sup>. CD28 enables and maintains the T-cell response, at least in part by increasing expression of cytokines which, in turn, is achieved by interaction between the primary ligands B7-1 (CD80) and B7-2 (CD86) on the surface of the antigen-presenting cell (APC)<sup>[17]</sup>. Upon activation, T-cells cause induction of expression of CTLA-4 and PD-1, which are inhibitory receptors, and the eventual relative balance of inhibitory and stimulatory signals will determine the outcome of the T-cell response<sup>[18]</sup>.

Phosphorylation of CTLA-4 at the YKVM motif results in sustained CTLA-4 expression at the cell surface which is ligated by B7-1 or B7-2 to block TCR/CD28-mediated activation of Akt, but does not abrogate the PI3K-regulated upregulation of anti-apoptotic Bcl-xL<sup>[18]</sup>. As opposed to this, ligation of PD-1 by PD-L1 or PD-L2 leads to the phosphorylation of ITSM and ITIM motifs within PD-1's cytoplasmic tail of PD-1 resulting in blocking of TCR/CD28-mediated activation of PI3K<sup>[18]</sup>. Following the migration to lymphoid activation organs by activated dendritic cells, molecules such as CTLA-4, B7-1, B7-2, and CD28 are important for the initial activation of naive T-cells of the clonal composition that forms the responding repertoire<sup>[17]</sup>. Further, due to interactions between ligands expressed on malignant cells and tissue macrophages, activated effectors which traffic back into peripheral tissues are influenced by PD-1/PD-L1- and PD-1/PD-L2-regulated signaling axes<sup>[17]</sup>.

In order to extravasate to various tissues organs or tumors, T-cells must have encountered their antigen previously – this initial encounter or “priming” takes place in lymphoid organs<sup>[19]</sup>. If the antigen is presented to the T-cell harboring the corresponding receptor, T-cell activation will occur by implicating the costimulatory molecules<sup>[19]</sup>. CTLA-4 regulates T-cells predominantly during initial T-cell activation by dendritic cells and other APCs (priming phase)<sup>[19]</sup>. Once T-cells have been primed and have acquired an immunological memory, their extravasation through tissue postcapillary venules is facilitated by inflammation<sup>[19]</sup>. In this context, by targeting T-cells more specifically that are already engaged in an ongoing effector T-cell response, PD-1 blocking will have a more restricted spectrum of T-cell activation compared with CTLA-4 blocking, hence anti-PD-1 or anti-PD-L1 antibodies would exhibit less immune adverse events than

anti-CTLA-4 antibodies<sup>[19]</sup>.

Usually, CTLA-4 and PD-1 inhibitory immune checkpoint pathways prevent an excess of T-cell effector activity, thus, restraining the immune system from any over-reaction to stimuli<sup>[20]</sup>. Indeed, T-cell exhaustion, which is a reversible inhibition of T-cell activation and proliferation, and evasion of tumor cell detection by the T-cell, can be caused by over-activity in either of the two inhibitory pathways (e.g., when there is abnormal expression of PD-1 and its ligands, PD-L1 or PD-L2)<sup>[20]</sup>. Interestingly, with anti-CTLA-1 or anti-PD-1 or anti-PD-L1 antibodies, this tumor-induced suppression of T-cell function can be reversed, and anti-tumor activity at the priming (CTLA-4) or tissue effector (PD-1) phase can be augmented<sup>[20]</sup>.

Wang et al. showed that nivolumab (BMS-936558) binds to PD-1 with high affinity and specificity, and effectively inhibits the interaction between PD-1 and its ligands<sup>[16]</sup>. In addition, nivolumab can enhance T-cell reactivity at very low concentrations (~1.5 ng/mL) in the presence of a T-cell receptor stimulus, but had no stimulatory effect in the absence of T-cell receptor stimulus<sup>[16]</sup>. Specifically, there was no significant release of inflammatory cytokines, including interferon  $\gamma$ , TNF  $\alpha$ , IL-2, IL-4, IL-6, or IL-10, from unstimulated whole blood after co-incubation with nivolumab, thus, demonstrating that nivolumab does not cause non-specific lymphocyte activation<sup>[16]</sup>. *Figure 1* depicts the mechanistic aspects of the systems pharmacology of nivolumab that explains its efficacy as an immuno-oncology agent.

## Nivolumab PK/PD metrics

In advanced melanoma, response rates are about 12% for anti-CTLA-4 and about 40% for anti-PD-1, and are remarkably durable, hence impacting on survival<sup>[21]</sup>. Nivolumab, when compared with dacarbazine, was found to be associated with significant improvement in progression-free and overall survival among previously-untreated patients having metastatic melanoma without a mutation in BRAF<sup>[22]</sup>. Unlike CTLA-4-targeting ipilimumab, anti-PD-1 nivolumab and pembrolizumab (Keytruda<sup>®</sup>) showed highly durable response rates and long-term safety<sup>[6]</sup>, validating the importance of PD-1 pathway blockade for treatment of malignant melanoma. Ansell et al., on observing that Reed-Sternberg cells in Hodgkin's lymphoma exploit the PD-1 pathway to evade immune detection in pre-clinical studies, administered 23 heavily-treated patients with relapsed or refractory Hodgkin's lymphoma with nivolumab at a dose of 3 mg/kg of body weight every 2 weeks and revealed that the progression-free survival at 24 weeks was 86%<sup>[23]</sup>.

Moreover, pre-treatment tumors from 10 patients revealed copy-number gains in *PD-L1* and *PD-L2* and enhanced the expression of both ligands. This study showed that in previously-treated relapsed or refractory Hodgkin's lymphoma patients, nivolumab demonstrated an acceptable safety profile and substantial therapeutic activity as well<sup>[23]</sup>. Lipson et al. studied three patients with colorectal cancer, renal cell cancer (RCC), and melanoma who achieved objective responses on an intermittent dosing regimen of BMS-936558, followed them for more than three years after cessation of therapy, and reported successful reinduction of anti-PD-1 therapy following delayed tumor progression<sup>[24]</sup>. Davar et al. have reported near complete response in a patient with advanced, heavily pre-treated, KRAS mutant pulmonary adenocarcinoma after administration of a single dose of nivolumab<sup>[25]</sup>.

## Nivolumab biomarkers

PD-1 has two ligands, PD-L1 and PD-L2, both of which are members of the B7 family of transmembrane proteins<sup>[26]</sup>. While the expression of PD-L2 is largely restricted to antigen-presenting cells, PD-L1 is expressed on many cell types such as APCs, B-cells, T-cells, epithelial cells, and monocytes, and is upregulated in response to proinflammatory cytokines such as IFN- $\gamma$  and IL-4 through signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 1 (IRF1)<sup>[26]</sup>. Unfortunately, PD-L1 is currently a weak biomarker in clinical practice due to (a) expression levels: tumors with higher expression of PD-L1 often correlate with poor prognosis in several malignant tumors as compared to tumors with low expression of PD-L1<sup>[27]</sup>; (b) tumor heterogeneity: there are wide intra- and inter-tumoral variations in the expression of PD-L1, indicating that sampling of tumor tissues may also impinge on the outcome of PD-L1 detection<sup>[26]</sup>; (c) low specificity: tumors that do not express detectable levels of PD-L1 on the cell surface can also respond to antibodies against PD-1, suggesting that the predictive value of PD-L1 expression may not be uniformly applicable to all types of cancer<sup>[26]</sup>; (d) inducible gene expression: PD-L1 can be expressed constitutively or can be induced by IFN- $\gamma$ <sup>[28,29]</sup> secreted by infiltrating lymphocytes or by IL-27<sup>[30]</sup>; and (e) potential drug resistance: resistance to PD-L1 inhibitors may be a cause of therapeutic failure, however, this has not been well-studied at the current time and needs systematic investigation<sup>[26]</sup>. PD-L1 has been reported to be modulated by MAPK and PI3K/Akt/mTOR oncogenic signaling pathways<sup>[31,32]</sup>.

Das et al. analyzed blood/tumor tissue from 45 patients undergoing single or combination (anti-PD-1 and anti-CTLA-4) immune checkpoint blockade and reported distinct functional and genomic signatures in purified human T-cells and monocytes *in vivo*. In this study, changes induced by therapy were less prominent in monocytes than in T-cells with such changes marked by alterations in coding genes, including non-coding RNAs and alternatively spliced transcripts<sup>[33]</sup>. Furthermore, it was also shown that PD-1 blockade led to alterations in genes associated with NK cell function and cytotoxicity whereas combination blockade led to alterations in chemokine genes and proliferation-associated genes, and that PD-1 receptor occupancy following anti-PD-1 therapy may be incomplete in the tumor T-cells<sup>[33]</sup>. The potential involvement of active janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling after nivolumab treatment (3 mg/kg body weight given every two weeks) is exemplified by the nuclear positivity of phosphorylated STAT3 in Reed-Sternberg cells in Hodgkin's lymphoma<sup>[33]</sup>.

Blocking of cell surface immune checkpoint proteins such as CTLA-4 and PD-1, as well as LAG-3 and TIM-3, by mAbs has conferred significant anti-tumor immunity; however, there is a preponderance of immune-related adverse reactions resembling auto-immune diseases, which resolve effectively with immunosuppressive drugs treatment<sup>[34]</sup>. Immunoarchitectural features such as expression of PD-1 and its ligands PD-L1 and PD-L2 as well as lymphocyte subpopulations and patterns of immune cell infiltration were studied for inter-relationships and potential correlations with clinical outcomes in 41 patients with melanoma, NSCLC, colorectal carcinoma, renal cell carcinoma (RCC), or castration-resistant prostate cancer who were part of an early-phase nivolumab trial and had evaluable pre-treatment tumor specimens<sup>[35]</sup>. It was concluded that tumor PD-L1 expression reflects an immune-active microenvironment and is closely correlated with response to anti-PD-1 blockade with nivolumab<sup>[35]</sup>.

Le et al. developed an interesting anti-PD-1 immunotherapy for colorectal cancer patients with microsatellite instability in a phase 2 study in order to evaluate the clinical activity of anti-PD-1 pembrolizumab and reported an objective response rate of 40% and progression-free survival rate of 78% in mismatch repair-deficient colorectal cancer patients, thus, concluding that PD-1 blockade is beneficial in tumors with mismatch repair deficiency<sup>[36]</sup>.

In the 2015 American Society of Clinical Oncology (ASCO) Annual Meeting, Eroglu et al. presented the data

on a retrospective analysis of over 1000 patients with desmoplastic melanoma (which has a higher mutational load than other sub-types of melanoma), who were treated with nivolumab, combination of nivolumab and ipilimumab, or pembrolizumab, and concluded that these patients with high mutation-burden tumors have higher response rates and favorable clinical outcomes to anti-PD/PD-L1 therapy compared to other patients with advanced melanoma<sup>[37]</sup>.

## Nivolumab disposition

Figure 2 elucidates key pharmacometric variables in anticancer efficacy of nivolumab such as target engagement, metabolism, systems pharmacology, and clearance. Given the large molecular weight of nivolumab (143.6 kDa), which is similar to other mAbs, no renal elimination is expected; however, nivolumab is expected to be cleared by proteolytic degradation through receptor-mediated endocytosis or non-specific endocytosis mainly in hepatic or reticuloendothelial cells<sup>[38]</sup>. It may be possible to speculate that nivolumab may exhibit target (PD-1)-mediated disposition, like other therapeutic proteins. This may constitute an interaction of the protein therapeutic with the pharmacological target receptor which is in a homeostatic equilibrium of synthesis and degradation<sup>[39]</sup>. Cao and Jusko reported a second-generation minimal physiologically-based pharmacokinetic (mPBPK) model that proffers a more mechanistic approach for the analysis of plasma mAb PK as compared to compartment models. This model generates parameters that furnishes insights into intrinsic distribution and elimination for many mAbs that were studied in man<sup>[40]</sup>. The metabolic pathway of nivolumab has not been characterized; however, it likely degrades via catabolic pathways into small peptides and amino acids in the same fashion as endogenous IgG<sup>[38]</sup>. In addition, nivolumab is not expected to be metabolized by liver cytochrome P450 (CYP450) or other drug-metabolizing enzymes (DMEs). In fact, due to the lack of cytokine modulation, nivolumab has no or low potential to modulate CYP enzymes, thus indicating a low risk of therapeutic protein-drug interactions<sup>[38]</sup>.

## Nivolumab pre-clinical and phase 1 clinical trial data

### Nivolumab pre-clinical data

Sanmamed et al. developed a humanized murine model (Rag2-/-IL2R $\gamma^{\text{null}}$  immunodeficient mice) whereby the mice were engrafted with human colorectal HT-29

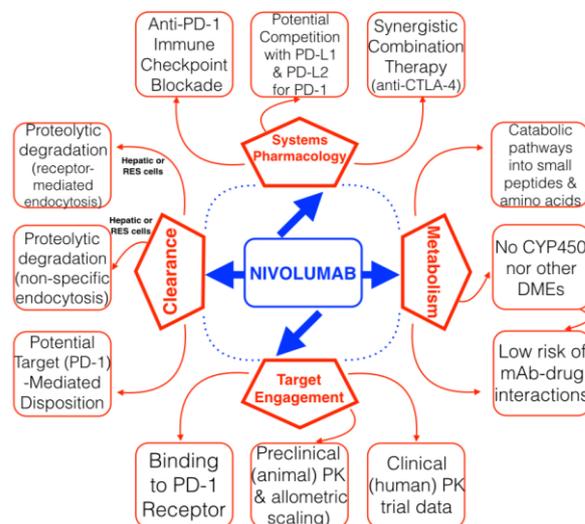


Figure 2 Pharmacometric variables in anticancer efficacy of immune checkpoint inhibitor nivolumab. Key pharmacometric variables impacting anticancer efficacy of immune checkpoint inhibitor nivolumab are described by (1) Target engagement: including efficacy of binding to PD-1 (programmed cell death-1) receptor, preclinical (animal) pharmacokinetics (PK) and allometric scaling, and clinical (human) PK trial data of nivolumab; (2) Metabolism: including breakdown by catabolic pathways into small peptides and amino acids, no role for Phase 1 CYP450 (cytochrome P450 enzymes) or other DMEs (drug-metabolizing enzymes) in metabolism and consequent low risk of interactions between mAb (monoclonal antibody) nivolumab and other concurrently administered drugs; (3) Systems Pharmacology: including blockade of immune checkpoint PD-1 by anti-PD-1 nivolumab, potential competition of nivolumab with ligands PD-L1 and PD-L2 for binding to PD-1, and potential of synergistic combination therapy, for example, with anti-CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) mAb ipilimumab; and (4) Clearance: including proteolytic degradation by receptor-mediated endocytosis or non-specific endocytosis in liver or cells of the RES (reticuloendothelial system), and potential target (PD-1)-mediated disposition constituting an interaction of the protein therapeutic with the pharmacologic target receptor which is in a homeostatic equilibrium of synthesis and degradation.

colorectal cancer cells and allogeneic human peripheral blood mononuclear cells (PBMCs), or with a patient-derived gastric carcinoma and PBMCs from the same patient, and anti-CD137 urelumab was sufficient to significantly slow tumor growth, increase numbers of activated human T-lymphocytes producing interferon- $\gamma$  and decrease numbers of human regulatory T-lymphocytes in the tumor xenografts<sup>[41]</sup>. It has been reported that single-dose of intravenous administration of (1 and 10 mg/kg) was found well-tolerated in cynomolgus monkeys with no effects on body weight or clin-

ical observations<sup>[16]</sup>. The  $C_{max}$  across monkey genders at a dose of 1 mg/kg was similar at 33.8  $\mu\text{g/mL}$  in male monkeys and 28.6  $\mu\text{g/mL}$  in female monkeys whereas the  $C_{max}$  at 10 mg/kg in male monkeys was 330  $\mu\text{g/mL}$ ; the corresponding half-lives ( $t_{1/2}$ ) were 124 hr, 139 hr, and 261 hr respectively; the corresponding total clearance (CL) values were 0.224, 0.250, and 0.172 mL/hr/kg respectively; the  $T_{max}$  was 0.25 hr in all these three cases<sup>[16]</sup>.

### Nivolumab phase 1 clinical trial data

In a first-in-human phase 1 clinical study<sup>[42]</sup>, 39 patients with advanced metastatic melanoma, colorectal cancer, castrate-resistant prostate cancer, non-small-cell lung cancer (NSCLC), or RCC, received a single-intravenous infusion of anti-PD-1 (MDX-1106, i.e., nivolumab) in dose-escalating 6-patient cohorts at 0.3, 1, 3, or 10 mg/kg, followed by a 15-patient expansion cohort at 10 mg/kg, and it was found that the treatment was well-tolerated and was associated with evidence of antitumor activity in all histology tissue except in castrate-resistant prostate cancer which was not studied in the 15-patient expansion cohort<sup>[24,42]</sup>. This study led to the design of the first multicenter trial wherein Topalian et al. enrolled 296 patients with advanced melanoma, NSCLC, castration-resistant prostate cancer, or renal cell carcinoma or colorectal cancer, to receive anti-PD-1 antibody (BMS-936558 also known as MDX-1106 or ONO-4538, i.e., nivolumab) at a dose of 0.1–10 mg/kg body weight for every two weeks for an 8-week treatment cycle with patients receiving up to 12 cycles<sup>[43]</sup>. In this study, grade 3 or 4 drug-related adverse events occurred in 14% of patients and there were three deaths from pulmonary toxicity with no maximum tolerated dose (MTD) being defined<sup>[43]</sup>. Additionally, objective responses were produced in approximately 1 in 4 to 1 in 5 patients with NSCLC, melanoma, or RCC, with the preliminary data suggesting a potential relationship between PD-L1 expression on tumor cells and objective response<sup>[43]</sup>.

Brahmer et al. conducted a multicenter Phase 1 trial of anti-PD-1 antibody (BMS-936559) at escalating doses ranging from 0.3 to 10 mg/kg body weight to 207 patients comprising NSCLC, RCC, melanoma, colorectal, ovarian, pancreatic, gastric and breast cancers and reported continued tumor regression (objective response rates of 6% to 17%) and prolonged stabilization of disease (rates of 12% to 41% at 24 weeks)<sup>[44]</sup>. In a randomized, double-blind phase 1 dose-escalation study involving 142 patients with metastatic melanoma who had not received treatment previously, combined inhibition of

T-cell checkpoint pathways by nivolumab (1 mg/kg body weight) and ipilimumab (3 mg/kg body weight) was associated with a high rate of objective response (61%) as compared to ipilimumab monotherapy (11%), with complete responses reported in 22% patients in the combination group versus none in the monotherapy group<sup>[45]</sup>.

McDermott et al. reported a Phase 1 study with expansion cohorts in 34 patients with previously-treated advanced RCC who were administered with intravenous nivolumab (1 or 10 mg/kg) in an outpatient setting once every two weeks for up to 96 weeks. In this study, 29% patients achieved objective responses (according to RECIST version 1.0) with median response duration of 12.9 months, 27% additional patients demonstrated stable disease lasting longer than 24 weeks, besides having the median for overall survival in all patients (71% with two to five prior systemic therapies) at 22.4 months, whereas one-, two-, and three-year survival rates were 71%, 48%, and 44%, respectively with the reversible grade 3 to 4 treatment-related adverse events occurring in 18% of patients<sup>[46]</sup>. Gettinger et al. administered nivolumab (1, 3, or 10 mg/kg) intravenously once every two weeks in 8-week cycles for up to 96 weeks to 129 patients with heavily pretreated advanced NSCLC and assessed tumor burden by RECIST (version 1.0) after each cycle<sup>[47]</sup>. In this study, median overall survival across doses was 9.9 months; 1-, 2-, and 3-year overall survival rates were 42%, 24%, and 18%, respectively, across doses, and 56%, 42%, and 27%, respectively, at the 3 mg/kg dose ( $n = 37$ ); however, three treatment-related deaths (2% of patients), each associated with pneumonitis, occurred in this study<sup>[47]</sup>.

In another phase 1 study, 90 patients with unresectable stage III or IV melanoma who were ipilimumab-naïve and had experienced progression after at least one prior therapy, or were ipilimumab-refractory and had experienced progression, received nivolumab at 1, 3, or 10 mg/kg every two weeks for 24 weeks and subsequently every 12 weeks for up to two years, with or without a multipeptide vaccine<sup>[48]</sup>. In this study, for both ipilimumab-refractory and -naïve patients, the RECIST 1.1 response rate was 25%, and nivolumab at 3 mg/kg with or without peptide vaccine was well-tolerated and induced responses lasting up to 140 weeks<sup>[48]</sup>. Wolchok et al. performed a phase 1 trial in 53 patients with advanced melanoma who were administered intravenous doses of nivolumab and ipilimumab for every three weeks for 4 doses, followed by nivolumab alone for every three weeks for 4 doses in a concurrent regimen. Alternatively, patients previously treated with ipilimumab received nivolumab every two weeks for up to

48 doses in a sequential treatment design<sup>[49]</sup>. In the concurrent regimen group (nivolumab 1 mg/kg plus ipilimumab 3 mg/kg), the objective response rate was 40%, and 53% of the patients had an objective response, all with tumor reduction of 80% or more, whereas in the sequential treatment group, the objective response rate was only 20%<sup>[49]</sup>.

## Nivolumab phase 2 and phase 3 clinical trial data

### Nivolumab phase 2 clinical trial data

In a phase 2 trial, Hamanishi et al. administered an intravenous infusion of nivolumab every two weeks at a dose of 1 or 3 mg/kg (constituting two 10-patient cohorts) to 20 patients with platinum-resistant ovarian cancer who were given up to 6 nivolumab treatment cycles (4 doses per cycle) and reported a median progression-free survival time of 3.5 months, median overall survival time of 20 months, disease control rate in all patients of 45%, the best overall response of 15%, grade 3 or 4 treatment-related adverse events in 8 (40%) patients and two patients with severe adverse events<sup>[50]</sup>. In a Phase 2, single-arm trial (CheckMate 063)[51] at 27 sites internationally on 117 previously-treated patients with advanced, refractory, squamous NSCLC, who were administered intravenous nivolumab (3 mg/kg) every 2 weeks, the median time to response was 3.3 months, median duration of response was not reached, and 17% of patients reported grade 3-4 treatment-related adverse events including fatigue, pneumonitis and diarrhea, reflecting clinically meaningful activity and a manageable safety profile<sup>[51]</sup>.

Motzer et al. conducted a randomized phase 2 trial involving 168 patients with metastatic RCC who were previously treated with agents targeting the vascular endothelial growth factor (VEGF) pathway<sup>[52]</sup>. These patients were administered nivolumab, either 0.3, 2, or 10 mg/kg intravenously once every 3 weeks; and it was reported that median progression-free survival was 2.7, 4.0, and 4.2 months respectively; median overall survival was 18.2, 25.5, and 24.7 months respectively; and objective response rates were 20%, 22%, and 20% respectively<sup>[52]</sup>. Interestingly, a combination of nivolumab and ipilimumab is still in a phase 2 clinical trial for recurrent glioblastoma reflecting the fact that checkpoint blockade immunotherapy for cancer has come of age<sup>[53]</sup>.

In the European Cancer Congress 2015, Stephen Hodi reported an open-label, randomized, phase 2 study of nivolumab given sequentially with ipilimumab in patients with advanced melanoma (CheckMate 064)<sup>[54]</sup>.

This study showed consistent efficacy advantages for nivolumab followed by ipilimumab as compared to ipilimumab followed by nivolumab in patients with advanced melanoma<sup>[54]</sup>. Saby George reported in the European Cancer Congress 2015, a randomized, phase 2 dose-ranging trial (CheckMate 010) of nivolumab in patients with metastatic renal cell carcinoma who were treated beyond progression, and concluded that these patients were able to safely continue treatment with nivolumab with some patients experiencing subsequent tumor shrinkage and extended survival<sup>[54]</sup>.

Topalian et al. enrolled 107 patients with advanced melanoma in a dose escalation, cohort expansion study to receive intravenous nivolumab (0.1, 0.3 and 1.0 mg/kg) in an outpatient setting every two weeks for up to 96 weeks and observed them for median overall survival (16.8 months), one-year survival (62%), two-year survival (43%), and reported acceptable long-term safety and durability of response that persisted after drug discontinuation<sup>[55]</sup>.

### Nivolumab phase 3 clinical trial data

Larkin et al. used a randomized, double-blind, phase 3 study to evaluate stage III or IV melanoma patients with nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone, and reported significantly longer median progression-free survival with nivolumab alone (6.9 months) or combination (11.5 months) than ipilimumab alone (2.9 months)<sup>[56]</sup>. In a randomized, controlled, open-label, phase 3 trial (CheckMate 037) at 90 various sites in 14 different countries, Weber et al. recruited 631 patients with unresectable or metastatic melanoma who had progressed after ipilimumab, or ipilimumab and a BRAF inhibitor if they were BRAF<sup>V600</sup> mutation-positive, and administered them with either an intravenous infusion of nivolumab (3 mg/kg every two weeks) or dacarbazine (1000 mg/m<sup>2</sup> every three weeks) or paclitaxel (175 mg/m<sup>2</sup> combined with carboplatin every three weeks)<sup>[57]</sup>. In this study, nivolumab led to a greater proportion of patients (31.7%) achieving an objective response than with the alternative chemotherapy regimens (10.6%); however, nivolumab resulted in grade 3 or 4 adverse events including increased lipase (1% of patients), increased alanine aminotransferase, anemia, and fatigue (1% of patients), and grade 3 or 4 drug-related serious adverse events (5% of patients), with no treatment-related deaths occurring<sup>[57]</sup>.

In a randomized, open-label, international, phase 3 study, 272 patients who were previously treated with advanced squamous-cell NSCLC were evaluated for the efficacy and safety of nivolumab (3 mg/kg body weight

every two weeks) as compared to docetaxel (75 mg/m<sup>2</sup> of body surface area every three weeks) respectively, and it was found that overall survival (9.2 months versus 6 months), overall survival rate at one year (42% versus 24%), response rate (20% versus 9%), and progression-free survival (3.5 months versus 2.8 months) were significantly better with nivolumab than with docetaxel, regardless of PD-L1 expression level<sup>[58,59]</sup>.

In another randomized, open-label, phase 3 study, 821 patients with advanced clear-cell RCC who had been previously treated with 1 or 2 anti-angiogenic therapy regimens were assigned in a 1:1 ratio to randomly receive either 3 mg of nivolumab per kg/body weight by intravenous route every 2 weeks or a 10 mg everolimus tablet taken orally once daily. In this study, the median overall survival was 25 months with nivolumab and 19.6 months with everolimus, the hazard ratio for death with nivolumab versus everolimus was 0.73 which met the pre-specified criterion for superiority, the objective response rate was greater with nivolumab (25%) than with everolimus (5%), median progression-free survival was 4.6 months with nivolumab and 4.4 months with everolimus, grade 3 or 4 treatment-related adverse events occurred in 19% of patients receiving nivolumab and in 37% of patients receiving everolimus, and the most common event with nivolumab was fatigue (in 2% of patients) and with everolimus was anemia (in 8% of patients)<sup>[60]</sup>.

Borghaei et al. recruited 582 patients with stage III or IV advanced nonsquamous NSCLC that progressed after previous platinum-based therapy in a Phase 3 randomized, controlled CheckMate 057 trial<sup>[61]</sup>. In this study, patients received either nivolumab (3 mg/kg of body weight every two weeks) or docetaxel (75 mg per square meter of body-surface area every three weeks)<sup>[61]</sup>. It was reported that the median overall survival was longer with nivolumab (12.2 months) as opposed to docetaxel (9.4 months), overall survival rate at one year was 51% with nivolumab versus 39% with docetaxel, the response rate was 19% with nivolumab versus 12% with docetaxel, and the rate of progression-free survival was higher with nivolumab (19%) versus docetaxel (8%)<sup>[61,62]</sup>. In addition, treatment-related grade 3 or 4 adverse events were reported in 10% of the patients in the nivolumab group, as opposed to 54% of those in the docetaxel group<sup>[61]</sup>. At the Plenary Session of the 2015 ASCO Annual Meeting, Jedd Wolchok reported the results of a randomized, double-blind, phase 3 trial (CheckMate 067) designed to evaluate nivolumab combined with ipilimumab, or

nivolumab alone, or ipilimumab alone, in melanoma<sup>[63]</sup>. In this study, 945 treatment-naïve patients were randomized 1:1:1 to nivolumab 1 mg/kg every 2 weeks + ipilimumab 3 mg/kg every 3 weeks for 4 doses followed by nivolumab 3 mg/kg every 2 weeks; nivolumab 3 mg/kg every 2 weeks + placebo; or ipilimumab 3 mg/kg every 3 weeks for 4 doses + placebo, until progression or unacceptable toxicity, with patients stratified by PD-L1 status, BRAF mutation status, and M-stage<sup>[63]</sup>. At a minimum follow-up of 9 months, it was found that progression-free survival was significantly improved for combined nivolumab + ipilimumab (11.5 months), or nivolumab alone (6.9 months), versus ipilimumab alone (2.9 months), and objective response rate was also significantly improved for combined nivolumab + ipilimumab (57.6%), or nivolumab alone (43.7%) versus ipilimumab alone (19%)<sup>[63]</sup>. The study concluded that nivolumab + ipilimumab, or nivolumab alone, showed superior clinical activity to ipilimumab alone<sup>[63]</sup>.

## Nivolumab safety

Nivolumab is known to be associated in a number of patients with immune-mediated side-effects including pneumonitis, colitis, hepatitis, nephritis/renal dysfunction, hypothyroidism and hyperthyroidism, pancreatitis, uveitis, demyelination, autoimmune neuropathy, adrenal insufficiency, facial and abducens nerve paresis, hypophysitis, diabetic ketoacidosis, hypopituitarism, Guillain-Barré syndrome, and myasthenic syndrome<sup>[64]</sup>. In such situations, nivolumab therapy may be withheld or discontinued depending on the grade of the specific immune-mediated adverse event at the discretion of the physician<sup>[64]</sup>. Shirai et al. have reported the case of an 81 year old woman with metastatic melanoma who developed myasthenia gravis and rhabdomyolysis after nivolumab immunotherapy, and underscored the importance of recognizing unexpected immune-related adverse events<sup>[65]</sup>. In a retrospective analysis of data pooled from 148 melanoma patients treated with nivolumab alone or nivolumab plus peptide vaccine, Freeman-Keller et al. reported immune-related adverse events in 68.2% of patients; however, grade III/IV adverse events including rash, asymptomatic elevation in amylase/lipase, and colitis, were rarely found<sup>[66]</sup>. Maurice et al. reported the case of a metastatic melanoma patient, initially treated with ipilimumab and then, with infusions of nivolumab, who developed subacute multifocal central nervous system (CNS) demyelination and succumbed to his CNS lesions 4 months later despite discontinuation of nivolumab<sup>[67]</sup>.

Kato et al.<sup>[68]</sup> have observed the exacerbation of psoriasis

riasis vulgaris during nivolumab for oral mucosal melanoma, whereas Matsumara et al.<sup>[69]</sup> have reported the exacerbation of psoriasis during nivolumab therapy for metastatic melanoma. Imafuku et al. reported severe rashes associated with vemurafanib administration following nivolumab therapy<sup>[70]</sup>. In addition, de Velasco et al. observed recovery in a 60-year-old male with metastatic clear cell RCC who also suffered from auto-immune uveitis and Jaccoud's arthropathy secondary in response to nivolumab treatment<sup>[71]</sup>. Abdel-Rahman et al. performed a meta-analysis of the risk of cutaneous toxicities associated with immune checkpoint inhibitors including ipilimumab, nivolumab, tremelimumab, pidilizumab, and pembrolizumab, and concluded an increased risk of all-grade skin rash, vitiligo, and pruritus associated with these inhibitors<sup>[72]</sup>. Somasundaram and Herlyn noted that future identification of therapy-response biomarkers, mobilization of tumor-reactive T-cell infiltration using cancer vaccines, or non-specific targeted-therapy drugs will minimize toxicity levels with immune-based therapies and provide long-term remissions in cancer patients<sup>[73]</sup>.

Importantly, nivolumab therapy can cause embryofetal toxicity if administered to a pregnant woman, hence, women of reproductive potential during nivolumab therapy should use effective contraception for at least 5 months after the last dose<sup>[64]</sup>. In addition, women are advised to discontinue breastfeeding during the treatment with nivolumab as the presence of nivolumab in human milk is unknown<sup>[64]</sup>. The safety and efficacy of nivolumab in pediatric and geriatric populations have not been established yet<sup>[64]</sup>.

According to the phase 3 study reported by Jedd Wolchok at the 2015 ASCO Plenary, and which was discussed earlier in this manuscript, Grade 3-4 drug-related adverse events occurred in 55.0%, 16.3%, and 27.3% of patients in the nivolumab + ipilimumab, nivolumab, and ipilimumab arms, respectively (most commonly diarrhea [9.3%, 2.2%, 6.1%], increased lipase [8.6%, 3.5%, 3.9%], increased alanine aminotransferase [8.3%, 1.3%, 1.6%], and colitis [7.7%, 0.6%, 8.7%]). Drug-related adverse events led to discontinuation in 36.4%, 7.7%, and 14.8% of patients in the nivolumab + ipilimumab, nivolumab, and ipilimumab arms, with 0, 1, and 1 drug-related deaths, respectively<sup>[63]</sup>. These data highlighted the significantly higher toxicity effect with the combinations of immune checkpoint inhibitors, i.e., nivolumab + ipilimumab<sup>[63]</sup>.

## Synergistic combination immunotherapies

Progress in understanding the immune system in general,

and the immune checkpoint modulation in particular, allows a new chapter to be written in the history of drug development<sup>[74]</sup>. Interestingly, clinical trials combining blockade of CTLA-4 and PD-1 may serve as a paradigm to guide future approaches to immuno-oncology combination therapy<sup>[75]</sup>. The CheckMate 067 trial data on combination of ipilimumab and nivolumab that was presented at ASCO 2015 by Wolchok has already been discussed here earlier<sup>[63]</sup>. Several other immunomodulatory pathways and also inhibitory factors that may be expressed or secreted by myeloid and stromal cells in the tumor microenvironment may be good putative targets for synergy with immune checkpoint blockade<sup>[76]</sup>. Sullivan et al. observed that with the ongoing research efforts that have highlighted the efficacy of small-molecule BRAF and mitogen-activated protein kinase (MAPK) - extracellular signal regulated kinase (ERK) or MEK inhibitors, in addition to immune checkpoint inhibitors, it may now be possible to better melanoma treatment approaches in particular, and cancer in general, by employing rational combinations of immunotherapies, molecularly-targeted therapies, and immunotherapies with molecularly-targeted therapies<sup>[77]</sup>. Given the plethora of putative immune pathway targets, it is important to consider which combinations should be accelerated in discovery programs and which subset of patients will likely be benefited by these therapies<sup>[76]</sup>.

Indeed, combinatorial therapies for melanoma, and other cancers, is not a distant dream of the future any more but is today fast becoming a reality in the clinical care setting with a rapidly-evolving evidence-base<sup>[78]</sup>. Notably, Fu et al. formulated an interferon  $\gamma$ -inducing cancer vaccine called TEGVAX (that combined GM-CSF and multiple toll-like receptor agonists to increase the number of activated dendritic cells) which retarded tumour growth in C57BL/6, Balb/c, and C3H/HeOJ mice; however, unexpectedly upregulated PD-L1 expression in the tumour microenvironment led to incomplete tumour elimination<sup>[79]</sup>. Combining nivolumab with TEGVAX elicited complete regression of established tumours in these murine models providing a mechanistic rationale for combination of interferon  $\gamma$ -inducing cancer vaccines and immune checkpoint blockade<sup>[79]</sup>. Fujimura et al. successfully treated two patients of metastatic melanoma with nivolumab in concert with contact immunotherapy by using contact-sensitizing agents including squaric acid dibutylester and diphencyprone<sup>[80]</sup>.

## Conclusions

Nivolumab, administered via intravenous infusion, has an acceptable safety profile and good efficacy. The encouraging literature on nivolumab lends credence to the promise of immune checkpoint blockade, not just in terms of its feasibility as an oncotherapeutic strategy, but also as a key tool of the future in the therapeutic arsenal against advanced cancers. However, despite our knowledge of the remarkable clinical benefits of nivolumab in metastatic cancers, only a few subsets of patients responded to antibody-based therapies including nivolumab or ipilimumab. Heterogeneity in lymphocyte infiltration and low frequency of anti-cancer-reactive T-cells in tumour lesions are partly responsible for the lack of response to antibody-based therapies<sup>[73]</sup>. Since PD-L1 is a weak biomarker as discussed earlier, it is difficult for the clinician to know precisely whether the patient will respond to nivolumab therapy or not. This can lead to significant “financial toxicity”<sup>[81]</sup> to the patient as immunotherapies are extremely expensive and can limit the use of these drugs or result in patient non-compliance in following up on scheduled infusion doses<sup>[82,83]</sup>. A potential reason for variability in response to immunotherapy may be due to pharmacogenomic differences in the population which may explain why only a selected patient population benefit from these therapies. Hence, the importance of the emerging field of immunopharmacogenomics in guiding patient selection and monitoring of anti-immune checkpoint treatment as recently discussed by Choudhury and Nakamura<sup>[84]</sup>. Interestingly, an automated PD-L1 immunohistochemistry assay is currently being investigated in clinical studies for use as an *in vitro* diagnostic in order to select and stratify patients for treatment with anti-PD-1 therapeutic antibody nivolumab<sup>[85]</sup>. In addition, the way forward to leverage maximum benefits for the cancer patient and to improve upon the existing clinical benefits with nivolumab may be to synergize both anti-PD-1 blockade with complementary targets in immune checkpoint pathways or other oncogenic signal transduction pathways.

Recently, the European Association of Urology renal cancer guidelines updated a recommendation on nivolumab and cabozantinib over the previous standard-of-care in patients who have failed one or more lines of VEGF targeted therapy<sup>[86]</sup>. With very interesting data being presented at ASCO 2015 as discussed earlier, it is now obvious that nivolumab shows better efficacy in patients with high mutation-burden tumours, and that

combination therapy of nivolumab plus ipilimumab, or only nivolumab, is more effective than only ipilimumab monotherapy. Additionally, PD-1 blockade is beneficial in colorectal tumours with mismatch repair deficiency as already discussed here. The US FDA has approved nivolumab for metastatic melanoma, NSCLC, and RCC, and, thus, these are currently the mainstay of therapeutic areas where nivolumab fits into clinical practice. As more clinical data emerges globally, it is almost certain that approvals for nivolumab will be visible in other cancer therapeutic areas including lymphoma, hepatocellular carcinoma, and colorectal carcinoma amongst others.

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## Conflict of interest

The author declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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