Commentary

Does PD1/PDL1 Immunotherapy Require Patient Tumor Subgroup Pre-Analysis in the Era of Precision Medicine?

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https://doi.org/10.31083/j.jmcm.2018.01.007

Abstract

Inter-individual variability of the therapeutic response of patients with cancer to anti-PD1 immunotherapy is a determinant for precision medicine. Anti-PD1 monoclonal antibodies (mAb) interact with the PD1 receptor expressed on T cells, hence preventing the recognition of PDL1 ligand on tumor cells and enhancing their cytotoxic effect. The Food & Drug Administration (FDA) as well as the European Medicines Agency (EMA) have approved anti-PD1 mAb for several human cancer therapies, including malignant melanoma, non-small cell lung cancer, renal cancer, urothelial cancer, and Hodgkin's lymphoma. Anti-PD1 mAb can increase overall survival or progression free survival, but in some subgroups of patients they have shown lower or no therapeutic effect. In recent years, expression levels of PDL1 in tumor cells have been recognized as a determinant for predicting the responsiveness to anti-PD1 mAb therapy, however other factors such as age, or somatic mutations might also play a role. Here we propose the pre-evaluation of PD1 receptor expression status as a prerequisite for patient selection for treatment with anti-PD1 mAb-based therapy.

Keywords

Immunotherapy; Cancer; Subgroups; PD1; Age

Submitted: November 20, 2017; **Accepted:** November 30, 2017

Cancer immunotherapy has currently gained a pivotal role in therapeutics of various malignancies and it was declared the breakthrough of the year in 2013 by *Science* [1]. The immune system controls tumor progression, and tumor cells activate multiple mechanisms to escape the immune system, including loss of tumor-associated antigens (TAAs) and/or major system histocompatibility complex (MHC), secretion of cytokines and neo-expression of inhibitory membrane molecules [2]. T cells are crucial for immune responses, in particular the "immune checkpoints", a type of on/off switches of T cell signaling, are fundamental to kill tumor cells without damaging the normal tissue [3]. These "on/off immune checkpoints switches" marked a relevant shift point towards novel and efficacious cancer treatment [4]. Immune checkpoint inhibitors target key signaling pathways such as CD28/cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) or programmed death 1 (PD1)/programmed death-ligands 1 and 2 (PDL1 and PDL2) to improve anti-tumor immune responses [5]. CTLA-4 enhances the immunosuppressive activity of regulatory T cells during priming and activation, whereas PD1/PDL1 pathways address T cell exhaustion and tolerance.

Among immune checkpoint inhibitors, ipilimumab (IPI) was the first mAb targeting CTLA-4 used in the treatment of malignant melanoma [6]. Nivolumab (NIVO) and pembrolizumab (PEM), were the first checkpoint inhibitors targeting PD1 that have demonstrated unprecedented clinical efficacy in several cancer types [7]. Consequently, the FDA has approved NIVO and/or PEM for the treatment of malignant melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), Hodgkin's lymphoma, squamous cell carcinoma of the head and neck, urothelial carcinoma, colorectal cancer, hepatocellular carcinoma, and gastric or gastroesophageal adenocarcinoma. The FDA has also approved three anti-PDL1 mAb: avelumab for the treatment of locally advanced or metastatic urothelial carcinoma and metastatic Merkel cell carcinoma, durvalumab only for patients with locally advanced or metastatic urothelial carcinoma, and atezolizumab for metastatic NSCLC and locally advanced or metastatic urothelial carcinoma (*https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm*). The immune checkpoint inhibitors currently approved by the FDA are summarized in Table 1. Approval of PD1/PDL1 inhibitors by Regulatory Agencies is based on clinical phase 3 studies as reflected in their antitumor activity, mainly improving the overall survival (OS) or progression/disease free survival (PFS/DFS) in the entire patients population that entered these clinical trials [8]. However, some significant differences in the antitumor activities were observed among patients' subgroups regarding PDL1 expression, demographic characteristics, or mutational status of tumor cells.

PDL1 expression is largely widespread on the surface of many different cell types: hematopoietic cells such as T cells, B cells, dendritic cells (DCs), macrophages, mesenchymal stem cells and bone marrow-derived mast cells, and on non-hematopoietic cells as vascular and stromal endothelial cells, pancreatic islet cells, astro-

Table 1. Immune-checkpoint inhibitors approved by FDA between 2014-2017. To the complete list see https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm

Drug Approved	Association?	Disease	Target	Genetic status	"Phenotypic" status	Date approval (dd/mm/yy)
Nivolumab	no	Hepatocellular carcinoma (HCC)	Anti-PD-1	–	–	22/09/2017
Pembrolizumab	no	Recurrent locally advanced or metastatic, gastric or gastroesophageal junction adenocarcinoma	Anti-PD-1	–	tumours expressing PD-L1	22/09/2017
Nivolumab	no	Mismatch repair deficient (dMMR) and microsatellite instability high (MSI-H) metastatic colorectal cancer	Anti-PD-1	–	–	01/08/2017
Pembrolizumab	no	Mismatch repair deficient (dMMR) and microsatellite instability high (MSI-H) solid tumours or colorectal cancer	Anti-PD-1	–	–	18/05/2017
Pembrolizumab	no	Locally advanced or metastatic urothelial carcinoma	Anti-PD-1	–	–	18/05/2017
Pembrolizumab	+Pemetrexed +Carboplatin	Metastatic non-squamous non-small cell lung cancer (NSCLC)	Anti-PD-1	–	–	10/05/2017
Avelumab	no	Locally advanced or metastatic urothelial carcinoma	Anti-PD-L1	–	–	09/05/2017
Durvalumab	no	Locally advanced or metastatic urothelial carcinoma	Anti-PD-L1	–	–	01/05/2017
Avelumab	no	Metastatic Merkel cell carcinoma	Anti-PD-L1	–	–	23/03/2017
Pembrolizumab	no	Refractory classical Hodgkin lymphoma (cHL)	Anti-PD-1	–	–	15/03/2017
Nivolumab	no	Locally advanced or metastatic urothelial carcinoma	Anti-PD-1	–	–	02/02/2017
Nivolumab	no	Recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN)	Anti-PD-1	–	–	10/11/2016
Pembrolizumab	no	Metastatic non-small cell lung cancer (NSCLC)	Anti-PD-1	–	tumours expressing PD-L1	24/10/2016
Atezolizumab	no	Metastatic non-small cell lung cancer (NSCLC)	Anti-PD-L1	EGFR + ALK + with tumour progression	–	18/10/2016
Pembrolizumab	no	Recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN)	Anti-PD-1	–	–	05/08/2016
Atezolizumab	no	Locally advanced or metastatic urothelial carcinoma	Anti-PD-L1	–	–	18/05/2016
Nivolumab	no	Classical Hodgkin lymphoma (cHL)	Anti-PD-1	–	–	17/05/2016
Pembrolizumab	no	Unresectable or metastatic melanoma	Anti-PD-1	–	–	18/12/2015
Nivolumab	no	Advanced renal cell carcinoma (RCC)	Anti-PD-1	–	–	23/11/2015
Ipilimumab	no	Cutaneous melanoma	Anti-CTLA-4	–	–	28/10/2015
Nivolumab	no	Metastatic non-small cell lung cancer (NSCLC)	Anti-PD-1	EGFR + ALK + with tumour progression	–	09/10/2015
Pembrolizumab	no	Metastatic non-small cell lung cancer (NSCLC)	Anti-PD-1	–	tumours expressing PD-L1	02/10/2015
Nivolumab	+Ipilimumab	Unresectable or metastatic melanoma	Anti-PD-1	BRAF V600 wild type	–	30/09/2015
Nivolumab	no	Metastatic squamous non-small cell lung cancer (NSCLC)	Anti-PD-1	–	–	04/03/2015
Nivolumab	no	Unresectable or metastatic melanoma	Anti-PD-1	BRAF V600 mutated	–	22/12/2014
Pembrolizumab	no	Unresectable or metastatic melanoma	Anti-PD-1	BRAF V600 mutated	–	04/09/2014

cytes, neurons, and keratinocytes. PDL1 is also expressed on placental syncytiotrophoblasts to induce fetal-maternal tolerance as human leukocyte antigen G (HLA-G) [9]. Pro-inflammatory signals induce PDL1 overexpression in cancer cells. PD1 becomes expressed on $CD4^+$ and $CD8^+$ T cells during initial antigen-mediated activation through the TCR; PDL1 is also expressed on B cells, monocytes, natural killer (NK) cells, and dendritic cells (DCs) [10]. The PD1/PDL1 interaction attenuates TCR-mediated signaling and impairs the activity of two signaling cascades co-required to initiate T cell activation: the PI3K/Akt and the Ras/MEK/Erk pathways. PTEN phosphorylation by CK2 and the consequent suppression of its phosphatase activity is one of the many mechanisms through which PD-1 inhibits the activation of the PI3K/Akt pathway. Moreover, PD1 inhibits Ras activation in the Ras/MEK/Erk cascade. These molecular mechanisms could suggest that the antitumor activity of anti-PD1 inhibitors might be influenced by PDL1 expression in tumor cells as well as by the biochemical mechanisms affecting the signaling cascade in tumor cells (i.e. the mutational status) [11]. Moreover, the impact of ageing on the immune system has been already recognized [12]. Morphological, cellular and biochemical changes responsible for "immunosenescence" diminish the effectiveness of the immune system in destroying tumor cells in elderly people [13, 14]. The different age of patients should be considered using immune checkpoint inhibitors.

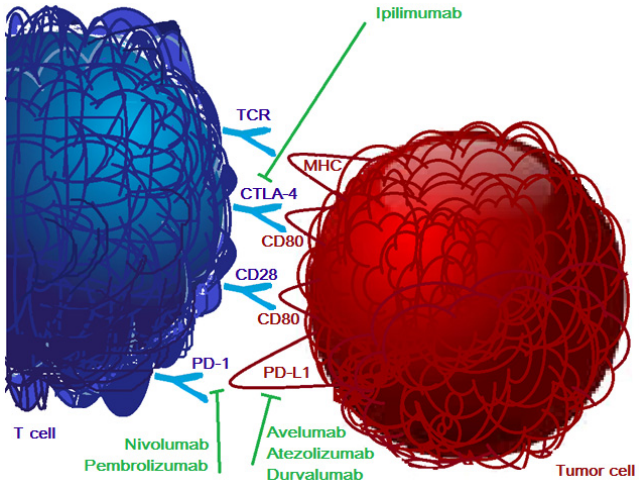

Fig. 1. Mechanism of action of approved immune checkpoint inhibitors. The induction of immunosuppression depends on the expression of negative co-stimulatory molecules such as CTLA-4 and PD-1 on T cell (in blue) and of PD-L1 on tumor cell (in red). Ipilimumab inhibits CTLA-4; nivolumab, pembrolizumab, avelumab, atezolizumab, and durvalumab affect PD-1/PD-L1 interaction. TCR: T- Cell Receptor; CTLA-4: cytotoxic T lymphocyte-associated antigen 4; CD: Cluster of Differentiation; PD-1: programmed death 1; PD-L1: programmed death-ligands 1; MHC: major histocompatibility complex

Metastatic melanoma was the first cancer treated with anti-PD1 drugs. NIVO and PEM were approved in melanoma patients by Regulatory Agencies without any request for PD1/PDL1 expression, mutational status of melanoma cells, or patient demographic characteristics. Recently, an update of the OS of the CheckMate 067 study was published [15]. This randomized, double-blind, phase 3 study compared NIVO alone or NIVO plus ipilimumab with ipilimumab alone in patients with metastatic melanoma [16]. The median OS (mOS) at 3 years was significantly different in the combinatory NIVO + IPI arm and NIVO alone versus IPI alone, respectively, but no significant differences were observed between NIVO + IPI versus NIVO (HR 0.85; 95% IC, 0.68 to 1.07). The median OS at 3 years was not reached in the NIVO + IPI group (95% CI, 38.2 months to not reached), was 37.6 months (95% CI, 29.1 to not reached) in the NIVO group, and was 19.9 months (95% CI, 16.9 to 24.6) in the IPI group [15]. The Hazard Ratio (HR) for death with NIVO + IPI vs IPI was 0.55 (95% CI, 0.45 to 0.69; $P < 0.001$) and with NIVO vs IPI was 0.65 (95% CI, 0.53 to 0.80; $P < 0.001$).

In contrast with the findings observed in the entire patients population, a subgroup analysis showed that OS was statistically different (HR 0.70) in the NIVO + IPI group compared to NIVO alone in tumor patients with PDL1 expression level $< 1\%$ but not in patients with PDL1 expression $\geq 5\%$ (HR 0.99) [15]. In patients with PDL1 expression $< 1\%$, the mOS was not reached (95% CI, 26.5 to not reached) in the combination NIVO + IPI group and was 23.5 months (95% CI, 13.0 to 36.5) in the NIVO group. In patients with PDL1 expression level $\geq 5\%$, mOS was not reached in both combination NIVO + IPI and NIVO group (95% CI, 39.1 months to not reached vs. 95% CI, 35.8 months to not reached, respectively). Notably, the addition of IPI to NIVO increased the side effects of therapy, hence rendering this combination therapy questionable in patients with tumor PDL1 expression level $\geq 5\%$.

A subgroup analysis based on the stratification according to BRAF mutational status demonstrated a statistical difference in OS in the NIVO + IPI arm compared to NIVO arm in patients with BRAF mutated (HR 0.69) but not in BRAF WT patients (HR 0.94). In the subgroup analysis of OS at 3 years, 68% of BRAF mutated patients were alive in the NIVO + IPI arm compared to 56% in the NIVO monotherapy arm. In patients without BRAF mutations, the percentage of living patients at 3 years was 53% and 50% in the NIVO + IPI and NIVO alone, respectively [15]. These data could suggest that the discrimination between PDL1 expression and BRAF wild type or mutated is relevant for the OS of patients with melanoma regardless of the anti-PD1 mAb used.

Two-phase-3 trials have compared the effect of NIVO versus docetaxel in NSCLC. In the CheckMate 057 study, a phase 3 study where patients with NSCLC progressed during or after platinum-based doublet chemotherapy, has reported an improved mOS in patients with advanced non-squamous NSCLC treated with NIVO [12.2 months (95% CI, 9.7 to 15.0) vs. 9.4 months (95% CI, 8.1 to 10.7) in the docetaxel group], as well as in the response rate (19% vs. 12%) [17]. At the interim analysis, NIVO was associated with greater efficacy than docetaxel both in terms of OS and PFS in subgroups defined according to pre-specified levels of PDL1 tumor-membrane expression ($\geq 1\%$, $\geq 5\%$, and $\geq 10\%$). In particular, mOS for patients with $\geq 1\%$, $\geq 5\%$, or $\geq 10\%$ PDL1 expression was 17.7 months (NIVO) vs 9.0 months (docetaxel), 19.4 vs. 8.1 months or 19.9 vs. 8.0 months, respectively. In the docetaxel arm, no major differences in response were observed, according to PDL1 expression. In a sub-group analysis of OS according to the smoking status, the study demonstrated that never smoked patients respond less to NIVO therapy (HR 1.02; 95% CI, 0.64 to 1.61), whereas a significant difference was observed in smoker patients treated with NIVO vs docetaxel (HR 0.70; 95% CI, 0.56 to 0.86). It has been reported that EGFR mutations increase in smoker patients. EGFR mutated tumors do not seem to benefit from NIVO therapy compared to docetaxel (HR 1.18; 95% CI, 0.69 to 2.00).

Patient's age could be detrimental for immune response. In the CheckMate 057 trial patients with $\geq$ 75 years of age appear not to benefit from NIVO therapy compared to docetaxel. Even if the subgroup analysis concerns a small size sample, this suggestion is supported by other studies with NIVO in NSCLC (CheckMate 057 [17]), renal cell carcinomas (CheckMate 025 [18]) and PEM in NSCLC (Keynote 010 [19]).

To date, only PEM was approved by Regulatory Agencies according to PDL1 expression levels in tumor cells. PEM was approved for NSCLC based on the KEYNOTE-010 clinical trial, a randomized phase 2/3 study evaluating the efficacy of PEM versus docetaxel for previously treated patients with PDL1 expression $\geq$ 1% [19] and on the KEYNOTE 024, a randomized phase 3 study comparing PEM with chemotherapy in previously untreated advanced NSCLC patients with PDL1 expression $\geq$ 50% [20]. For the KEYNOTE-010 trial, mOS was 10.4 months (95% CI, 9.4 to 11.9) for the PEM 2 mg/kg group, 12.7 months (95% CI, 10.0 to 17.3) for the 10 mg/kg PEM group, and 8.5 months (95% CI, 7.5 to 9.8)for the docetaxel group. In the KEYNOTE 024 trial the estimated OS rate at 6 months was 80.2% in the PEM arm vs 72.4% in the chemotherapy arm. In the KEYNOTE-010 trial, no significant differences in OS were observed in patients with EGFR mutated tumors.

In conclusion, immunotherapy with anti-PDL1 inhibitors is profoundly changing the management of patients with cancer. These immunotherapeutic antitumor drugs have been approved by Regulatory Agencies for the treatment of different types of cancer thanks to the significant improvements in clinical parameters such as OS and PFS. Approval has been generally based on the clinical activity in the entire population that entered these registered clinical trials. However, in the era of precision medicine, a more precise strategy could be applied to pre-select patients that could truly benefit from immunotherapy. The subgroup analysis of the registered trials shows significant differences among tumors exhibiting different levels of PDL1 expression, somatic mutation and even patient age. Even if extrapolated final conclusions from subgroup analysis could be misleading for definitive decisions, the subgroup analysis encourages prospective clinical trials to better define patients who can actually benefit from immunotherapy. In the era of precision medicine, the assessment of tumor characteristics in patients appears to become a pre-requisite in order to administer the proper PD1 inhibitor to the well-defined individual patient.

Acknowledgments

This work was supported by "Application of advanced nanotechnology in the development of innovative cancer diagnostic tools," AIRC 5 $\times$ 1000 Special Program Molecular Oncology (no. 12214).

Conflict of Interest

All the authors declare no conflicts of interest.

Author Contributions

Lucia Scarabel analysed the literature data. Giuseppe Toffoli revised critically the literature data. All authors discussed the results and contributed to the final writing of the manuscript.

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