Commentary

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

Lucia Scarabel, Giuseppe Toffoli*

Experimental and Clinical Pharmacology Unit, CRO Aviano National Cancer Institute, IRCCS, via F. Gallini 2, Aviano (PN) 33081, Italy

*Correspondence: gtoffoli@cro.it (Giuseppe Toffoli)

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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

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 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 mAbs: 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-
| 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 | –             | –                   | 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  | 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 |
| Pembrolizumab | no           | Locally advanced or metastatic urothelial carcinoma of the head and neck (SCCHN) | Anti-PD-1 | –             | tumours expressing PD-L1 | 24/10/2016 |
| Pembrolizumab | 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 placent
tal sncityophoblasts 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, nat-
ural killer (NK) cells, and dendritic cells (DCs) [10]. The PD1/PDL1
interaction attenuates TCR-mediated signaling and impairs the activ-
ity of two signaling cascades co-required to initiate T cell activation: the
PI3K/Akt and the Ras/MEK/Erk pathways. PTEN phosphory-
lation 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 mechan-
isms 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
“immunosenesence” diminish the effectiveness of the immune sys-
tem in destroying tumor cells in elderly people [13, 14]. The differ-
tent age of patients should be considered using immune checkpoint
inhibitors.

![Mechanism of action of approved immune checkpoint inhibitors](image)

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 histocompatibil-
ity 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 charac-
teristics. 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% CI, 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 popu-
lation, 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 ≥ 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 ≥ 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,
hereby rendering this combination therapy questionable in patients
with tumor PDL1 expression level ≥ 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-
expression (≥ 1%, ≥ 5%, and ≥ 10%). In particular, mOS for patients with
≥ 1%, mOS was not reached (95% CI, 26.5 to not reached) in the
combination 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.
Patient’s age could be detrimental for immune response. In the CheckMate 057 trial patients with ≥ 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 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 ≥ 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 ≥ 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 observed in patients with EGFR mutated tumors.

In the KEYNOTE-010 trial, no significant differences in OS were observed in patients with EGFR mutated tumors.

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**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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