PD-1/PD-L1 Pathway in Breast Cancer

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Summary
The programmed cell death-1 receptor (PD-1) is an immune checkpoint inhibitor which is expressed on the surface of immune effector cells. It is activated mainly by PD-L1 which can be expressed by all human cells. The PD-1/PD-L1 pathway plays a subtle role in maintaining peripheral T-lymphocyte tolerance and regulating inflammation. In cancer, the expression of PD-L1 seems to be one of the major immune escape mechanisms. Many studies have shown efficacy of blocking PD-1 or PD-L1 with specific antibodies like pembrolizumab or atezolizumab. In breast cancer, potential response was demonstrated in metastatic triple-negative breast cancers.

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Introduction
Solid tumors are initiated by a combination of mutations within their genetic information. Mutations may not only induce proliferation and invasion but also de novo antigens by changing DNA read-out. Every foreign antigen alerts the immune system, leading to an immune reaction directed against it. Evidently, in existing solid tumors, this antigen-directed immune reaction is initially ineffective or disabled or both, a phenomenon called immune escape.

PD-1/PD-L1 Pathway

PD-1 is an inhibitory immune checkpoint inhibitor which is expressed on the surface of T-cells, B-cells, natural killer T-cells,
monocytes, and dendritic cells, but not resting T-cells (fig. 2). The PD-1 pathway plays a subtle role in maintaining peripheral T-lymphocyte tolerance and regulating inflammation [9].

PD-1 was originally isolated from a T-cell hybridoma undergoing T-cell receptor activation-induced cell death, hence its name, programmed cell death-1 [10]. Despite its name, PD-1 does not induce cell death directly but reduces cell growth factors as well as survival signals. PD-1 binds 2 ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC) [11, 12]. Activation of PD-1 by PD-L1 or -L2 induces downregulation of T-cell activity, reduced cytokine production, T-cell lysis, and induction of tolerance to antigens [13–16]. In vitro blockade of PD-1 with monoclonal antibodies led to a 2-fold increase in cytokine production [17]. However, the in vivo activity also depends on T-cell motility as well as the duration of the interaction with antigen-presenting cells and target cells [18]. When T-cells have been activated by their TCR, PD-1 is expressed simultaneously to offer the attacked cell a way of escaping the immune response. PD-1 decreases once the immune response has eliminated the pathologic antigen [19]. An important role of the PD1/PD-L1 pathway has been shown in diabetes [20], cardiomyopathy [21], human immunodeficiency virus infection [22], lupus [23] and other autoimmune diseases [24], as well as in solid tumors.

Indeed, the blockade of immune checkpoints using respective monoclonal antibodies has been shown to trigger efficient antitumor responses not only in classical ‘immunogenic’ tumor types such as melanoma and renal cell carcinoma, but also in many other solid tumors including lung, colorectal, ovarian, esophageal, bladder, and breast cancer.

**PD-1/PD-L1 Pathway in Solid Tumors**

In solid tumors, the PD-1/PD-L1 inhibitory pathway can be (mis-)used to silence the immune system by increasing the expression of PD-L1 on the tumor cell surface [25]. PD-L1 expression has been associated with large tumor size, high grade, high proliferation, estrogen receptor-negative status, and HER2-positive status [26], and it is inversely correlated with survival in ovarian [27, 28] and breast cancer [29, 30]. PD-L1 is expressed in 20% of triple-negative breast cancers (TNBCs) [31]. This indicates that although antitumor immunity is elicited against many solid tumors, it is counterbalanced by immunosuppressive factors. It was shown in vivo that PD-L1 increases tumorigenesis and invasiveness and makes tumor cells less susceptible to specific CD8+ T-cells [32]. Melanoma tumor growth is widely suppressed in PD-1 knockout mice. Furthermore, it was shown in vivo that a blockade of the PD-1/PD-L1 pathway using specific antibodies leads to stronger tumor regression in cellular immunotherapies [33].

Translational research indicates that interferon-gamma, secreted by tumor-infiltrating cytotoxic T-cells, leads to an upregulation of PD-L1 on the surface of melanoma cells that activates the PD-1 receptor to prevent immune recognition and destruction of melanoma cells.

The goal of immune checkpoint inhibitors such as anti-CTLA-4 and anti-PD-1/anti-PD-L1 is to ‘release the brakes’ and enhance T-cell activation by blocking negative pathways.

**PD-1-Directed Treatment**

The first PD-1 antibody examined in humans was nivolumab. In 2 phase I trials with multiple tumor entities it was shown that despite the fact that only a low number of patients responded to the treatment, those few responders had an impressive long-lasting
effect in terms of tumor remission [34]. Especially melanoma, non-
small cell lung cancer, and kidney cancer patients showed promising
results with response rates of up to 33%. However, even in these early studies, immune-related adverse events grade 3/4 were shown in 5% of the patients, which is now known as immune-related toxicity. These events included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis, and were not correlated to
dose. Even lethal events due to toxicity were reported. Hence, it is
mandatory for clinicians to be able to deal with this new toxicity profile [35].

Another antibody – later named pembrolizumab – was tested in
multiple phase I–III trials in solid tumors (mainly melanoma and
lung cancer), showing similar results in terms of the number of res-
ponding patients, duration of response, efficacy, and toxicity com-
pared to nivolumab.

These interesting results naturally raise the question of whether
PD-1/PD-L1 pathway-directed therapy can also be of benefit in
breast cancer patients. Breast cancer has several subtypes that can
be analyzed by immunohistochemistry or with gene expression pro-
files. Especially in the estrogen-, progesterone-, and HER2-neg-
ative subtype (TNBC), in which many mutations occur that may
give rise to neoantigens, we see immunogenic potential. The pres-
ence of tumor-infiltrating lymphocytes within the tumor tissue
[36] as well as the prognostic value of immunity-related gene sig-
natures in TNBC are proof of this hypothesis [37]. Especially in
TNBC with its immune gene signature, PD-L1 expression can be
detected in a higher proportion of tumors [38].

Single-agent pembrolizumab was tested in 27 heavily pretreated
patients with metastatic PD-L1-positive TNBC within the phase Ib
study, KEYNOTE-012. The antibody pembrolizumab was given in-
travenously at 10 mg/kg every 2 weeks. Tumor samples were
screened for PD-L1 expression using a prototype immunohisto-
chemistry assay. Patients with distinctive stromal or ≥ 1% tumor
cell nest PD-L1 staining were eligible because some data suggested
that patients with PD-L1 overexpressing tumors have improved
clinical outcomes with anti-PD-1-directed therapy. There was a
clinical benefit rate of approximately 20%: 1 complete response
(CR), 4 partial responses (PR), and 7 cases of stable disease (SD); 3
patients remained on pembrolizumab for at least 11 months. 1
treatment-related death due to disseminated intravascular coagula-
tion was reported. Again, patients who experienced a benefit with
pembrolizumab showed long duration of response (median 17.9
weeks (range 7.3–32.4 weeks)) [39]. In this small cohort, the re-
ported side effects were comparable to those reported from mel-
anoma and lung cancer trials. In the future, a combination with cy-
totoxic therapies (e.g., chemotherapy or radiation) might prove
more effective than PD-1 inhibition alone. However, to date, there
are only preliminary data with no efficacy data available as yet.

Atezolizumab is a human anti-PD-L1 antibody with a modified
Fc region to avoid antibody-dependent cytotoxicity or comple-
ment-dependent cytotoxicity induction [40]. A total of 54 TNBC
patients treated within a phase I study reached a 19% objective
response rate. The duration of response ranged from 0.1 to > 41.6
weeks.

Furthermore, the anti-PD-1 nivolumab (BMS-936558/MDX-
1106) and the anti-PD-L1 durvalumab (MEDI4736) are currently
under investigation in breast cancer.

Is PD-L1 Expression in Tumors a Predictive
Biomarker?

Since only a minority of cancer patients showed clinically rele-
vant tumor remission after anti-PD-1 treatment, a predictive factor
is required to determine patients who will derive a benefit from a
checkpoint inhibitor strategy. There are several unresolved issues
regarding PD-L1 analysis: variable detection antibodies, differing
immunohistochemistry cut-offs, tissue preparation, processing
variability, primary versus metastatic biopsies, oncogenic versus
induced PD-L1 expression, and staining of tumor/stroma versus
immune cells. However, despite the problems of different assays
and undefined standards, it was shown in vivo that responses to
antibody therapy were greater in tumors with high PD-L1 expres-
sion [41]. Trials conducted in melanoma patients reported that pa-
tients with high PD-L1 expression had a greater chance of response
than those with low expression. However, there was a remarkable
number of false-negative results in tumors with low PD-L1 expres-
sion even with low cut-offs; furthermore, even if high PD-L1 ex-
pression was found, 70% of melanoma tumors did not respond to

Hence, PD-L1 expression does not seem to be a reliable predic-
tive marker as it would exclude patients from receiving an effective
treatment they may in fact respond to.

Conclusion

Immunomodulation seems to be a promising strategy in solid
tumors. High immunogenicity has been described in breast cancer
subtypes with a high proliferation index (TNBC, HER2). Immune
checkpoints are one of the major mechanisms of immune escape.
Expression of PD-L1 on tumor cells leads to lower activity of CD8+
T-cells. Antibodies against PD-1 or PD-L1 are being investigated in
clinical trials. First results are promising but only a subset of pa-
tients (20%) respond to immune checkpoint inhibitory treatment.
Predictive markers are urgently needed to select those patients with
the best chance for an effective treatment [42].

Disclosure Statement

The authors did not provide a disclosure statement.
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