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PD-1 and PD-L1 Immune Checkpoint Blockade to Treat Breast Cancer

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Summary

Immune checkpoint inhibition represents a major recent breakthrough in the treatment of malignant diseases including breast cancer. Blocking the programmed death receptor-1 (PD-1) and its ligand, PD-L1, has shown impressive antitumor activity and may lead to durable long-term disease control, especially in the triple-negative subtypes of breast cancer (TNBC). Although immune checkpoint blockade is generally well tolerated, specific immune-related adverse events (irAEs) may occur. This review summarizes the clinical efficacy, perspectives, and future challenges of using PD-1/PD-L1-directed antibodies in the treatment of breast cancer.

Introduction

The immune system plays an integral role in cancer development and therefore potentially offers novel targeted therapies. Complex interactions of tumor cells, immune effector cells, stromal cells, and soluble factors are crucial for disease progression and/or eradication of tumor cells [1]. Hence, the modulation of immunogenic regulators (checkpoints) is a promising approach to treat malignant disease [2]. The programmed death receptor-1 (PD-1) and its ligand, PD-L1, are increasingly recognized as powerful targets to enhance tumor-directed cytotoxic T-cell function. The PD-1-blocking antibodies pembrolizumab and nivolumab obtained Food and Drug Administration (FDA) approval for the treatment of advanced melanoma in 2014, and of non-small-cell lung cancer (NSCLC) in 2015 [3–7].

As breast cancer is also capable of stimulating immune responses, targeting the immune system is an encouraging strategy for its treatment. Triple-negative breast cancer (TNBC) in particular seems highly immunogenic because tumor-infiltrating lymphocytes (TILs), which have been demonstrated to positively correlate with response to cytotoxic therapy and prognosis, are predominantly present within hormone receptor (HR)-negative subtypes [8–11]. Encouraging results from phase I trials using checkpoint inhibitors directed against PD-1/PD-L1 have been reported, and phase II and III trials are currently ongoing. In this review, we aim to summarize recent data on PD-1/PD-L1 antibodies to treat breast cancer. While our focus lies on clinical experience and challenges, we also cover the underlying preclinical rationale of these highly promising agents.

Biology and Preclinical Rationale of Targeting PD-1 and PD-L1

Although the immune system protects its host against malignant tumor cells, it can also promote cancer development by selecting for tumor cell clones that escape immune surveillance [12, 13]. Interaction between cancer progression and immune response occurs in 3 phases. In the initial elimination phase, an acute inflammatory response activates immune effector cells (macrophages, dendritic cells, natural killer cells) that migrate into the tumor microenvironment. However, some tumor cell clones may still survive (immunosurveillance), shifting inflammation to a chronic equilibrium phase that may last for a period of many years. Finally, the tumor escapes from immune detection (escape phase), result-

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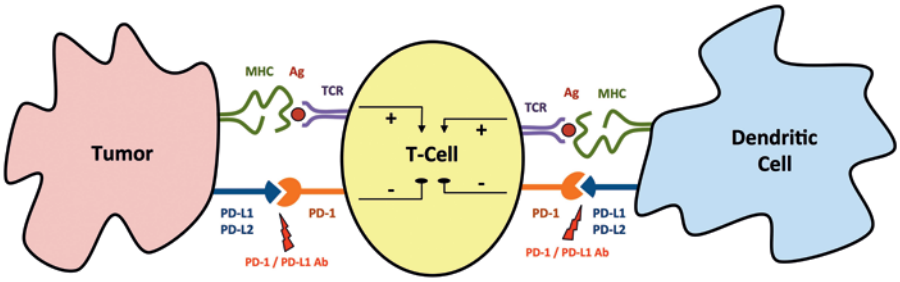

Fig. 1. Simplified illustration of the complex interaction between PD-1 and PD-L1. To initiate an antitumor immune response, tumor-specific antigens (Ag) are presented to T cells via the major histocompatibility complex (MHC). Interaction of the programmed death receptor-1 (PD-1) with its ligands (PD-L1/PD-L2) induces a negative control signal on T-cell activity that enables tumor cells to escape immune defense. Blocking the PD-1/PD-L1 axis with specific antibodies (Ab) promotes antitumor immune activity. TCR = T-cell receptor.

ing in autonomous outgrow and metastatic spread. Modulation of immune-regulating checkpoints aims at impeding immune escape and enhancing tumor-directed immune responses.

PD-1 is an immune checkpoint receptor that is expressed by activated lymphocytes (T and B cells, natural killer cells, monocytes, dendritic cells, myeloid cells, thymocytes). Interaction with its ligands PD-L1 or PD-L2 induces a negative control signal that limits T cell activity. PD-L1 suppresses autoimmunity and is constitutively expressed by T and B cells, dendritic cells, macrophages, mesenchymal stem cells, and mast cells [14]. It is also upregulated in multiple solid malignancies including breast cancer [15–18]. Figure 1 illustrates a condensed snapshot of the complex interaction between PD1 and PD-1/PD-L1 that occurs at multiple steps of an antitumor immune response and enables tumor cells to evade the immune defense [19].

Preclinical in vivo models have shown that blocking the PD-1/PD-L1 axis promotes T cell-mediated antitumor immune activity and that PD-1-deficient mice develop various spontaneous autoimmune diseases [20–22]. A number of antibodies directed against PD-1 (nivolumab, pembrolizumab, pidilizumab, PDR001) or its ligand PD-L1 (atezolizumab, durvalumab, avelumab, BMS-936559) are currently under clinical investigation. Table 1 summarizes ongoing clinical trials, identified at ClinicalTrials.gov.

There are several reasons why most current trial protocols focus on TNBC:

- PD-L1 expression is highest in TNBC (approximately 20–30% of all TNBCs express PD-L1) [15, 23].
- A significant infiltration of TILs that facilitate immune response has been reported in TNBC [8–11, 24–26].
- Loss of PTEN correlates with HR-negative breast cancer and leads to upregulation of PD-L1 [27, 28].
- TNBC is associated with a higher mutational burden that can produce immunogenic neoantigens [27, 29].
- Apart from chemotherapy, treatment alternatives for TNBC are limited, which is in contrast to HR-positive or human epidermal growth factor receptor 2 (HER2)-positive breast cancer.

Clinical Experiences in Targeting PD-1 and PD-L1 for Breast Cancer Treatment

The humanized monoclonal antibody pembrolizumab is highly selective for PD-1. KEYNOTE is a series of clinical trials to determine whether pembrolizumab is effective in the treatment of various cancers. In the phase I trial KEYNOTE-12, Nanda et al. [30] recently found clinical activity and an acceptable safety profile of pembrolizumab given intravenously at 10 mg/kg every 2 weeks to women with PD-L1-positive TNBC. The expression rate for PD-L1 was 59% and 32 patients were enrolled. Only 5 patients (16%) had toxicities grade ≥ 3. Although most patients were heavily pretreated, the overall response rate was 19%, with durations of response of up to 47 weeks (median duration not yet reached). Currently, there are ongoing phase II (KEYNOTE-86, NCT02447003) and phase III clinical trials (KEYNOTE-119, NCT02555657) that will evaluate pembrolizumab as a monotreatment for TNBC while other phase I–III studies investigate the combination of pembrolizumab with chemotherapy (table 1).

Atezolizumab (MPDL3280A) is a humanized monoclonal antibody that binds to PD-L1. Emens et al. [31] presented results of a phase I trial in patients with metastatic TNBC. Atezolizumab administered at 15 mg/kg, 20 mg/kg, or a 1200-mg flat dose every 2 weeks was well tolerated, and only 11% of a heavily pretreated population experienced adverse events of grade ≥ 3 (adrenal insufficiency, neutropenia, nausea, vomiting, and 1 pulmonary hypertension event in a patient with an atrial septal defect). Among 21 patients whose data were ready for efficacy evaluation at the time of data presentation, 3 patients had partial remission and 2 patients had complete remission. Additionally, 3 patients appeared to have progressive disease but later showed evidence of durable nonclassical responses ('pseudoprogression'). Overall, the 24-week progression-free survival rate was 33%.

Adams et al. [32] investigated the combination of nanoparticle albumin-bound paclitaxel (nab-paclitaxel; 125 mg/m^2, days 1, 8, 15, q4w) with atezolizumab (800 mg, q2w) in 32 patients with metastatic TNBC. The most common treatment-related toxicity was neutropenia (53% all grades; 41% grade 3–4). No dose-limiting toxicity or drug-related deaths occurred. Among the 24 patients who were evaluable at the time of data analysis, 1 had complete remission and 16 had partial response. In addition, 3 patients developed new lesions and were therefore scored as having progressive disease, but remained on treatment with prolonged biologic response. Treatment efficacy was observed in both PD-L1-positive and PD-L1-negative patients. An ongoing phase III trial (IMpassion) is currently evaluating the combination of atezolizumab and nab-paclitaxel in previously untreated patients with metastatic TNBC (NCT02425891).

Table 1. Ongoing clinical trials with antibodies directed against PD-1/PD-L1 to treat breast cancer

Antibody	Target	Study identifier (study name)	Phase	Breast cancer subtype	Combination with
Pembrolizumab (MK3475)	PD-1 PD-L1	NCT02555657 (KEYNOTE-119)	III	advanced TN	
		NCT02555657 (KEYNOTE-355)		advanced TN	mono-CTX
Atezolizumab (MPDL3280A)		NCT02425891 (IMpassion)		advanced TN	nab-paclitaxel
Pembrolizumab (MK3475)	PD-1	NCT02648477	II	advanced HER2+	doxorubicin/aromatase inhibitor
		NCT02447003 (KEYNOTE-086)		advanced TN	
		NCT02752685		advanced HER2–	nab-paclitaxel
		NCT02644369 (INSPIRE)		advanced TN	
Atezolizumab (MPDL3280A)	PD-L1	NCT02530489 (TN first line)		neoadjuvant TN	nab-paclitaxel
Durvalumab (MEDI4736)	PD-L1	NCT02669914		brain metastases	
		NCT02536794		advanced HER2–	tremelilumab
		NCT02685059 (GeparNuevo)		neoadjuvant TN	neoadjuvant CTX
PDR001	PD-1	NCT02404441	I–II	advanced TN	
		NCT02404441		advanced TN	
Pembrolizumab (MK3475)	PD-1	NCT02129556 (PANACEA)		advanced HER2+	trastuzumab
		NCT02657889 (KEYNOTE-162)		advanced TN	mono-CTX
		NCT02513472		advanced TN	eribulin
		NCT02452424		advanced TN	PLX3397
Atezolizumab (MPDL3280A)	PD-L1	NCT02543645		advanced TN	varlilumab
		NCT02708680		advanced TN	entinostat
Avelumab (MSB0010718C)	PD-L1	NCT02554812		advanced TN	PF-05082566, PF-04518600
Durvalumab (MEDI4736)	PD-L1	NCT02484404		advanced TN	olaparib/cediranib
		NCT02734004		advanced gBRCA+/HER2–	olaparib
		NCT02489448		neoadjuvant TN	neoadjuvant CTX
Nivolumab (BMS-936558)	PD-1	NCT02309177	I	advanced HER–	nab-paclitaxel
Pembrolizumab (MK3475)	PD-1	NCT02622074 (KEYNOTE-172)		neoadjuvant TN	neoadjuvant CTX
		NCT01975831		advanced non-TN	tremelilumab
		NCT02622074 (KEYNOTE-173)		neoadjuvant TN	neoadjuvant CTX
		NCT02452424		advanced TN	enoblituzumab
Atezolizumab (MPDL3280A)	PD-L1	NCT01375842		advanced	
		NCT02605915		advanced HER2+	trastuzumab + pertuzumab/T-DM1
Avelumab (MSB0010718C)	PD-L1	NCT01772004		advanced	
Durvalumab (MEDI4736)	PD-L1	NCT02628132		advanced TN	paclitaxel
		NCT02649686		advanced HER2+	trastuzumab

TN = Triple negative, HER2 = human epidermal growth factor receptor 2, gBRCA = germline BRCA CTX = chemotherapy, nab-paclitaxel = nanoparticle albumin-bound paclitaxel, T-DM1 = trastuzumab-emtansin.

Avelumab (MSB0010718C) is a human anti-PD-L1 antibody. In a phase 1 trial, presented by Dirix et al. [33], 168 patients with metastatic or advanced breast cancer of any subtype received 10 mg/kg avelumab every 2 weeks. Adverse events of any grade occurred in 71% of the patients, with fatigue (20%), nausea (14%), and infusion-related reactions (12%) being the most common. 14% of the patients experienced toxicities of grade ≥ 3 (fatigue, anemia, increased γ-glutamyl transferase (GGT)/autoimmune hepatitis, and

arthralgia) and there were 2 treatment-related deaths (acute liver failure, respiratory distress). 9 patients responded to treatment (1 complete response and 8 partial responses). 5 of these 9 responses were ongoing at the time of data analysis. Responders were among all subtypes of breast cancer. There was a numerical higher response rate in PD-L1-expressing TNBC patients.

Managing Toxicity and Side-Effects of PD-1- and PD-L1-Directed Treatment

The spectrum of immune-related adverse events (irAEs) differs from the toxicity known from other anticancer drugs. Although the huge majority of events are mild (grade 1–2) and reversible, clinicians should be aware of the toxicity profile of PD-1 checkpoint inhibitors to avoid delay in diagnosis and treatment [34]. irAEs can affect any organ system, but typically include the skin, the gastrointestinal (GI) tract, and the hepatic, endocrine and respiratory systems [35]. Other rare events such as uveitis, pancreatitis, hematological events, neurologic adverse events, and nephritis have also been reported [36–40]. In general, irAEs are manageable by the use of immunosuppressive therapy (e.g. glucocorticoids) without impeding the antitumor response. Whether checkpoint blockade can trigger an underlying autoimmune disorder is unclear as these patients have been excluded from clinical trials.

The most common toxicities are skin-related events. Reticular, maculopapular, erythematous rash and/or pruritus is frequent and typically involves the trunk and extremities [35, 41]. Rash and other low-grade dermatologic events can be treated with topical glucocorticoids and oral antipruritics (mainly antihistamines). Oral mucositis and dry mouth are also common and can be treated using oral corticosteroid rinses and lidocaine [7, 35]. Other dermatologic events include urticaria, vitiligo, and palmoplantar erythrodysesthesia [34]. Grade 3–4 events are rare; however, Stevens-Johnson syndrome and toxic epidermal necrolysis requiring hospitalization, discontinuation of checkpoint blockade, and intravenous corticosteroid treatment have been reported [35].

Diarrhea or colitis begins approximately after 6 weeks of checkpoint blockade and occurs in 10–20% of the patients, with a relatively low rate of grade 3–4 events (1–2%) [34]. Early symptoms can present as watery or bloody diarrhea, abdominal pain, fever, weight loss, and nausea or vomiting. *Clostridium difficile* and other infectious etiologies should be excluded and colonoscopy may be helpful to confirm or rule out colitis. Intravenous corticosteroids, hydration, and electrolyte management are required in severe cases. In patients who are refractory to corticosteroids, treatment with infliximab can be considered [42, 43].

Increased liver function test values are seen in approximately 5% of the patients; they are generally asymptomatic and mainly of grade 1–2 [5, 44]. As the onset of elevated liver enzyme levels is highly variable, hepatic function should be monitored before each treatment cycle. Management includes an oral corticosteroid or oral mycophenolate mofetil if the liver function test values do not decrease [35].

Endocrinopathies that can affect the pituitary, adrenal, and thyroid glands often present with non-specific symptoms such as headache, fatigue, weight gain or loss, and nausea. Although hypophysitis has rarely been reported in patients treated with PD-1/PD-L1-blocking agents and thyroiditis occurs in less than 10% of the patients, severe cases have been described [7, 44, 45]. Diagnosis is made by characteristic laboratory findings. In addition, radiographic changes such as an enlargement of the pituitary gland may occur [46, 47]. Thus, monitoring of the thyroid stimulation hormone (TSH) during checkpoint blockade is recommended [34]. Treatment consists of corticosteroids and, if necessary, hormonal supplementation. The very rare case of an adrenal crisis must be considered if dehydration, hypotension, and electrolyte imbalances occur [34].

For the respiratory system, the leading symptoms of non-infectious pneumonitis are dry and unproductive cough, dyspnea, and tachypnea. Diagnostic procedures include imaging (computed tomography (CT) scans), lung function tests, and a bronchoscopy in moderate to severe cases to exclude infectious etiologies (especially viral or atypical bacterial germs). Treatment consists of corticosteroids and, in severe or refractory cases, immunosuppressive agents such as mycophenolate mofetil, infliximab, or cyclophosphamide [34].

Future Challenges of PD-1- and PD-L1-Directed Treatment

The patterns of response to immune checkpoint blockade may differ from classical response criteria, such as the Response Evaluation Criteria in Solid Tumors (RECIST) [2]. The time to achieve clinical response to treatment may be prolonged and can manifest after an initial increase in tumor burden or the onset of new tumor lesions [48]. In contrast to chemotherapy, where stable disease is often regarded as transient, achievement of stable disease by the use of immunotherapeutic agents may be viewed as an indicator of a meaningful therapeutic effect [49]. Therefore, Wolchok et al. [49] and Nishino et al. [50] proposed guidelines for the evaluation of immune therapy activity in solid tumors. These immune-related response criteria continue to be refined, and further prospective evaluation is warranted.

In the era of precision oncology, predictive factors that forecast the efficacy of immune checkpoint therapy are essential to identify patients who are most likely to benefit from PD-1/PD-L1-directed therapy. Additionally, biomarkers that monitor tumor-specific immune responses as well as irAEs are warranted. A recent meta-analysis of patients with malignant melanoma or NSCLC demonstrated a significant association of PD-L1 expression and response to PD-1/PD-L1-directed treatment [51]. Nevertheless, PD-L1-negative patients may still respond to PD-1 blockade. Therefore, assessment of PD-L1 expression to identify patients for PD-1/PD-L1-directed therapy should be considered with caution and is not yet ready for clinical routine [2]. Biological and technical challenges have to be considered and standardization is required as different

antibodies and cut-off values have been used for immunohistochemistry (IHC) staining in recent trials [14]. Furthermore, PD-L1 expression is a dynamic marker which can change in response to disease progression and treatment [52, 53]. Examples of other biomarkers that are currently under investigation are mutational load, neoantigens, the presence of TILs, inflammatory gene signatures, and blood-based immune biomarkers [16, 54–57].

Combination approaches, such as adding other immunotherapeutic, cytotoxic, or targeted agents to PD-1/PD-L1 antibodies may enhance checkpoint inhibition. However, it is still unclear if any specific combination is superior to single-agent treatment [2]. The optimal dose and schedule of immune checkpoint blockade need to be determined in future clinical trials.

Conclusions

Early clinical trials of using antibodies directed against PD-1 and PD-L1 to treat breast cancer patients demonstrated exciting clinical activity. However, given the complexity of breast cancer biology and immune responses to breast cancer, many questions remain to be answered. Examples are optimal dosing, scheduling, combination approaches, response criteria, and biomarkers for immunotherapy. Clinical experience with respect to the management of irAEs is warranted. As immunotherapies may establish durable long-term disease control, this approach holds great promise to significantly improve the outcome of breast cancer patients.

Disclosure Statement

All authors declare that they have no conflicts of interest.

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