

# Targeted immunotherapy with a checkpoint inhibitor in combination with chemotherapy: A new clinical paradigm in the treatment of triple-negative breast cancer

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## ABSTRACT

The treatment of several solid and hematologic malignancies with immune checkpoint inhibitors (against programmed death receptor-1/ligand-1 [PD-1/PD-L1]) has dramatically changed the cancer treatment paradigm. However, no checkpoint inhibitors were previously approved for the treatment of triple-negative breast cancer (TNBC), a difficult-to-treat disease with a high unmet therapeutic need. Based on IMpassion130 clinical trial (NCT02425891), the Food and Drug Administration (FDA) has recently granted an accelerated approval for atezolizumab (TECENTRIQ®), a monoclonal antibody drug targeting PD-L1, plus chemotherapy (Abraxane; nab®-Paclitaxel) for the treatment of adults with PD-L1-positive, unresectable, locally advanced or metastatic TNBC. The FDA has also approved the Ventana diagnostic antibody SP142 as a companion test for selecting TNBC patients for treatment with atezolizumab. In the present review, we briefly discuss the importance of this breakthrough as the first cancer immunotherapy regimen to be approved for the management of breast cancer.

KEY WORDS: Breast cancer; triple-negative breast cancer; immune checkpoint inhibitors; predictive biomarkers; PD-L1

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## REVIEW

Recent advances in the development of cancer immunotherapy using immune checkpoint inhibitors against either programmed death receptor-1 (PD-1) or its ligand PD-L1 have revolutionized treatment of several solid tumors [1-4]. The interaction between PD-1 on T-cells and its ligands PD-L1 and PD-L2 on cancer cells promotes T-cell exhaustion and conversion of T effector cells to immunosuppressive T regulatory (Treg) cells (Figure 1) [5]. Immune checkpoint inhibitors (against either PD-1 or PD-L1) block the suppressor PD-1/PD-L1 axis contributing to the reactivation of cytotoxic T effector cells and consequently enhancing the anticancer activity of the immune system (Figure 1) [5].

Stunning successes of monoclonal antibody-based immune checkpoint inhibitors against PD-1/PD-L1 (e.g., nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, and ipilimumab) have been achieved in various cancers [6]. These include non-small cell lung carcinoma (NSCLC), renal, bladder, head and neck, gastric/gastroesophageal junction (GEJ),

microsatellite instable (MSI-H) colorectal, cervical, hepatocellular and Merkel cell carcinoma, as well as in malignant melanoma (both pediatric and adult) and classical Hodgkin's lymphoma [6,7]. In addition, an anti-PD-1 agent pembrolizumab has been approved for all solid MSI-H cancers regardless the histotype ("tumor agnostic approach") [7-10].

Triple-negative breast cancer (TNBC) is a complex and highly aggressive subtype of breast cancer lacking estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2) receptors, thereby making it difficult to treat [11]. It carries the highest metastatic potential and has the poorest clinical outcome among all the subtypes of breast cancer [11]. Due to the advances in molecular characterization of TNBC, various novel therapeutic targets including poly ADP-ribose polymerase-1 (PARP-1) inhibitors, tyrosine kinase inhibitors, immune checkpoints, anti-androgens, and epigenetic targets have come into focus [11].

Although breast cancer has been initially considered a "non-immunogenic" cancer, numerous studies have now shown PD-L1 expression in both cancer and inflammatory cells (tumor infiltrating lymphocytes [TILs]). PD-L1 positivity in cancer or inflammatory cells has been reported across the breast cancer histotypes [12-26]. In particular, ER-negative breast cancers (TNBC and HER2 positive) have been shown to be "immunogenic" and potentially amenable for the trials

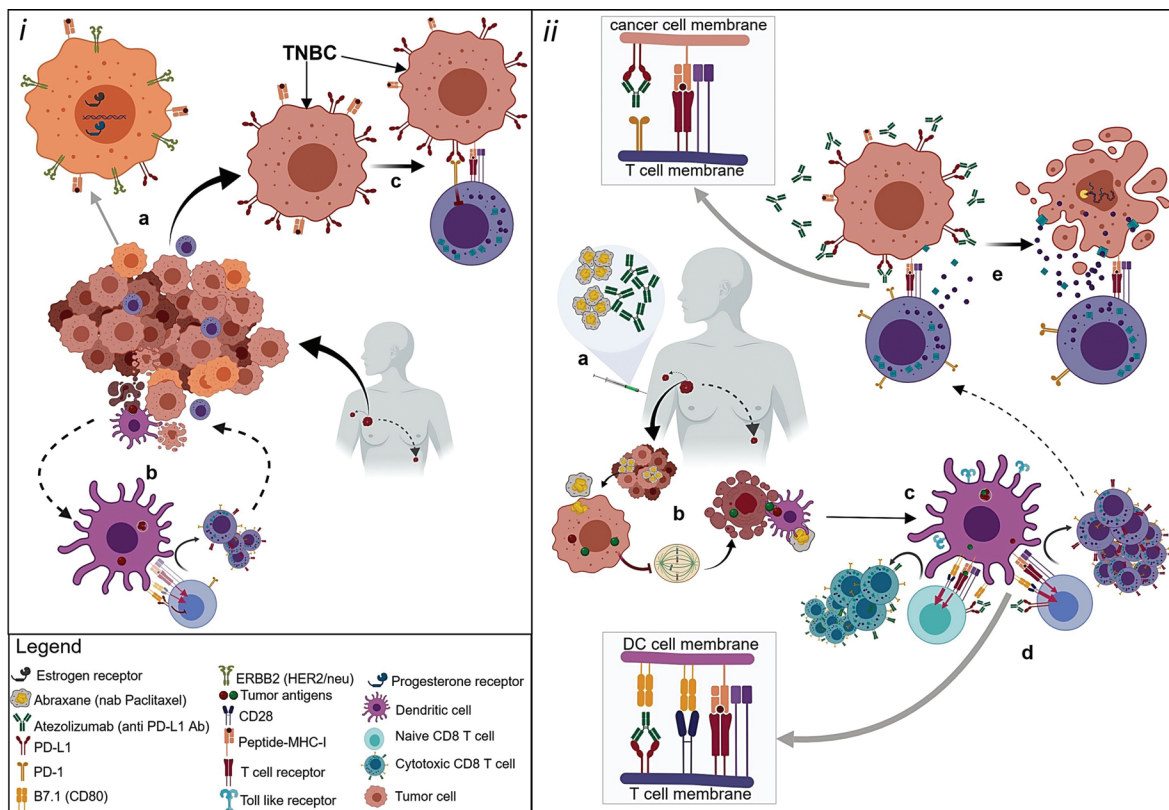
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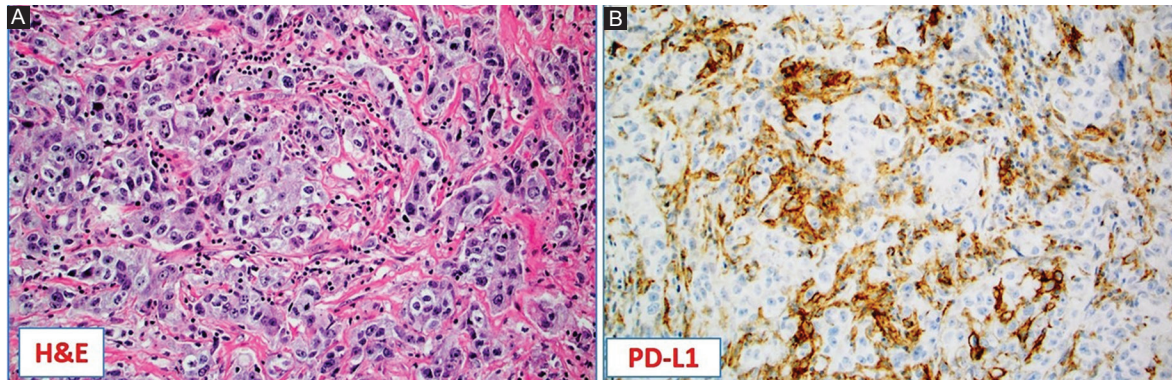
with anti-PD-1/PD-L1 agents [12,16,17,20,26-35]. TNBC is thought to have relatively high PD-L1 expression, predominantly in inflammatory (immune) cells and occasionally in cancer cells (Figure 2A and B) [14,16-18,20,29-31,36-39]. A recent systematic review of Zhang *et al.* [40], based on analysis >2500 breast cancers, revealed PD-L1 positivity in the range 21-56% (Table 1). In such studies, the tumor proportion score (TPS) is defined as “the percentage of viable tumor cells showing partial or complete membrane staining ( $\geq 1+$ ) relative to all viable tumor cells present in the sample (positive and negative)”; source: [https://www.accessdata.fda.gov/cdrh\\_docs/pdf15/p150013b.pdf](https://www.accessdata.fda.gov/cdrh_docs/pdf15/p150013b.pdf), accessed: March 10, 2019). It is noteworthy that, in most PD-L1-positive breast cancers, PD-L1 expression is not diffuse (>50% of positive cancer cells

or TPS <50%) but rather focal or patchy and limited to a small proportion of cancer cells [14].

There are numerous ongoing clinical trials with immune checkpoint inhibitors combined with other treatment modalities in breast cancer [41]. Of note has been atezolizumab which selectively targets PD-L1 to prevent interaction with the receptors PD-1 and B7-1 (a costimulatory cell-surface protein) to reverse T-cell suppression. It had previously been approved as a single-agent for the treatment of metastatic urothelial/bladder carcinoma and non-small-cell lung cancer [42,43]. This monoclonal antibody drug is clinically effective and has a good safety profile in patients with other solid tumors [1], so it was a good candidate for potential use in the management of patients with TNBC. The most remarkable results have been recently



**FIGURE 1.** The possible mechanism of action of anti-programmed death receptor-1/ligand-1 (PD-1/PD-L1) drugs (e.g., atezolizumab) and nab-paclitaxel in metastatic triple-negative breast cancer (TNBC). (i) Metastatic breast cancers are dynamic environments undergoing proliferation and apoptosis along with immune cell infiltration. Breast cancers are heterogeneous populations which may or may not (e.g., TNBC) be influenced by ERBB2/human epidermal growth factor receptor 2 (HER2) and/or nuclear estrogen receptor (ER) and progesterone receptor (PR) signaling (a). Apoptotic tumor cells are phagocytosed, and tumor-specific antigens are expressed on the major histocompatibility complex (MHC) molecules by tumor-infiltrating antigen presenting cells (APC) that may activate antigen-specific cytotoxic T cell (CTL) responses by engaging their T cell receptors. Costimulatory signals through B7 on APCs and CD28 on T cells are necessary for CTL differentiation and proliferation. T cells express PD-L1 and its receptor PD-1. The interaction of PD-L1 with B7.1 suppresses T cell differentiation into CTL (b). Metastatic TNBC expresses PD-L1 in abundance. The engagement of PD-L1 with PD-1 blocks CTL effector function allowing unchecked tumor progression (c). (ii) Metastatic TNBC positive for PD-L1 respond to combination therapy with atezolizumab (monoclonal anti-PD-L1 antibody) and Abraxane (nab-paclitaxel) (a). Abraxane enhances expression of tumor-specific antigens and induction of apoptosis (G2/M phase arrest), thereby facilitating antigen presentation via APCs (b). APCs are activated via nab-paclitaxel and enhance toll-like receptor (TLR) expression contributing to efficient antigen presentation as well as costimulation by B7 family (c). Suppressive signal in activating T cells is inhibited as atezolizumab blocks the interaction of PD-L1 with B7.1. Thus augmenting signal induction for activation, differentiation, and proliferation of tumor-specific CTL clones (d). Metastatic TNBC expresses PD-L1 in abundance. Disrupting the engagement of PD-L1 with its receptor PD-1 by anti-PD-L1 antibody allows CTLs to secrete perforins that make holes in the target cell membrane allowing granzymes to enter and induce caspase-dependent apoptosis of these aggressive cancers (e).



**FIGURE 2.** Programmed death ligand-1 (PD-L1) as a biomarker for triple-negative breast cancer (TNBC). (A) Hematoxylin and Eosin (H&E) slide of the high-grade, TNBC not otherwise specified (NOS) with abundant tumor infiltrating lymphocytes (TILs) [×10]; (B) TILs were strongly positive for PD-L1 by immunohistochemistry (clone SP142, Ventana). Note the lack of PD-L1 expression in the cancer cells (×10).

**TABLE 1.** A list of the available diagnostic anti-PD-L1 antibodies and their status in regards to the FDA approval

Antibody (Manufacturer) [54]	FDA status	Cancer subtype
22c3 (Dako Agilent)	Companion diagnostics*	NSCLC Bladder cancer Cervical cancer Gastric and GEJ cancer
SP142 (Ventana)	Companion diagnostics Complementary diagnostics**	Bladder cancer Breast cancer NSCLC
SP263 (Ventana)	Complementary diagnostics	Bladder cancer
28-8 (Dako Agilent)	Complementary diagnostics	NSCLC Bladder cancer Melanoma HNSCC
73-10 (Dako Agilent/Merck)	Not approved	Bladder cancer Merkel cell carcinoma

\*Companion diagnostics: "A medical device, often an *in vitro* device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product." (Source: FDA, accessed January 25, 2019), \*\*Complementary diagnostics: "A test that aids in the benefit–risk decision–making about the use of the therapeutic product, where the difference in benefit–risk is clinically meaningful" (Sources: ASCO/American Society of Clinical Oncology/, 2016 and [55].

NSCLC: Non-small cell lung cancer; GEJ cancer: Gastroesophageal cancer; HNSCC: Head and neck squamous cell carcinoma; FDA: Food and Drug Administration

achieved in the clinical trial IMpassion130 (NCT02425891) [44]. This study led the Food and Drug Administration (FDA) to grant accelerated approval for atezolizumab (TECENTRIQ®, anti-PD-L1 agent) in combination with chemotherapy using a solvent-free, nanoparticle albumin-bound (nab) formulation of paclitaxel (nab®-Paclitaxel; Abraxane) for the treatment of patients with TNBC (date of approval: March 8, 2019). The approval was based on the randomized trial involving 902 TNBC patients with unresectable, locally advanced, and/or metastatic TNBC without prior treatment for the metastatic disease. Patients were randomized (1:1) to receive atezolizumab plus chemotherapy with Abraxanevs. placebo plus Abraxane. The possible rationale for combining the checkpoint inhibitor

with taxane-based chemotherapy that inhibits mitosis was that it may enhance tumor-antigen release and antitumor responses to immune checkpoint inhibition. Additionally, solvent-free taxanes might also activate toll-like receptors and promote dendritic cell activity (Figure 1) [45,46].

In the IMpassion130 trial, prior to treating patients with atezolizumab, tumor samples were centrally assessed by immunohistochemistry for the presence of PD-L1 expression (SP142 clone, Ventana). PD-L1 expression was assessed in tumor-infiltrating (immune) cells using two-tier system: "a percentage of tumor area" <1% (=PD-L1 negative) or ≥1% (=PD-L1 positive). The study revealed that the patients whose cancers were positive for PD-L1 (~41%) and received atezolizumab did significantly better than those treated with nab-paclitaxel: Median progression-free survival (PFS) was 7.2 months with atezolizumab compared with 5.5 months of the patients treated with placebo-nab-paclitaxel only. In the PD-L1-positive subgroup, the response rate was ~59% with atezolizumab–nab-paclitaxel in comparison with ~43% in the placebo–nab-paclitaxel subgroup. Notably, 10% of the patients in the atezolizumab–nab-paclitaxel group achieved a complete response as compared with only ~1% of those in the placebo–nab-paclitaxel group [44]. Importantly, the atezolizumab plus nab-paclitaxel combination group yielded no new safety concerns as the safety profile appeared consistent with the known profiles of each component drug. Indeed, the most common side effects with the combined treatment were hair loss, fatigue, tingling or numbness in the hands and feet, nausea and vomiting, diarrhea, anemia, constipation, cough, headache, neutropenia, and reduced appetite, which were present among 20% or more of patients.

One of the important findings in the IMpassion130 trial is the efficiency of combined therapy (immunotherapy + conventional chemotherapy). The combined treatment has its rationale at the molecular level as illustrated in the case of breast cancer in Figure 1. This approach has also been clinically verified to be successful in other cancers like NSCLC. Thus, the FDA in September 2017 approved the first combination of chemotherapy



and immunotherapy for patients with metastatic, non-squamous NSCLC. The approval included pembrolizumab (KEYTRUDA®) in combination with pemetrexed and carboplatin. The approval was based on results obtained in the KEYNOTE-021 Phase 2 clinical trial that demonstrated that non-squamous NSCLC patients treated with the combination therapy exhibited a 55% therapeutic response rate in comparison with 29% with chemotherapy alone [47]. A recent update on this study (24 months follow-up) confirmed the initial data with significant improvements in PFS and objective response rate (~57% in combined group vs. 30% in chemotherapy group) in the patients treated with combined immunotherapy (pembrolizumab)-chemotherapy (pemetrexed-carboplatin) [48]. More recently, the FDA on March 18, 2019 has granted accelerated approval for the use of atezolizumab in combination with carboplatin and etoposide chemotherapy for the first-line treatment of adult patients with extensive-stage SCLC (ES-SCLC). Approval was based on the IMpower133 (NCT02763579), a randomized (1:1), multicenter, double-blind, placebo-controlled trial in 403 patients with ES-SCLC. Patients receiving atezolizumab with chemotherapy showed improved median overall survival of 12.3 months compared to 10.3 months for those on placebo with chemotherapy (hazard ratio [HR] 0.70; 95% CI: 0.54, 0.91;  $p = 0.0069$ ). Furthermore, patients on the checkpoint inhibitor plus chemotherapy had a median PFS of 5.2 months compared with 4.3 months in the placebo with chemotherapy arm of the trial (HR 0.77; 0.62, 0.96;  $p = 0.0170$ ) [49]. This approval has changed the standard first-line therapy for the first time in several decades for this cancer [50]. Despite concerns over the cost-effectiveness of checkpoint inhibitor-based immunotherapy [51], these recent FDA approvals (e.g., for NSCLC, ES-SCLC, and TNBC as discussed herein) have firmly established

the use of checkpoint inhibitors together with conventional chemotherapy as a novel clinical paradigm for the treatment of otherwise difficult-to-treat cancers.

It is clinically important to identify patients who are likely to respond to checkpoint inhibitor-based immunotherapy. Although a quite difficult task given the complex tumor microenvironment and interactions between the cancer and various immune cells such as T-lymphocytes, B-lymphocytes, dendritic, and antigen-presenting cells (Figure 1), several biomarkers have nonetheless been identified to reliably predict the effectiveness of the anti-PD-1/PD-L1 checkpoint inhibitors in cancer patients. These include PD-L1 status (the target protein/antigen for atezolizumab), tumor mutational burden (load) [TMB/TML], microsatellite instability status (MSI) and the number of TILs (Tables 1 and 2). PD-L1 expression in cancer or immune cells as detected by immunohistochemistry has been shown to be one of the most reliable predictive biomarkers as confirmed in the IMpassion130 (NCT02425891) study. For detection purposes, four out of five diagnostic antibodies have been validated as either companion or complementary diagnostics (Table 1). Indeed, the current FDA approval of atezolizumab in the treatment of TNBC applies only to patients whose breast cancers express PD-L1 in an FDA-approved test (i.e., the Ventana diagnostic antibody SP142).

This recent FDA approval of atezolizumab plus chemotherapy for the treatment of adults with PD-L1-positive, unresectable, locally advanced, or metastatic TNBC represents the first cancer immunotherapy regimen to be approved for the management of breast cancer. It is truly a landmark therapeutic development for patients with TNBC given the limited treatment options available for this heterogeneous, but a highly

**TABLE 2.** The status of predictive biomarkers to immune checkpoint inhibitors in cancers with approved anti-PD-1/PD-L1 agents and breast cancer

Predictive biomarker to checkpoint inhibitor	Cancer type	Status in breast cancer
PD-L1 expression (TC and/or IC)*	NSCLC Bladder cancer HNSCC Cervical cancer Gastric/GEJ cancer	21-56% in ER-negative breast cancers (TNBC and HER2-positive)
PD-L1 (CD274) gene amplification [56,57]	Classical HL (36%) RCC (Sarcomatoid) (6%) HNSCC (~3%) NSCLC (~2%)	~1-2%
High tumor mutational load (burden) [58,59]	Melanoma NSCLC Bladder Merkel cell carcinoma Hepatocellular carcinoma	Predominantly low
MSI-H or MMR-D	Tumor-agnostic**	Low (~1%)
Increased TILs	Renal cell carcinoma Colorectal carcinoma Gastric/GEJ cancer	Common in ER-negative breast cancers (TNBC and HER2-positive)

\*Assessed by immunohistochemistry, \*\*Tumor-agnostic approach implies a treatment based on a specific genomic/molecular/abnormality in a cancer, rather than its anatomic or histologic origins [60]; good examples of such an approach include immune checkpoint inhibitors and tropomyosin kinase receptor (TRK) inhibitors (e.g., larotrectinib). TC: Tumor cells; IC: Inflammatory (immune) cells; ER: Estrogen receptor; GEJ: Gastroesophageal junction; NSCLC: Non-small cell lung carcinoma; HNSCC: Head and neck squamous cell carcinoma; TNBC: Triple-negative breast cancer; RCC: Renal cell carcinoma; HL: Hodgkin's lymphoma; MSI-H: Microsatellite instability high; MMR-D: Mismatch repair deficient; TILs: Tumor infiltrating lymphocytes

aggressive subtype of breast cancer [52]. Chemotherapy alone had been the mainstay of treatment for many years for TNBC and so the approval of this checkpoint inhibitor combination for people with PD-L1-positive TNBC disease fulfills an unmet medical need. Hopefully, several additional ongoing trials with immune checkpoint inhibitors other than atezolizumab [41] will endorse the results from the IMpassion130 (NCT02425891) study. Additional efforts are also required to optimize predictive biomarkers in TNBC (PD-L1 antibody selection, Table 1, threshold for positivity, cancer vs. immune cells expression, further dissection of the breast cancer immune microenvironment, Figure 1) [53] and to maximize the effectiveness of these important class of immune-targeting therapeutic agents.

## DECLARATION OF INTERESTS

ZG is employed by Caris Life Sciences, which commercially offers testing for PD-L1. Other authors declare no conflict of interests.

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