BOSNIAN JOURNAL OF
BASIC MEDICAL SCIENCES
Journal of the Association of Basic Medical Sciences of FBIH

REVIEW ARTICLE

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

Farhan S. Cyprian[1], Saghir Akhtar[1], Zoran Gatalica[2], Semir Vranic[1]*

[1]College of Medicine, Qatar University, Doha, Qatar

[2]Caris Life Sciences, Phoenix, Arizona, United States of America

***Corresponding author:** Semir Vranic, College of Medicine**,** Qatar University

PO Box 2713, Doha, Qatar. Phone: +974 4403 7873.

E-mail: semir.vranic@gmail.com and svranic@qu.edu.qa

Submitted: 25 March 2019 /**Accepted:** 26 March 2019

DOI: http://dx.doi.org/10.17305/bjbms.2019.4204

ABSTRACT

The treatment of several solid and hematologic malignancies with immune checkpoint inhibitors (against 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), 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. 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.

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

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 has 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 (illustrated in Figure 1) (5).

Stunning successes of monoclonal antibody-based immune checkpoint inhibitors against PD-1/PD-L1 (e.g. nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, ipilumab) have been achieved in various cancers (6). These include non-small cell lung carcinoma (NSCLC), renal, bladder, head and neck, gastric/gastroesophageal (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 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 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 PARP (poly ADP ribose polymerase) 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). 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 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-B) (14, 16-18, 20, 29-31, 36-39). A recent systematic review of Zhang et al. (40), based on analysis >2,500 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 (≥ 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, 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 was a good candidate for potential use in the management of patients with TNBC. The most remarkable results have been recently 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 Abraxane vs. 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 that 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, 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 progress-free survival 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 small cell lung cancer (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 0.70; 95% CI: 0.54, 0.91; p=0.0069). Furthermore, patients on the checkpoint inhibitor plus chemotherapy had a median progression-free survival 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 tumor infiltrating lymphocytes (TIL) (summarized in 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 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 fulfils 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/see 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.

REFERENCES

1. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-7.
2. Danilova L, Wang H, Sunshine J, Kaunitz GJ, Cottrell TR, Xu H, et al. Association of PD-1/PD-L axis expression with cytolytic activity, mutational load, and prognosis in melanoma and other solid tumors. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(48):E7769-E77.
3. Taube JM, Young GD, McMiller TL, Chen S, Salas JT, Pritchard TS, et al. Differential Expression of Immune-Regulatory Genes Associated with PD-L1 Display in Melanoma: Implications for PD-1 Pathway Blockade. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(17):3969-76.
4. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(19):5064-74.
5. Swoboda A, Nanda R. Immune Checkpoint Blockade for Breast Cancer. Cancer treatment and research. 2018;173:155-65.
6. Wei SC, Duffy CR, Allison JP. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discov. 2018;8(9):1069-86.
7. Sun C, Mezzadra R, Schumacher TN. Regulation and Function of the PD-L1 Checkpoint. Immunity. 2018;48(3):434-52.
8. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509-20.
9. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409-13.
10. Vranic S. Microsatellite instability status predicts response to anti-PD-1/PD-L1 therapy regardless the histotype: A comment on recent advances. Bosnian journal of basic medical sciences. 2017;17(3):274-5.
11. Lee A, Djamgoz MBA. Triple negative breast cancer: Emerging therapeutic modalities and novel combination therapies. Cancer treatment reviews. 2018;62:110-22.
12. Solinas C, Gombos A, Latifyan S, Piccart-Gebhart M, Kok M, Buisseret L. Targeting immune checkpoints in breast cancer: an update of early results. ESMO open. 2017;2(5):e000255.
13. Joneja U, Vranic S, Swensen J, Feldman R, Chen W, Kimbrough J, et al. Comprehensive profiling of metaplastic breast carcinomas reveals frequent overexpression of programmed death-ligand 1. J Clin Pathol. 2017;70(3):255-9.
14. Dill EA, Gru AA, Atkins KA, Friedman LA, Moore ME, Bullock TN, et al. PD-L1 Expression and Intratumoral Heterogeneity Across Breast Cancer Subtypes and Stages: An Assessment of 245 Primary and 40 Metastatic Tumors. Am J Surg Pathol. 2017;41(3):334-42.
15. Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al-Qudaihi G, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia. 2006;8(3):190-8.
16. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. Cancer immunology research. 2014;2(4):361-70.
17. Barrett MT, Lenkiewicz E, Malasi S, Basu A, Yearley JH, Annamalai L, et al. The association of genomic lesions and PD-1/PD-L1 expression in resected triple-negative breast cancers. Breast Cancer Res. 2018;20(1):71.

18. Sobral-Leite M, Van de Vijver K, Michaut M, van der Linden R, Hooijer GKJ, Horlings HM, et al. Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. Oncoimmunology. 2018;7(12):e1509820.
19. Stovgaard ES, Dyhl-Polk A, Roslind A, Balslev E, Nielsen D. PD-L1 expression in breast cancer: expression in subtypes and prognostic significance: a systematic review. Breast Cancer Res Treat. 2019.
20. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2965-70.
21. Vranic S, Palazzo J, Sanati S, Florento E, Contreras E, Xiu J, et al. Potential Novel Therapy Targets in Neuroendocrine Carcinomas of the Breast. Clin Breast Cancer. 2018.
22. Vranic S, Palazzo J, Swensen J, Xiu J, Florento E, Gatalica Z. Theranostic molecular profiling of pleomorphic ductal carcinoma of the breast. Breast J. 2019;25(1):175-6.
23. Vranic S, Feldman R, Gatalica Z. Apocrine carcinoma of the breast: A brief update on the molecular features and targetable biomarkers. Bosn J Basic Med Sci. 2017;17(1):9-11.
24. Thompson ED, Taube JM, Asch-Kendrick RJ, Ogurtsova A, Xu H, Sharma R, et al. PD-L1 expression and the immune microenvironment in primary invasive lobular carcinomas of the breast. Mod Pathol. 2017;30(11):1551-60.
25. Cimino-Mathews A, Thompson E, Taube JM, Ye X, Lu Y, Meeker A, et al. PD-L1 (B7-H1) expression and the immune tumor microenvironment in primary and metastatic breast carcinomas. Human pathology. 2016;47(1):52-63.
26. He J, Huo L, Ma J, Zhao J, Bassett RL, Sun X, et al. Expression of Programmed Death Ligand 1 (PD-L1) in Posttreatment Primary Inflammatory Breast Cancers and Clinical Implications. Am J Clin Pathol. 2018;149(3):253-61.
27. Zawlik I, Gablo N, Szymanska B, Pawlowska Z, Chudobinski C, Chalubinska-Fendler J, et al. Immune checkpoints in aggressive breast cancer subtypes. Neoplasma. 2016;63(5):768-73.
28. Ali HR, Glont SE, Blows FM, Provenzano E, Dawson SJ, Liu B, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. Annals of oncology : official journal of the European Society for Medical Oncology. 2015;26(7):1488-93.
29. Wimberly H, Brown JR, Schalper K, Haack H, Silver MR, Nixon C, et al. PD-L1 Expression Correlates with Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy in Breast Cancer. Cancer immunology research. 2015;3(4):326-32.
30. Altan M, Kidwell KM, Pelekanou V, Carvajal-Hausdorf DE, Schalper KA, Toki MI, et al. Association of B7-H4, PD-L1, and tumor infiltrating lymphocytes with outcomes in breast cancer. NPJ Breast Cancer. 2018;4:40.
31. Tray N, Taff J, Singh B, Suh J, Ngo N, Kwa M, et al. Metaplastic breast cancers: Genomic profiling, mutational burden and tumor-infiltrating lymphocytes. Breast. 2018;44:29-32.
32. Barroso-Sousa R, Barry WT, Guo H, Dillon D, Tan YB, Fuhrman K, et al. The immune profile of small HER2-positive breast cancers: a secondary analysis from the APT trial. Ann Oncol. 2019.
33. Tawfik O, Kimler BF, Karnik T, Shehata P. Clinicopathological correlation of PD-L1 expression in primary and metastatic breast cancer and infiltrating immune cells. Hum Pathol. 2018;80:170-8.
34. Pelekanou V, Barlow WE, Nahleh ZA, Wasserman B, Lo YC, von Wahlde MK, et al. Tumor-Infiltrating Lymphocytes and PD-L1 Expression in Pre- and Posttreatment Breast Cancers in the SWOG S0800 Phase II Neoadjuvant Chemotherapy Trial. Molecular cancer therapeutics. 2018;17(6):1324-31.
35. Hou Y, Nitta H, Wei L, Banks PM, Parwani AV, Li Z. Evaluation of Immune Reaction and PD-L1 Expression Using Multiplex Immunohistochemistry in HER2-Positive Breast

Cancer: The Association With Response to Anti-HER2 Neoadjuvant Therapy. Clinical breast cancer. 2018;18(2):e237-e44.
36. Mori H, Kubo M, Yamaguchi R, Nishimura R, Osako T, Arima N, et al. The combination of PD-L1 expression and decreased tumor-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget. 2017;8(9):15584-92.
37. AiErken N, Shi HJ, Zhou Y, Shao N, Zhang J, Shi Y, et al. High PD-L1 Expression Is Closely Associated With Tumor-Infiltrating Lymphocytes and Leads to Good Clinical Outcomes in Chinese Triple Negative Breast Cancer Patients. International journal of biological sciences. 2017;13(9):1172-9.
38. Davis M, Tripathi S, Hughley R, He Q, Bae S, Karanam B, et al. AR negative triple negative or "quadruple negative" breast cancers in African American women have an enriched basal and immune signature. PLoS One. 2018;13(6):e0196909.
39. Gil Del Alcazar CR, Huh SJ, Ekram MB, Trinh A, Liu LL, Beca F, et al. Immune Escape in Breast Cancer During In Situ to Invasive Carcinoma Transition. Cancer Discov. 2017;7(10):1098-115.
40. Zhang M, Sun H, Zhao S, Wang Y, Pu H, Wang Y, et al. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. Oncotarget. 2017;8(19):31347-54.
41. Esteva FJ, Hubbard-Lucey VM, Tang J, Pusztai L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. The Lancet Oncology. 2019;20(3):e175-e86.
42. Tecentriq (atezolizumab): summary of product characteristics. Welwyn Garden City, United Kingdom: Roche Registration 2018.
43. Tecentriq (atezolizumab) South San Francisco, CA: Genentech; 2018.
44. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018;379(22):2108-21.
45. Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. Cancer immunology research. 2015;3(5):436-43.
46. Yardley DA. nab-Paclitaxel mechanisms of action and delivery. J Control Release. 2013;170(3):365-72.
47. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. The Lancet Oncology. 2016;17(11):1497-508.
48. Borghaei H, Langer CJ, Gadgeel S, Papadimitrakopoulou VA, Patnaik A, Powell SF, et al. 24-Month Overall Survival from KEYNOTE-021 Cohort G: Pemetrexed and Carboplatin with or without Pembrolizumab as First-Line Therapy for Advanced Nonsquamous Non-Small Cell Lung Cancer. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2019;14(1):124-9.
49. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. The New England journal of medicine. 2018;379(23):2220-9.
50. Pacheco J, Bunn PA. Advancements in Small-cell Lung Cancer: The Changing Landscape Following IMpower-133. Clinical lung cancer. 2019.
51. Zhou K, Zhou J, Huang J, Zhang N, Bai L, Yang Y, et al. Cost-effectiveness analysis of atezolizumab plus chemotherapy in the first-line treatment of extensive-stage small-cell lung cancer. Lung cancer. 2019;130:1-4.
52. Heimes AS, Schmidt M. Atezolizumab for the treatment of triple-negative breast cancer. Expert opinion on investigational drugs. 2019;28(1):1-5.
53. Gruosso T, Gigoux M, Manem VSK, Bertos N, Zuo D, Perlitch I, et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. J Clin Invest. 2019.
54. Tsao MS KK, Dacic S, Yatabe Y, Hirsch FR. IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer. Aurora, Colorado: Editorial Rx Press 2017.

55. Scheerens H, Malong A, Bassett K, Boyd Z, Gupta V, Harris J, et al. Current Status of Companion and Complementary Diagnostics: Strategic Considerations for Development and Launch. Clin Transl Sci. 2017;10(2):84-92.
56. Goodman AM, Piccioni D, Kato S, Boichard A, Wang HY, Frampton G, et al. Prevalence of PDL1 Amplification and Preliminary Response to Immune Checkpoint Blockade in Solid Tumors. JAMA Oncol. 2018;4(9):1237-44.
57. Roemer MG, Advani RH, Ligon AH, Natkunam Y, Redd RA, Homer H, et al. PD-L1 and PD-L2 Genetic Alterations Define Classical Hodgkin Lymphoma and Predict Outcome. J Clin Oncol. 2016;34(23):2690-7.
58. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome medicine. 2017;9(1):34.
59. Gupta S, Vanderbilt CM, Cotzia P, Arias-Stella JA, 3rd, Chang JC, Zehir A, et al. Next-Generation Sequencing-Based Assessment of JAK2, PD-L1, and PD-L2 Copy Number Alterations at 9p24.1 in Breast Cancer: Potential Implications for Clinical Management. J Mol Diagn. 2019;21(2):307-17.
60. Flaherty KT, Le DT, Lemery S. Tissue-Agnostic Drug Development. American Society of Clinical Oncology educational book American Society of Clinical Oncology Meeting. 2017;37:222-30.

TABLES

TABLE 1. A list of the available diagnostic anti-PD-L1 antibodies and their status in regards to the Food and Drug Administration (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.

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
Microsatellite instability high (MSI-H) or mismatch repair deficient (MMR-D)	Tumor-agnostic**	Low (~1%)
Increased tumor infiltrating lymphocytes (TIL)	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 receptors (TRK) inhibitors (e.g. larotrectinib).

TC = tumor cells; IC = inflammatory (immune) cells; ER = Estrogen receptor; GEJ = Gastroesophageal junction carcinoma; NSCLC = Non-small cell lung carcinoma; HNSCC = Head and neck squamous cell carcinoma; TNBC = Triple-negative breast cancer; RCC = Renal cell carcinoma; HL = Hodgkin lymphoma.

Figures

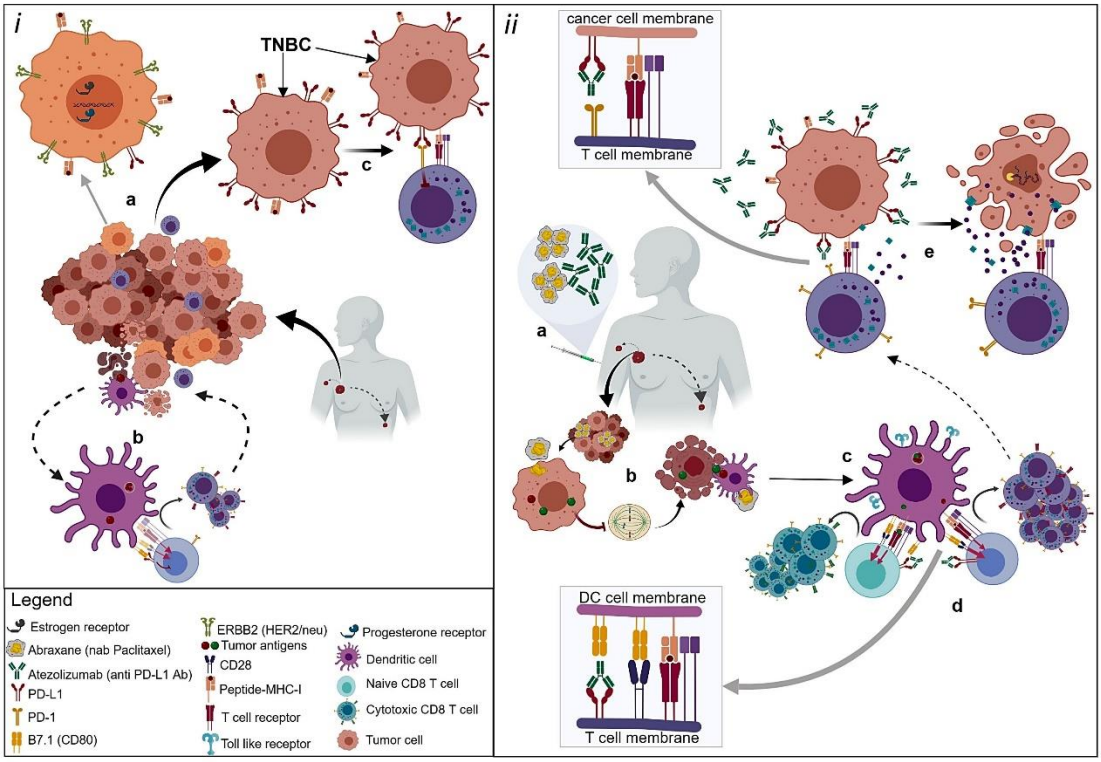

FIGURE 1. **The possible mechanism of action anti-PD-1/PD-L1 drugs (e.g. atezolizumab) and nab-paclitaxel in metastatic triple-negative breast cancer (TNBC).**

i: Metastatic breast cancers are dynamic environments undergoing proliferation and apoptosis along with immune cell infiltration. Breast cancers are a heterogeneous populations which may or may not (TNBC) be influenced by ERBB2/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 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 cell express Program death

ligand-1 (PD-L1) and its receptor PD-1. The interaction of PD-L1 with B7.1 suppresses T cell differentiation into CTL (b). Metastatic TNBC express 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 co-stimulation 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 express 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).

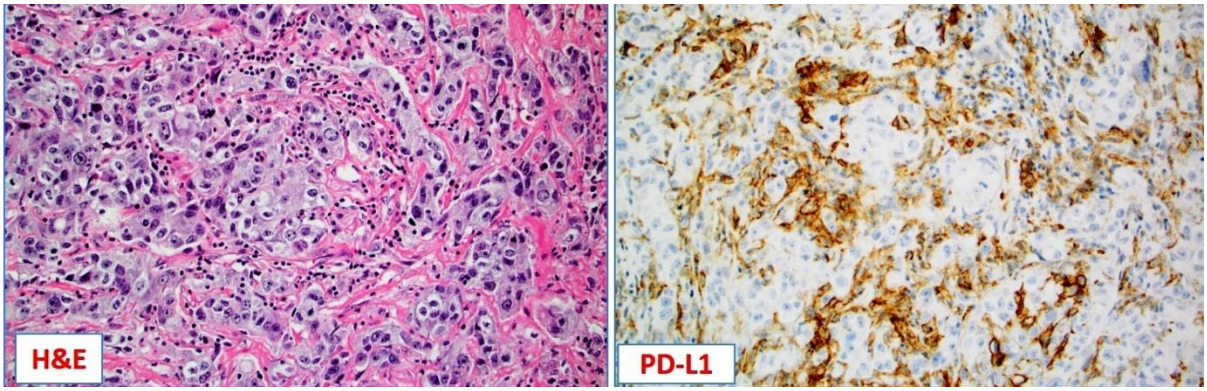

FIGURE 2. **PD-L1 as a biomarker for TNBC.**

(A): Hematoxylin and Eosin (H&E) slide of the high-grade, triple-negative breast cancer (TNBC) not otherwise specified (NOS) with abundant tumor infiltrating lymphocytes (TILs) (10x); (B) TILs were strongly positive for PD-L1 by immunohistochemistry (clone SP142, Ventana). Note the lack of PD-L1 expression in the cancer cells (10x).