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HOT TOPICS IN BREAST CANCER (K HUNT, SECTION EDITOR)

PD-L1 Testing in Patients with Breast Cancer: Controversies and Current Practice

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Abstract

Purpose of Review Atezolizumab has emerged as a novel treatment in patients with triple-negative breast cancer showing unprecedented survival gain. This breakthrough benefit is exclusively observed in PD-L1-positive patients, but PD-L1 positivity still lacks a reliable definition. In this review, we discuss the current data on PD-L1 testing and its impact on the treatment of patients.

Recent Findings The VENTANA SP142 assay was used in the trial that led to the approval of atezolizumab in the first-line setting. However, recent data show that this assay appears to be less sensitive to detect PD-L1 expression compared with other assays. Also, the intra- and inter-pathologist variability is substantial when performing various assays.

Summary PD-L1 testing remains a challenge in clinical practice. The variability and performance of assays may be an issue, and clinicians should avoid the interchangeability between assays. More studies are needed to elucidate the best way to use PD-L1 testing.

Keywords Breast cancer · PD-L1 testing · Immunotherapy · Immune checkpoint inhibitors

Introduction

Triple-negative breast cancer (TNBC) accounts for 15–20% of all breast cancers and is defined by the lack of expression of the estrogen, progesterone, and HER2 receptors [1]. It is known as an aggressive breast cancer subtype and long-term outcomes are poor, with overall survival (OS) ranging between 12 and 16 months in the metastatic setting [2]. Even in the era of genomic and targeted therapy, this disease subtype still lacks drugs that specifically target the tumor cells, and chemotherapy remains the standard treatment for TNBC [1, 2].

Recently, immunotherapy has debuted as an active agent in breast cancer and atezolizumab, a PD-L1 inhibitor, demonstrated breakthrough results in patients with metastatic TNBC, leading to the first approval of an immune checkpoint inhibitor in breast cancer. In the IMpassion130 randomized phase III trial, 902 TNBC patients received either *nab*-paclitaxel alone or *nab*-paclitaxel plus atezolizumab in the first-line setting [3••]. After a median follow-up of approximately 12 months, patients who received combination therapy achieved a slight improvement in progression-free survival (7.2 versus 5.5 months [HR 0.80, 95% CI 0.69–0.92]), with a non-significant trend towards improved OS (21.3 versus 17.6 months [HR 0.84, 95% CI 0.69–1.02]) in the intention-to-treat population [3••]. The statistical design of the trial did not allow formal OS calculation specifically in PD-L1-positive patients due to its hierarchical design, allowing for comparison of survival in the PD-L1-positive group only if a statistically significant difference was observed in the intention-to-treat population. However, in the preplanned analysis, patients with PD-L1-expressing immune effector cells within the tumor experienced a 9.5-month increase in OS with the addition of atezolizumab (HR 0.62, 95% CI 0.45–0.86) [3••]. A recent update at 18 months demonstrated that the survival benefit for the PD-L1-positive patients was upheld, with an absolute benefit of 7 months. To date, this is the largest OS benefit observed with a single agent in patients with advanced TNBC [4•].

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In IMpassion130, PD-L1 positivity was defined by the presence of PD-L1 in the tumor-infiltrating immune cells using the VENTANA SP142 immunohistochemistry (IHC) assay, and its expression was graded according to the percentage of tumor area with immune cells staining positive for PD-L1: IC3 (≥ 10%), IC2 (≥ 5% and < 10%), IC1 (≥ 1% and < 5%), and IC0 (< 1%) [3••, 4•].

The phase II KEYNOTE 086 study also demonstrated activity of the anti-PD-1 agent pembrolizumab given as monotherapy in the first-line setting for patients with TNBC [5•]. The overall response rate was 21.4% (95% CI 13.9–31.4) and the disease control rate was 23.8% (95% CI 15.9–34.0) with a median duration of response of 10.4 months. Unlike IMpassion130, PD-L1 positivity was determined by the combined positive score, which is the ratio of all PD-L1-expressing cells (tumor cells, lymphocytes, macrophages) to the number of all tumor cells, using the DAKO PDL1 22C3 IHC assay [5•].

However, recent data have shown poor reproducibility of PD-L1 staining in real-world practice [6]. Discordance between different pathologists and discordance between the commercially available staining assays highlight the need for a better understanding of the characteristics of each assay, as well as the need for standardization of the PD-L1 measurement for the best use of these drugs [6]. In this review, we discuss the challenges of PD-L1 testing and the clinical implications of this variability for the treatment of patients with breast cancer.

Differences Between Commercial Assays

Data in Lung Cancer

The Blueprint PD-L1 Immunohistochemistry (IHC) Assay Comparison Project is an industrial-academic collaborative partnership that provided analytical and clinical comparability of four PD-L1 IHC assays—22C3, Dako PD-L1 IHC 28-8 pharmDx, SP142, and VENTANA PD-L1 (SP263)—in patients with lung cancer [7]. An initial study evaluated 39 patients through 3 independent pathology experts who analyzed the percentages of tumor and immune cells staining positive at any intensity and demonstrated that the 22C3, 28-8, and SP263 assays had comparable results, while the SP142 assay stained fewer tumor cells [7]. Considering these findings, the Blueprint Project launched phase 2 of the study that included a greater number of lung cancer cases as well as additional expert pathologists. This larger study confirmed the interchangeability of the 22C3, 28-8, and SP263 assays, whereas the SP142 assay showed less sensitivity to detect PD-L1 expression [8••]. Of note, a very poor concordance between assays was observed when evaluating stained tumor-infiltrating immune cells. In addition, Rimm et al. consolidated these findings in a prospective study that included 90 cases of non-small cell lung cancer, showing that the SP142 assay was associated with statistically significant lower levels of PD-L1 staining than the other 3 assays for both tumor cells and tumor-infiltrating immune cells [6]. Table 1 shows the concordance rate between assays.

Data in Breast Cancer

Similar to the Blueprint Project, Sun et al. evaluated the expression of PD-L1 using different immunohistochemical antibodies in 218 patients with TNBC [9]. PD-L1 expression in cancer cells was analyzed by various cutoff values (1%, 5%, 10%, and 50%) and expression in immune cells was analyzed as negative, low-positive, and high-positive. E1L3N antibody showed the highest expression rates, with 14.7%, 14.7%, 11.0%, and 2.3% for the cutoff values (1%, 5%, 10%, and 50%, respectively) of the tumor cells, and also a higher rate (37.6%) of immune cell positivity. Conversely, SP142 showed the lowest expression rate in cancer cells (11.5%, 11.0%, 6.9%, and 0.5%) for all cutoff values, respectively, and the lowest expression in immune cells (19.3%). Hence, the concordance rate between the 28-8 and E1L3N assays was high in both cancer cells and immune cells while the SP142 assay showed low concordance rates with the other two antibodies [9].

In a more recent study presented at the 2019 European Society of Medical Oncology (ESMO) Breast Cancer Annual Congress, Scott et al. also performed a concordance analysis between four commercially available assays for TNBC [10••]. A single pathologist evaluated the concordance of the proportion of stained tumor cells, stained immune cells, and tumor occupied by immune cells in 196 TNBC samples using the SP263, SP142, 28-8, and 22C3 assays. While the SP263, 28-8, and 22C3 assays were consistent in PD-L1 patient classification, the SP142 assay was shown to be less sensitive, identifying almost 20% fewer PD-L1-expressing tumor cells and immune cells (Table 1) [10••].

In this context, the interchangeability between assays should be carefully considered and clinicians should be aware of the possibility of underestimated PD-L1 positivity in patients with TNBC when using the SP142 assay.

Intra- and Inter-observer Reproducibility Assessment of PD-L1

Immunohistochemistry testing includes subjective interpretation, particularly for PD-L1 staining, since it is expressed on both tumor and tumor-infiltrating immune cells. Studies assessing the reproducibility of interpretation and scoring of PD-L1 expression between pathologists are scarce and most of them involve either few pathologists or a small number of

Table 1 SP142 PD-L1 assay concordance rate in lung and breast cancer studies at 1% cutoff

Study	*n*	SP142 concordance with 28-8	SP142 concordance with 22C-3	SP142 concordance with SP263	SP142 concordance with E1L3N
Lung cancer					
Blueprint 1 [7]	39	63.2%	63.2%	86.8%	NR
Blueprint 2 [8••]	81	NNR	NNR	NNR	NR
Rimm et al.* [6]	90	1.270	0.970	NR	− 1.216
Breast cancer					
Sun et al. [9]	218	53.5%	NR	NR	53.7%
Scott et al. [10••]	196	80.6%	80.6%	78.1%	NR

*Mean difference after pairwise assay comparison by 13 pathologists

NR, not reported; *NNR*, not numerically reported

tissue samples [11]. Cooper et al. evaluated the intra- and inter-observer reproducibility of PD-L1 in lung cancer using 22C3 and showed an overall intra-observer agreement of 90%. A significant rate of disagreement between pathologists was observed (17%), mainly when a cutoff of 50% PD-L1 positivity was considered [12].

In breast cancer, data on PD-L1 reproducibility are even more scant. Rimm et al. reanalyzed data from the National Comprehensive Cancer Network (NCCN) study and evaluated PD-L1 staining variability, using the IMpassion130 scoring by 13 pathologists, showing a low agreement of 70% [13•]. Although this study used lung tissue, it demonstrated that standardization of PD-L1 expression using breast cancer study criteria would have one-third of the pathologists identifying dramatically fewer cases as PD-L1 positive with the SP142 assay [13•].

Thus, intra- and inter-observer reproducibility for PD-L1 staining presents a challenge for anti-PD-1/PD-L1 checkpoint inhibitors in routine clinical practice.

Differences Between PD-L1 in Primary Tumor Versus Metastatic Site

Recent studies have demonstrated that PD-L1 expression levels change within the TNBC microenvironment over the course of the disease, particularly in response to treatment [14, 15].

In early-stage disease, PD-L1 expression remains stable in the tumor microenvironment before and after neoadjuvant chemotherapy. However, in the metastatic setting, TNBC samples often have fewer tumor-infiltrating immune cells, lower PD-L1 levels, and decreased expression of genes associated with cytotoxic T cell activity when compared with primary tumor samples [15]. Szekely et al. evaluated primary and metastatic samples of patients with ER-positive or ER-negative metastatic breast cancer and observed a substantial decrease in PD-L1 expression at the metastatic site compared with the primary site evaluated by the E1L3N assay [16••]. In the cohort of 39 patients who had available tissue whole sections, the median stromal PD-L1 positivity in metastases and the primary tumor was 14% and 52% ($p = 0.0004$), respectively. In a second cohort that included only tissue microarrays, PD-L1 staining at the metastatic site and the primary tumor was 7% and 22% ($p = 0.03$), respectively. In addition, the significant difference between PD-L1 positivity between cohorts suggests that tissue microarrays may underestimate the true rates of PD-L1 positivity. Depletion of most immune cell types and immune effector functions was observed in metastatic tissue through immune gene profiling, with immune metagenes of activated T cells, CD8 cells, T-helper and regulatory cells, cytotoxic cells, dendritic and mastoid cells significantly downregulated in metastatic sites compared with primary tumors. Interestingly, the immune predictive signature Nanostring PanCancer Immune Profiling assay (Nanostring Technologies, Inc.) demonstrated a lower probability of response to immunotherapy in metastases, corresponding to an immune inert environment in metastatic sites [16••].

Preliminary data have also demonstrated the heterogeneity of PD-L1 expression between different anatomical sites in metastatic disease, with lymph nodes presenting a higher positivity of PD-L1 staining and liver presenting the lowest PD-L1 positivity on tumor-infiltrating immune cells [17].

Clinical Implications of PD-L1 Positivity in Breast Cancer

Clinical Features and Prognostic Implications of PD-L1 Positivity

PD-L1 positivity has been associated with more aggressive clinicopathologic features. Large tumor size, high

grade, negative hormonal receptors, and high Ki67 expression have been demonstrated to be associated with elevated levels of PD-L1. In addition, PD-L1 expression is more frequent in basal-like and ERBB2-enriched subtypes [18•].

When considering the overall breast cancer population in Sabatier et al.'s study, PD-L1 was not associated with better long-term outcomes, such as metastasis-free survival or OS. However, when patients with more aggressive biologic subtype were evaluated, PD-L1 expression significantly correlated with better response to chemotherapy and improved long-term outcomes. These findings suggest that PD-L1 expression is associated with the degree of functional immune cell infiltration and reflects an intrinsic immune anti-tumoral activity [18•].

PD-L1 Expression and Predictive Role in Response to Checkpoint Inhibitors

In contrast to data in lung and urothelial cancer that have demonstrated an important predictive role of PD-L1 expression in response to checkpoint inhibitors, the predictive value in breast cancer is still unclear [19, 20].

In the IMpassion130 study, only patients with tumors with greater than 1% of PD-L1-positive immune cells (185/451 patients) derived significant OS benefit from atezolizumab (HR for OS = 0.62; 95% CI 0.45–0.86), whereas no significant improvement was observed in the overall patient population (HR for OS = 0.84; 95% CI 0.69–1.02). Patients with PD-L1-positive TNBC also benefited from a higher objective response rate (58.9% versus 42.6%, $p = 0.002$) and a longer duration of response (8.5 months versus 5.5 months; HR 0.60 CI 95% 0.43–0.86) when compared with PD-L1-negative patients [3••].

Conversely, in the phase II KEYNOTE 086 trial that evaluated pembrolizumab monotherapy in previously treated patients, there was no significant difference in clinical outcomes between patients with PD-L1-positive and PD-L1-negative tumors. The overall response rate was 5.7% in patients with a combined positive score ≥ 1% and 4.7% in PD-L1-negative patients. The median duration of response was not reached in PD-L1-positive patients and was 4.4 months in the PD-L1-negative population. Similar progression-free survival rates were observed in both cohorts regardless of PD-L1 expression [5•].

The JAVELIN trial, a phase Ib trial that evaluated the anti-PD-L1 avelumab, also failed to demonstrate PD-L1 expression in tumor cells as a predictive marker at different thresholds, but did show a trend of increased drug activity when PD-L1 expression was assessed exclusively in tumor-associated immune cells [21].

Conclusions

Immune checkpoint inhibitors have emerged as potential agents that may substantially increase survival and quality of life in patients with breast cancer, especially patients with TNBC. A better understanding of the mechanisms of response and resistance to these drugs are urgently needed for the development of more reliable predictive biomarkers that will help identify the subgroups of patients who are more likely to derive benefit from anti-PD-1/PD-L1 blockade. To date, no available biomarker has satisfactorily demonstrated a trustworthy sensitivity for establishment in daily clinical practice. As discussed, PD-L1 staining still lacks reproducibility and the use of the SP142 assay remains a challenge for clinicians, since it may underestimate patients who could potentially benefit from the drug.

In real-world practice, interchangeability between assays should be carefully considered since these may not reflect the results obtained in clinical trials; the best approach to testing (e.g., primary breast tumor, metastatic site of disease) and assay remains unclear. Additional studies to validate alternative strategies, such as the development of a more sensitive model combining PD-L1 expression with tumor-infiltrating immune cell levels or the use of mRNA to quantify PD-L1 expression, are warranted.

Compliance with Ethical Standards

Conflict of Interest Sara Tolaney reports receiving institutional research support from Merck, Bristol-Myers Squibb, Exelixis, Eli Lilly, Pfizer, Novartis, AstraZeneca, Eisai, Nektar, Odenate, Sanofi, Immunomedics, and Genentech; and has served on advisory boards for Genentech, Eli Lilly, Novartis, Pfizer, Nektar, Immunomedics, Nanostring, Daiichi-Sankyo, Bristol-Meyers Squibb, Sanofi, Athenex, AstraZeneca, Eisai, Puma, and Merck. Pedro Exman and Ana Garrido-Castro declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Trivers KF, Lund MJ, Porter PL, Liff JM, Flagg EW, Coates RJ, et al. The epidemiology of triple-negative breast cancer, including race. Cancer Causes Control. 2009;20(7):1071–82. https://doi.org/10.1007/s10552-009-9331-1.
2. Gonzalez-Angulo AM, Timms KM, Liu S, Chen H, Litton JK, Potter J, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin

Cancer Res. 2011;17(5):1082–9. https://doi.org/10.1158/1078-0432.CCR-10-2560.

3.•• 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. https://doi.org/10.1056/NEJMoa1809615. **Impassion130 is a phase III trial that led to the first FDA approval of an immune checkpoint inhibitor. This trial also defined a predictive biomarker of response to atezolizumab.**

4.• Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. IMpassion130: updated overall survival (OS) from a global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab (atezo) + nab-paclitaxel (nP) in previously untreated locally advanced or metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2019;37(15_suppl):1003. https://doi.org/10.1200/JCO.2019.37.15_suppl.1003. **This abstract presents the updated analyses of the Impassion130 study after 18 months of follow-up.**

5.• Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. Ann Oncol. 2019;30(3):397–404. https://doi.org/10.1093/annonc/mdy517. **The phase II trial KEYNOTE-086 evaluated the efficacy of pembrolizumab as monotherapy for metastatic TNBC.**

6. Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. JAMA Oncol. 2017;3(8):1051–8. https://doi.org/10.1001/jamaoncol.2017.0013.

7. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, et al. PD-L1 Immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. J Thorac Oncol. 2017;12(2):208–22. https://doi.org/10.1016/j.jtho.2016.11.2228.

8.•• Tsao MS, Kerr KM, Kockx M, Beasley MB, Borczuk AC, Botling J, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint Phase 2 Project. J Thorac Oncol. 2018;13(9):1302–11. https://doi.org/10.1016/j.jtho.2018.05.013. **This study demonstrated the significant diferences between PD-L1 assays in lung cancer, showing that the interchange between distinct assays should not be performed.**

9. Sun WY, Lee YK, Koo JS. Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med. 2016;14(1):173. https://doi.org/10.1186/s12967-016-0925-6.

10.•• Scott M, Scorer P, Barker C, Al-Masri H. Comparison of patient populations identified by different PD-L1 assays in in triple-negative breast cancer (TNBC). Ann Oncol. 2019;30(Supplement_3). https://doi.org/10.1093/annonc/mdz095.009. **This study compared the performance of the assays in patients with triple-negative breast cancer.**

11. Phillips T, Simmons P, Inzunza HD, Cogswell J, Novotny J Jr, Taylor C, et al. Development of an automated PD-L1 immunohistochemistry (IHC) assay for non-small cell lung cancer. Appl Immunohistochem Mol Morphol. 2015;23(8):541–9. https://doi.org/10.1097/PAI.0000000000000256.

12. Cooper WA, Russell PA, Cherian M, Duhig EE, Godbolt D, Jessup PJ, et al. Intra- and interobserver reproducibility assessment of PD-L1 biomarker in non-small cell lung cancer. Clin Cancer Res. 2017;23(16):4569–77. https://doi.org/10.1158/1078-0432.CCR-17-0151.

13.• Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, et al. Reanalysis of the NCCN PD-L1 companion diagnostic assay study for lung cancer in the context of PD-L1 expression findings in triple-negative breast cancer. Breast Cancer Res. 2019;21(1):72. https://doi.org/10.1186/s13058-019-1156-6. **This paper looked at different assays of TNBC.**

14. 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. Mol Cancer Ther. 2018;17(6):1324–31. https://doi.org/10.1158/1535-7163.MCT-17-1005.

15. 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. Hum Pathol. 2016;47(1):52–63. https://doi.org/10.1016/j.humpath.2015.09.003.

16.•• Szekely B, Bossuyt V, Li X, Wali VB, Patwardhan GA, Frederick C, et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol. 2018;29(11):2232–9. https://doi.org/10.1093/annonc/mdy399. **This is a very important study that shows how different the micro-enviroment is in primary tumors and metastasis, mainly considering the immunological landscape.**

17. Li Y, Chang C-W, Tran D, Denker M, Hedge P, Molinero L. Abstract PD6-01: prevalence of PDL1 and tumor infiltrating lymphocytes (TILs) in primary and metastatic TNBC. Cancer Res. 2018;78(4 Suppl):Abstract nr PD6-01.

18.• Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR, et al. Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget. 2015;6(7):5449–64. https://doi.org/10.18632/oncotarget.3216. **This study shows the prognostic and predictive value of PD-L1 in patients with triple-negative breast cancer.**

19. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823–33. https://doi.org/10.1056/NEJMoa1606774.

20. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017;389(10064):67–76. https://doi.org/10.1016/S0140-6736(16)32455-2.

21. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat. 2018;167(3):671–86. https://doi.org/10.1007/s10549-017-4537-5.