Programmed death 1 (PD-1) and PD-1 ligand (PD-L1) distribution in triple negative breast cancer (TNBC)

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Abstract

Background: Recent data indicate a promising response to immune checkpoint inhibition in patients with metastatic TNBC. Ample research showed that PD-L1, a PD-1 ligand, is expressed in multiple tumor types, including TNBC, and may be a predictor of response to PD-1/PD-L1 blockade. Quantification of the stromal composition, particularly PD-1 and PD-L1 expression, continues to be controversial in its relationship to immune checkpoint inhibition in several cancer types, and it remains unclear whether PD-L1 expression is necessary to predict response. Here, we aimed to determine the distribution of PD-1 and PD-L1 in a large set of centrally ascertained specimens of TNBC.

Methods: The study cohort consisted of 993 tumor samples (both primary and metastatic TNBC) analyzed for either PD-1 or PD-L1 expression in one laboratory (Caris Life Sciences; Phoenix, AZ). Estrogen receptor (ER) and progesterone receptor (PR) status was assessed by immunohistochemistry (IHC). HER2/Neu expression or amplification was assessed by either IHC or in-situ hybridization. PD-1 and PD-L1 expression were confirmed using IHC with validated antibodies. For PD-L1, clone SP142 (Roche Diagnostics) was utilized and a sample was considered positive if there was ≥ 5% membranous staining of tumor cells. For PD-1, clone EH21.1 (BD Biosciences) was used. Tumor infiltrating lymphocytes (TILs) expressing PD-1 were counted and a sample was considered positive if there was at least one PD-1 positive TIL per 40x microscopic field.

Results: The median age in this cohort was 56 years (range: 22 – 88). A total of 363 TNBC specimens were tested for PD-1 via IHC. One hundred fifty eight (158; 43.5%) were negative for PD-1 expression. Two hundred five (205; 56.5%) were positive for PD-1. Of those that were PD-1 positive, 116 (56.6%), were in samples from a primary site (breast) and 89 (43.4%) in samples from a metastatic site. A total of 630 TNBC specimens were tested for PD-L1 via IHC. Five hundred seventy four (574; 91.1%) were negative for PD-L1. Fifty-six (56; 8.9%) were positive for PD-L1. Of those that were PD-L1 positive, were equally distributed between primary site and metastatic sites (28/324, and 28/306, respectively).

Conclusion: In this retrospective analysis, we describe, to the best of our knowledge, the distribution of PD-1 and PD-L1 expression in one of the largest datasets reported in TNBC. Unlike prior reports showing a high PDL-1 expression in excess of 50% in TNBC, this analysis show a low distribution of PD-L1 positivity. Our cohort represents a biased sample as those were unselected patients with recurrent breast cancer. Additionally, other factors can be implicated, including a change in the antibody used. These findings call for future standardization of the PD-L1 assay, particularly if further exploration showed PD-L1 to be a predictive or prognostic biomarker in mTNBC, particularly in relationship to therapy with immune checkpoint blockade.

Background

Triple negative breast cancers (TNBCs) have poor prognoses due to the lack of effective therapeutic targeting agents .

Immune checkpoint inhibition, through the use of monoclonal antibodies targeting checkpoint proteins on tumor cells or immune cells, have shown promising results in many cancer types, and may also show durable responses in TNBCs.

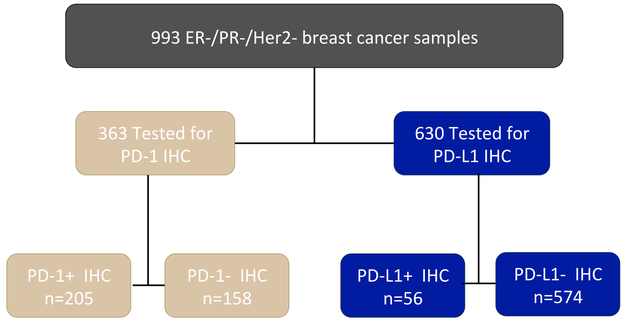
Recent data from the phase 1b KEYNOTE-012 trial demonstrated the utility of pembrolizumab (PD-1 inhibitor; Merck) in advanced TNBC. Of 32 TNBC patients, 37.5% showed a decrease in tumor burden and the overall disease control rate was 25.9%.[1] In addition, combination therapy using atezolizumab (PD-L1 inhibitor; Roche) and nab-paclitaxel showed a 66.7% response rate in patients with TNBC[2]. These promising data suggest utility for immune checkpoint inhibition as a beneficial therapy in advanced TNBC.

Confounding is the role of PD-1 and PD-L1 in determining responses to these immune checkpoint inhibitors. It remains unclear if expression of these markers is predictive of response to immune checkpoint inhibition. Additionally, the rate of PD-1/PD-L1 positivity in TNBCs is unknown. This study retrospectively assessed the distribution of PD-1 and PD-L1 expression in a cohort of TNBC tumors tested at a central laboratory.

Methods

993 TNBC consecutive breast tumor samples were tested for expression of the estrogen receptor (ER), progesterone receptor (PR), Her2/Neu, PD-1 (BD Biosciences; clone EH21.1) and/or PD-L1 (Roche Diagnostics; clone SP142) expression in a central laboratory (Caris Life Sciences). A breast tumor sample was considered positive for PD-1 expression if there was one PD-1-positive tumor infiltrating lymphocyte (TIL) per 40X microscopic field. A breast tumor sample was considered positive for PD-L1 expression if there were ≥ 5% membranous staining of tumor cells. Expression frequencies were calculated and compared using the Fisher's exact test, with $p < 0.05$ considered statistically significant.

Study Cohort



Results

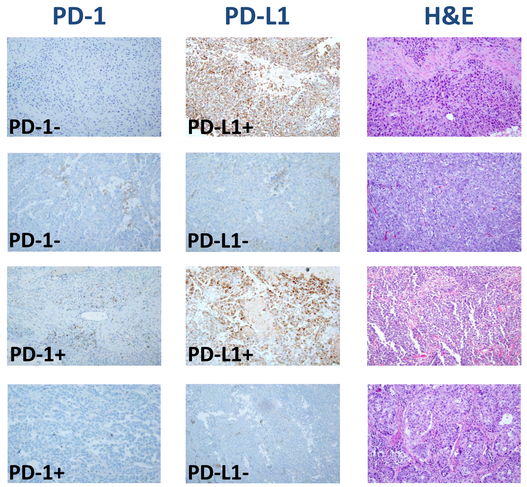


Figure 1 – Representative images of PD1 and PD-L1 staining in the study cohort. Images are at 20X magnification.

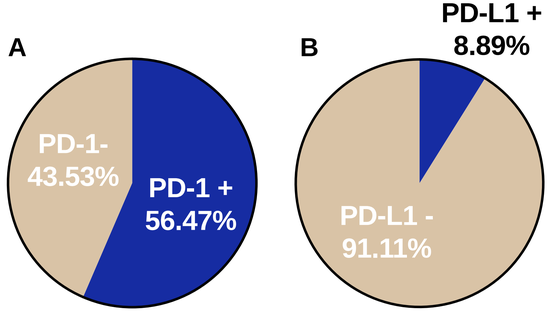


Figure 2 – PD-1 and PD-L1 expression via IHC in a TNBC cohort. (A) Percentages of samples harboring PD1 positive and negative tumor infiltrating lymphocytes (TILs)in the TNBC study cohort. (B) Percentages of PD-L1 positive and negative tumor samples in the study cohort.

Results

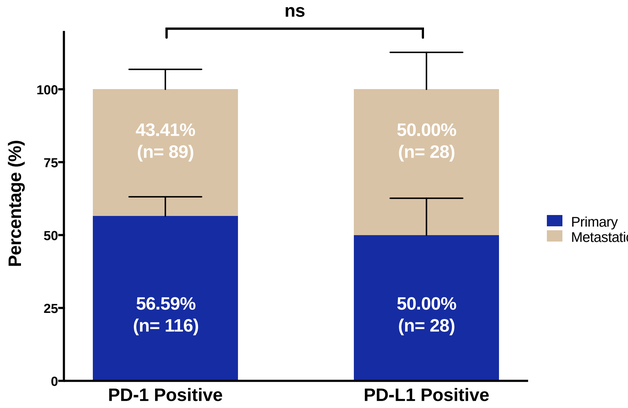


Figure 3 – PD-1 and PD-L1 expression between primary site and metastatic TNBCs are similar. Frequency comparisons calculated using a two-sided Fisher's exact test. Calculated p-value: p = 0.4488.

Conclusions

- PD-1 expression was shown in 56.47% of our TNBC cohort, consistent with recently published I-SPY-1[3] study.
- Based on our threshold, we showed that 8.89% of TNBCs are PD-L1 positive. These results are slightly lower than the Sun et. al study[4], which showed an 11% positive rate PD-L1 positive rate in TNBCs using the SP142 antibody. This is also significantly lower than the 58.6% rate shown in the KEYNOTE-012 trial[1].
- To date, there has been a lack of uniformity in assessing PD-1 and PD-L1 positivity in breast cancer.
- These data highlight the need for consensus around importance of determining the appropriate thresholds for positive response to PD-1/PD-L1 inhibitor therapies.
- Future studies correlating PD-1/PD-L1 expression with responses to immune checkpoint inhibitor therapies are necessary for appropriate threshold determination.

References

1. Nanda, R., Chow, L. Q. M., Dees, E. C., Berger, R., Gupta, S., Geva, R., et al. (2016). Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *Journal of Clinical Oncology*, 1–10.
2. Adams S, Diamond J, Hamilton E, et al. Safety and clinical activity of atezolizumab (anti-PDL1) in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer. Presented at: San Antonio Breast Cancer Symposium; December 8-12, 2015; San Antonio, TX. Abstract P2-11-06.
3. Vidula et. al. (2015). Programmed cell death 1 (PD-1) receptor and programmed death ligand 1 (PD-L1) expression in primary breast cancer (BC); correlations with clinical characteristics and patient outcomes. J Clin Oncol 33, 2015 (suppl; abstr 1090).
4. Sun, W. Y., Lee, Y. K., & Koo, J. S. (2016). Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. *Journal of Translational Medicine*, 1–12.