Programmed death ligand-1 protein expression difference in basal like and non-basal like triple negative breast cancer and its association with disease free survival and overall survival: A systematic review

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Abstract

The study aims to summarize the literature and explore the strength of evidence for PD-L1 expression difference in basal like TNBC and non-basal like TNBC, and association of PD-L1 expression with disease free survival and overall survival in each group. A systematic search of the original research literature through November 29th, 2020, reported according to PRISMA guideline. Eligible studies investigated must have a primary outcome and at least one secondary outcome. Two reviewers independently searched, selected, and assessed quality of studies and risk of bias. Any discrepancies will be resolved by consensus or by consulting a third and fourth author. A total of 6813 articles were screened from which five articles were selected and assessed for quality of studies and risk of bias. Of 5 articles, no similar findings are found regarding the level of PD-L1 expression and its correlation with recurrence and overall survival. There is not enough substantial evidence to support the difference PD-L1 protein expression level in basal and non-basal like TNBC and its association with recurrence and overall survival. Hence, further studies are needed specifically to focus on this problem.

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Introduction

Breast cancer (BC) is known as a heterogeneous disease. BC is classified into five different groups based on gene expression profiling: luminal A and B, Her-2 positive, normal breast-like-tumors, and basal-like breast cancer (BLBC).[1-2] Each breast cancer subtype displays significant differences in incidence, age at diagnosis, stage, biological behaviour, and prognosis.[3]

Nowadays, basal-like breast cancer subtype is more commonly known as triple negative breast cancer (TNBC) in view of the fact that most BLBC lack of estrogen receptor (ER), progesterone receptor (PR) and Her2-Neu receptor.[4] The incidence rate of TNBC is only 15-20% of all BC. However, due to highly aggressive behaviour, high incidence of recurrence and metastasis, and no response to hormonal or targeted therapy, it is categorized as the worst prognosis BC subtype.[5]

Triple negative breast cancer (TNBC) itself is a highly variable disease. Molecular and gene expression profile of TNBC is being researched extensively to get a full comprehension of TNBC profile, understand the biological profile and clinical characteristics, and develop a personalized treatment.[6]

Discordance is found when TNBC is referred as basal-like breast cancer (BLBC), because not all TNBC are basal-like TNBC (based on first-generation cDNA microarrays only 75-80% of TNBC are basal-like, 15-20% of TNBC is non-basal like).[3] The basal-like subtype is derived from the basal layer of breast epithelial cells, which is positive for basal myoepithelial markers such as either EGFR, CK 5/6, CK14, or P-cadherin.[6]

A study by Lehmann *et al*., (2011) further classified TNBC based on gene expression profile into six subtypes: basal like-1 (BL-1), basal like-2 (BL-2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), dan luminal androgen receptor (LAR). Prat *et al*. (2013) showed us that basal and non-basal-like classification is the main biological difference seen in patients with TNBC, despite the diversity of TNBC.[7]

Microarray-based gene profiling techniques are distinctly sophisticated, highly cost and only available in research center. Thus, it is rarely used in clinical practice. IHC assay, on the other hand, is more accessible and relatively has lower cost.[8]

Immunotherapy has been a new promising treatment strategy for several solid tumors such as lung cancer and melanoma, but with limited breast usage because BC is considered less immunogenic. Nevertheless, TNBC is considered more immunogenic than other BC subtype, with higher TILs and high PD-L1 expression within the tumor than another BC subtype. Tumor-infiltrating lymphocytes (TILs) are lymphocytic cell population cells that infiltrate tumor tissue and have been described in many malignancies including BC.[9] The high level of mutations in TNBC, hence higher level of tumor neoantigens within TNBC will activate neoantigen-specific T cells to act as antitumor makes TNBC more immunogenic than other BC subtype.[10,11.]

Immune-checkpoint inhibitors (ICI) is currently a new hope for TNBC treatment, although monotherapy administration shows low efficacy, with response rates only 5-23% in treatment naïve PD-L1–positive patients.[12,13.]

ICI usage needs more research in clinical trial format, reinforced with strong preclinical and translational research to improve patient clinical response.[8]

In the heterogeneity of TNBC, classification and identification of subtype that are likely to respond to immunotherapy are essential to give a personalized treatment to this group.[13]

Our immune system is a delicate balance between inhibitory and stimulatory pathways. This balance is needed to ensure the immune system is in perfect control in encountering threat such as infection, allergic reaction, and neoplasm growth. One of the inhibitory axes is programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1).[12]

PD-1 is a member of the B7-CD28 family of co-stimulatory molecules. PD-1 in immune cells primarily binds to PD-L1 and PD-L2, which present on tumor cells' surface and tumor-infiltrating immune cells, induced by interferon-γ (IFN-γ) other cytokines. PDL-1 when binds to PD-1 will give signals to suppress cytotoxic T-lymphocytes response to the tumor, such as decreased expression of markers in the cell-surface, diminished T-lymphocytes proliferation and cytokine production.[14]

PDL-1 has been researched in many studies alongside TIL for its usage as prognostic marker with conflicting results, but most of these studies consider the TNBC as equal to BLBC.[15] The results of these studies have been summarized in several meta-analyses. Most of the meta-analysis are concluded that PD-L1 correlated with poor prognosis but currently no specific review regarding PD-L1 expression in basal-like and non-basal like TNBC.[16-20.]

This study aims to summarize the literature and explore the strength of evidence for PD-L1 expression difference in basal like TNBC and non-basal like TNBC, and association of PD-L1 expression to recurrence and overall survival group.

Materials and Methods

According to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement checklist.[21]

Search strategy

We searched all the research article using the PubMed, PubMed Central, Google Scholar, Science Direct, EuroPMC database using keywords listed in Table 1. The last search in each of the databases was conducted on November 29th, 2020. Multiple article checks were performed on the five databases following PRISMA guideline, as seen in Figure 1.

Studies were included in this review if met the following inclusion criteria: the study must be a research article within publication range 2010-2020. The article must represent the study question (P: TNBC patients with ER-, PR- and Her2Neu- defined by IHC. I: basal like TNBC, defined by breast cancer patients that exhibit ER-PR-, Her2Neu-, EGFR and/or CK5/6 expressions by IHC, C: Non-basal like TNBC, patients that exhibit ER-, PR, Her2Neu- but no expressions either EGFR or CK5/6 by IHC, O: analyze the PD-L1 expression by IHC/protein expression level in each group as the primary outcome, and its association to the secondary outcome: disease-free survival/ recurrence free survival and overall survival).

The reviewers evaluated the titles and abstracts for all studies identified through the PRISMA search strategy.[21] Two primary reviewers (FH and BH) screened titles and abstracts through a total of 6813 articles and removed duplications. For the articles that remained after the initial screen, 15 full texts were reviewed for eligibility. When there are differences between 2 authors, third and fourth author (AH and YA) were consulted to decide.

Full texts were evaluated when there was insufficient information in the title and abstract to decide inclusion and exclusion. We also contacted the corresponding authors to obtain any additional information, if necessary. Studies that sub-classified TNBC based on gene expression profile, and those who assume basal-like TNBC are equal to all TNBC also be excluded. The studies were excluded if their designs were review articles, systematic reviews, or meta-analyses. Due to language limitation, study written in a language other than English will be excluded.

Outcome definitions

Primary study outcomes could be defined as PD-L1 protein expression level in both basal-like and non-basal like TNBC group. Secondary outcomes of interest are DFS/RFS and/or OS of both groups. Disease-free survival or recurrence free survival were defined as the period between diagnosis and relapse of breast cancer. Overall survival was defined as the period between diagnosis date of breast cancer-related death.

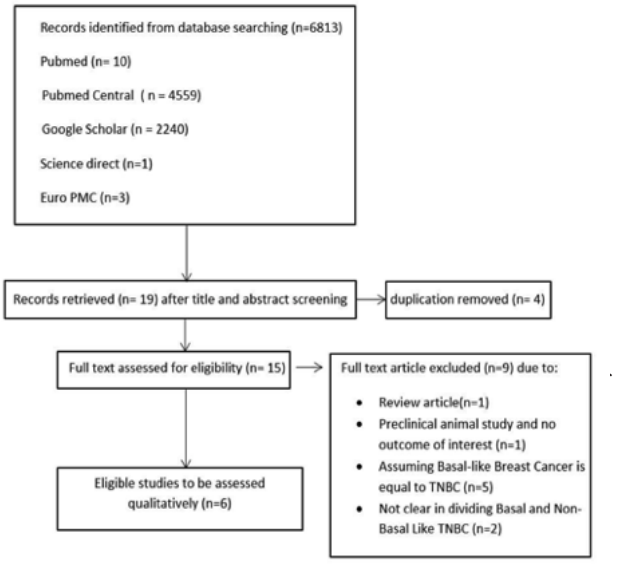

Figure 1. PRISMA strategy used for searching strategy.

Results

Literature search

A total of 6813 articles were identified through the search strategy. Figure 1 shows the result of the PRISMA strategy search in this systematic review. Most articles were excluded because the study assumes basal like breast cancer is equal to TNBC, especially in the result analysis. At the end of our literature search, six studies are being screened for their quality and risk of bias, and in this assessment, we found 1 study is fair in quality and has high risk of bias. In the end and only five articles are included in this systematic review.[23-27] Details of quality assessment and risk of bias assessment are mentioned in the supplement data.

Characteristics of the included studies

The main characteristics of the five studies included in this systematic review are summarized in Tables 2 and 3. All studies were all only subset of the main study, all conducted in cohort retrospective with TMA used for tissue sampling, with the number of patients is between 104-152 patients. In 5 studies, basal-like TNBC accounts for the majority of TNBC patients. The eligibility criteria were mostly the availability of tissue micro-array/TMA to be studied. Tissue Micro Array is a technique which allows a single histologic slide to contain many small representative tissue samples derived from hundreds of different cases, hence it will increase the productiveness to analyze multiple specimens at the same time.[24]

Primary outcome

Our study compiled only several subsets of studies with different PD-L1 protein expression levels in basal-like TNBC and non-basal like TNBC. In Table 2, we could see that each study is using different antibodies with a different scoring system. All of them were

Table 1. Literature search strategy.

Database	Keyword	Result
PubMed	"Triple negative breast neoplasm" OR "triple negative breast cancer" AND "basal like" OR non "basal-like" AND "PD-L1" OR "programmed cell death ligand-1" AND "disease free survival" OR "recurrence free survival" OR "overall survival" (PubMed generated keywords in supplemental file)	10
PubMed Central	"Triple negative breast neoplasm" OR "triple negative breast cancer" AND "basal like" OR non "basal-like" AND "PD-L1" OR "programmed cell death ligand-1" AND "disease free survival" OR "recurrence free survival" OR "overall survival" (PubMed Central generated keywords in supplemental file)	4559
Google Scholar	Triple negative breast cancer basal like non basal like PD-L1 programmed cell death 1 disease free survival recurrence free survival overall survival	2240
Science Direct	Triple negative breast cancer AND (basal like OR non basal like) AND programmed cell death ligand 1 AND prognosis	1
Euro PMC	"Triple negative breast cancer" AND "basal like" AND "non basal like" AND "programmed cell death ligand-1"AND"prognosis"	3

Table 2. Characteristics of eligible studies.

	Author	Patients' characteristics	Study design	Eligibility criteria	Length of follow up
1	Bae *et al*., 2016	465 all breast ca patients diagnosed from 2001-2013: 109 are TNBC patients comprised of 81 patients are basal-like TNBC and 28 patients are non-basal like	Cohort retrospective	Inclusion criteria: tissue punches were evaluable for TMA for this study	41.0 months
2	Guo *et al*., 2016	183 TNBC patients from 1999-2008: 116 patients are basal-like TNBC, 67 patients are non-basal like	Cohort retrospective	Inclusion criteria: primary operable breast cancer, no family history for breast or ovary cancer, no prior treatments before surgery, mastectomies, or lumpectomies specimens with sufficient tissue for TMA	76.4 months
3	Beckers *et al*, 2016	161 TNBC patients	Cohort retrospective	Blocks are available for TMA, confirmed TNBC in repeated testing	55 months
4	Wang *et al*, 2017	443 all primary breast ca patients diagnosed within period 1988-1995: 104 TNBC patients comprised of 69 patients are basal-like TNBC, and 35 patients are non-basal like TNBC	Cohort retrospective	Inclusion criteria: the paraffin blocks and tissue cores should be available for TMA	87 months
5	Ren *et al*., 2018	195 TNBC patients underwent curative operations from May 2002-May 2012: 152 patients are basal-like TNBC, and 43 patients are non-basal like	Cohort retrospective	Exclusion criteria: a case with insufficient paraffin-embedded tumor tissue for TMA or those with preoperational neoadjuvant treatment	Follow up time began from the date of surgery until June 30th 2016

TNBC, triple negative breast cancer; TMA, tissue micro-assay.

Table 3. Evaluation of PD-L1 expression in eligible studies.

	Author	Methods PD-L1 protein expression assessment	Antibodies used for detecting PD-L1 protein expression	Scoring system for PD-L1 protein expression	PD-L1 protein expression grouping
1	Bae *et al.*, 2016	Two independent observers performed IHC scoring in a blinded manner, and the consensus was reached by repeated examination when results were discordant	Rabbit monoclonal antibody (clone E1L3N, cell signaling technology)	PD-L1 expression in membranous and cytoplasmic staining was considered positive. H-score (0-300): staining (0=negative; 1=weak; 2=moderate; 3=strong) x proportion of positive staining. PD-L1 expression categorized based on H-score (low expression=0-99; high expression=100-300) using modified Muenst scoring method	High or low expression
2	Guo *et al.*, 2016	Slides were assessed by two experts blind to the patient's history and histological findings	Rabbit monoclonal antibody (SP142, Ventana, Tucson AZ)	Tumor and immune cells were attributed separate scores on a four-point scale as follows: 0(no staining), 1+(cytoplasmic and/or weak membranous staining in <10% of the positive cells), 2+ (weak to moderate cytoplasmic and/or membranous staining in ≥10% of the positive cells) or 3+ (strong cytoplasmic and/or membranous staining in ≥10% of the positive cells). PD-L1 scores in patients with multiple specimens were based on the highest score	Negative or positive
3	Beckers *et al.*, 2016	IHC scoring was evaluated in consensus viewing by two observers; one is a specialist in breast pathologist	IHC, rabbit PD-L1 monoclonal Ab, clone E1L3N (cell signaling technology, Danvers, MA, USA)	Tumors/patients were classified as PD-L1-positive if there was ≥1% tumoral membranous or cytoplasmic PD-L1 expression or ≥1% Immune cells in the stromal compartment were positive. Further, it is assessed in a membranous, cytoplasmic and stromal compartment with the following grading: absent(0), Low(1+), Intermediate(2+), Strong (3+)	Negative or positive
4	Wang *et al.*, 2017	An experienced breast pathologist performed IHC scoring in a blinded manner	SP142 rabbit monoclonal (Biocare)	H score method (0-300)=staining intensity (0-3) x proportion of positive (membranous)staining tumor cells (0-100%). Tumor cell expression was categorized into low to high expression levels based on scores below the upper 75th quartile. PD-L1 expression in TIL not scored	High or low expression
5	Ren *et al.*, 2018	Two experienced pathologists did IHC scoring.	Rabbit monoclonal, Ventana code 263	Positive: membrane staining, cutoff value ≥25%	Negative or Positive

IHC, immunohistochemistry; PD-L1, programmed cell death-ligand 1; TNBC, triple negative breast cancer; TMA, tissue micro-assay.

using TMA for tissue assessment.[25-29] The primary and secondary outcome of compiled studies are summarized in Table 4.

In studies by Wang *et al*. (2017) and Bae *et al*. (2016), PD-L1 expression is dichotomized into two groups: high and low expression. Both were using H- scores to assess PD-L1 protein expression and with a similar cutoff for positivity, albeit the two studies were using different antibodies (SP 142 and E1L3N in each). In the first study by Wang, *et al*. (2017) the PD-L1 expression is high in basal-like TNBC (67% of all samples) in contrast with non-basal like TNBC (31% of all samples) P-value 0.013. This study clearly defined the basal-like and non-basal-like TNBC by EGFR and CK5/6 expression, and further analyzed its association with PD-L1 expression in each group. The finding is in contrast if compared to a second study by Bae *et al*. (2016) in which low expression of PD-L1 is majority group (70.4%) in basal-like TNBC (P<0.001). However, the non-basal group also mostly have low expression of PD-L1 (96.42%) P-value <0.464.

Both studies by Guo *et al.* (2016) and Ren, et al. (2018) are not mainly focus on TNBC heterogeneity. We pulled out data within these two studies that only congruent with this systematic review. They divide PD-L1 protein expression into negative or positive but also based on different antibodies and different scoring system. In conclusion, none of them stated significant difference between PD-L1 protein expression in basal-like TNBC and non-basal like TNBC, supported by similar findings by Beckers *et al.* (2015).

With the intention of objectivity, 4 in 5 studies are assessed by two or more different pathologists, only 1 study by Wang *et al*. (2017) assessed by one breast pathologist in a blinded manner. No analysis for inter-reader concordance in all five studies.[25-29]

Secondary outcome

A study by Wang *et al*. (2017) clearly defined the basal-like and non-basal Like TNBC. The PD-L1 expression in tumor cells is positively associated with recurrence, meaning the higher PD-L1 expression in tumor cells of basal-like TNBC, the lower likely the patient to have a recurrence. PD-L1 status in basal-like TNBC is showing the only trend towards better overall survival but not statistically significant. In non-basal like groups, the PD-L1 expression is not associated with recurrence or overall survival in this study.[28]

In contrary, a study by Bae, *et al.* (2016) concluded PDL-1 expression variance in basal-like and non basal like TNBC has no significant association with both recurrence and overall survival.[24] Guo et al. and Ren *et al.* (2018) also noted that the expression of PD-L1 by either immune cells or tumor cells was not significantly associated with overall survival in neither basal-like and non-basal like TNBC.[27,29]

In addition, a study by Beckers *et al. (*2016) stated that, although no statistical difference between PD-L1 expressions in all compartment of basal-like TNBC and non-basal like TNBC, overall PDL-1 expression more than 5% in cytoplasmic TNBC tumor cells has significant correlation to lower risk of breast cancer death.[25]

Discussion

TNBC still has grim prognosis until now. The heterogeneity in TNBC is also making the clinicians and researchers difficult to determine personalized therapy.[30]

TNBC has been long assumed to be equal to BLBC, but these two subtypes are not the same thing. Most basal-like are TNBC, it is true, but in other subtypes such as Luminal A, Luminal B, Her-2 enriched we also find basal-like breast cancer. In TNBC, as men-

Table 4. Primary and secondary outcome.

	Author	Primary outcome: PD-L1 level in basal TNBC vs non-basal TNBC group	Secondary outcome: recurrence/survival
1	Bae *et al.*, 2016	In basal like TNBC group: low PD-L1 expression protein in 70.40%, high expression in 29.60% P value <0.001 in non-basal like TNBC group: low PD-L1 protein expression in 96.42%, high expression in 3.6% P<0.464	PDL expression in basal-like TNBC does not correlate with DFS P=0.476) nor OS (P 0.173). There was no correlation in non-basal-like TNBC either in DFS (P=0.650) and OS (P=0.847)
2	Guo *et al.*, 2016	No significant difference in the proportion of PD-L1 protein expressed in tumor cell and immune cell between basal-like (n=116) and non-basal-like (n=67) breast cancer subtype (13.8 vs 13.4% of all TNBC, P=0.94)	Expression of PD-L1, by either immune cells or tumor cells was not significantly associated with overall survival in neither TNBC, basal-like nor non-basal like. Recurrence is not analyzed
3	Beckers *et al.,* 2016	No association of basal-like TNBC with PD-L1 expression in membranous (P=0.4182), cytoplasmic (P=0.643) nor stromal compartment (P=0.7484). No mention of PD-L1 expression in non-basal like TNBC	Membranous PD-L1 is not associated with outcome. Cytoplasmic expression of PD-L1 ≥5% correlated to lower risk of BC death
4	Wang *et al.*, 2017	In basal-like TNBC group: low PD-L1 protein expression in 67%, high expression in 33% in non-basal like TNBC: low PD-L1 expression in 69%, high expression in 31% (P-value 0.013)	PD-L1 status in basal-like TNBC group is associated with RFS (hazard ratio=0.39, 95% CI=0.22-0.86, P=0.018) with High PD-L1 level is associated with better RFS. In association with OS, the PD-L1 status in basal-like TNBC shows the only trend towards better OS. In non-basal like TNBC, PD-L1 positive status is not significant for either RFS or OS
5	Ren *et al.*, 2018	Analysis with IHC/protein expression: no significant difference in the proportion of PD-L1 protein expressed tumors between basal-like (n=152) and non-basal-like (n=43) breast cancer subtype (7.20 vs 4.65% of all TNBC, P=0.737)	No significant association between overall PD-L1 protein expression and patient survival. Recurrence is not mentioned

IHC, immunohistochemistry; PD-L1, programmed cell death-ligand 1; TNBC, triple negative breast cancer.

tioned before, only 75-80% expressed basal features and 20-25% are non-basal like.[7,30]

The usage of immunologic marker for predicting prognosis in TNBC has been started for several years. Many have resulted from these studies, in which one of the most highlights are PD-L1, either using IHC assay, miRNA level of CD274 gene expression.[26,29,31-33]

Studies in PD-L1 protein expression in TNBC and its correlation with prognosis (DFS/RFS/OS) are many, with inconsistent results.[17-20,27] This is mainly due to tumor heterogeneity, and most studies are using TMA in their studies with different reagents and methods to assess PD-L1 protein expression.[29-30]

It is also known that each of anti-PD-1 and anti-PD-L1 agents in clinical trials uses their own company IHC assay and reagent, thus complicating the PD-L1 expression assessment and decreasing the comparability of PD-L1 as a prognostic marker in studies. Several studies comparing different PD-L1 antibodies for IHC in BC have found varied result with different antibodies (such as SP263, 28-8, 22C3, SP142, E1L3N) with the lowest staining found in IHC assay using SP 142. FDA has approved SP 263, 28-2 and 22C3 as they showed comparable result.[31] In future studies, PD-L1 IHC clone's choice would end up using the clone by which ICI/ Immune Checkpoint Inhibitors shows effect in clinical trials than which clone shows consistent results.[33-35]

Recently in November 2020, the FDA granted accelerated approval of the PD-L1 inhibitor keytruda (pembrolizumab) in combination with chemotherapy to treat people with locally recurrent or metastatic TNBC. Their tumors express PD-L1.[36] They are based on clinical trial coded KEYNOTE-355 (NCT02819518). This also accompanied with approval use of PD-L1 IHC 22C3 pharm DX as a companion diagnostic for selecting patients with TNBC for pembrolizumab.[37] This approval of pembrolizumab weigh the consideration of using PD-L1 IHC 22C3 pharm DX as a future standard antibody in assessing PDL-1.

This systematic review found that inconsistent results are probably due to different antibodies, different scoring systems, and different grouping of PD-L1 protein expression (high/low *vs* negative/positive), hence data could not be pooled, and the meta-analysis cannot be done. The highest cutoff point found in a study by Ren, *et al* .(2016) which considered 25% PD-L1 protein level in membranous staining as positive following strict criteria made from the antibody producer, which is different from another study (usually PD-L1 protein level more than 1% or 5% is already considered positive). The author also mentioned this scoring system probably the answer of why the PD-L1 protein positivity in their study is low (only in 13.6% of TNBC patients) and no association found of PD-L1 protein with overall survival. Interestingly this study also noted that PD-L1 mRNA is higher in basal-like TNBC (P=0.033).[29]

The combination of TMA usage and IHC assay for assessing PD-L1 protein level is also noted for their lack in covering TNBC heterogeneity, a lack in which many studies try to cover using multicores of patients' tissue.[38] Subjectivity in assessing PD-L1 protein positivity also noted. We only compiled studies that mentioned that PD-L1 protein expression assessment is in blind manner or by 2 pathologists or more to reduce this subjectivity risk.

While incomparability of PD-L1 protein expression using IHC assay is noted, IHC assay is still used to assess PD-L1 protein expression and determining ICI application in daily practice and many studies. Analyzing the PD-L1 protein level and clinical outcomes in cohort fashion was more reasonable because protein is more stable than mRNA over long periods. Not all mRNA is translated into protein.[39]

Currently, no study focuses on assessing the difference of PD-L1 expression in the basal like and non-basal like TNBC. To our knowledge, this is the first systematic review made to this purpose. In 5 subset of studies included in our review, mostly stated that PD-L1 protein expression level is higher in basal-like TNBC than non-basal like. In non-basal like group, the PD-L1 protein expression mostly low, with no correlation with prognosis. This is probably due to a relatively smaller sample of non-basal like a group compared to the basal-like group. Further, another study with larger samples of TNBC is needed, with proportional sample of both group (basal and non-basal-like group) and prospective cohort method for better prognostic evaluation.

If it is proven, then it could be another essential matter to guide the subsequent studies evaluating PD-L1 protein expression in TNBC and next clinical trial that we should consider exclusion of non-basal like group since this group could dilute the study result. However, the PD-L1 expression in each group and its association with prognosis remains unclear due to different reagents, methods and stratification used for evaluating PD-L1 expression in IHC assay. Further, agreement of methods and antibody in assessing PD-L1 protein level is needed.

This study also have several limitations. We were searching only 5 databases, more searching in other databases probably will provide more specific studies. We also could not ensembled data due to diversification of antibodies, cut-off, and method in assesing PD-L1, hence we could not establish a meta-analysis.

Conclusions

In conclusion, further studies specifically address this PD-L1 protein level differences in Basal like and non-basal like TNBC and its correlation with prognosis are needed, with larger sample and proportional sample of both groups to further highlighted the differences between the two group and prospective cohort method for better prognostic evaluation. We also noted that agreement of methods and antibody in assessing PD-L1 protein level is needed to elevate the use of PDL-1 as prognostic marker in TNBC.

References

1. Perou CM, Sørile T, Eisen MB, et al. Molecular portraits of human breast tumors. Nature 2000;406:747-52.
2. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS 2001;98:10869-74.
3. Prat A, Adamo B, Cheang MCU, et al. Molecular characterization of basal like and non basal like triple negative breast cancer. Oncologist 2013;18:123-33.
4. Wang DY, Jiang Z, Ben-David Y, et al. Molecular stratification within triple-negative breast cancer subtypes. Sci Rep 2019;9:1-10.
5. Chang-Qing Y, Jie L, Shi-Qi Z, et al. Recent treatment progress of triple negative breast cancer. Prog Biophys Mol Biol 2020;151:40-53.
6. Polónia A, Pinto R, Cameselle-Teijeiro JF, et al. Prognostic value of stromal tumor infiltrating lymphocytes and programmed cell death-ligand 1 expression in breast cancer. J Clin Pathol 2017;70:860-7.
7. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:1-17.
8. Majumder A, Jagani R, Basu A. Double-positive in triple-negative? how significant is basal cytokeratin expression in breast

cancer? Med J Armed Forces India 2020;76:63-70.
9. Teixeira L, Rothé F, Ignatiadis M, Sotiriou C. Breast cancer immunology. Oncol Times 2016;38:18-9.
10. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumor-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40-50.
11. Keenan TE, Tolaney SM. Role of immunotherapy in triple-negative breast cancer. J Natl Compr Cancer Netw 2020;18:479-89.
12. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
13. Gibson J. Anti-PD-L1 for metastatic triple-negative breast cancer. Lancet Oncol 2015;16:e264.
14. Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the b7-1 costimulatory molecule to inhibit t cell responses. Immunity 2007;27:111-22.
15. Matikas A, Zerdes I, Lövrot J, et al. Prognostic implications of PD-L1 expression in breast cancer: systematic review and meta-analysis of immunohistochemistry and pooled analysis of transcriptomic data. Clin Cancer Res 2019;25:5717-26.
16. Guo Y, Yu P, Liu Z, et al. Prognostic and clinicopathological value of programmed death ligand-1 in breast cancer: A meta-analysis. PLoS One 2016;11:1-11.
17. Li X, Li M, Lian Z, et al. Prognostic role of programmed death ligand-1 expression in breast cancer: a systematic review and meta-analysis. Target Oncol 2016;11:753-61.
18. Kim HM, Lee J, Koo JS. Clinicopathological and prognostic significance of programmed death ligand-1 expression in breast cancer: A meta-analysis. BMC Cancer 2017;17:1-11.
19. Wang C, Zhu H, Zhou Y, et al. Prognostic value of PD-L1 in breast cancer: a meta-analysis. Breast J 2017;23:436-43.
20. Zhang M, Sun H, Zhao S, et al. Expression of PD-L1 and prognosis in breast cancer: A meta-analysis. Oncotarget 2017;8:31347-54.
21. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: The prisma statement. PLoS Med 2009;6:1-6.
22. Wells G, Shea B, O'Connell D, et al. The newcastle-ottawa scale (nos) for assessing the quality of nonrandomised studies in meta-analysis. The Ottawa Hospital Research Intitute 2014.
23. Benford D, Halldorsson T, Jeger MJ, et al. The principles and methods behind EFSA's guidance on uncertainty analysis in scientific assessment. EFSA J 2018;16:1-234.
24. Jawhar NMT. Tissue microarray: a rapidly evolving diagnostic and research tool. Ann Saudi Med 2009;29:123-7.
25. Beckers RK, Selinger CI, Vilain R, et al. Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumor-infiltrating lymphocytes and improved outcome. Histopathology 2016;69:25-34.
26. Bae SB, Cho HD, Oh MH, et al. Expression of programmed death receptor ligand 1 with high tumor-infiltrating lymphocytes is associated with better prognosis in breast cancer. J Breast Cancer 2016;19:242-51.
27. Guo L, Li W, Zhu X, et al. PD-L1 expression and cd274 gene alteration in triple-negative breast cancer: implication for prognostic biomarker. Springerplus 2016;5:1-8.
28. Wang ZQ, Milne K, Derocher H, et al. PD-L1 and intratumoral immune response in breast cancer. Oncotarget 2017;8:51641-51.
29. Ren X, Wu H, Lu J, et al. Pd1 protein expression in tumor infiltrated lymphocytes rather than PD-L1 in tumor cells predicts survival in triple-negative breast cancer. Cancer Biol Ther 2018;19:373-80.
30. Ali HR, Glont SE, Blows FM, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumors and associated with infiltrating lymphocytes. Ann Oncol 2015;26:1488-93.
31. Sabatier R, Finetti P, Mamessier E, et al. Prognostic and predictive value of PD-L1 expression in breast cancer. Oncotarget 2015;6:5449-64.
32. Stovgaard ES, Dyhl-Polk A, Roslind A, et al. PD-L1 expression in breast cancer: expression in subtypes and prognostic significance: a systematic review. Breast Cancer Res Treat 2019;174:571-84.
33. Dogra A, Mehta A, Doval DC. Are basal-like and non-basal-like triple-negative breast cancers really different? J Oncol 2020;2020:1-9.
34. Karnik T, Kimler BF, Fan F, et al. PD-L1 in breast cancer: comparative analysis of 3 different antibodies. Hum Pathol 2018;72:28-34.
35. Qin T, Zeng YD, Qin G, et al. High PD-L1 expression was associated with poor prognosis in 870 Chinese patients with breast cancer. Oncotarget 2015;6:33972-81.
36. Food and Drug Administration. Fda grants accelerated approval to pembrolizumab for locally recurrent unresectable or metastatic triple negative breast cancer; 2020.
37. Cortes J, Cescon DW, Rugo HS, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (keynote-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. J Clin Oncol 2020;396:1817-28.
38. Wang X, Teng F, Kong L, et al. PD-L1 expression in human cancers and its association with clinical outcomes. OncoTargets Ther 2016;9:5023-39.
39. Schalper KA, Velcheti V, Carvajal D, et al. In situ tumor PD-L1 mrna expression is associated with increased TIand better outcome in breast carcinomas. Clin Cancer Res 2014;20:2773-82.