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DIFFERENCES IN STROMAL TIL$_s$ GRADE and PD-L1 EXPRESSION IN TNBC AND NON-TNBC AT PROF.DR.I.G.N.G. NGOERAH GENERAL HOSPITAL

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ABSTRACT

Breast carcinoma is type of cancer with high incidence and mortality rates in women worldwide. Breast carcinoma is divided into two categories, *Invasive Breast Carcinoma of No Special Type* (IBC-NST) and specific-type breast carcinoma. Breast carcinoma is divided into four surrogate molecular subtypes, TNBC and non-TNBC (Luminal A, Luminal B, *HER2 overexpressive*). TIL$_s$ are mononuclear immune cells that infiltrate tumor tissue in breast carcinoma. PD-1 and its ligand PD-L1, are immune checkpoint inhibitors to regulate the body's immune response. The aim of study was to prove the difference in TIL$_s$ grade and PD-L1 expression between TNBC and non-TNBC.This study was an observational analytic study with a cross-sectional study design. The study sample consisted of 42 IBC-NST patients who underwent biopsy/MRM and had histopathological and immunohistochemical (ER, PR, HER2, Ki67) examinations, comprising 21 non-TNBC patients and 21 TNBC patients at RSUP Prof.dr.I.G.N.G. Ngoerah, from January 1, 2021 to December 31, 2023. In this study, the youngest age was 33 years old and the oldest was 76 years old, with an average age of 50.17±10.57 years. Analysis using *chi-square* showed a significant difference in TIL$_s$ grade and PD-L1 expression between TNBC and non-TNBC, with a p-value of 0.014 ($p < 0.05$) and a prevalence ratio (PR) of 1.58 (95% CI: 1.07 – 2.35). From *chi-square* analysis, a statistically significant relationship was obtained between TNBC and non-TNBC with age ($p<0.001$), histological grade type ($p=0.031$), TIL$_s$ ($p=0.014$), and PD-L1 expression ($p = 0.014$).In conclusion, there was a significant difference in the degree of TIL$_s$ and PD-L1 expression between patients with TNBC and non-TNBC at Prof.dr.I.G.N.G. Hospital. Ngoerah.

Keywords: invasive breast cancer., TIL$_s$., PD-L1 expression.

INTRODUCTION

Breast cancer is one type of cancer with high incidence and mortality rates among women globally.

According to data from Global Cancer Statistics obtained from the IARC, breast cancer ranked second after lung cancer in terms of incidence in 2022. The estimated number of new cases was 2.3 million (11.6% of all cancer cases). Furthermore, breast cancer ranked fourth in terms of mortality, with an estimated 666,000 deaths (6.9%) of all cancer-related deaths[1]. National Institute of Health Research and Development's data shows that the highest number of cancer cases in Indonesia is breast cancer 19.18%[2]. Based on cancer registry in Indonesia, breast cancer also ranks first with 65,858 cases out of a total of 396,914 new cases, with estimated number of deaths is more than 22,000 cases[3].

There is a significant variation in breast cancer survival rates worldwide, with estimated 5-year survival ranging from around 80% in high-income countries to below 40% in low-income countries[4].

The most common type of breast cancer is carcinoma. This cancer is highly heterogeneous with varied morphological and biological features, thus exhibiting different behaviors, responses to therapy, and prognoses. There are two genes that can increase the risk of developing breast carcinoma, BRCA1 and BRCA2. Reproductive history, the absence of a breastfeeding, diet, endogenous steroid hormones, oral contraceptives, hormone replacement therapy), obesity, alcohol consumption, physical activity, and smoking can also increase the risk of breast cancer[5].

In general, breast carcinoma is grouped into two main categories: invasive breast carcinoma of no special type (IBC-NST) and specific types. Furthermore, breast carcinomas are divided into four surrogate molecular subtypes: Luminal A, Luminal B, *HER2 overexpressive*, and TNBC[5].

The classification of IBC surrogate molecular subtypes is useful for determining targeted therapy and predicting prognosis. Approximately 75% of breast carcinoma cases are categorized as Hormone Receptor (HR) positive (ER and PR positive in Luminal A and Luminal B), showing a good response to hormonal therapy (*selective estrogen receptor modulators* (SERMs) and *aromatase inhibitors* (AI_s). Meanwhile, *HER2 targeted therapy* is used as the primary treatment for *HER2 overexpressive*[6].

On the other hand, due to the aggressiveness and heterogeneity of TNBC, there is no specific targeted therapy. Luminal A breast carcinoma has a better prognosis compared to the Luminal B. Meanwhile, *HER2 overexpressive* is better compared to the TNBC type[7]. The success in *hormonal theraphy* and *HER2 targeted therapy* has shifted researchers' focus to TNBC[8]. TNBC, a group of breast carcinomas that exhibit highly heterogeneous molecular characteristics, accounts for 5-10% of all breast carcinoma that does not express ER, PR, and HER2[9].

This heterogeneity in TNBC has led to difficulties in finding suitable molecular targets in preclinical studies and is reflected in the absence of specific targeted therapies found in clinical trials for TNBC patients[10]. TNBC patients' do not respond to *hormonal therapy* or *HER2 targeted therapy* due to the absence of related receptor markers, with standard non-surgical therapy is specific chemotherapy. However, less than 30% of TNBC achieve a complete response, resulting in recurrence and mortality rates that remain higher than non-TNBC subtypes[11].

TIL_s are mononuclear immune cells that infiltrate tumor tissue, consisting of stromal TIL_s and intratumoral TIL_s. In IBC-NST with low TIL_s (involving < 10% of the stroma), there is a significant correlation with lymph node metastasis[12].

Another study found high stromal TIL_s in tumor nests and stroma, especially in high-grade TNBC without medullary features (78.10%) compared to TNBC with medullary features (61.33%). Evidence suggests that high stromal TIL_s in both non-TNBC and TNBC breast carcinomas are associated with a better prognosis[13].

TNBC with high TIL_s correlate with a better prognosis and better treatment response. *HER2 overexpressive* with high TIL_s show a correlation with better prognosis and treatment response. Meanwhile, Luminal A and B, which generally have low TIL_s, have a better prognostic impact. The study in Luminal-type breast cancer patients was inconsistent with the relationship observed in TNBC and *HER2 overexpressive*[14].

Nowadays, many studies have been conducted to find innovative therapeutic strategies to improve the survival of TNBC patients. One of these is immunotherapy with anti PD-1 and anti PD-L1. PD-L1 is an immune checkpoint inhibitor that can be found on normal cells and some cancer cells. Currently, anti-PD-1/PD-L1 therapy is a recommended standard therapy for many advanced or metastatic tumors (e.g., lung carcinoma, urothelial carcinoma, classic Hodgkin's lymphoma, head and neck carcinoma, breast carcinoma, etc)[15].

In addition, the target for this immunotherapy is not limited to TNBC patients but also for other molecular subtypes of IBC (non-TNBC) that are resistant to *hormonal targeted therapy* and *HER2 overexpressive therapy*[8].

In routine diagnostics, PD-L1 expression can be measured using IHC examination, and various commercially available assays use numerous antibody clones for PD-L1 detection, as well as several expression scores and cut-off values. Due to these different IHC staining and assessment methods, each is specifically used to evaluate the clinical efficacy of Immune Checkpoint Inhibitors (ICI_s) in clinical studies and to determine a direct comparison of the predictive value of various IHC antibody clones and PD-L1 scores/cut-off[16].

Research has shown that PD-L1 is expressed in 32.4% of locally advanced IBC cases, showing a significant correlation with older age groups, high-grade tumors, and high pre-treatment TIL_s density. PD-L1 expression (antibody clone Cat No BSB 2651) was higher in the *HER2 overexpressive* (45.5%) than in TNBC (44.4%). The research results indicate that PD-L1 could be a new target in the treatment of high-grade breast carcinoma patients and the TNBC group[17].

Meanwhile, in other research, the positive PD-L1 expression rate (antibody clone 22C3 same compared with 28-8) in Luminal A was 16.7% Tumor Proportion Score (TPS), 26.2% Immune Cell Score (ICS), and 21.4% Combined Positive Score (CPS); the positive PD-L1 expression rate in Luminal B was 11.1% TPS, 44.4% ICS, and 22.2% CPS; the positive PD-L1 expression rate in *HER2 overexpressive* was 0% TPS, 50.0% ICS, and 50.0% CPS; and the positive PD-L1 expression rate in TNBC was 42.9%[18].

Research in India using the antibody clone CAL10 found PD-L1 positivity in 14.67% of patients (score 1 in 6% and score 2 in 8.67% of patients). PD-L1 expression correlated positively with higher tumor grade (grade 3). This study found higher positive PD-L1 expression in TNBC cases compared to Luminal A, Luminal B, and HER2 overexpressive types[19].

The above studies show inconsistent prevalence, where one study found a higher prevalence of PD-L1 positive cases in HER2 compared to TNBC cases, while other studies proved the opposite[17,18,19]. Considering the potential of PD-L1 immunotherapy in IBC-NST, it is necessary to conduct research on PD-L1 expression in IBC-NST cases treated at RSUP Prof. dr. I.G.N.G. Ngoerah Hospital, as baseline data for further research related to anti-PD-L1 therapy, both in TNBC and non-TNBC cases.

The aim of this study was to prove the difference in TIL_s grade and PD-L1 expression between TNBC and non-TNBC, provide benefits and additional information for clinicians in management, and provide explanation as well as educate IBC-NST patients who are currently undergoing therapy.

2 LITERATURE REVIEW

2.1 Anatomy and Histology of the Breast

Anatomically, the breast is covered by skin, beneath which lies subcutaneous fatty tissue and fascia. The fascia is attached to the pectoralis major and serratus anterior muscles, which are located directly above the ribs (from the second to the sixth ribs)[20]. Breast tissue extends from the area of the 2nd or 3rd intercostal space down to the inframammary fold (6th or 7th intercostal space), and lies transversely from the sternum to the anterior axillary line. Breast tissue also extends into the axillary area, forming the "axillary tail of Spence"[21].

The functional unit of the breast consists of the terminal duct-lobular unit (TDLU) and the large duct system. The TDLU is formed by lobules composed of acini (terminal ductules). The ductal and lobular system of the breast is lined by secretory epithelium in the inner layer and surrounded by myoepithelial cells (basal cells) on the outer layer. This bilayer (two-layered epithelium) structure is crucial for distinguishing between benign and malignant lesions. Luminal epithelial cells are cuboidal in shape with eosinophilic cytoplasm and relatively uniform-sized nuclei. Myoepithelial cells can sometimes be difficult to observe and often have varied morphology, ranging from flattened to epithelioid-shaped cells with abundant and clear cytoplasm[22].

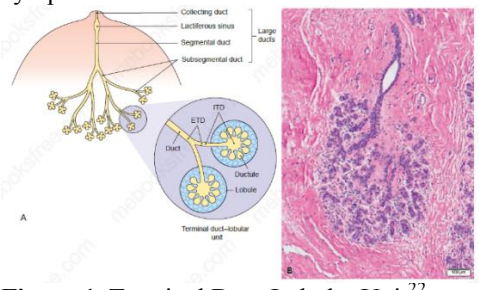

Figure 1. Terminal Duct Lobular Unit[22]

2.2 Invasive Breast Cancer

Breast carcinoma is a heterogeneous disease that varies clinically, in biological characteristics, morphology, genetics, and molecular pathology among individual breast cancer patients. These different subtypes of breast carcinoma can be identified using gene expression profiles that possess distinct genetic and molecular pathological characteristics[23].

Patients typically present with a palpable lump (approximately 2 to 3 cm), skin retraction, nipple inversion, white or reddish nipple discharge, and changes in the color and texture of the skin around the lump may also be found. In more advanced cases, ulceration can occur. Half of the patients presenting in this condition have regional axillary lymph node metastasis[5].

Patients with TNBC typically present at a younger age compared to patients with non-TNBC. TNBC patients are usually associated with larger tumor size, higher clinical stage, high histological grade, a greater number of lymph node metastases, and a higher rate of recurrence compared to non-TNBC cases. The most common sites of metastasis in TNBC cases are the lungs, liver, and brain[24].

Macroscopically, lesions in invasive breast carcinoma can be quite difficult to distinguish from in situ carcinoma lesions. The tumor is generally visible and palpable, with a greyish-white to tan color, and an irregular shape with indistinct borders against the surrounding breast tissue. Upon palpation, the lump may feel firmer or harder, and when cut with a knife, it will have a sandy/gritty sensation[25].

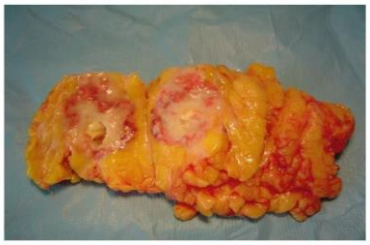

Figure 2. Macroscopic appearance of invasive breast carcinoma[26]

Invasive breast carcinoma has varied histopathological features depending on the subtype. Breast carcinomas are classified into specific subtypes if more than 90% of the tumor-forming components consist of histological features specific to a particular variant. If the specific histological features are between 10-90%, the tumor is categorized as "mixed-IBC NST and carcinoma with specific subtype", and if less than 10% of the tumor-forming cells, the tumor categorized as Invasive Breast Carcinoma of no special type (IBC-NST)[5].

Invasive Breast Carcinoma of no special type (IBC-NST) is the most common subtype encountered, where the tumor lacks specific morphological features. Architecturally, the tumor cells are arranged in cords, trabeculae, or can also be solid, with an infiltrative growth pattern accompanied by a desmoplastic reaction[5].

In general, specific breast carcinomas can be grouped based on their histological subtypes as follows[5]:

1. Invasive Lobular Carcinoma (ILC)
2. Tubular Carcinoma.
3. Cribriform Carcinoma
4. Mucinous Carcinoma
5. Mucinous Cystadenocarcinoma
6. Invasive Micropapillary Carcinoma
7. Carcinoma with Apocrine Differentiation
8. Metaplastic Carcinoma

The criteria used for histological grading is the Nottingham modification of the Scarff-Bloom Richardson criteria by evaluating the percentage of tubular formation, nuclear pleomorphism, and proliferation through mitotic count. Each parameter is scored from 1 to 3. Grade 1 breast carcinoma is assigned when the total score is 3-5, grade 2 when the total score is 6 or 7, and grade 3 when the total score is 8 or 9[5].

Table 1. Histological Grading Criteria for Breast Cancer based on the Nottingham Modification of the Scarff-Bloom Richardson Criteria[5]

Parameter	Score
Tubular formation	
More than 75%	1
10-75%	2
<10%	3
Nuclear pleomorphism	
Nuclear Size <1.5 times the nucleus of a normal epithelial cell, relatively uniform, nucleoli not visible	1
Nuclear Size 1.5-2 times the nucleus of a normal epithelial cell, nuclear pleomorphism moderate, nucleoli visible, small in size	2
Nuclear Size 2 times the nucleus of a normal epithelial cell, nuclear pleomorphism severe, prominent nucleoli, vesicular chromatin	3
Mitosis (Dependent on the microscopic field area)*	1-3

*Microscope with a diameter of 0.65, with a field area of 0.332 (score 1 ≤ 12 mitoses; score 2 13-24 mitoses; score 3 ≥25 mitoses).

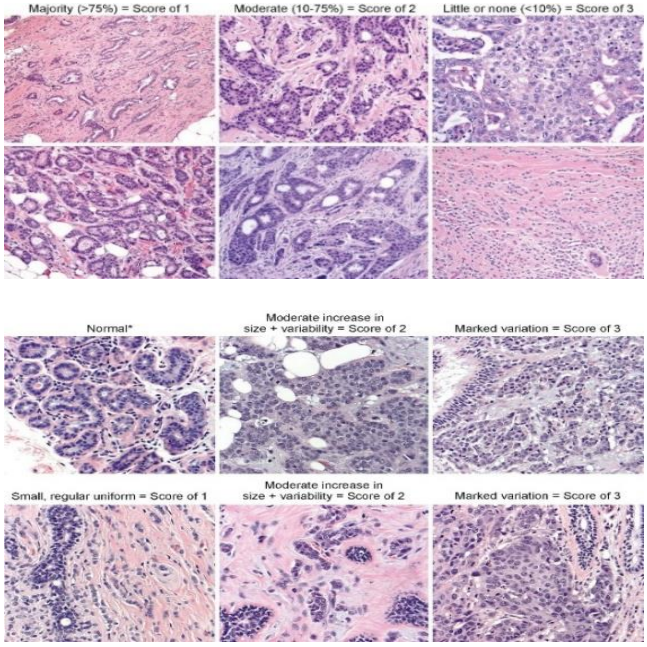

Figure 3. *Nottingham Grade: Tubular* Formation and Nuclear Pleomorphism[27].

With the advancement of transcriptomic analysis technology using microarrays, breast cancer has proven to be highly heterogeneous at the molecular level, with a wide range of gene expression patterns that cause breast cancer. Gene expression profiling studies of breast cancer can classify it into 4 main intrinsic subtypes with different prognoses: Luminal A, Luminal B, *HER2-overexpressive*, and TNBC[5].

Table 2. Molecular subtype Invassive Breast Cancer[22]

Molecular subtype				
Immunopro file	Luminal A	Luminal B	HER2 overexpre ssive	Basal-like
ER, PR	ER+ and PR high +	ER+ and PR low or intermed iate+	ER-, PR-	ER-, PR-
HER2	HER2 -	HER2+ or HER2-	HER2+	HER2-
0thers	Low Ki67(<1 4%)	Ki67≥14 %		CK5/6 and or EGFR+

The latest consensus uses a cut-off value of 20% to differentiate high and low Ki67. The majority of phenotypes in TNBC (80%) fall into the Basal-Like (BL) molecular subtype. TNBC and BL share many similarities, including genomic instability, mostly high grade, high proliferation, EGFR expression, high frequency of BRCA1 mutations, and p53 mutations[10].

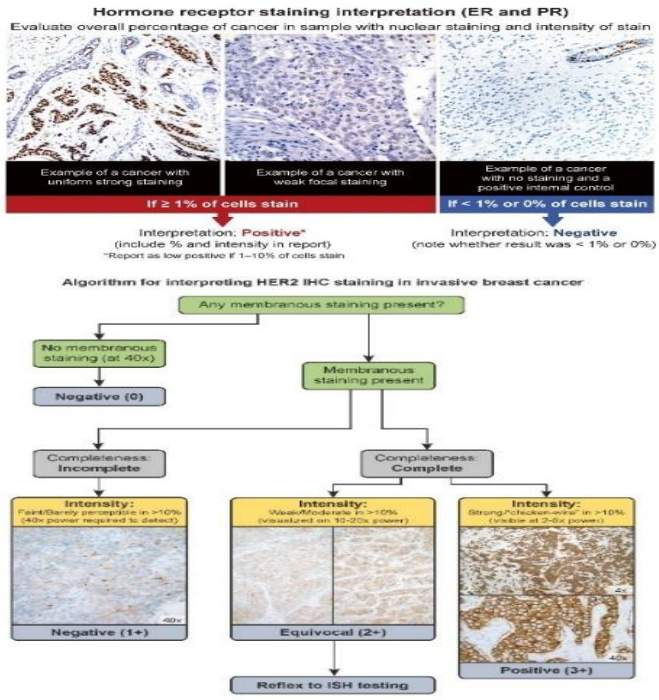

Figure 4. Interpretation of ER, PR, and HER2 in IBC NST[27].

The prognosis of invasive breast carcinoma is significantly influenced by various variables. Some prognostic markers can also be predictive factors for therapeutic response, such as ER and HER-2 status. Several standard prognostic factors include patient age, disease stage, histological grade, tumor type, surgical margin status, and lymphovascular invasion status[5].

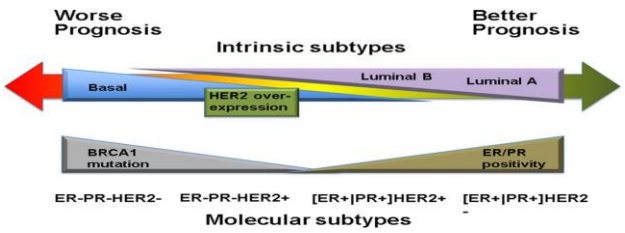

Figure 5. Prognosis of invasive breast carcinoma based on intrinsic subtypes[7].

2.3 *Tumor Infiltrating Lymphocytes (TIL$_s$)*

Tumor-infiltrating lymphocytes (TIL$_s$) are mononuclear immune cells that infiltrate tumor tissue and have been described in most types of solid tumors. TIL$_s$ are divided into stromal and intratumoral compartments. The TIL$_s$ population consists of cytotoxic T cells (CD8+) and helper T cells (CD4+), B cells, and follicular dendritic cells[28].

Stromal TIL$_s$ infiltrate the stromal tissue located near the tumor cells, localized diffusely in the stroma between the carcinoma cells. Immune cells in the stroma are considered tumor-infiltrating cells. Intratumoral TIL$_s$ actively infiltrate the centers ("nests") of tumor cells, having direct contact with the tumor cells[28].

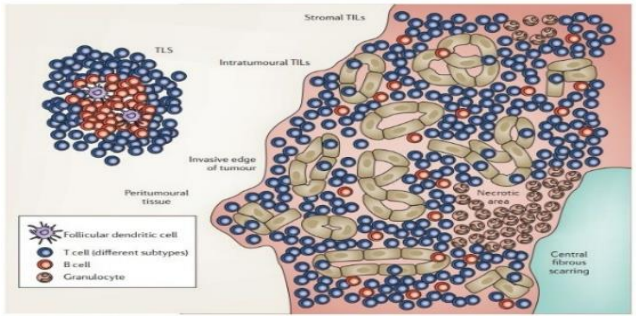

Figure 6. Diagram of the immune microenvironment in breast carcinoma[28].

The steps for evaluating TIL$_s$ in breast carcinoma used quantification be performed on H&E-stained tissue sections at 20-40x magnification with a 10x eyepiece on the most representative tumor block from the core biopsy or surgical specimen. TIL$_s$ should be assessed in the stroma between the carcinoma areas, and all mononuclear cells (lymphocytes and plasma cells) should be included. Stromal TIL$_s$ should be scored as a percentage of the stromal area only. Carcinoma cells, peritumoral follicular aggregates, and tertiary lymphoid structures with germinal centers are not included in the stromal TIL$_s$ assessment[5].

The reported area is the average stromal TIL$_s$ area, not just focusing on hotspots. Stromal TIL$_s$ are categorized into 3 groups: mild TIL$_s$ (0-10% stromal TIL$_s$), moderate TIL$_s$ (20-40% stromal TILs), and dense TILs (50-90% stromal TIL$_s$)[29]. TIL$_s$ interpretation can be assessed by visual estimation using the recommendations by the Receiving Operator Curve in 2017. TIL$_s$ density is assessed as a percentage of stromal TIL$_s$ obtained from five highest TIL$_s$-infiltrated HPFs selected, and the average of these five fields is taken. Stromal TIL$_s$ density is divided into two categories: low (≤37.5%) and high (>37.5%)[30]. Some other studies divide stromal TIL$_s$ into two categories: low TIL$_s$ (≤60%) and high TIL$_s$ (>60%)[31].

2.4 Programmed Death-Ligand 1 (PD-L1)

PD-L1 is a protein that acts as a brake or block to keep the body's immune response under control (immune checkpoint inhibitor), and it can be found on normal cells and some cancer cells. PD-1 and PD-L1 are considered factors that inhibit the immune response against cancer cells[32]. Under physiological conditions, the interaction between PD-1 and PD-L1 is crucial for maintaining immune tolerance against excessive immune cell activity. However, PD-L1 expression also becomes a mechanism for malignant cells to evade the immune system[33].

The PD-L1 structure is a 290-amino acid protein receptor encoded by CD274, consisting of 7 exons and located on chromosome 9 in humans. The exons comprise 2 extracellular domains, IgV-like and IgC-like domains, a transmembrane domain, and a cytoplasmic (intracellular) domain. The short intracellular domain of PD-L1 consists of 30 amino acids, and its function is unknown. The protein is constitutively expressed on many cell types, including Antigen Presenting Cells (APCs), T cells, B cells, monocytes, and epithelial cells, and its expression is upregulated in response to pro-inflammatory cytokines such as IFN-γ and IL4 via *Signal Transducer and Activator of Transcription 1* (STAT1) and *IFN Regulatory Factor-1* (IRF-1)[33].

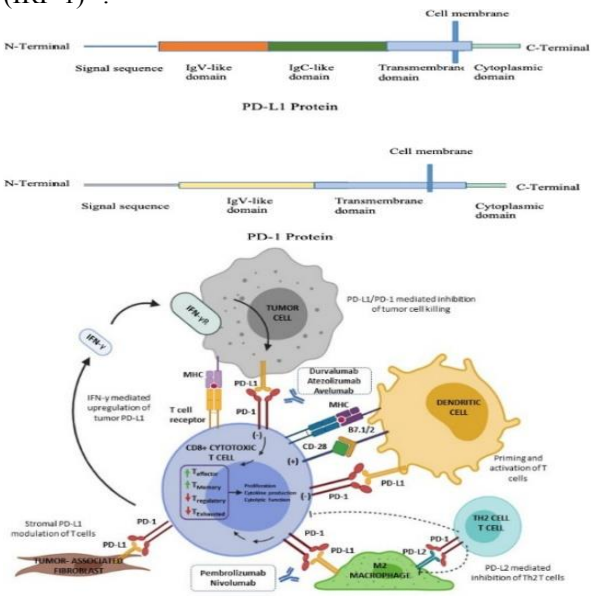

Figure 7. PD-1 and PD-L1 structure and its mechanism[33]

PD-L1 plays a role in inhibiting T cell function in peripheral tissues, while PD-L2 functions to suppress T cell activation in lymphoid organs. Low PD-L1 expression is typically found on lymphocytes, *Antigen Presenting Cells* (APCs),

the cornea, syncytiotrophoblasts, and Langerhans islet cells, where it plays a role in tissue homeostasis against inflammatory responses. High PD-L1 expression is observed in the thymus and dendritic cells, which are locations of interaction between PD-L1 and its receptor PD-1, thus preventing T cell proliferation and differentiation[33].

PD-L1 expression in breast carcinoma can be assessed by pathologists using different scoring approaches, consisting of *Tumor Proportion Score* (TPS), *Immune Cell score* (ICS), and *Combined Positive Score* (CPS). TPS is calculated based on the percentage of invasive carcinoma cells showing partial or complete membrane staining of any intensity. ICS is calculated by summing the PD-L1 positive Immune Cells (IC) with membrane and/or cytoplasmic staining divided by the total number of tumor cells present. CPS is calculated by summing the PD-L1 positive Tumor Cells (TC) and IC divided by the total number of tumor cells and multiplied by 100%. PD-L1 expression was calculated using all three scoring approaches with a cut-off of 1% according to previous research[18].

Several established PD-L1 clones currently used for breast carcinoma include Sp142 and Sp263. This study used the PD-L1 clone 28-8 because other research used the 28-8 antibody and found the comparison between PD-L1 clones 22C3 and 28-8 to be equivalent[34].

MATERIAL AND METHODS

Research Subjects

This research is using an *analytical observational* study with a *cross-sectional design*. The sample size for this research is 42 cases of invasive breast carcinoma of no special type whose specimens were examined at the Prof. dr.I.G.N.G. Ngoerah General Hospital from January 1, 2021, to December 31, 2023, with the ethical clearance number 0809/UN14.2.2.VII.14/LT/2025 and with the Prof.dr.I.G.N.G. Ngoerah General Hospital number DP.04.03/D.XVII.2.2.2/225374/2025, and who met the inclusion criteria established by the researchers. Samples were collected using *consecutive sampling technique*.

The inclusion criteria used are paraffin block from incisional biopsies or MRM with a histopathological diagnosis of IBC-NST of all surrogate molecular subtypes, who have not received chemotherapy or radiotherapy prior to MRM and paraffin blocks must contain tumor mass to assess PD-L1 expression.

Research Variabel

The variables in this study consist of dependent variables (PD-L1 Expression and Stromal TIL_s Grade) and Independent Variable (IBC-NST TNBC and Non-TNBC). Variabel Operational definition in this research consist of:

1.Invasive Breast Carcinoma of No Special Type (IBC-NST) is an invasive breast malignancy originating from the glandular epithelial component, where ≥90% does not exhibit a specific histological pattern/lacks specific features, and includes the molecular subtypes Luminal A, Luminal B, HER-2 overexpressive, and TNBC[19].

2. Stromal tumor-infiltrating lymphocytes (TIL_s) are a mononuclear lymphocytic reaction that infiltrates the stromal tissue located near the tumor mass using H&E staining at 400x magnification performed by the researcher and 2 anatomical pathology specialists. Stromal TIL_s are categorized into two groups: low TIL_s (≤60%) and high TIL_s (>60%)[31].

3. PD-L1 expression in tumor cells (Tumor Proportion Score/TPS) was assessed using immunohistochemical staining with the monoclonal antibody clone 28-8, performed by the researcher and 2 anatomical pathology specialists. Immunohistochemical staining on tumor cells showing membrane and/or cytoplasmic staining, which could be homogeneous or heterogeneous, and brown in color with weak to strong intensity, was evaluated. TPS is the proportion obtained by dividing the total number of tumor cells positive for PD-L1 by the total number of tumor cells, using high magnification (400x) and considered positive if ≥ 1% and negative if < 1%[34].

Statistical analysis

The data were processed using the Statistical Package for the Social Sciences (SPSS) 27.0 for Windows program with the following steps:

1. Descriptive analysis covering sample characteristics.
2. The difference in PD-L1 expression between the IBC-NST TNBC and Non-TNBC groups was analyzed using the Chi-square test.
3. The difference in the degree of stromal TIL_s between the IBC-NST TNBC and Non-TNBC groups was analyzed using the Chi-square test.
4. The significance level (α) was set at a probability of $p < 0.05$.

RESULTS

During the study period, 42 samples meeting the research criteria were obtained, consisting of 21 TNBC and 21 non-TNBC. The age characteristics of the research samples were as follows: 5 (11.9%) in their 30s, 18 (42.8%) in their 40s, 13 (30.9%) in their 50s, 4 (9.6%) in their 60s, and 2 (4.8%) in their 70s. The youngest sample in this study was 33 years old and the oldest was 76 years old, with a mean age of 50.17±10.57 years.

Based on the histopathological diagnosis characteristics, all research samples were diagnosed with IBC-NST. Based on grading characteristics, the research samples included 2 (4.8%) with grade 1, 16 (38.1%) with grade 2, and 24 (57.1%) with grade 3. Based on TIL_s grade characteristics, the research samples had 31 (73.8%) with low TIL_s and 11 (26.2%) with high TIL_s. Regarding PD-L1 expression, 31 (73.8%) samples showed negative PD-L1 expression and 11 (26.2%) showed positive PD-L1 expression. The characteristics of the research samples, including age decade, histological type, TIL_s grade, and PD-L1 expression, are presented in Table 3.

Table 3. Characteristics of Research Samples

Variable	Group		Total	p
	Non TNBC	TNBC		
Age				
Decade 3	5 (23,8%)	0 (0)	5 (11,9%)	
Decade 4	16 (76,2%)	2 (9,5%)	18 (42,9%)	<
Decade 5	0 (0)	13 (61,9%)	13 (31,0%)	0,001
Decade 6	0 (0)	4 (19,0%)	4 (9,5%)	
Decade 7	0 (0)	2 (9,5%)	2 (4,8%)	
Histological grade				
Grade 1	2 (9,5%)	0 (0)	2 (4,8%)	0,031
Grade 2	11 (52,4%)	5 (23,8%)	16 (38,1%)	
Grade 3	8 (38,1%)	16 (76,2%)	24 (57,1%)	
TIL_s grade				
Low	19 (90,48%)	12 (57,14%)	31 (73,81%)	0,014
High	2 (9,52)	9 (42,86)	11 (26,19%)	
PD-L1 expression				
Negative	19 (90,48%)	12 (57,14%)	31 (73,81%)	0,014
Positive	2 (9,52)	9 (42,86)	11 (26,19%)	

Table 4. Characteristics of stromal TIL_s and PD-L1 Expression

Variabel	Total
Derajat stromal TIL_s	
Rendah	31 (73,81%)
Tinggi	11 (26,19%)
Ekspresi PD-L1	
Negatif	31 (73,81%)
Positif	11 (26,19%)

Chi-square test was used to analyze the TIL_s grade between TNBC and non-TNBC patients based on a 2x2 cross-tabulation. The results of the analysis are presented in Table 4 below. Based on Table 4, there was a statistically significant difference in TIL_s grade between TNBC and non-TNBC patients, with a p-value of 0.014 ($p < 0.05$) and a prevalence ratio (PR) of 1.58 (95% CI: 1.07 – 2.35).

Table 5. TIL_s Grade between TNBC and Non-TNBC Patients

Variable	TIL_s grade		PR	CI 95%	p
	Low	High			
Non-TNBC	19 (90,48%)	2 (9,52%)	1,58	1,07 – 2,35	0,014
TNBC	12 (57,14%)	9 (42,86%)			

Low TIL_s grade is defined as ≤ 60% mononuclear cells in the stroma surrounding the tumor **(Figure 8.A)** at 400x magnification, and high TIL_s grade is defined as > 60% mononuclear cells in the stroma surrounding the tumor **(Figure 8.B)** at 400x magnification.

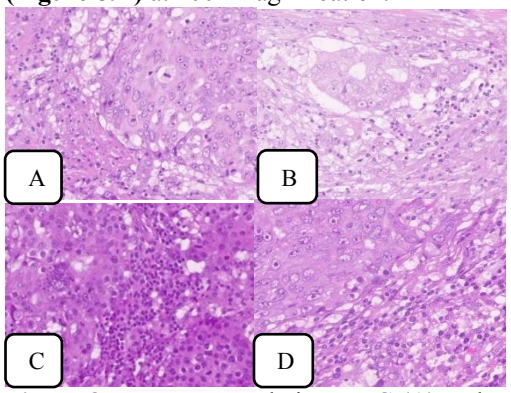

Figure 8. Low TIL_s grade in TNBC (A) and non-TNBC (B) High TIL_s grade in TNBC (C) and non-TNBC (D) H&E400x magnification

The Chi-Square test was used to analyze PD-L1 expression between TNBC and non-TNBC patients based on a 2x2 cross-tabulation. The results of the analysis are presented in Table 5 below. Based on Table 5.3, there was a statistically significant difference in PD-L1 expression between TNBC and non-TNBC patients, with a p-value of 0.014 ($p < 0.05$) and a prevalence ratio (PR) of 1.58 (95% CI: 1.07 – 2.35).

Table 6. PD-L1 Expression between TNBC and Non-TNBC Patients

Variable	PD-L1 expression		PR	CI 95%	p
	Negative	Positive			
Non-TNBC	19 (90,48%)	2 (9,52%)	1,58	1,07 – 2,35	0,014
TNBC	12 (57,14%)	9 (42,86%)			

Positive PD-L1 expression was defined as ≥ 1% of stained tumor cells **(Figure 9.A)**, and negative expression was defined as < 1% of stained tumor cells **(Figure 9.B).**

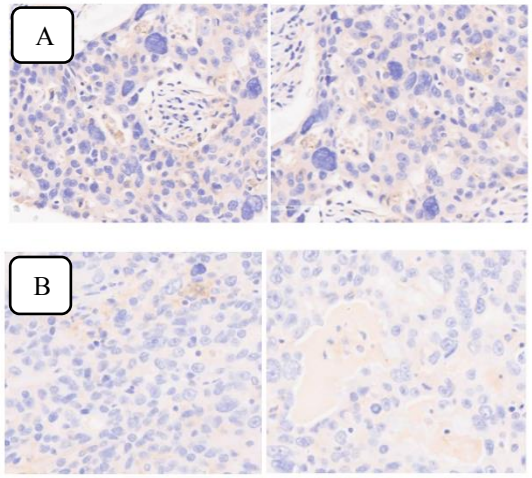

Figure 9. Positive PD-L1 in TNBC (A) and negative PD-L1 in non-TNBC (B) H&E 400x magnification.

DISCUSSION

Characteristics of TIL_s Grade in the Research Samples

In Zhao's study, high TIL_s were found in tumor nests and stroma, especially in high-grade TNBC cases without medullary features, compared to TNBC cases with medullary features. This study found that high stromal TIL_s in both non-TNBC and TNBC breast carcinoma were associated with a better prognosis[13].

In TNBC and *HER2-overexpressive*, high TIL_s correlate with better prognosis and treatment response. This is because the TNBC and HER2-overexpressive subtypes are more immunogenic, in which they are more effective at stimulating the body's immune response to attack cancer cells. This is due to the fact that immune cells, especially T-CD8+, can more easily penetrate and destroy tumor cells in the TNBC and *HER2-overexpressive* subtypes. Meanwhile, Luminal A and B which generally have low TIL_s, are said to have a better prognostic impact. This is because Luminal A and B generally have positive hormone receptors (ER and/or PR), and often also have negative HER2 receptors and low Ki-67, which affects the number of immune cells penetrating the tumor, resulting in fewer. In that study, TIL_s in Luminal-type breast carcinoma patients were inconsistent with the relationship observed in TNBC and HER2-overexpressive types[14].

Consistent with previous research, this study found low TIL_s in the Luminal A and B subtypes, while the *HER2-overexpressive* had samples with both low and high TIL_s. The TNBC subtype showed highly variable TIL_s, and this difference was statistically significant[13,14].

In this study, 42 samples were histopathologically diagnosed as IBC-NST. The number of non-TNBC (Luminal A, Luminal B, and *HER2 overexpressive*) was 21 patients (50%), and the number of TNBC was 21 patients (50%). Low TIL_s grade was found in 31 patients, specifically 19 (45.2%) non-TNBC and 12 (28.6%) TNBC. High TIL_s grade was found in 11 patients, specifically 2 (4.8%) non-TNBC and 9 (21.4%) TNBC.

Characteristics of PD-L1 Expression in the Research Samples

One study found PD-L1 expressed in 32.4% of locally advanced IBC cases, showing a significant correlation with older age groups, high-grade tumors, and high pre-treatment TIL_s density. PD-L1 expression on tumor cells using antibody clone Cat No BSB 2651 was higher in HER2-overexpressive (45.5%) than TNBC (44.4%)[17]. This might be because HER2 and TNBC subtypes (lacking estrogen or progesterone receptors and not expressing HER2) actively suppress anti-tumor immune responses, which may contribute to faster growth and wider spread, leading to high PD-L1 expression that can induce an immunosuppressive tumor microenvironment and inhibit the immune system's ability to fight cancer cells[35,36].

Meanwhile, another study using the 22C3 antibody clone found positive PD-L1 expression in non-TNBC Luminal A cases at 16.7% (TPS), 26.2% (ICS), and 21.4% (CPS); in Luminal B at 11.1% (TPS), 44.4% (ICS), and 22.2% (CPS); positive PD-L1 expression in HER2-overexpressing cases at 0% (TPS), 50.0% (ICS), and 50.0% (CPS); and positive PD-L1 expression in TNBC cases at 42.9% (TPS)[18].

In this study, 42 samples were histopathologically diagnosed as IBC-NST, and then PD-L1 examination was performed using the 28-8 antibody clone. Positive PD-L1 expression was found in 11 patients, specifically 9 (21.4%) TNBC and 2 (4.8%) non-TNBC (*HER2 overexpressive* only). The other 31 patients had negative PD-L1 expression, specifically 19 (45.2%) non-TNBC (Luminal A, Luminal B, and *HER2overexpressive*) and 12 (28.6%) TNBC.

This study then performed a statistical analysis of TIL_s grade and PD-L1 expression between TNBC and non-TNBC patients using the *chi-square* test based on a 2x2 cross-tabulation. The results of this statistical analysis showed a significant difference in both TIL_s grade and PD-L1 expression between non-TNBC and TNBC patients, with a p-value of 0.014 ($p < 0.05$) and a prevalence ratio (PR) of 1.58 (95% CI: 1.07 – 2.35).

Nowadays, the researchers have not found other studies in Bali that assessed the difference in TIL_s grade or PD-L1 expression between TNBC and non-TNBC. The study found that high TIL_s in TNBC and *HER2 overexpressive* cases correlated with better prognosis and treatment response[14]. Meanwhile, Luminal A and Luminal B with low TIL_s were also said to have a good prognosis. Another study stated that high TIL_s in both TNBC and non-TNBC cases have a better prognosis. In this study, high TIL_s in TNBC and *HER2 overexpressive* might have a good prognosis, and further research is needed[13].

Meanwhile, PD-L1 expression in Luminal A and B was investigated because previous research indicated that a

larger sample size is needed to determine positive PD-L1 expression in Luminal A and B[18]. In this study, due to time and sample limitations, negative PD-L1 expression was found in Luminal A and B cases. This may still require further investigation.

CONCLUSION AND RECOMMENDATIONS

Based on the results of this study, it can be concluded that there is a significant difference in TIL_s grade and PD-L1 expression between TNBC and non-TNBC patients at Prof. dr. I.G.N.G. Ngoerah General Hospital.

TIL_s grade can be used as an examination in IBC-NST cases, as it has prognostic and predictive value in these cases, and need further investigation. PD-L1 immunohistochemistry can be considered as an additional examination in IBC-NST cases, considering the potential for PD-L1 immunotherapy in these cases. Further research with prospective study designs or multicenter studies is needed to support the consistency of the research results.

CONFLICT OF INTEREST

There were no conflicts of interest in this study.

REFFERENCES

1. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., Jemal, A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May-Jun;74(3):229-263. doi: 10.3322/caac.21834. Epub 2024 Apr 4. PMID: 38572751.Pangribowo, S. (2019). *Beban Kanker di Indonesia. Pusat Data Dan Informasi Kesehatan Kementerian Kesehatan RI*. p.1–16.
2. KEMENKES. (2022). *Kanker Payudara Paling Banyak di Indonesia, Kemenkes Targetkan Pemerataan Layanan Kesehatan*. https://sehatnegeriku.kemkes.go.id. Diakses 20 Desember 2024.
3. Najafi, S., Sadeghi, M., Abasvandi, F., Shajari, M. R., Mohebi, K., & Ghandchi, H. (2019). Prognostic factors influencing prognosis in early breast cancer patients. *Przeglad Menopauzalny*, *18*(2), 82–88. https://doi.org/10.5114/pm.2019.86833.
4. Rakha, E., Toss, M., & Quinn, C. (2022). Specific cell differentiation in breast cancer: A basis for histological classification. In *Journal of Clinical Pathology* (Vol. 75, Issue 2, pp. 76–84). BMJ Publishing Group. https://doi.org/10.1136/jclinpath-2021-207487.
5. Jallah, J. K., Dweh, T. J., Anjankar, A., & Palma, O. (2023). *A Review of the Advancements in Targeted Therapies for Breast Cancer*. *2*(10). https://doi.org/10.7759/cureus.47847.
6. Dai, D., Zhong, Y., Wang, Z., & Yousafzai, N. A. (2019). The prognostic impact of age in different molecular subtypes of breast cancer : a population-based study. 2, 1–14. https://doi.org/10.7717/peerj.7252.
7. Salmaninejad A, Valilou SF, Shabgah AG, et al. (2019). PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy. *J Cell Physiol*, *234*, 16824–37.
8. Thomas, A., Reis-Filho, J. S., Geyer, C. E., & Wen, H. Y. (2023). Rare subtypes of triple negative breast cancer: Current understanding and future directions. In *npj Breast Cancer* (Vol. 9, Issue 1). Nature Research. https://doi.org/10.1038/s41523-023-00554-x.
9. Zhao, S., Zuo, W., Shao, Z., & Jiang, Y. (2020). Molecular subtypes and precision treatment of triple-negative breast cancer. *8*(7), 1–14. https://doi.org/10.21037/atm.2020.03.194.
10. Garrido-Castro, A.C., Lin, N.U., P. K. (2019). Insights into molecular classifications of triple-negative breast cancer: improving patient selection for treatment. *Cancer Discov.*, 176–98.
11. Bandyopadhyay, A., & Krishna, M. P. (2023). Tumor Infiltrating Lymphocytes (TILs) and Tumor Budding in Invasive Breast Carcinoma: Correlation with Known Prognostic Parameters. *Archives of Breast Cancer*, *10*(3), 241–247. https://doi.org/10.32768/abc.2023103241-24.
12. Zhao, Y., Huang, T., Jin, X., Gong, X. M., & Lu, Y. Z. (2023). Clinicopathologic Features and Immune Cell Subtypes Analysis of Tumor-infiltrating Lymphocytes Rich Invasive Breast Carcinoma of No Special Type. *Applied Immunohistochemistry and Molecular Morphology*, *31*(6), 354–362. https://doi.org/10.1097/PAI.0000000000001125.
13. Rafał, P., & Kunc, M. (2024). Tumor-Infiltrating Lymphocytes (TILs) in Breast Cancer : Prognostic and Predictive Significance across Molecular Subtypes. 12(4):763. DOI:10.3390/biomedicines12040763.
14. Wilky, B.A. (2019). Immune checkpoint inhibitors: The linchpins of modern immunotherapy. *Immunol Rev*, *290(1)*, 6–23.
15. Gonzalez-Ericsson, P.I. et al. (2020). *The Journal of Pathology - 2020 - Gonzalez- Ericsson - The path to a better biomarker application of a risk management*. 250(5):667-684. doi: 10.1002/path.5406.
16. Hussein, M. A., Ismail, H. A., Saqr, M., & Nouh, M. A. (2022). Expression of PD-L1 in Locally Advanced Breast Cancer and Its Impact on Neoadjuvant Chemotherapy Response. 23, 2095–2103. https://doi.org/10.31557/APJCP.2022.23.6.2095.
17. Alhesa, A., Awad, H., Bloukh, S., Al-balas, M., El-sadoni, M., Qattan, D., Azab, B., & Saleh, T. (2022). PD-L1 expression in breast invasive ductal carcinoma with incomplete pathological response to neoadjuvant chemotherapy. International Journal of Immunopathology and Pharmacology, 36, 1–16. https://doi.org/10.1177/03946320221078433.
18. Punhani, P., & Ahluwalia, C. (2023). Expression of Programmed Death Ligand 1 (PD-L1) in Breast Cancer patients in India and its Correlation with Prognostic Parameters. *Archives of Breast Cancer*, *10*(3), 280–290. https://doi.org/10.32768/abc.2023103280-290.

19. Rivard, A.B., Galarza-Paez, L., P. D. (2023). Anatomy, Thorax, Breast. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan.2023 Jul 17.* https://www.ncbi.nlm.nih.gov/books/NBK519575/
20. Policlinico, U. I. (2020). Breast Cancer Surgery During the COVID-19 Pandemic: An Observational Clinical Study of the Breast Surgery Clinic at Ospedale Policlinico San Martino - Genoa, Italy. *1667-1673. doi: 10.21873/invivo.11959.*
21. Collins, L.C. (2018). *Normal Anatomy and Histology. Biopsy Interpretation of the Breast, Second Edition.p35-38.*
22. Erber, R., & Hartmann, A. (2020). *Histology of Luminal Breast Cancer*. 327–336. https://doi.org/10.1159/000509025
23. Qiu, J., Xue, X., Hu, C., Xu, H., Kou, D., Li, R., & Li, M. (2016). J o u r n a l o f C a n c e r Comparison of Clinicopathological Features and Prognosis in Triple-Negative and Non-Triple Negative Breast Cancer. 7(1). https://doi.org/10.7150/jca.10944.
24. Hicks and Lester. (2021). Diagnostic Pathology: Breast 3rd ed.p324-331.
25. Stolnicu. (2018). *Practical Atlas of Breast Pathology*.2nd ed.p.36-38.
26. Allison, K.H. (2025). *Invasive breast carcinoma: General overview.*WHO bluebook classification online.
27. Savas, P., Virassamy, B., Ye, C., Salim, A., Mintoff, C.P., Caramia, F., Salgado, R., Byrne, D.J., Teo, Z.L., Dushyanthen, S., Byrne, A., Wein, L., Luen, S.J., Poliness, C., Nightingale, S.S., Skandarajah, A.S., Gyorki, D.E., Thornton, C.M., Beavis, P.A., Fox, S.B.; Kathleen Cuningham Foundation Consorti, L. S. (2018). Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nat Med*, 986–993.
28. Salgado, R., Denkert, C., Demaria, S., Sirtaine, N., Klauschen, F., Pruneri, G., Wienert, S., Van den Eynden, G., Baehner, F.L., Penault-Llorca, F., Perez, E.A., Thompson, E.A., Symmans, W.F., Richardson, A.L., Brock, J., Criscitiello, C., Bailey, H., Ignatiadis, M., Floris, G., Sparano, J., Kos, Z., L. S. (2015). The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol.*, 259–271.
29. Pelekanou, V., Carvajal-hausdroft, D.E., Altan, M., Wasserman, B.(2017). Breast Cancer Research.19:19. doi:10.1186/s13058-017-0884.
30. Evangelou, Z. O. I., Papoudou-bai, A., Karpathiou, G., Kourea, H., Kamina, S., Goussia, A., Harissis, H., Peschos, D., & Batistatou, A. (2020). *PD-L1 Expression and Tumor-infiltrating Lymphocytes in Breast Cancer : Clinicopathological Analysis in Women Younger than 40 Years Old*. *647*, 639–647. https://doi.org/10.21873/invivo.11818.
31. Kurozumi, S., Inoue, K., Matsumoto, H., Fujii, T., & Horiguchi, J. (2019). Clinicopathological values of PD-L1 expression in HER2-positive breast cancer. *Scientific Reports*, 1–8. https://doi.org/10.1038/s41598-019-52944-6.
32. Kythreotou, A., Siddique, A., Mauri, F. A., Bower, M., & Pinato, D. J. (2018). *PD-L1*. 189–194. https://doi.org/10.1136/jclinpath-2017-204853.
33. Guhl1, G. S. · M. M., Solomayer1, · Erich‐Franz, Wagenpfeil3, · Gudrun, ·, Hammadeh1, M. E., Juhasz‐Boess4, · Ingolf, Endrikat1, · Jan, Kasoha1, · Mariz, & Bohle2, · Rainer Maria. (2021). *Immunohistochemical assessment of PD‐L1 expression using three different monoclonal antibody*.(p. :1689–1695).
34. Badve, S. S., Penault-Llorca, F., Reis-Filho, J. S., Deurloo, R., Siziopikou, K. P., D'Arrigo, C., & Viale, G. (2022). Determining PD-L1 Status in Patients with Triple-Negative Breast Cancer: Lessons Learned from IMpassion130. *Journal of the National Cancer Institute*, *114*(5), 664–675. https://doi.org/10.1093/jnci/djab121.
35. Medić-Milijić, N., Jovanić, I., Nedeljković, M., Marković, I., Spurnić, I., Milovanović, Z., Ademović, N., Tomić, T., Tanić, N., & Tanić, N. (2024). Prognostic and Clinical Significance of PD-L1, EGFR and Androgen Receptor (AR) Expression in Triple-Negative Breast Cancer (TNBC) Patients. *Life*, *14*(6), 1–14. https://doi.org/10.3390/life14060682.