EXPRESSION OF PROGRAMMED DEATH-LIGAND I(PD-L1) IN TRIPLE NEGATIVE BREAST CARCINOMA-A CROSS SECTIONAL STUDY AT TERTIARY CARE HOSPITAL

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BELAGAVI – 590010

KARNATAKA.

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KAHER J.N. MEDICAL COLLEGE BELAGAVI

Dr. Vijayalaxmi Dhorigol M.D
Professor & Head
Department of Pathology
J. N. Medical College,
Nehru Nagar,
Belagavi-590010

Professor & Head
Department of Pathology
J.N. Medical College,
BELAGAVI.

Date: 24-03-2025
Place: Belagavi.

Dr. (Mrs) N.S.Mahantashetti M.D.(Pead)
Principal
J. N. Medical College,
Nehru Nagar,
Belagavi-590010

PRINCIPAL
Jawaharlal Nehru Medical College
BELAGAVI

Date: 24-03-2025
Place: Belagavi

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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu rincipal@jnmc.edu

Ref No: MDC/PG/ Date: 19-03-2025

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Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BN0122010
Postgraduate Student,
2022-23 Batch,
Department of Pathology
J. N. Medical College, Belagavi.

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JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref No.MDC/JNMCIEC/ 136

Date: 21/03/2023

To
REG NO. BN0122010
PG Student in Pathology
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

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(Dr. Smita Sonoli)
Member Secretary
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi

LIST OF ABBREVATIONS

S. No	Abbreviation	Expansion
1	AUC	Area Under the Curve
2	BL	Basal-Like
3	BRCA	Breast Cancer Gene
4	CAP/TGF- β	Common Activation Pathway / Transforming Growth Factor Beta
5	CMTM6	CKLF Like MARVEL Transmembrane Domain Containing 6
6	CPS	Combined Positive Score
7	CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
8	DAB	3,3'-Diaminobenzidine
9	EDTA	Ethylenediaminetetraacetic Acid
10	ER	Estrogen Receptor
11	FFPE	Formalin-Fixed, Paraffin-Embedded
12	H&E	Hematoxylin and Eosin
13	HER2	Human Epidermal Growth Factor Receptor 2
14	IC	Immune Cells
15	IDC	Invasive Ductal Carcinoma
16	IHC	Immunohistochemistry
17	IM	Immunomodulatory
18	JAK/STAT	Janus Kinase / Signal Transducer and Activator of Transcription
19	LAP/TGF- β	Latency-Associated Peptide / Transforming Growth Factor Beta
20	LAR	Luminal Androgen Receptor
21	M	Mesenchymal
22	MBC	Metastatic Breast Cancer
23	MBR	Modified Bloom-Richardson
24	MRD	Minimal Residual Disease
25	MSL	Mesenchymal Stem-Like
26	NSCLC	Non-Small Cell Lung Cancer

27	PARP	Poly (ADP-ribose) Polymerase
28	PD1	Programmed Cell Death Protein 1
29	PDL1	Programmed Death-Ligand 1
30	PI3K/AKT	Phosphoinositide 3-Kinase / Protein Kinase B
31	PR	Progesterone Receptor
32	SMA	Smooth Muscle Actin
33	STAT3	Signal Transducer and Activator of Transcription 3
34	TDLU	Terminal Duct Lobular Unit
35	TILs	Tumor-Infiltrating Lymphocytes
36	TME	Tumor Microenvironment
37	TNBC	Triple-Negative Breast Carcinoma
38	WHO	World Health Organization
39	cDNA	Complementary DNA
40	pCR	Pathologic Complete Response

ABSTRACT

Title: "**EXPRESSION OF PROGRAMMED DEATH-LIGAND I(PD-L1) IN TRIPLE NEGATIVE BREAST CARCINOMA-A CROSS-SECTIONAL STUDY AT TERTIARY CARE HOSPITAL**"

Background: Triple-negative breast cancer (TNBC) is an aggressive subtype lacking ER, PR, and HER2 expression. Programmed death-ligand 1 (PD-L1) plays a role in immune evasion and is a potential therapeutic target. The PD-1/PD-L1 axis allows tumor cells to evade immune surveillance, making PD-L1 expression a promising biomarker for immunotherapy. However, variations in PD-L1 detection methods and scoring criteria impact its clinical interpretation[8]. This study evaluates PD-L1 expression in TNBC using immunohistochemistry and its correlation with clinicopathological parameters.

Objectives: To assess immunohistochemical expression of PD-L1 in TNBC utilizing immunohistochemical antibodies and to correlate PDL1 expression with other clinicopathological parameters.

Methods: A hospital based cross sectional study was conducted in the Department of Pathology from January 2023 to December 2024 that evaluated 50 operated cases of breast carcinoma specimens received in the histopathology laboratory at KLE's DR PRABHAKAR KORE CHARITABLE HOSPITAL AND DR PRABHAKAR KORE HOSPITAL AND RESEARCH CENTER, BELAGAVI.

Results: PD-L1 positivity (CPS ≥10) was observed in 40% of cases. A significant correlation was found with high tumor grade ($p = 0.001$), perineural invasion ($p = 0.001$), and infiltrative margins ($p = 0.007$). No significant association was noted with tumor laterality, quadrant involvement, lymphovascular invasion, or lymph node metastasis. Younger patients (≤40 years) exhibited higher PD-L1 positivity (71.43%), though not statistically significant ($p = 0.061$).

Conclusions: PD-L1 expression is linked to aggressive tumor features, supporting its role as a prognostic biomarker and therapeutic target. Further research is needed to validate these findings and optimize immunotherapy strategies for TNBC.

Key word: Triple-negative breast cancer, PD-L1, Prognostic biomarker.

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INTRODUCTION

Breast cancer is one of the most common cancers in women and is responsible for a large proportion of cancer morbidity and mortality worldwide [1]. Rather than being one disease, it is a heterogeneous group of neoplasms that are subdivided into different subtypes with distinct molecular characteristics and clinical behaviors [2]. Rapid progress in genomic and molecular technologies has clarified the classification of breast tumors and has improved predictions of disease course and better matching of patients to targeted therapies [3]. Within these categories, triple-negative breast cancer (TNBC) is specifically notable because it lacks the expression of the estrogen receptor and progesterone receptor and amplification or overexpression of human epidermal growth factor receptor 2 (HER2) [4]. Although TNBC accounts for about 10–20% of all breast cancers, it is associated with a disproportionately high fraction of breast cancer–associated deaths, primarily because of its aggressive progression and limited scope of effective targeted treatments [5]. In addition, TNBC frequently demonstrates a propensity for early recurrence and tends to metastasize to organs such as the lungs and brain [6].

The treatment of TNBC is challenging because it lacks specific hormonal and HER2 targets, making the adoption of established endocrine therapies or HER2-directed agents impossible [7]. Thus, chemotherapy remains the first systemic therapy for this subtype, but again, the response varies because TNBC itself is molecularly heterogeneous [8]. Large-scale gene expression analyses have shown multiple distinct subgroups within TNBC, characterized by different signaling pathways and cellular features, with opportunity for novel interventions targeted to specific molecular drivers [9]. However, the prognosis remains poorer for TNBC than for other types of breast cancer, encouraging further work to identify more accurate biomarkers and targeted approaches [10].

In the past decade, studies have increasingly emphasized the role of the immune system in mediating TNBC progression and treatment responses [11]. Tumors infiltrated with higher levels of immune cells, including tumor-infiltrating lymphocytes (TILs), often have a better response to chemotherapy and increased survival in patients [12]. This observation has generated interest in manipulating tumor–immune interactions to enhance the body's natural anti-tumor defense mechanisms [13]. Among numerous immunoregulatory pathways, the PD-1 receptor and its ligand, PD-L1, is particularly unique due to their capability to inhibit immune surveillance and confer the ability to evade destruction in tumor cells [14].

The PD-1/PD-L1 axis is a key checkpoint that maintains immune balance under normal conditions, preventing autoimmunity by downregulating overly active T cells [15]. PD-1, located on activated T cells and other immune cells, interacts with PD-L1, which can be expressed by both immune and tumor cells [16]. When bound, this interaction dampens T-cell proliferation and effectiveness, thereby helping tumors avoid immunologic attack [17]. TNBC appears more likely than some other breast cancer subtypes to exploit this pathway, making PD-L1 an appealing target for therapeutic intervention [18].

Clinical trials testing antibodies that block PD-1 or PD-L1 in TNBC have produced promising results, especially for individuals with advanced disease, and have demonstrated prolonged remissions in certain cases when combined with chemotherapy [19]. However, many patients do not enjoy sustained benefits from these therapies, highlighting the need for accurate biomarkers that can identify likely responders. Although PD-L1 expression has been examined as such a biomarker through immunohistochemical (IHC) assays, results vary, partly due to inconsistencies in detection methods, scoring criteria, and threshold definitions for positivity [20]. Furthermore, the precise location of PD-L1—whether on tumor cells or on infiltrating immune cells—can differentially influence prognosis and therapeutic response.

Standardizing PD-L1 testing remains complicated, as studies often utilize different staining antibodies and cut-off points, which can yield conflicting interpretations of PD-L1 status [21]. Hence, more uniform analytical approaches are needed before PD-L1 testing can reliably inform treatment decisions. Additionally, some TNBCs display complex immune landscapes involving multiple immunosuppressive pathways; thus, PD-L1 alone may not fully predict a tumor's sensitivity to checkpoint inhibition. The search for improved markers includes assessing tumor mutational burden, identifying specific gene signatures, and analyzing the presence and function of TILs in the tumor microenvironment.

TNBC tumors frequently possess high genomic instability, potentially producing a larger array of tumor-specific antigens that could elicit robust immune responses. Yet, if a tumor also expresses high PD-L1 levels, it may override this immune activity by inactivating T cells [22]. Unraveling the relationship between these factors could shed light on why some patients demonstrate lasting remissions with immunotherapy while others show limited or no benefit. Further insights might arise from combined treatment regimens that incorporate immune checkpoint inhibitors with other modalities, such as Poly (ADP-ribose) polymerase inhibitors or anti-angiogenic agents, with the hope of magnifying immune-mediated tumor clearance.

In a tertiary care setting, patients generally present with more advanced or complex disease. Investigating PD-L1 expression in TNBC at such a center provides a useful perspective on its real-world prevalence and whether it correlates with clinical or pathological characteristics like grade, lymph node status, or overall stage [23]. Better understanding these relationships may advance the clinical utility of PD-L1 as both a prognostic and predictive tool, guiding the integration of immunotherapies into more personalized management algorithms.

Although certain studies propose that high PD-L1 expression may be indicative of a more immunoactive microenvironment that could respond favorably to checkpoint blockade, other findings link PD-L1 overexpression to heightened tumor aggressiveness and decreased survival. Reconciling these discrepancies requires standardized IHC protocols and deeper immunological profiling to gauge the intricacies of tumor–host interactions. Future research may examine additional immune checkpoint pathways and analyze the interplay of various immunosuppressive cell populations, ultimately aiming to refine immunotherapy for TNBC [24].

This current cross-sectional evaluation of PD-L1 expression in TNBC could help clarify how commonly PD-L1 upregulation occurs in this subtype, and whether it can be tied to specific histopathological or clinical features. This study aims to provide evidence that can help in the refinement of therapy choices in TNBC by characterizing PD-L1 levels in tumor cells and surrounding stroma correlating them with patient demographics and disease parameters. With the ever-widening pool of immunotherapeutic options and combination approaches being the new standard of care, a deeper understanding of PD-L1's significance will be important in optimizing patient outcomes [25].

Finally, TNBC remains a challenging cancer in oncology as it has a high metastatic potential and only a narrow range of effective treatment modalities. Identifying PD-L1 as an important axis of immune escape for cancer cells underscores the more general role that immunological responses may play in molding its course. By systematically evaluating the expression of PD-L1 and its link to disease characteristics, clinicians will have a better indication of patients likely to benefit from checkpoint inhibitors. This would therefore bring clinicians much closer to a future in which TNBC is treated according to the profile of each patient's tumor.

AIMS AND OBJECTIVES

1. **Primary Objective:**

    - To study immunohistochemical expression of PDL1 in triple negative breast cancer.

2. **Secondary Objectives:**

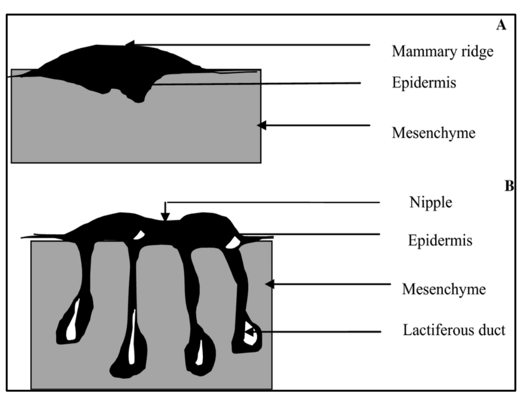
    - Correlate PD-L1 expression with standard clinicopathological parameters (e.g., tumor grade, stage).

REVIEW OF LITERATURE

The female breast is a multifaceted organ composed of glandular, fibrous, and fatty tissues. While its primary task is milk production, it also fulfils significant roles in immune surveillance and hormonal regulation.

1.Embryological Development

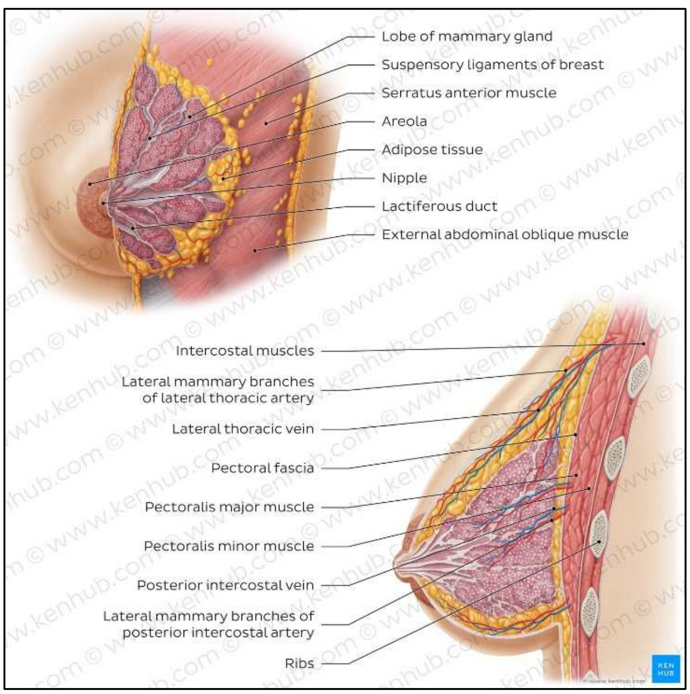
The mammary glands develop from milk lines (mammary ridges) appearing between the fourth and sixth weeks of embryonic life. These lines stretch from the axilla to the inguinal region, with most of their length regressing to leave only the chest region, which forms the definitive breast in both genders. Primary bud formation occurs around the sixth week by epithelial buds grow into the underlying mesenchyme, forming approximately 15–20 lobular buds that will evolve into the lactiferous ducts. Mesenchymal interaction follows primary bud formation by surrounding mesenchyme differentiates into connective tissue, adipose tissue, and supportive fibrous tissue. During this process, nipple formation begins with the mammary area initially appearing as a shallow depression known as the mammary pit. Later in fetal or neonatal life, this pit elevates to form the nipple-areola complex, contributing to the final structural development of the mammary gland. Disruptions in this process can lead to uncommon congenital variations [26] .



(Figure 1: Embryologic Development of the Breast. Source: Tripathy S, Singh S, Das SK. Potential of breastmilk in stem cell research. Cell Tissue Bank.)

2. Anatomy of the Breast

Positioned on the anterior chest wall over the pectoralis major muscle, each breast typically extends from the lateral margin of the sternum to approximately the mid-axillary line. In the vertical plane, it spans from the second to the sixth rib. A section of breast tissue called the axillary tail of Spence projects into the axilla and is clinically significant in surgical and oncological assessments. The external features of the breast include the nipple and the areola. The nipple is generally located near the fourth intercostal space in a non-ptotic breast and serves as the opening site for the lactiferous ducts. Surrounding the nipple is the areola, a pigmented region that contains Montgomery's glands—modified sebaceous glands that secrete lubricating substances, particularly during lactation, to protect the nipple and maintain skin integrity[27].

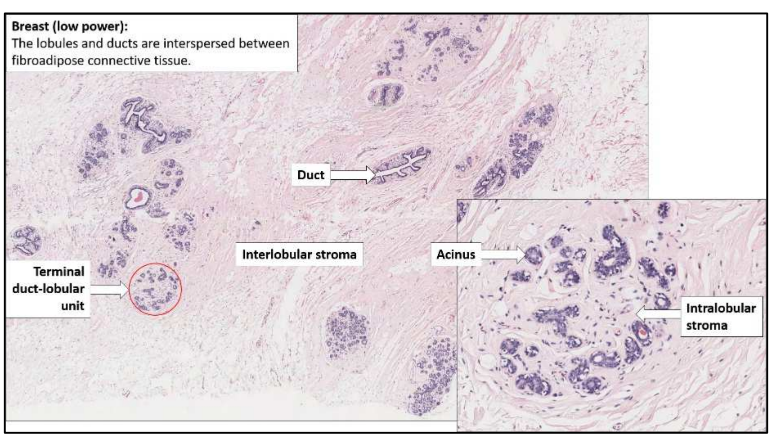


(Figure 2: Structure of the Female Breast. Source: Kenhub)

The structural composition of the breast consists of three key components: glandular tissue, fibrous tissue, and adipose tissue. The glandular tissue, or parenchyma, is organized into 15–20 lobes, each of which drains through a lactiferous duct that opens onto the nipple. These lobes are further subdivided into lobules, where milk production occurs. The fibrous tissue, or supportive stroma, includes Cooper's ligaments, which extend between the skin and deep fascia, playing a crucial role in maintaining the shape and structural integrity of the breast. Surrounding these elements is adipose tissue, which fills the spaces around the glandular components and largely determines the size and contour of the breast[28].

3. Histological Structure

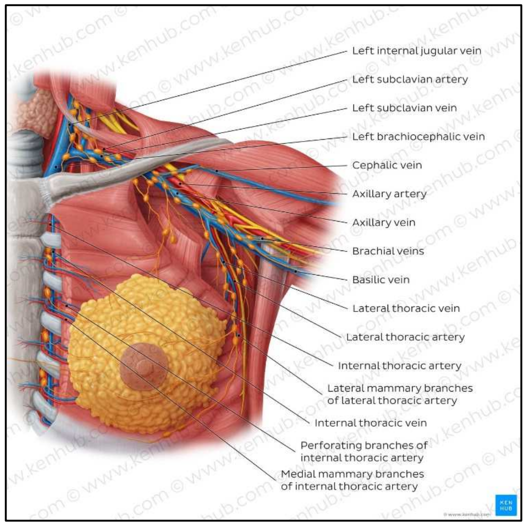
The Terminal Duct Lobular Unit (TDLU) is the fundamental functional component of the breast, consisting of terminal ducts and lobules. Terminal ducts are small ducts that directly connect to the lobules, which are clusters of milk-secreting glands. These lobules are lined by two distinct cell layers: luminal epithelial cells, responsible for milk production, and myoepithelial cells, which contract to aid in milk ejection. These cells are enclosed within a basement membrane and specialized stromal tissue, forming the essential microenvironment for lactation[29].



The epithelial and stromal components of the breast contribute to both normal function and pathological processes. Luminal epithelial cells, typically hormone-sensitive, remain relatively inactive except during lactation when they secrete milk. Myoepithelial cells, situated between luminal cells and the basement membrane, express smooth muscle actin (SMA) and facilitate milk ejection.The stromal framework consists of fibrous connective tissue and immune cells such as lymphocytes, macrophages, and dendritic cells. Alterations in the stromal environment can influence tumor expansion and metastatic progression in breast cancer[30].

4. Vascular Supply

The arterial supply of the breast is derived from multiple sources, ensuring adequate perfusion to different regions. The internal thoracic (internal mammary) artery primarily supplies the medial areas of the breast. The lateral thoracic artery, branching from the axillary artery, provides blood to the lateral portion and can be particularly prominent in women. Additionally, the thoracoacromial artery (pectoral branch), another branch of the axillary artery, contributes to the vascularization of the upper aspect of the breast. The posterior intercostal arteries, typically from the second to fourth intercostal spaces, give off small perforating branches that further support the breast's blood supply[31].



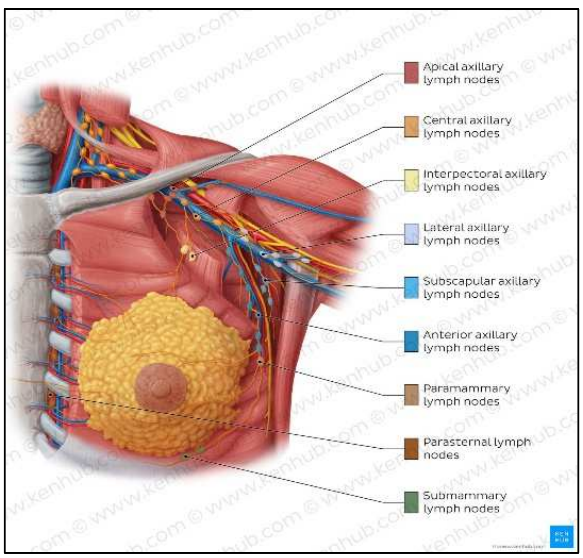
(Figure 4: Arterial and Venous Supply of the Breast. Source: Kenhub)

Venous drainage of the breast follows pathways similar to its arterial supply. Superficial veins form a subcutaneous network that primarily drains into the axillary and internal thoracic veins. Meanwhile, deep veins accompany the corresponding arteries and eventually drain into the axillary, subclavian, and internal thoracic veins. A notable venous plexus exists within the breast, which can create collateral channels, playing a crucial role in the potential hematogenous spread of malignancies in advanced breast cancer[32].

5. Lymphatic Drainage

Lymphatic drainage plays a crucial role in the spread of breast cancer, with the axillary lymph nodes serving as the primary site for metastasis. Understanding these pathways is essential for procedures such as sentinel lymph node biopsy and axillary dissection, as well as for assessing prognosis in breast cancer patients. The majority of lymphatic fluid from the breast is directed toward the axillary lymph nodes, highlighting their clinical significance in disease progression and management.

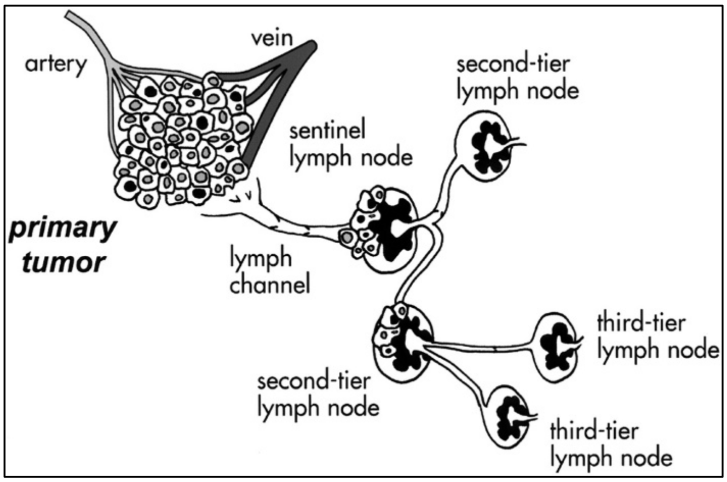
The lymphatic drainage of the breast follows several key pathways. The axillary lymph nodes are the most significant group and are categorized into pectoral (anterior) nodes, which receive lymph from the lateral breast; subscapular (posterior) nodes along the subscapular vessels; humeral (lateral) nodes near the brachial vessels; central nodes embedded within axillary adipose tissue; and apical nodes at the axillary apex, which drain into the subclavian trunk. Additionally, the internal mammary (parasternal) lymph nodes run along the internal thoracic vessels, primarily draining the medial portions of the breast. The posterior intercostal nodes are involved in draining deeper breast tissues. In advanced malignancies, cross-communication between the lymphatic networks of the opposite breast or abdominal nodes can facilitate metastatic spread, emphasizing the importance of thorough evaluation in breast cancer staging[33].



(Figure 5: Lymphatic Drainage Pathway.Source:Kenhub)

6. Sentinel Lymph Node

The sentinel lymph node is the first node or group of nodes receiving lymphatic flow from a primary tumor. Its examination (sentinel lymph node biopsy) is crucial for staging and treatment strategies. A positive node suggests metastatic involvement, while a negative node may obviate the need for more extensive nodal dissection[34] .



(Figure 6: Sentinel Lymph Node Mapping. Source: jnm.snmjournals.org)

7. Innervation

The breast receives its **sensory innervation** primarily from the anterior and lateral cutaneous branches of the fourth to sixth intercostal nerves. These nerves provide general sensation to the breast tissue, with particularly dense innervation around the nipple and areola. This specialized sensory network plays a crucial role in the oxytocin-driven reflex, which is essential for milk ejection during lactation[35].

Sympathetic innervation of the breast occurs via autonomic fibers that travel along blood vessels, regulating vascular tone and the smooth muscle of the nipple-areolar complex. This innervation contributes to nipple erection, a response triggered by various stimuli. Although the direct impact of sympathetic innervation on the breast's immune microenvironment remains unclear, sympathetic activity can influence vascular permeability and indirectly modulate immune cell trafficking, potentially affecting inflammatory and pathological processes within the breast[35,36].

8. Historical Aspects of Breast Cancer

The understanding of breast cancer has evolved significantly over time. Traditionally, classifications were based on histopathology and hormone receptor status, forming the foundation of clinical decision-making. However, a major breakthrough came with molecular profiling studies, notably by Perou et al., which identified distinct intrinsic subtypes—Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like—redefining breast cancer as a heterogeneous disease with unique biological and therapeutic implications[37,38].

Several milestones have shaped breast cancer diagnosis, treatment, and immunohistochemistry. Molecular classification was revolutionized by Perou et al.'s cDNA microarray profiling, which established gene-expression signatures that provide prognostic and predictive insights. This led to targeted therapies, including HER2 inhibitors for HER2-driven disease and, more recently, immune checkpoint inhibitors for PD-L1–positive tumors. In immunohistochemistry (IHC), the identification of PD-L1 as a biomarker has driven the development of standardized IHC assays, though challenges persist in assay reproducibility and clinical interpretation. These advancements continue to refine breast cancer management, guiding more personalized and effective treatment strategies.[39,40]

9. Epidemiology of Breast Cancer

Breast cancer incidence varies worldwide, with TNBC accounting for a minority yet disproportionately lethal fraction of cases .In a population-based study from the California Cancer Registry, TNBC represented 4.6% of breast cancer diagnoses but was associated with younger age, higher grade, and poorer 5-year survival compared to other subtypes. TNBC has a higher incidence among African American women and tends to present at later stages. The aggressive clinical behaviour observed in these populations underscores the urgency of improving early detection and developing novel interventions.[41,42]

10. Risk Factors for Breast Cancer

Several factors contribute to the risk of developing Triple-Negative Breast Cancer (TNBC), including genetic, hormonal, lifestyle, and environmental influences. Genetic predisposition, particularly BRCA1/2 mutations, is strongly linked to TNBC, with the basal-like subtype**,** as described by Perou et al.**,** frequently overlapping with BRCA1-driven tumors. While hormonal risk factors such as prolonged estrogen exposure are less relevant due to the lack of ER/PR expression in TNBC, other factors, including obesity and reproductive history**,** can still influence overall breast cancer risk. Environmental exposures**,** such as occupational hazards and radiation**,** have also been identified as contributors to breast cancer development. Additionally, epidemiological studies highlight that TNBC disproportionately affects certain demographic groups, including African American women and younger patients, emphasizing the need for targeted screening strategies to improve early detection and intervention.[43,44]

11. Patterns of Spread in Breast Carcinoma

Breast cancer progression involves local invasion, lymphatic spread, and hematogenous metastasis, with Triple-Negative Breast Cancer (TNBC) exhibiting a particularly aggressive pattern. Local invasion typically extends into adjacent tissues, affecting the overlying skin or the underlying pectoralis major muscle, with TNBC known for its rapid local expansion. Lymphatic metastasis frequently involves the axillary lymph nodes, with spread to the apical (infraclavicular) nodes indicating a poorer prognosis. Additionally, internal mammary chain involvement may be clinically silent, emphasizing the importance of advanced imaging for accurate staging. Hematogenous metastasis allows tumor cells to disseminate through the bloodstream, commonly targeting the bones, lungs, liver, and brain. In TNBC, early visceral metastasis is a concern, underscoring the urgent need for systemic therapies to manage disease progression effectively.[45,46]

12. WHO Classification of Breast Cancer

The current WHO (5th edition, 2019) classification integrates histopathological features with molecular insights. Standard subtypes include invasive ductal carcinoma (no special type), lobular carcinoma, and other rarer histologies [47]. Metaplastic breast carcinoma (MBC) is one such rare subtype. Jinkala et al. explored MBC and found that 54% of cases expressed PD-L1, suggesting possible responsiveness to immunotherapies in this rare but aggressive subgroup [48].

13. Molecular Classification of Breast Cancer

Breast cancer taxonomy underwent a significant transformation with the work of Perou et al., who identified intrinsic molecular subtypes, including Luminal A, Luminal B, HER2-Enriched, and Basal-like categories [49]. While Basal-like tumors frequently overlap with Triple-Negative Breast Cancer (TNBC), they are not entirely synonymous, as some TNBC cases may exhibit molecular features distinct from the basal phenotype [50].

Expanding on this classification, Lehmann et al. further refined TNBC into six distinct subtypes—Basal-like 1 (BL1), Basal-like 2 (BL2), Immunomodulatory (IM), Mesenchymal (M), Mesenchymal Stem-like (MSL), and Luminal Androgen Receptor (LAR)—each characterized by unique gene-expression profiles and varying therapeutic susceptibilities [51]. This framework was later validated clinically by Masuda et al., who demonstrated that these subtypes exhibit differential responses to neoadjuvant chemotherapy, reinforcing the need for personalized treatment strategies in TNBC management [52].

14. Overview of Triple-Negative Breast Cancer (TNBC)

TNBC lacks expression of oestrogen receptor (ER), progesterone receptor (PR), and HER2, comprising 10–20% of all breast cancer diagnoses [53]. It is particularly aggressive and disproportionately affects younger and African American women, often presenting at advanced stages [54]. Moreover, it exhibits poor long-term prognosis compared to other subtypes, emphasizing the urgent need for novel therapeutic approaches [55].

15. The Immune Microenvironment of the Breast

Beyond lactation, the breast hosts an immune network comprising resident lymphocytes, macrophages, dendritic cells, and other immune cells [56]. These cells are pivotal for protection against infections, regulation of inflammation, and surveillance for malignant transformations [57].

In breast carcinoma, the immune milieu can either constrain tumor progression or foster it, depending on local signaling pathways and checkpoint mechanisms [58].

An essential regulatory system of the immune response is governed by the PD-1/PD-L1 axis [59]. Cancer cells may upregulate PD-L1 to reduce T-cell activity, thereby sidestepping immune-mediated destruction [60].

- PD-1 Receptor: Found on T-cells, B-cells, and NK cells [61].
- PD-L1 Ligand: Expressed by multiple cell types, including tumor cells and tumor-infiltrating immune cells [62].

When PD-L1 binds to PD-1 on T-cells, the T-cell's antitumor functions can be inhibited, facilitating tumor survival [63].

16. Significance of PD-L1 in Oncology

Programmed death-ligand 1 (PD-L1) has emerged as a crucial immune checkpoint molecule that enables tumors to evade host immune responses [64]. Its expression has been documented across multiple tumor types, including TNBC, and correlates with varied clinical outcomes [65].

The success of immune checkpoint inhibitors in other malignancies, such as melanoma and non–small cell lung cancer (NSCLC), spurred interest in applying similar strategies to breast cancer, particularly TNBC [66].

17. TNBC and Immune Microenvironment

TNBC often harbors a robust immune infiltrate. Zhang et al. highlighted the role of "tumor-educated" B cells expressing PD-L1 and LAP/TGF-β, which can suppress T-cell activity [67].

Tumor-Infiltrating Lymphocytes (TILs) play a crucial role in the tumor microenvironment of Triple-Negative Breast Cancer (TNBC), with high TIL density being associated with better prognosis and higher pathological complete response (pCR) rates following chemotherapy [68]. Studies, including Park et al., have demonstrated that patients exhibiting both high TIL levels and PD-L1 positivity experience significantly improved 5-year disease-free survival [69].

18. PD-L1 Expression in TNBC

PD-L1 expression in Triple-Negative Breast Cancer (TNBC) varies across tumor and immune cells, influencing both prognosis and therapeutic strategies [70]. Studies, including Kim et al., have reported that approximately 46% of TNBC cases exhibit PD-L1 positivity, a significantly higher rate compared to other breast cancer subtypes (~22%) [71].

Clinicopathological correlations indicate that PD-L1–positive TNBC is often high-grade and characterized by a dense immune infiltrate, suggesting an active tumor immune microenvironment [72]. However, the prognostic significance of PD-L1 expression remains inconsistent across studies [73].

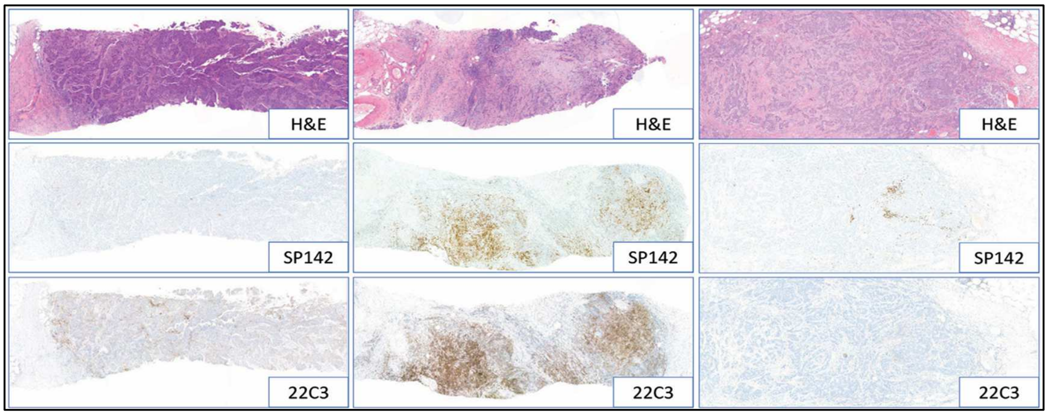
Additionally, PD-L1 localization differs among TNBC cases, with some tumors displaying PD-L1 predominantly on tumor cells, while others show expression mainly on infiltrating immune cells [74]. This distinction is clinically relevant, as it influences eligibility for PD-L1–targeting immunotherapies and highlights the necessity for standardized scoring systems to guide treatment decisions effectively [75].

19. Reporting Methodology of PD-L1 in TNBC

Two clinically approved assays, **SP142 CDx (Ventana)** and **22C3 PharmDx** (Agilent), are routinely employed, each using distinct scoring algorithms and positivity thresholds [76].

- SP142 CDx (Ventana) assay: Approved for atezolizumab therapy and employs the immune cell (IC) scoring algorithm, where PD-L1 positivity is defined as ≥1% PD-L1-stained tumor-associated immune cells in the tumor microenvironment (TME) [77].

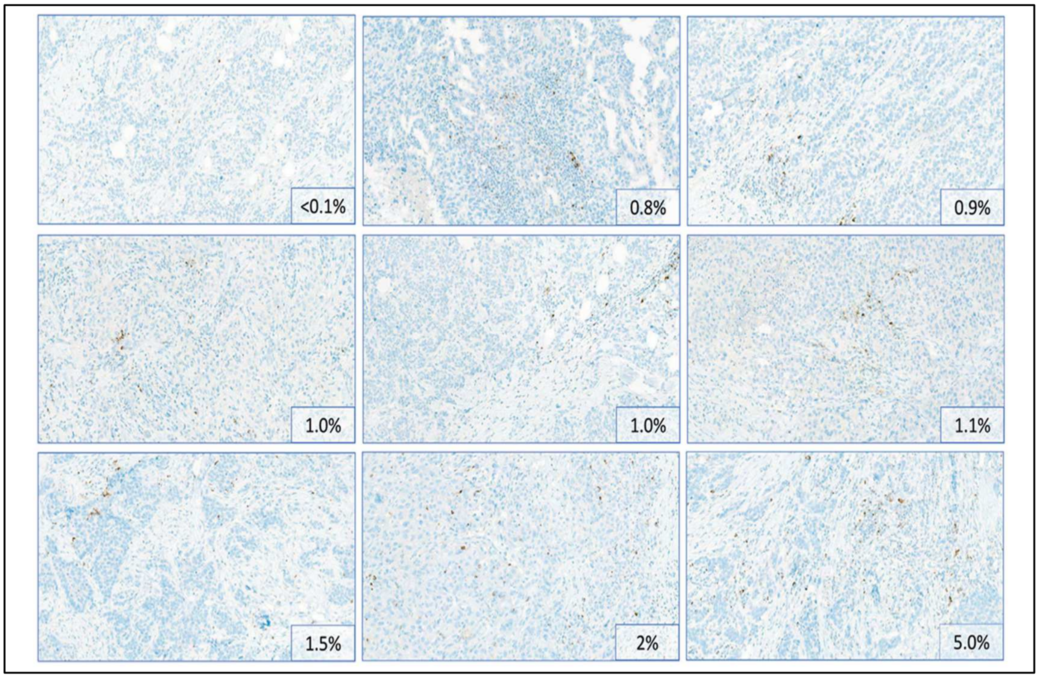
- 22C3 PharmDx assay: Validated for pembrolizumab therapy, uses the combined positive score (CPS) algorithm, with a threshold of CPS ≥10 for PD-L1 positivity [78].



(*Figure 7: PD-L1 status using two PD-L1 tests. Source: Study conducted by Adelina Baltan, Simona Costache, Abeer M Shaaban* Corrado D'Arrigo*)*

The SP142(Ventana) assay evaluates PD-L1 expression in immune cells only, excluding tumour cells from scoring. The percentage of tumour-associated immune cells staining positive for PD-L1 within the tumour area (excluding necrosis) is calculated. All immune cells, including lymphocytes, macrophages, and multinucleated giant cells, are considered, whereas spindle cells (fibroblasts, endothelial cells) and nuclear staining are excluded[77].

Interpretation criteria: Immune Cell Scoring (IC) for SP142:



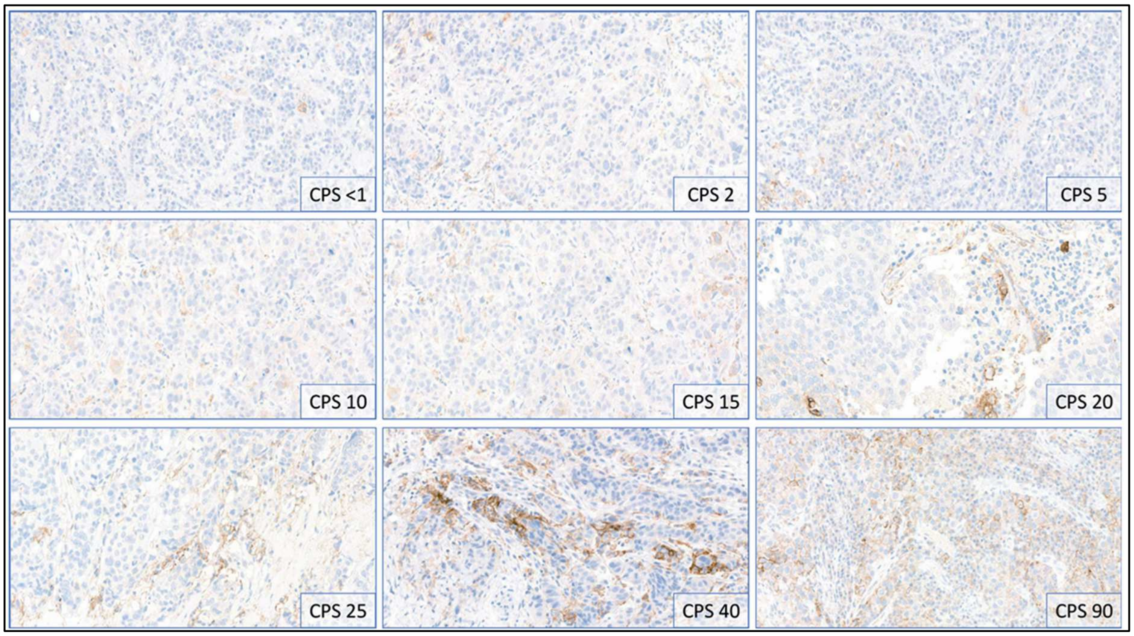
(*Figure 8: Varying amounts of IC staining. Source: Study conducted by Adelina Baltan, Simona Costache, Abeer M Shaaban* Corrado D'Arrigo*)"*

- **IC0:** <1% PD-L1+ ICs (Negative)
- **IC1:** 1–5% PD-L1+ ICs
- **IC2:** 5–10% PD-L1+ ICs
- **IC3:** >10% PD-L1+ ICs (Strongly Positive)
- Cut off for positivity: ≥1% PD-L1-positive immune cells.

The 22C3 PharmDx assay, in contrast, includes both tumour cells and immune cells in its assessment. CPS is defined as the sum of PD-L1-positive tumour cells and immune cells (lymphocytes, macrophages, and multinucleated giant cells), divided by the total number of viable tumour cells, multiplied by 100. Only membranous staining of tumour cells is counted, whereas both membranous and cytoplasmic staining of immune cells are accepted[78].

$$CPS = \frac{\text{PD-L1-positive tumor cells} + \text{immune cells} \times 100}{\text{Total viable tumor cells}}$$

Positive if CPS ≥10. ”



(*Figure 9: Varying amounts of CPS staining. Source: Study conducted by Adelina Baltan, Simona Costache, Abeer M Shaaban* Corrado D’Arrigo*)*

Interpretation criteria: Tumour Cell & IC Scoring (CPS) for 22C3:

- **CPS <1:** Negative
- **CPS 1-9:** Low Expression
- **CPS ≥10:** Positive

Given the discordance between assays, studies have indicated that SP142(Ventana) underestimates PD-L1 positivity compared to 22C3. Consequently, cases that are SP142-negative but 22C3-positive may not derive benefit from atezolizumab therapy. Thus, selection of assay depends on the targeted immune checkpoint inhibitor[78].

20. Molecular Mechanisms Regulating PD-L1 Expression

Genetic and epigenetic factors play a crucial role in PD-L1 regulation in Triple-Negative Breast Cancer (TNBC), influencing tumor immune evasion and treatment responses. Studies have shown that silencing or knockout of PD-L1 can significantly enhance T-cell responses, restoring immune-mediated tumor suppression.

CMTM6, a recently identified regulator of PD-L1 stability, has been found to prolong PD-L1 expression on tumor cells, with its overexpression correlating with worse clinical outcomes. Additionally, signaling pathways, such as PI3K/AKT and JAK/STAT, contribute to PD-L1 regulation.

Research by Shi et al. demonstrated that STAT3 activity promotes PD-L1 upregulation, while CMTM6 knockdown reduces STAT3 signaling, leading to a decrease in cell-surface PD-L1 and enhanced T-cell proliferation. These findings highlight potential therapeutic targets for modulating immune checkpoint activity in TNBC [79].

21. Prognostic and Predictive Value of PD-L1 in TNBC

PD-L1 expression in Triple-Negative Breast Cancer (TNBC) has been extensively studied as a predictive biomarker for immunotherapy response. Multiple investigators have suggested that high PD-L1 levels often correlate with active immune infiltration and improved responses in early-stage TNBC.

Additionally, PD-L1 positivity has been linked to better outcomes with immune checkpoint inhibitors, reinforcing its clinical relevance. However, the predictive role of PD-L1 is complex, as its expression can overlap with other immune checkpoints or be influenced by molecular features of the tumor. Despite these complexities, PD-L1 assessment is becoming an essential tool for identifying TNBC patients most likely to benefit from immunotherapy, aiding in personalized treatment strategies [80].

22. Therapeutic Implications

PD-L1 inhibitors, such as Atezolizumab and Pembrolizumab, have emerged as promising therapeutic options for Triple-Negative Breast Cancer (TNBC), particularly in advanced or metastatic cases.

Checkpoint blockade targeting PD-L1, when combined with chemotherapy, has demonstrated improved clinical outcomes, drawing from early successes in melanoma, non-small cell lung cancer (NSCLC), and bladder cancer. Beyond monotherapy, combination strategies are being actively explored to enhance efficacy.

- PARP inhibitors, when paired with PD-L1 blockade, may synergize by increasing tumor immunogenicity, making cancer cells more susceptible to immune attack.
- Dual checkpoint blockade, involving anti-PD-L1 and anti-CTLA4 agents, has shown stronger T-cell activation compared to monotherapy, though at the cost of higher toxicity.

Ongoing clinical trials continue to refine these approaches, aiming to optimize patient selection and therapeutic benefit in TNBC [81].

23. Regional and Global Studies

Several key studies from indexed journals have contributed to the understanding of PD-L1 expression in Triple-Negative Breast Cancer (TNBC) across diverse populations.

- Bauer et al. utilized the California Cancer Registry to highlight TNBC's distinct epidemiologic profile, emphasizing its aggressive nature and demographic disparities.
- In Asian cohorts, Kim et al. reported higher PD-L1 positivity among TNBC patients, suggesting potential regional or genetic influences on immune checkpoint expression.
- Meanwhile, research on emerging regions, including studies on metaplastic breast carcinoma (MBC) and other rare subtypes, underscores the global effort to clarify PD-L1's role in heterogeneous tumor populations.

Population-based comparisons suggest that variations in PD-L1 prevalence may stem from genetic factors, intrinsic tumor biology, or assay-related differences. Further multi-center investigations are needed to better define these disparities and refine global treatment guidelines for PD-L1-targeted therapies in TNBC [82].

24. Challenges and Future Directions

Diagnostic Limitations & Emerging Therapies

Subjective IHC interpretation, variable scoring cutoffs, and tumor heterogeneity hinder the universal implementation of PD-L1 as a biomarker. Novel markers like CMTM6, proptosis-related genes, or TIL composition may augment PD-L1–based stratification [83].

Prospects for Personalized Treatment

Future research will likely expand combination regimens, personalize treatment based on molecular subtype or immune signature, and investigate epigenetic modifications.

Validating predictive models that integrate clinical factors, biomarkers, and lab data is a key step toward precision oncology [84].

This literature review underscores the complexity of TNBC, highlighting PD-L1's pivotal role in immune evasion and potential value as a therapeutic target.

Significant variability in PD-L1 expression, scoring methods, and clinical outcomes across diverse cohorts emphasizes the need for standardized diagnostic protocols and well-designed clinical trials.

Ultimately, leveraging immunotherapy in TNBC may improve survival, especially when combined with other targeted interventions, but more uniform methodologies and robust, multi-institutional research are needed [85].

MATERIALS AND METHODS

Study Design and Settings

This hospital-based cross-sectional study was carried out in the Department of Pathology at KLE's Dr. Prabhakar Kore Charitable Hospital and Dr. Prabhakar Kore Hospital and Research Center, Belagavi. The study period extended from January 2023 to December 2024.

Ethical Consideration

Ethical clearance was obtained from the JNMC Institutional Ethics Committee prior to commencement of the study. (Ref No. MDC/JNMCIEC/136)

Sample Size and Sampling Technique

A sample size of 30 cases was initially calculated using MedCalc software, considering an alpha (α) of 0.05 and beta (β) of 0.20 to ensure statistical validity. However, during the study period, a **total of 50** consecutive cases that met the inclusion criteria were enrolled, enhancing the study's statistical power and reliability of the findings.

1. **Inclusion Criteria**
    - Immunohistochemically confirmed triple-negative breast carcinoma (TNBC).
2. **Exclusion Criteria**
    - Inadequate biopsy specimens.
    - Improperly fixed tissue samples.
    - Cases in which estrogen receptor (ER), progesterone receptor (PR), and HER2 status were not evaluated.

Data Collection

All patients who presented with breast carcinoma and consented to participate were included. Detailed clinical information—including hormone receptor status and relevant demographic data—was retrieved from the Medical Records Department (MRD).

Operated breast carcinoma specimens were received in the KLE's histopathology laboratory. Each specimen was grossed according to standard pathological protocols, and findings such as tumor size, consistency, and the presence or absence of necrosis were noted. Representative tissue sections were taken for histopathological examination. Hematoxylin and eosin (H&E)-stained slides were analyzed under a light microscope to determine histological type and grade of the tumor based on established classification criteria (e.g., WHO classification for breast tumors).

Histopathological Evaluation

- **Gross Examination:** Specimens were inspected for tumor size, shape, cut surface appearance, and any additional pathologic features. The grossing notes were recorded meticulously.

- **Microscopic Examination:** H&E-stained slides were evaluated to confirm the diagnosis of breast carcinoma. Tumor grade, lymphovascular invasion, and other histopathological parameters were noted. Only those cases confirmed as triple-negative (ER-negative, PR-negative, HER2-negative on immunohistochemistry) were included in the study.

PD-L1 Immunohistochemical Analysis

Paraffin-embedded tissue blocks from confirmed TNBC cases were sectioned at 3–4 µm thickness and mounted on coated glass slides. The sections were then deparaffinized in xylene and rehydrated through graded alcohols.

1. **Antigen Retrieval:** Antigen retrieval was performed using an E.D.T.A buffer in a pressure cooker as per the protocol recommended by the antibody manufacturer.

2. **Blocking Endogenous Peroxidase:** Endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide for 10–15 minutes at room temperature.

3. **Primary Antibody Incubation:** The sections were incubated with a primary monoclonal anti-PD-L1 antibody (clone: AN921-2M Rabbit Monoclonal Antibody-Ready to use) for the duration of 1 hour.

4. **Secondary Antibody and Detection:** Following thorough washing, a secondary antibody (Goat Anti-Rabbit IgG) was applied. The colorimetric detection was carried out using a substrate-chromogen solution (DAB—3,3'-diaminobenzidine) to visualize the antigen-antibody reaction.

5. **Counterstaining and Mounting:** The sections were counterstained with hematoxylin, dehydrated, and mounted with a coverslip.

6. **Interpretation of PD-L1 Staining:** PD-L1 expression in triple-negative breast carcinoma (TNBC) was evaluated using immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tissue. We used Combined Positivity Score (CPS) method for interpretation of PD-L1 positivity. Use of on-slide controls, such as tonsillar tissue, is used to monitor staining consistency [55].

Statistical Analysis

All data obtained—clinical, histopathological, and immunohistochemical—were coded and entered into a Microsoft Excel spreadsheet. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 27. Descriptive statistics were expressed as frequencies and percentages for categorical variables. Associations between PD-L1 expression and various clinicopathological parameters were evaluated using the chi-square test. A p-value of <0.05 was considered statistically significant. This systematic approach to data collection, histopathological evaluation, and immunohistochemical assessment ensured a comprehensive analysis of PD-L1 expression among TNBC cases within the study period.

RESULTS

In this cross-sectional study total 50 cases of TNBCs which met inclusion criteria were evaluated for immunohistochemical expression of PD-L1 along with standard clinicopathological parameters (e.g., tumor grade, stage).

The age of the youngest patient was 33 years whereas oldest was 73years. The Mean age ± Standard Deviation (SD) was 52.06 ± 9.93 years. Tumor laterality was more common in the left breast [29 cases (58%)] than the right breast [21 cases (42%)]. 30 cases (60%) had lump duration of 13–24 months, while 20 cases (40%) had lump duration of 1–12 months (Mean: 13.24 ± 6.07 months). The upper outer quadrant was the most commonly involved [23 cases (46%)], followed by the central [10 cases (20%)]. Invasive ductal carcinoma (IDC) was the predominant histological type, accounting for 98% of cases, while papillary neoplasm was observed in 2% of cases. In TNBC patients, Grade III was the most common Modified Bloom-Richardson grade [25 cases (50%)], followed by Grade II [16 cases (32%)]. Lymphovascular invasion was present in 38 cases (76%), perineural invasion was observed in 16 cases (32%), lymph node metastasis was present in 23 cases (46%), distant organ metastasis was rare, occurring in only 1 case (2%). Regarding lymphocytic response, 26 cases (52%) had a low response, while 24 cases (48%) had a moderate response. Infiltrating margins were ill-defined in 41 cases (82%). T3N0M0 was the most frequently encountered tumor stage, with a mean tumor size of 4.3cms. Positive PD-L1 expression (CPS ≥10) was observed in 20 cases (40%) out of 50 TNBC cases. The frequency of various parameters are given in Table 1 to 10.

Table 1: Frequency Distribution of Age in TNBC patients

Age groups	No of patients	Frequency (%) of patients
<=40yrs	7	14.00
41-50yrs	15	30.00
51-60yrs	17	34.00
>=61yrs	11	22.00
Total	50	100.00
Mean	52.06	
SD	9.93	

Table 2: Frequency Distribution of Tumor Laterality in TNBC Patients

Sides	No of patients	Frequency % of patients
Left	29	58.00
Right	21	42.00
Total	50	100.00

Table 3: Frequency Distribution of Lump Duration in TNBC Patients

Duration of lump	No of patients	Frequency % of patients
1-12months	20	40.00
13-24months	30	60.00
Total	50	100.00
Mean	13.24	
SD	6.07	

Table 4: Frequency Distribution of Quadrant Involvement in TNBC Patients

Quadrant involved	No of patients	Frequency % of patients
Lower Outer	5	10.00
Lower Inner	8	16.00
Central	10	20.00
Upper Outer	23	46.00
Upper Inner	4	8.00
Total	50	100.00

Table 5: Frequency Distribution of TNBC Patients by Histological Type

Histological type	No of patients	Frequency % of patients
Invasive Ductal Carcinoma	49	98
Papillary Neoplasm	1	2.00
Total	50	100.00

Table 6: Frequency Distribution of TNBC Patients by Modified Bloom-Richardson Grade

Modified Bloom Richardson grade	No of patients	Frequency % of patients
Grade I	9	18.00
Grade II	16	32.00
Grade III	25	50.00
Total	50	100.00

Table 7: Frequency Distribution of TNBC Patients by Lymphovascular Invasion, Perineural Invasion, Lymph Node Metastasis, and Distant Organ Metastasis

	No of patients	Frequency % of patients
Lymphyvascular invasion		
Absent	12	24.00
Present	38	76.00
Perineural Invasion		
Absent	34	68.00
Present	16	32.00
Lymph Node Metastasis		
Absent	27	54.00
Present	23	46.00
Distant Organ Metastasis		
Absent	49	98.00
Present	1	2.00
Total	50	100.00

Table 8: Frequency Distribution of TNBC Patients by Lymphocytic Response and Infiltrating Margins

	No of patients	Frequency % of patients
Lymphocytic Response		
Low	26	52.00
Moderate	24	48.00
Infilterating margins		
Ill-defined	41	82.00
Well-defined	9	18.00
Total	50	100.00

Table 9: TNM Classification-wise Frequency Distribution of TNBC Patients

TNM Classification	No of patients	Frequency % of patients
T stage		
T 1	7	14.00
T 2	11	22.00
T 3	20	40.00
T 4	12	24.00
N stage		
N 0	27	54.00
N 1	7	14.00
N 2	16	32.00
M stage		
M 0	49	98
M 1	1	2
Total	50	100.00

Table 10: Frequency Distribution of TNBC Patients Based on PD-L1 Expression (CPS Score)

PDL1 Expression-CPS	No of patients	Frequency % of patients
Negative [CPS: 1-9]	30	60.00
Positive [CPS>=10]	20	40.00
Total	50	100.00

Figure 10: Frequency Distribution of Age in TNBC patients

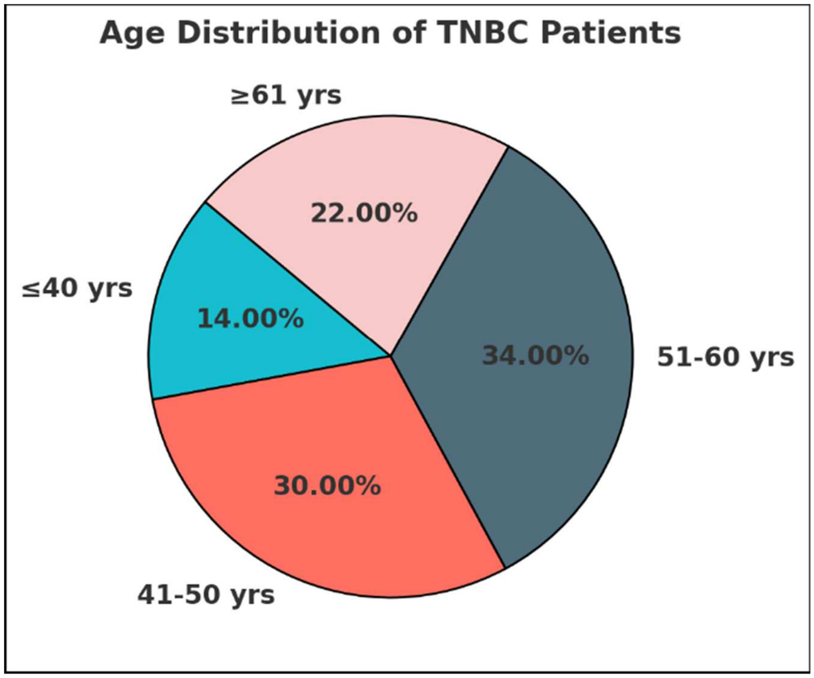


Figure 11: Frequency Distribution of Tumor Laterality in TNBC Patients

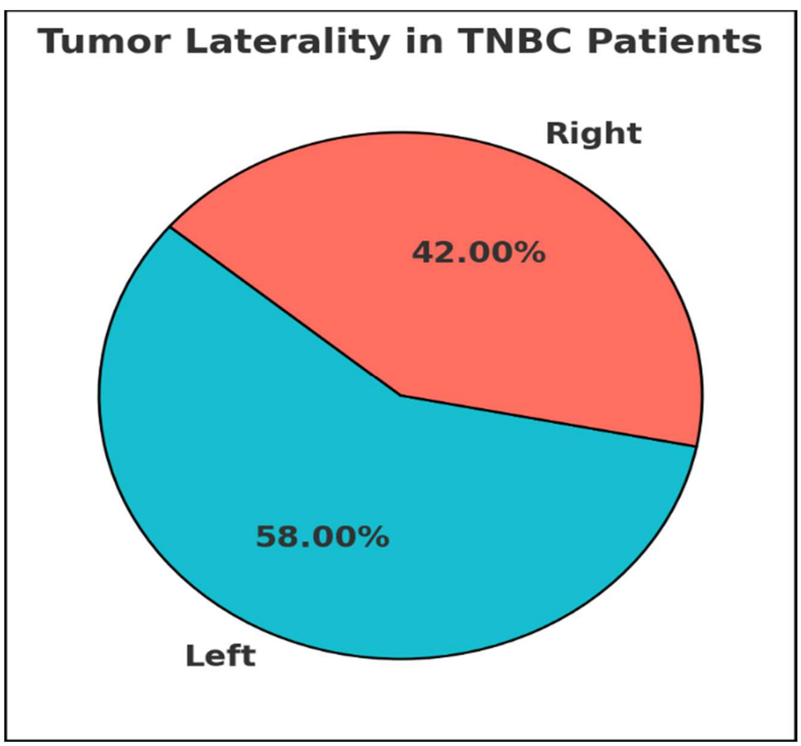


Figure 12: Frequency Distribution of Lump Duration in TNBC Patients

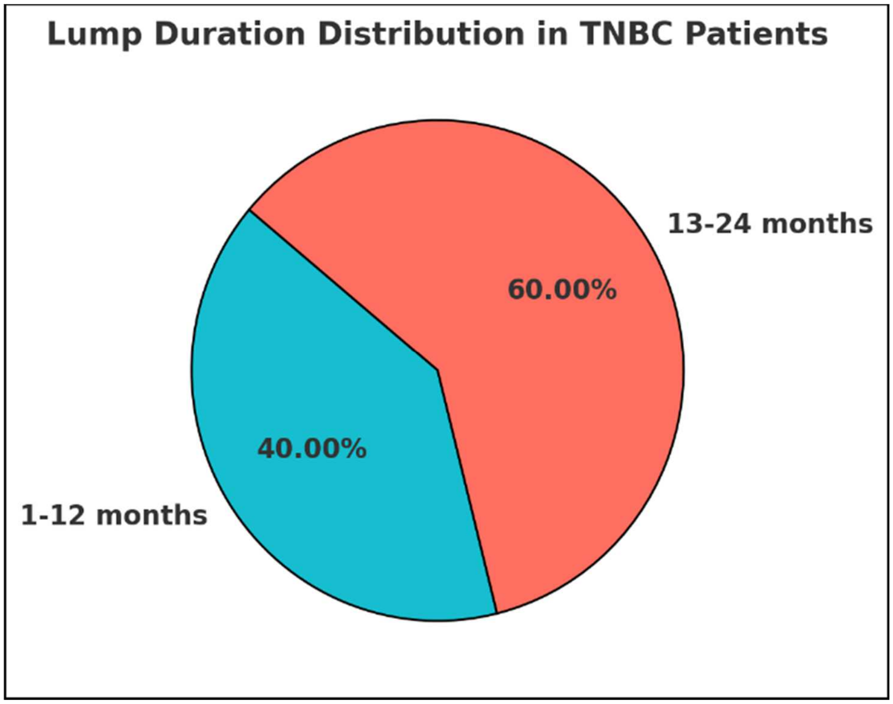


Figure 13: Frequency Distribution of Quadrant Involvement in TNBC Patients

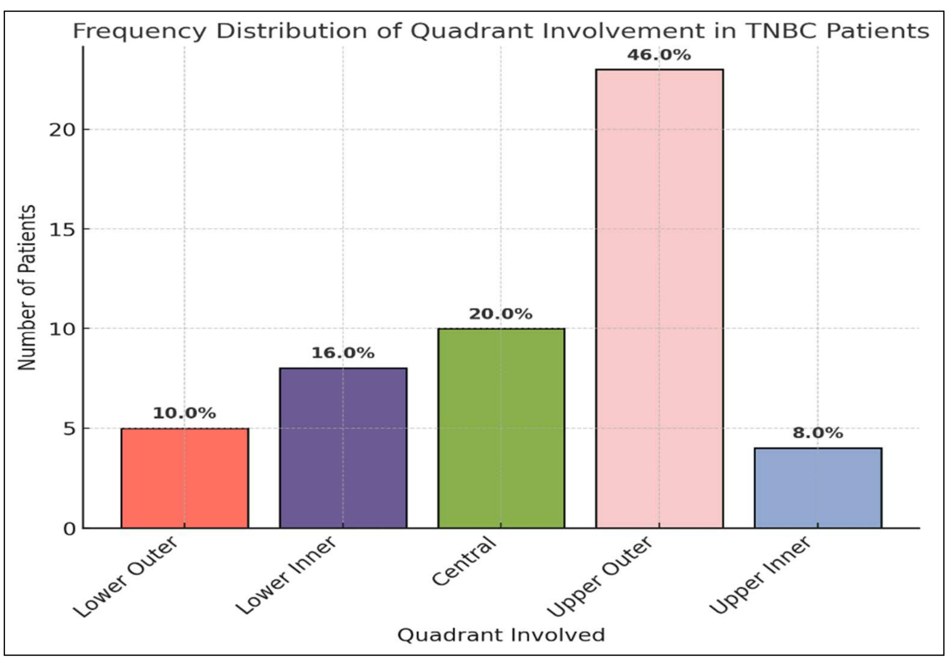


Figure 14: Frequency Distribution of TNBC Patients by Histological Type

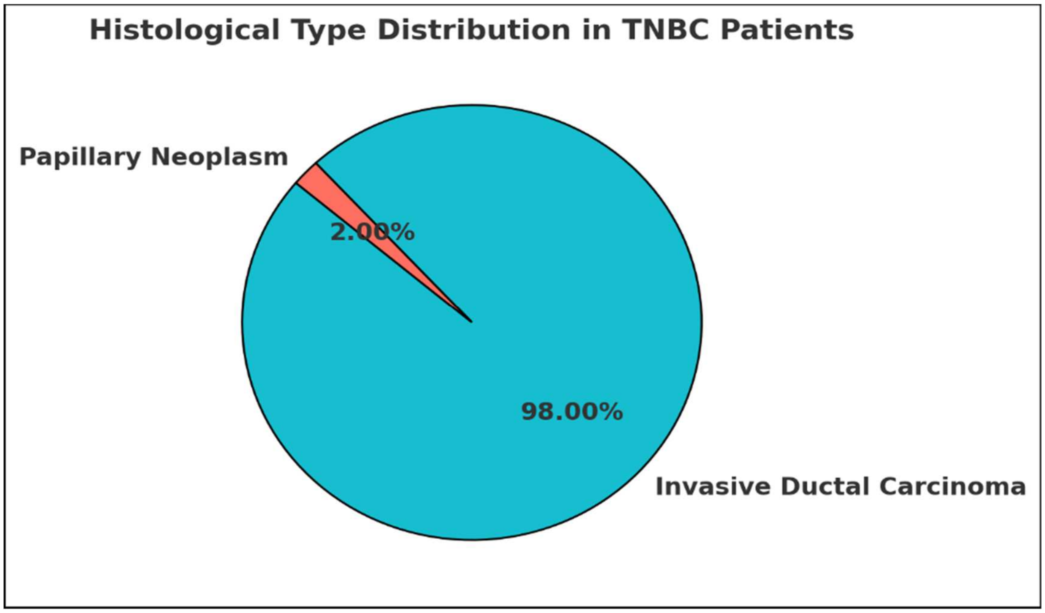


Figure 15: Frequency Distribution of TNBC Patients by Modified Bloom-Richardson Grade

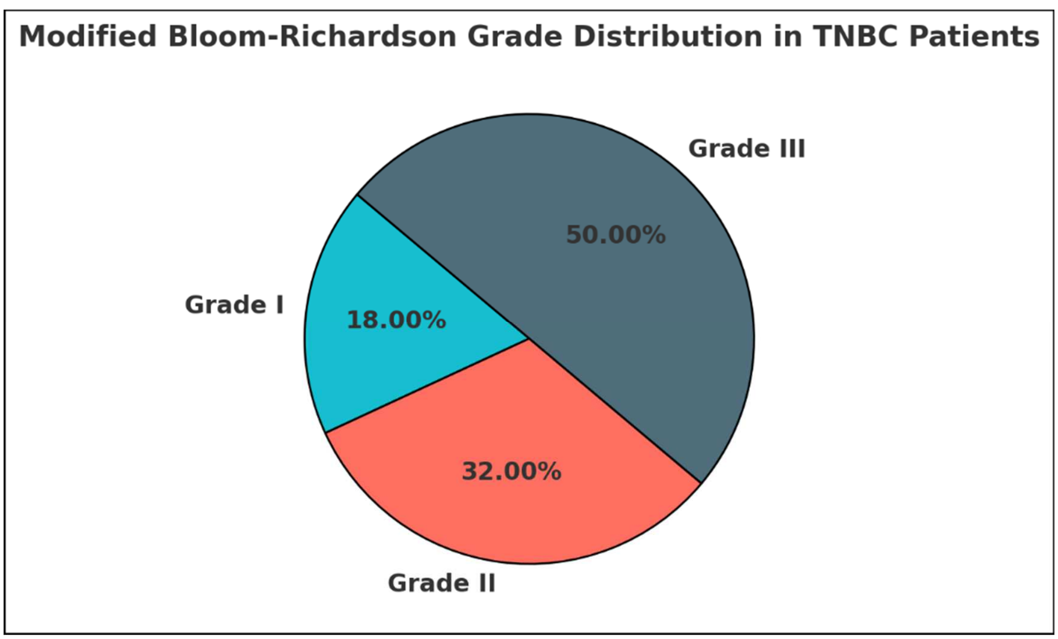


Figure 16: Frequency Distribution of TNBC Patients by Lymphovascular Invasion, Perineural Invasion, Lymph Node Metastasis, and Distant Organ Metastasis

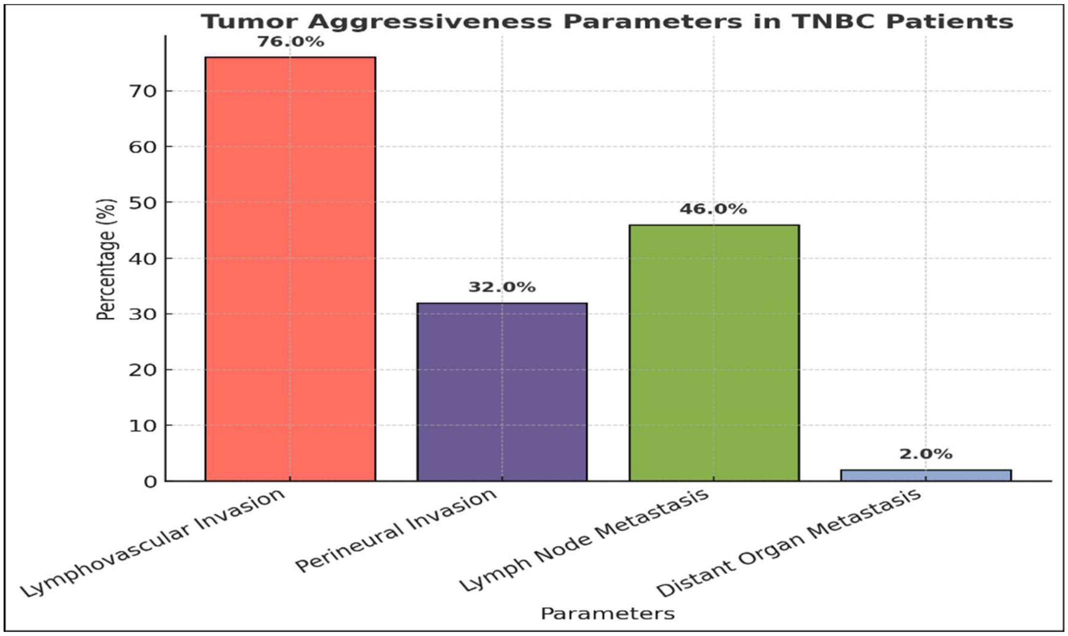


Figure 17: Frequency Distribution of TNBC Patients by Lymphocytic Response and Infiltrating Margins

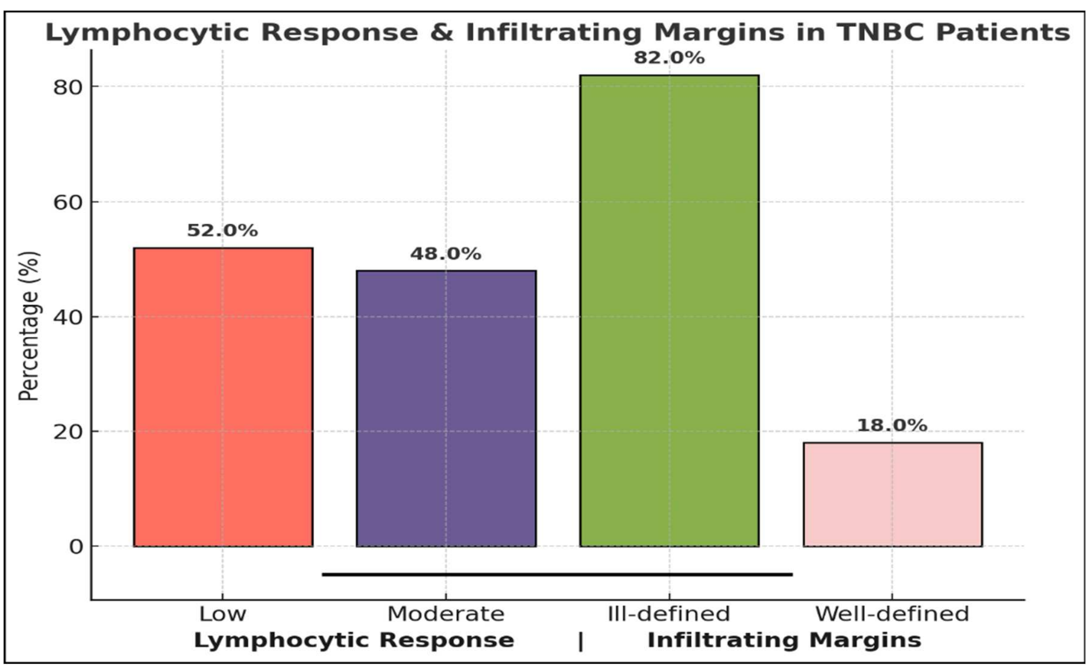


Figure 18: TNM Stage-wise Frequency Distribution of TNBC Patients

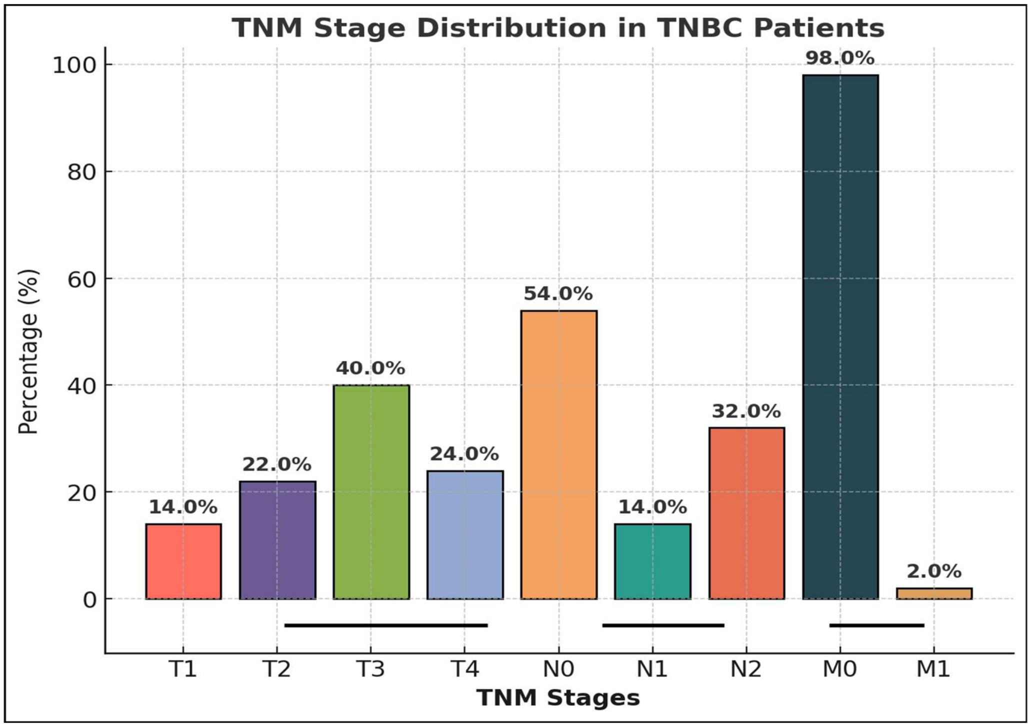


Figure 19: Frequency Distribution of TNBC Patients Based on PD-L1 Expression (CPS Score)

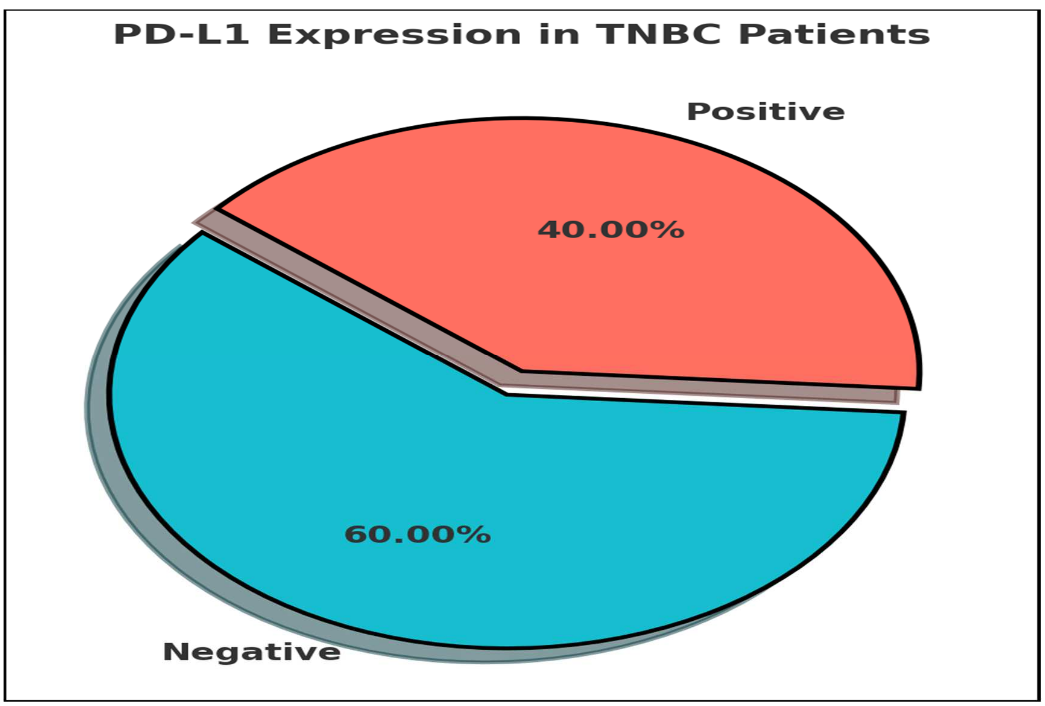


Table 11: Association between PDL1 expression with determinants including Age, side, size of lump, duration of lump & quadrant

Profile	PDL1 expression					Chi-square	p-value
	Negative	%	Positive	%	Total		
Age groups							
<=40yrs	2	28.57	5	71.43	7	7.3600	0.0610
41-50yrs	11	73.33	4	26.67	15		
51-60yrs	8	47.06	9	52.94	17		
>=61yrs	9	81.82	2	18.18	11		
Sides							
Left	18	62.07	11	37.93	29	0.1230	0.7260
Right	12	57.14	9	42.86	21		
Duration of lump							
1-12months	9	45.00	11	55.00	20	3.1250	0.0770
13-24months	21	70.00	9	30.00	30		
Quadrant involved							
Lower Outer	3	60.00	2	40.00	5	0.1950	0.9960
Lower Inner	5	62.50	3	37.50	8		
Central	6	60.00	4	40.00	10		
Upper Outer	14	60.87	9	39.13	23		
Upper Inner	2	50.00	2	50.00	4		
Total	30	60.00	20	40.00	50		

Table 11 represents the association between PD-L1 expression and various clinicopathological determinants, including age, tumor laterality, lump duration, and quadrant involvement. Among different age groups, PD-L1 positivity was highest (71.43%) in patients ≤40 years, while the lowest positivity (18.18%) was observed in patients ≥61 years. Although the Chi-square test ($\chi^2 = 7.36$, $p = 0.061$) suggests a borderline association, it does not reach statistical significance. Similarly, lump duration showed a trend, where PD-L1 positivity was higher (55.00%) in patients with a lump duration of 1-12 months compared to 30.00% in those with a duration of 13-24 months ($\chi^2 = 3.125$, $p = 0.077$). However, tumor laterality ($p = 0.726$) and quadrant involvement ($p = 0.996$) did not show a significant correlation with PD-L1 expression. Overall, while age and lump duration exhibited a potential trend toward PD-L1 positivity, no determinant showed a strong statistically significant association ($p > 0.05$) in this study.

Figure 20: Association between PDL1 expression with determinants including Age, side, size of lump, duration of lump & quadrant

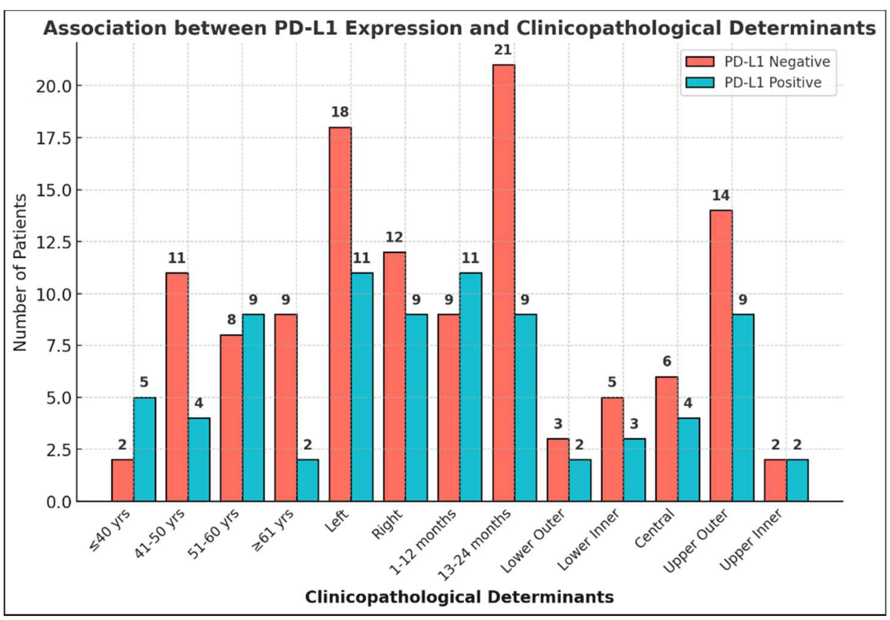


Table 12: Association between PDL1 expression with determinants including determinants histological type & modified bloom Richardson grade

Profile	PDL1 expression					Chi-square	p-value
	Negative	%	Positive	%	Total		
Histological type							
Invasive Ductal Carcinoma	29	59.183	20	40.816	49	0.000	1.000
Papillary Neoplasm	1	100.00	0	0.00	1		
Modified bloom Richardson grade							
Grade 1	9	100	0		0	27.09	0.001
Grade 2	15	93.75	1	6.25	16		
Grade 3	6	24	19	76	25		
Total	30	60.00	20	40.00	50		

Table 12 presents the distribution of PD-L1 expression across different histological types and Modified Bloom Richardson (MBR) grades in a cohort of 50 patients. For histological types, Invasive Ductal Carcinoma (IDC) shows a distribution where 40.82% of the cases are PD-L1 positive and 59.18% are PD-L1 negative, while Papillary Neoplasm shows 100% PD-L1 negativity. The chi-square test (χ^2 = 0.000, p = 1.000) indicates no statistically significant association between histological type and PD-L1 expression. In contrast, for MBR grades, Grade 1 tumors are all PD-L1 negative (100%), Grade 2 tumors show 93.75% PD-L1 negativity with 6.25% positivity, and Grade 3 tumors exhibit a significant shift towards PD-L1 positivity (76%). The chi-square test for MBR grade (χ^2 = 27.09, p = 0.001) reveals a statistically significant relationship between MBR grade and PD-L1 expression, with higher tumor grades being associated with a higher percentage of PD-L1 positivity. These findings suggest that PD-L1 expression is more prevalent in higher MBR grade tumors, which could have implications for targeted immunotherapies in breast cancer treatment.

Figure 21: Association between PDL1 expression with determinants including determinants histological type & modified bloom Richardson grade

Table 13: Association between PDL1 expressions and Tumour Characteristics

	PDL1 expression					Chi-square	p-value
	Negative	%	Positive	%	Total		
Lymphy vascular invasion							
0	8	66.67	4	33.33	12	0.2920	0.5890
1	22	57.89	16	42.11	38		
Perineural invasion							
Negative	26	76.47	8	23.53	34	12.0100	0.0010*
Positive	4	25.00	12	75.00	16		
Lymph node metastasis							
Negative	17	62.96	10	37.04	27	0.2150	0.6430
Positive	13	56.52	10	43.48	23		
Distant organ metastasis							
Negative	30	61.22	19	38.78	49	1.5310	0.2160
Positive	0	0.00	1	100.00	1		
Lymphocytic response							
Low	13	50.00	13	50.00	26	2.2570	0.1330
Moderate	17	70.83	7	29.17	24		
Infilterating margins							
Ill-defined	21	51.22	20	48.78	41	7.3170	0.0070*
Well-defined	9	100.00	0	0.00	9		
Total	30	60.00	20	40.00	50		

Table 13 represents the association between PD-L1 expression and key tumor characteristics, including lymphovascular invasion, perineural invasion, lymph node metastasis, distant organ metastasis, lymphocytic response, and infiltrating margins. While PD-L1 positivity was slightly higher in patients with lymphovascular invasion (42.11%) compared to those without (33.33%), this association was not statistically significant ($p = 0.589$). Similarly, PD-L1 positivity was observed in 43.48% of patients with lymph node metastasis and 37.04% of those without, but this difference was not significant ($p = 0.643$).

However, perineural invasion showed a highly significant association with PD-L1 expression ($p = 0.001$), with 75.00% of perineural invasion cases exhibiting PD-L1 positivity, compared to only 23.53% in patients without perineural invasion. Likewise, infiltrating margins were significantly associated with PD-L1 expression ($p = 0.007$), as PD-L1 positivity was found in 48.78% of tumors with ill-defined margins, while all tumors with well-defined margins (100%) were PD-L1 negative. Although PD-L1 positivity was more common in patients with low lymphocytic response (50.00%) than in those with moderate response (29.17%), this trend did not reach statistical significance ($p = 0.133$).

Overall, the findings suggest that PD-L1 expression is significantly associated with more aggressive tumor features, particularly perineural invasion and ill-defined margins, while other tumor characteristics did not show a strong statistical correlation.

Figure 22: Association between PDL1 expressions and Tumor Characteristics

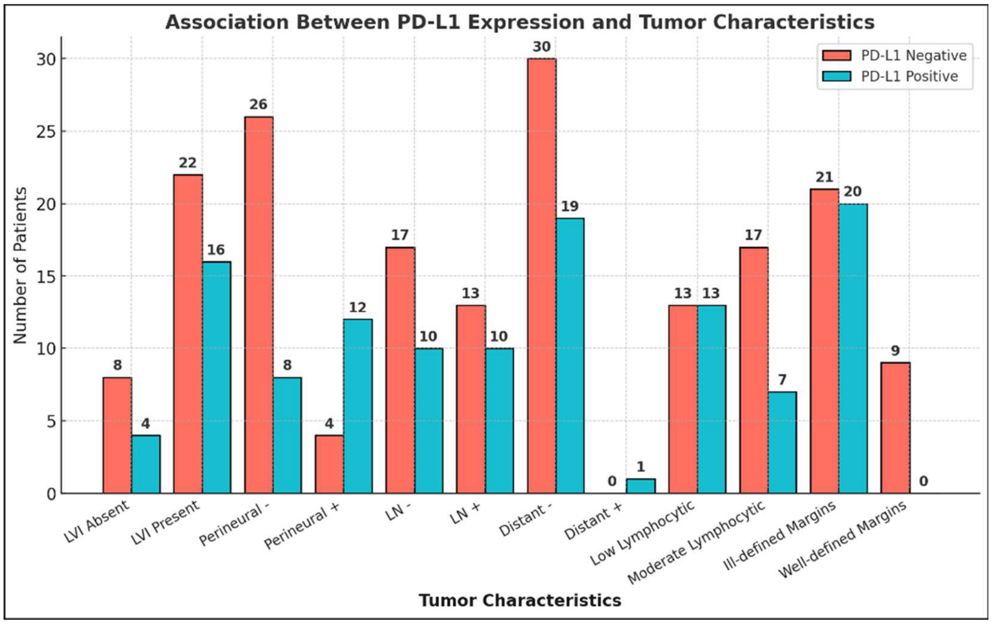


Figure 23: ROC for histological type in prediction of PDL1 expression

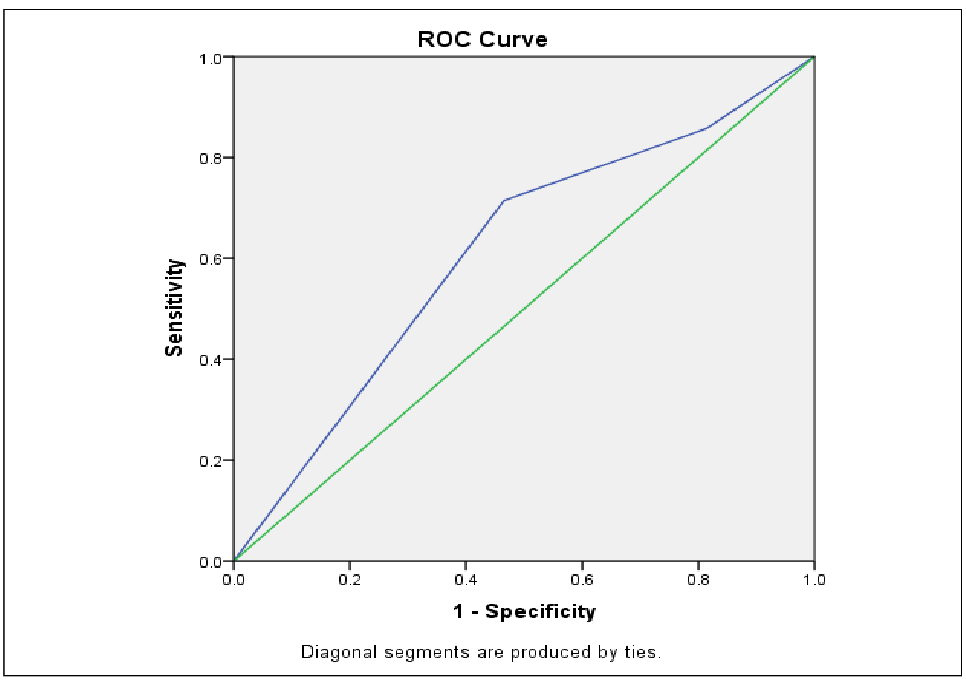


Table 14: Area under curve

Area	Std. Error	p-value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.6130	0.1160	0.3420	0.3850	0.8410

Table 14 presents the Area Under the Curve (AUC) for histological type in predicting PD-L1 expression, along with its standard error, p-value, and 95% confidence interval. The AUC value of 0.613 suggests that histological type has a weak predictive ability for PD-L1 expression, as it is only slightly better than random chance (AUC = 0.5 indicates no discrimination). The standard error of 0.116 reflects variability in the AUC estimate, while the p-value of 0.342 indicates that the predictive relationship is not statistically significant ($p > 0.05$). The 95% confidence interval (0.385 – 0.841) suggests that while the true AUC value could fall within this range, its broad spread highlights uncertainty in the model's discriminative power. Overall, these findings indicate that histological type alone is not a strong predictor of PD-L1 expression, and additional clinicopathological factors may be needed to improve predictive accuracy.

Figure 24: ROC for Modified Bloom Richardson grade in prediction of PDL1 expression

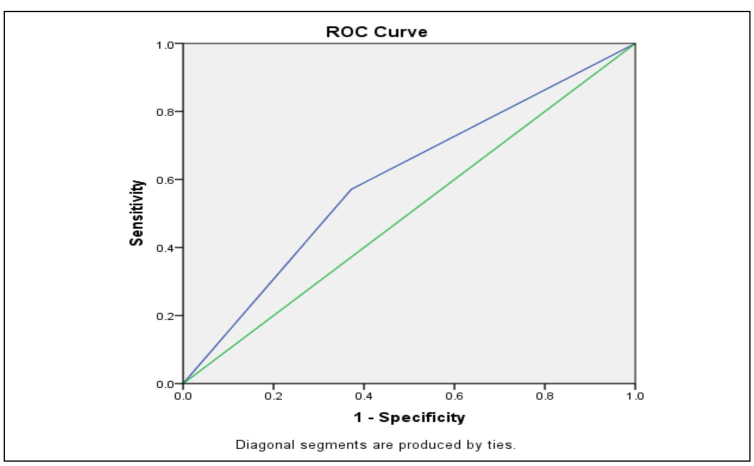


Table 15: Area under curve

Area	Std. Error	p-value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.6000	0.1170	0.4020	0.3700	0.8300

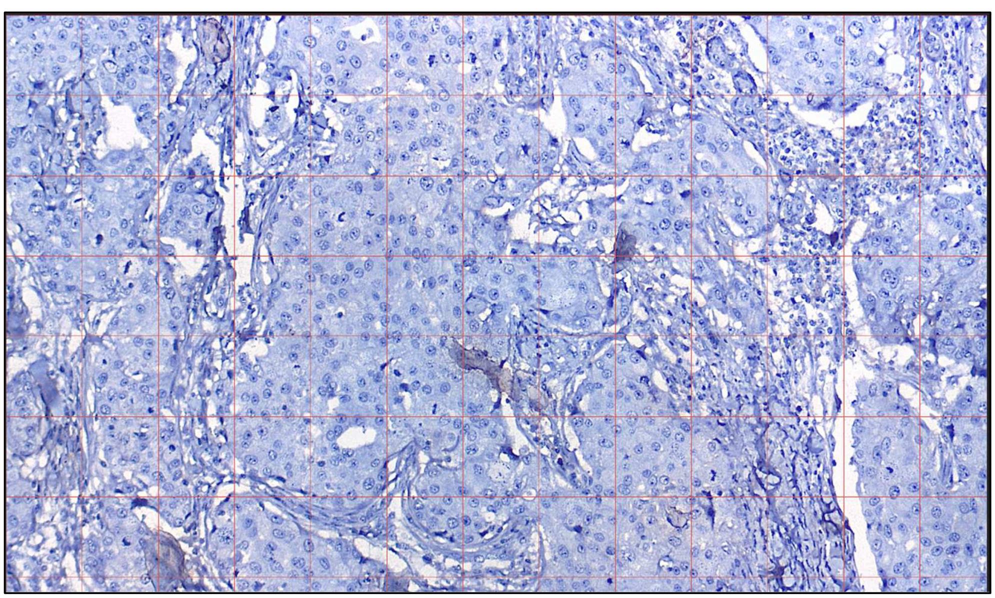
Table 15 presents the Area Under the Curve (AUC) for Modified Bloom-Richardson (MBR) grade in predicting PD-L1 expression, along with its standard error, p-value, and 95% confidence interval. The AUC value of 0.600 suggests that MBR grade has a weak discriminative ability, performing only slightly better than random chance (AUC = 0.5 indicates no predictive power). The standard error of 0.117 reflects some variability in the AUC estimate, while the p-value of 0.402 indicates that the association between MBR grade and PD-L1 expression is not statistically significant ($p > 0.05$). The 95% confidence interval (0.370 – 0.830) highlights the uncertainty in the model's predictive strength, with a broad range suggesting that the true AUC value is highly variable. Overall, these findings indicate that MBR grade alone is not a strong predictor of PD-L1 expression, and additional clinicopathological factors may be needed to enhance its predictive accuracy.

Table 16: Summary of all numerical parameters

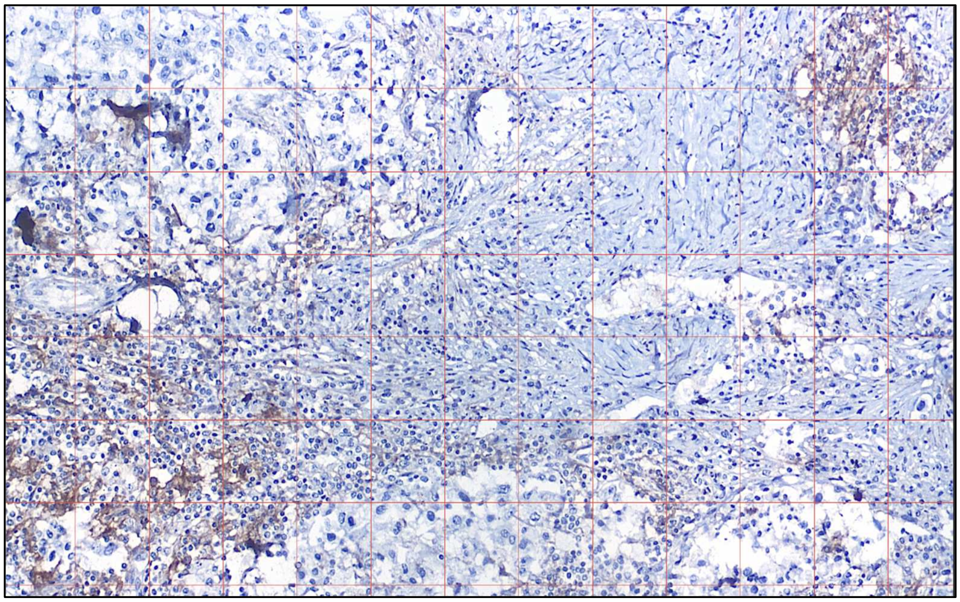
Summary	Age (Yrs)	Duration of Lump (months)	Tumor Size (mm)	Ki-67 %
Minimum	33.00	2.00	13.10	15.00
Maximum	73.00	24.00	104.40	29.00
Range	40.00	22.00	91.30	14.00
Mean	52.06	13.24	43.95	20.62
Median	52.00	14.00	38.34	20.00
Std.Dev.	9.93	6.07	24.19	4.08

The age distribution is fairly consistent, with most patients around 52 years old**.** Lump duration varies widely**,** suggesting differences in disease awareness or progression rates. Tumor sizes show high variability, indicating a diverse tumor burden among patients**.** Ki-67 expression remains relatively stable, with a small range of variability.

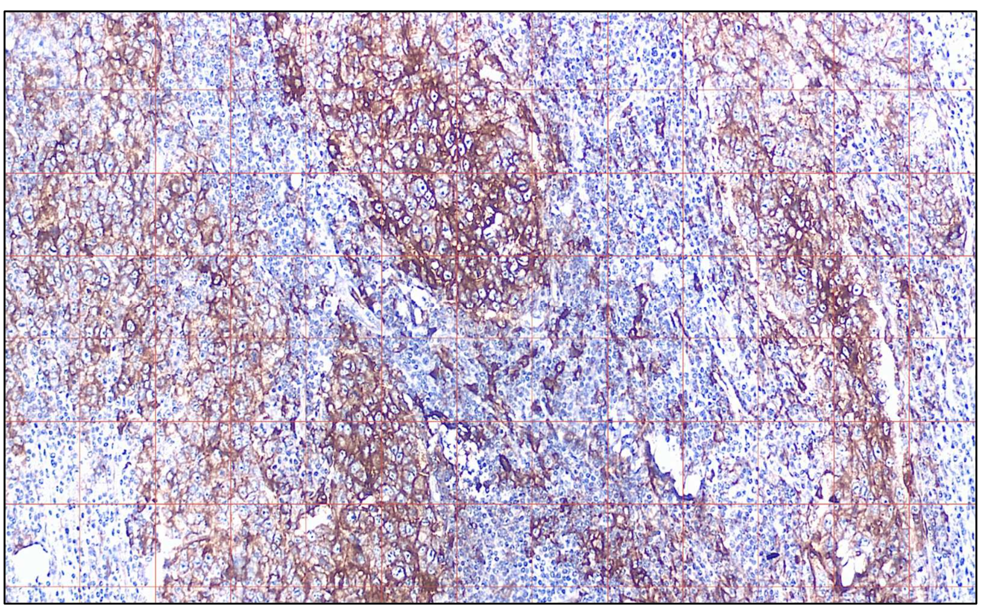
Photomicrograph 1: PD-L1 expression in TNBC, CPS <1: Negative (IHC stain X100)



Photomicrograph 2: PD-L1 expression in TNBC: Low expression (IHC stain X100)



Photomicrograph 3: PD-L1 expression in TNBC, CPS 80: Positive (IHC stain X100)



DISCUSSION

This study aimed to evaluate the immunohistochemical expression of PD-L1 in triple-negative breast cancer (TNBC) cases and its association with clinicopathological parameters. Our findings contribute to the growing body of literature on the prognostic and potential therapeutic implications of PD-L1 in TNBC.

PD-L1 Expression and Clinicopathological Parameters

In our study, PD-L1 positivity (CPS ≥10) was observed in 40% of TNBC cases, a rate consistent with findings by Mittendorf et al., who reported PD-L1 expression in 38% of TNBC cases[86], and Adams et al. , who found PD-L1 positivity in 41% of their cohort[87]. Similarly, Schalper et al. observed a PD-L1 positivity rate ranging between 30-50% in different TNBC subtypes [88]. These findings reinforce the variability in PD-L1 expression and its potential role in immune checkpoint blockade therapies.

The age distribution of PD-L1-positive cases showed a trend toward younger patients, with the highest positivity rate (71.43%) in those aged ≤40 years. Although the chi-square test did not reach statistical significance ($p = 0.061$), our observations align with findings by Sabatier et al., who reported a higher prevalence of PD-L1 expression in younger TNBC patients[89]. Conversely, Beckers et al. found no significant correlation between age and PD-L1 expression, indicating the need for larger studies to clarify this association[90].

Tumor laterality and quadrant involvement did not show significant associations with PD-L1 expression ($p = 0.726$ and $p = 0.996$, respectively). This is consistent with the results of Ali et al., who found that tumor location does not significantly influence PD-L1 expression[91].

Histological and Tumor Grading Associations

Histologically, invasive ductal carcinoma (IDC) was the predominant type (98% of cases), which is in agreement with Liu et al., who found IDC in 95% of TNBC cases[92]. While our study did not find a significant correlation between histological type and PD-L1 expression (p = 1.000), studies by Vranic et al.and Thomas et al. suggest that some histological subtypes, such as metaplastic carcinoma, exhibit higher PD-L1 expression than IDC[93,94].

The Modified Bloom-Richardson (MBR) grade showed a statistically significant association with PD-L1 expression (p = 0.001), with Grade III tumors exhibiting the highest PD-L1 positivity (76%). Our findings are in line with those of Cimino-Mathews et al., who reported that higher-grade tumors demonstrate greater PD-L1 expression[95]. Similarly, Schalper et al. found that PD-L1 positivity was significantly higher in Grade III tumors compared to Grade I and II, reinforcing the link between tumor aggressiveness and immune evasion[96].

Tumor Microenvironment and PD-L1 Expression

Among tumor aggressiveness parameters, perineural invasion (p = 0.001) and infiltrating margins (p = 0.007) showed significant associations with PD-L1 expression. PD-L1 positivity was significantly higher in tumors with perineural invasion (75%) and ill-defined margins (48.78%). This supports the findings of Chen et al., who demonstrated a strong correlation between PD-L1 expression and aggressive histopathological features in TNBC[97]. Similarly, Schalper et al. noted that tumors with high PD-L1 expression exhibit more invasive characteristics and greater immune cell infiltration[98].

Interestingly, lymphovascular invasion (p = 0.589) and lymph node metastasis (p = 0.643) did not show significant associations with PD-L1 expression. While some studies, such as those by Sun et al. and Paré et al., have suggested a link between PD-L1 expression and metastatic potential[99,100], others, like Adams et al., found no significant correlation[87]. The lack of statistical significance in our study may be due to the relatively small sample size.

Predictive Value of PD-L1 Expression

Receiver operating characteristic (ROC) curve analysis for histological type (AUC = 0.613, p = 0.342) and MBR grade (AUC = 0.600, p = 0.402) indicated weak predictive ability for PD-L1 expression. This is in agreement with the study by Dieci et al., who found that PD-L1 expression alone is not a strong predictor of clinical outcomes in TNBC and should be used in conjunction with other biomarkers[101].

Implications for Immunotherapy

The observed PD-L1 positivity rate of 40% in TNBC patients suggests that a significant subset of patients may benefit from immune checkpoint inhibitors such as anti-PD-1/PD-L1 therapies. Studies by Emens et al. and Schmid et al. demonstrated that PD-L1-positive TNBC patients show improved response rates to checkpoint inhibitors like atezolizumab and pembrolizumab[102,103]. The strong association between PD-L1 expression and high-grade tumors (Grade III) further supports the potential of immunotherapy in aggressive TNBC cases.

However, given that PD-L1 negativity was observed in 60% of cases, additional immune-related biomarkers such as tumor mutational burden (TMB) and tumor-infiltrating lymphocytes (TILs) should be explored to identify responders to immunotherapy[104,105].

CONCLUSION

This study provides valuable insights into the prevalence and clinical relevance of PD-L1 expression in TNBC. The key findings include:

1. PD-L1 positivity was observed in 40% of TNBC cases, with a trend toward younger patients, similar to findings by Mittendorf et al. and Sabatier et al. [86,89].

2. Tumor grade (MBR Grade III) showed a significant association with PD-L1 positivity ($p = 0.001$), consistent with the results of Cimino-Mathews et al. and Schalper et al.[95,96].

3. Perineural invasion ($p = 0.001$) and ill-defined tumor margins ($p = 0.007$) were significantly associated with PD-L1 expression, in agreement with Chen et al. and Schalper et al.[97,98].

4. No significant associations were found between PD-L1 expression and tumor laterality, quadrant involvement, lymphovascular invasion, or lymph node metastasis, supporting findings by Ali et al. (2018) and Adams et al.[91,87].

5. ROC analysis showed that histological type and MBR grade alone have weak predictive value for PD-L1 expression, reinforcing the need for additional biomarkers[101].

Clinical Implications and Future Directions

The findings reinforce the role of PD-L1 as a potential therapeutic target in TNBC, particularly in high-grade and invasive tumors. Given the lack of strong predictors for PD-L1 expression, future studies should explore molecular and genetic profiling to identify additional determinants of PD-L1 positivity. Furthermore, prospective clinical trials evaluating the efficacy of PD-L1 inhibitors in TNBC subgroups with high PD-L1 expression are warranted.

This study contributes to the growing evidence on PD-L1 in TNBC but is limited by its small sample size and cross-sectional design. Larger, multi-center studies with molecular subtyping are needed to validate these findings and enhance personalized treatment approaches in TNBC.

SUMMARY

This study evaluated PD-L1 expression in triple-negative breast cancer (TNBC) and its association with clinicopathological factors. PD-L1 positivity (CPS ≥10) was observed in 40% of cases, with a trend toward younger patients and significant associations with high tumor grade (MBR Grade III, $p = 0.001$), perineural invasion ($p = 0.001$), and ill-defined margins ($p = 0.007$). However, no significant correlations were found with tumor laterality, quadrant involvement, lymphovascular invasion, or lymph node metastasis. ROC analysis indicated weak predictive value for PD-L1 expression based on histological type and tumor grade. The findings highlight PD-L1 as a potential therapeutic target in TNBC, particularly in aggressive cases, but underscore the need for additional biomarkers and larger studies to refine patient selection for immunotherapy.

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ANNEXURES I

PROFORMA

- NAME:
- AGE:
- BRIEF CLINICAL HISTORY
- DATE OF COLLECTION
- PAST HISTORY -

H/O Carcinoma Breast
H/O Carcinoma Endometrium
H/O Carcinoma Ovary
Others

- FAMILY HISTORY (Ca Breast) o Mother o Sister o Daughter
- WO MEDICAL ILLNESS

Diabetes Mellitus
Hypertension
Any Other

- LOCAL EXAMINATION (Breast)o Site o Side o Size o Skin changes o Axillary lymph node involvement o Nipple, Areola o Others

•INVESTIGATIONS GROSS FINDINGS Size
Quadrant involved
Margins
Nipple and areola Skin involvement
H and E findings Histological type
Grade
Necrosis
Fibrosis
In situ component
Lympho-vascular involvement
Lymph node metastasis

IHC -

0 HER2

0 PD-LI

OTHERS - Clinical Staging

Treatment advised

(Hormonal/Chemotherapy/Radiotherapy)

ANNEXURES II

Consent Form

"EXPRESSION OF PROGRAMMED DEATH - LIGAND I(PD-LI) IN TRIPLE NEGATIVE BREAST CARCINOMA- A CROSS SECTIONAL STUDY AT TERTIARY CARE HOSPITAL"

Introduction: Breast cancer is commonest cancer globally. The triple negative breast carcinoma is one of the types of breast carcinoma. The purpose of this study is to evaluate expression of PD-LI in triple negative breast carcinoma.

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer the best of your knowledge. You will be interviewed regarding your present, past, family history and your clinical manifestation. The block will be taken from representative areas of carcinoma. These blocks will be used to study expression of PD-LI marker.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will not get any benefits by participating in this study.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the principal investigator.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777

Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

ANNEXURES III

Consent Statement

I am making a voluntary decision to participate in the study "EXPRESSION OF PROGRAMMED DEATH - LIGAND I(PD-LI) IN TRIPLE NEGATIVE BREAST CARCINOMA- A CROSS SECTIONAL STUDY AT TERTIARY CARE HOSPITAL". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURES IV

WHO (2019) Classification of Breast Tumors

Epithelial Tumors

- Ductal carcinoma in situ (DCIS)
- Lobular carcinoma in situ (LCIS)
- Invasive carcinoma (NST, No Special Type)
- Invasive lobular carcinoma
- Tubular carcinoma
- Mucinous carcinoma
- Cribriform carcinoma
- Medullary carcinoma
- Metaplastic carcinoma

Salivary Gland-type Tumors

- Adenoid cystic carcinoma
- Secretory carcinoma
- Mucoepidermoid carcinoma

Neuroendocrine Tumors

- Neuroendocrine tumor (NET)
- Neuroendocrine carcinoma (NEC)
- Small cell carcinoma
- Large cell neuroendocrine carcinoma

Fibroepithelial Tumors

- Fibroadenoma
- Phyllodes tumor (benign, borderline, malignant)

Mesenchymal Tumors

- Angiosarcoma

- Liposarcoma
- Myofibroblastoma

Hematolymphoid Tumors

- Lymphoma

Tumors of the Nipple

- Paget disease

Molecular Classification

- Luminal A
- Luminal B
- HER2-enriched
- Triple-negative/basal-like

ANNEXURES V

Nottingham Grading System: A Semi-Qualitative Method for Assessing Histological Grade in Invasive Breast Cancer

- **Tubule Formation (Glandular Differentiation)**
    - The extent to which tumor cells form tubular structures similar to normal breast glands.
    - Score 1: >75% tubule formation
    - Score 2: 10–75% tubule formation
    - Score 3: <10% tubule formation
- **Nuclear Pleomorphism**
    - The degree of variation in the size and shape of tumor cell nuclei.
    - Score 1: Small, uniform nuclei, minimal pleomorphism
    - Score 2: Moderate nuclear enlargement and pleomorphism
    - Score 3: Marked variation in nuclear size and shape, severe pleomorphism
- **Mitotic Count**
    - The number of mitotic figures (dividing cells) observed in a defined area under a high-power microscope field (usually 10 high-power fields).
    - Score 1: Low mitotic activity
    - Score 2: Intermediate mitotic activity
    - Score 3: High mitotic activity

Final Grading (Total Score = Sum of the Three Scores)

- **Grade 1 (Well Differentiated):** Total score of 3–5
- **Grade 2 (Moderately Differentiated):** Total score of 6–7
- **Grade 3 (Poorly Differentiated):** Total score of 8–9

ANNEXURES VI

Methods of Measurement of Proliferation in Breast Cancer

Mitotic Index: Assessed histologically by counting mitotic figures in tumor cell populations.

Proportion of Cells in S Phase of the Cell Cycle: Measured using **flow cytometry**, which evaluates the fraction of actively dividing cells.

Immunohistochemical Method: Involves the detection of proteins such as **Ki-67**, which is expressed by actively proliferating cells.

Proliferative Activity of Tumor Cells: Measured using the **Thymidine Labeling Index (TLI)**, which assesses the incorporation of labeled thymidine into DNA during cell division.

ANNEXURE VII

Scoring Criteria (College of American Pathologists [CAP]) by Immunohistochemistry (IHC) for Estrogen Receptor (ER), Progesterone Receptor (PR), and HER2

Estrogen Receptor (ER) Scoring Criteria

- **Positive**: >10% of cancer cells have nuclear staining (any intensity).
- **Low Positive**: 1–10% of cancer cells have nuclear staining (any intensity).
- **Negative**: <1% or 0% of invasive cancer cells have nuclear staining (report on controls in cases with ≤10% staining).

Progesterone Receptor (PR) Scoring Criteria

- **Positive**: ≥1% of invasive cancer cells have nuclear staining of any intensity.
- **Low Positive**: Optional reporting category.
- **Negative**: <1% or 0% of invasive cancer cells have nuclear staining (report on controls in cases with ≤10% staining).

HER2 Scoring Criteria

- **Positive**: Circumferential membrane staining that is complete, intense, and present in >10% of tumor cells.
- **Equivocal**: Weak-to-moderate complete membrane staining observed in >10% of tumor cells.
- **Negative**:
    - Incomplete membrane staining that is faint/barely perceptible and present in >10% of tumor cells (1+).
    - No staining observed or membrane staining that is incomplete, faint/barely perceptible, and in ≤10% of tumor cells (0).

In Situ Hybridization for HER2

- **Positive**: HER2/CEP17 ratio **≥2.0** and average HER2 copy number **≥4.0 signals/cell**.
- **Negative**: HER2/CEP17 ratio **<2.0** and average HER2 copy number **<4.0 signals/cell**.

ANNEXURE VIII

PD-L1 REPORTING FORMAT (CPS) IN TRIPLE-NEGATIVE BREAST CANCER (TNBC)

Patient Information

- Patient Name: ____________________________
- Age/Sex: ____________________________
- Hospital/Clinic Name: ____________________________
- Sample Type: ☐ Biopsy ☐ Surgical Resection
- Date of Sample Collection: _______________
- Date of Report: _______________

PD-L1 Testing Details

- Test Method: Immunohistochemistry (IHC)
- Antibody Used: ☐ 22C3 ☐ SP263 ☐ 28-8 ☐ Other: _________
- Scoring Method: Combined Positive Score (CPS)
- Tumor Type: Triple-Negative Breast Cancer (TNBC)

PD-L1 CPS Calculation

$$CPS = \frac{\text{PD-L1-positive tumor cells} + \text{immune cells} \times 100}{\text{Total viable tumor cells}}$$

Positive if CPS ≥10.

- PD-L1 Stained Tumor Cells: _________
- PD-L1 Stained Immune Cells (Lymphocytes/Macrophages): _________
- Total Viable Tumor Cells Counted: _________
- Calculated CPS Score: __________

PD-L1 CPS Interpretation

CPS Score	PD-L1 Expression Status	Clinical Significance
CPS <1	Negative	Not eligible for PD-1/PD-L1 inhibitors
CPS 1–9	Low Expression	Consider immunotherapy in select cases
CPS ≥10	Positive	Eligible for pembrolizumab (FDA-approved cutoff for TNBC)

- Final PD-L1 Status: ☐ Negative ☐ Low Expression ☐ Positive
- CPS Score: ___________
- Eligibility for Immunotherapy: ☐ Yes ☐ No

Pathologist's Certification

- Pathologist Name: ______________________________
- Accredited Laboratory: ______________________________
- Signature: ______________________________
- Date: ______________________________

This PD-L1 CPS report helps determine immunotherapy eligibility (pembrolizumab) in TNBC, with CPS ≥10 indicating PD-L1 positivity and suitability for treatment.

ANNEXURE IX

pTNM Classification for Breast Cancer (AJCC 8th Edition)

Tumor (T) – Primary Tumor

- pT0: No evidence of primary tumor
- pTis (DCIS/Lobular Carcinoma in Situ): Ductal carcinoma in situ or lobular carcinoma in situ
- pTis (Paget's Disease): Paget's disease of the nipple without underlying invasive carcinoma
- pT1: Tumor ≤20 mm
  - pT1a: >1 mm but ≤5 mm
  - pT1b: >5 mm but ≤10 mm
  - pT1c: >10 mm but ≤20 mm
- pT2: Tumor >20 mm but ≤50 mm
- pT3: Tumor >50 mm
- pT4: Tumor of any size with direct extension to the chest wall and/or skin
  - pT4a: Extension to the chest wall
  - pT4b: Ulceration and/or ipsilateral satellite nodules in the skin
  - pT4c: Both T4a and T4b features
  - pT4d: Inflammatory carcinoma

Nodes (N) – Regional Lymph Node Involvement

- pN0: No regional lymph node metastasis
- pN1: Metastasis in 1–3 axillary lymph nodes or internal mammary nodes detected by sentinel lymph node biopsy
  - pN1mi: Micrometastases (0.2–2 mm)
  - pN1a: Metastasis in 1–3 axillary lymph nodes (>2 mm)
  - pN1b: Metastasis in internal mammary nodes
  - pN1c: Combination of N1a and N1b
- pN2:
  - pN2a: Metastasis in 4–9 axillary lymph nodes
  - pN2b: Metastasis in clinically detected internal mammary nodes without axillary involvement

- pN3:
    - pN3a: Metastasis in ≥10 axillary nodes or infraclavicular nodes
    - pN3b: Metastasis in both axillary and internal mammary nodes
    - pN3c: Metastasis in supraclavicular lymph nodes

Metastasis (M) – Distant Metastasis

- pM0: No clinical or radiographic evidence of distant metastasis
- cM0 (i+): No evidence of distant metastasis but isolated tumor cells in blood, bone marrow, or non-regional lymph nodes
- pM1: Distant metastasis confirmed by biopsy

ANNEXURE X

KEY TO MASTER CHART

S. No	Serial Number
LI	Lower Inner
UI	Upper Inner
LO	Lower Outer
UO	Upper Outer
IDC	Invasive Ductal Carcinoma
P	Present
A	Absent
Mo	Moderate
Lo	Low

ANNEXURE XI

MASTER CHART

Serial_Number	Biopsy_Number	Age_(Yrs)	Sex	Side	Duration_of_Lump(MONTH	Quadrant	Histological_Type	Modified Bloom Richardson	Lymphyvascular_Invasion	Perineural_Invasion	Lymph_Node_Metastasis	Distant_Organ_Metastasis	Lymphocytic_Response	Infilterating_Margins	PD-L1_Expression-CPS	T_stage	N_stage	M_stage	Tumor_Size_cm	Mitotic_Index_per_10HPF	Necrosis	KI-67_%
1	957/22	54	F	Right	15	LO	IDC	III	P	P	P	A	Mo	ID	10	T3	N2	M0	5.1 x 5.8	7	P	17
2	1238/2	48	F	Left	10	C	IDC	II	P	A	P	A	Mo	ID	<1	T3	N2	M0	3.4 x 2.	3	P	18
3	2103/2	56	F	Left	12	UO	IDC	I	A	A	P	A	Mo	WD	<1	T1	N1	M0	5.0 x 5.8	2	P	18
4	2464/2	65	F	Right	7	C	IDC	II	A	A	A	A	Mo	ID	1	T2	N0	M0	4.8 x 2.	1	P	16
5	2735/2	47	F	Right	14	LI	IDC	II	P	A	P	A	Lo	ID	<1	T4	N2	M0	5.2 x 4.	1	P	21
6	2906/2	47	F	Left	22	LI	IDC	III	P	A	A	A	Lo	ID	1	T4	N0	M1	4.0 x 5.	8	P	27
7	3136/2	65	F	Right	2	UI	IDC	I	P	A	P	A	Lo	WD	<1	T1	N2	M0	3.4 x 4.	2	P	23
8	4361/2	57	F	Right	8	UO	IDC	III	P	A	A	P	Lo	ID	40	T4	N0	M0	4.8 x 5.	6	A	25
9	244/23	58	F	Right	13	LI	IDC	II	A	A	A	A	Mo	ID	5	T4	N0	M0	4.4 x 5.	3	A	15
10	549/23	46	F	Right	23	UO	IDC	III	A	P	A	A	Mo	ID	30	T3	N0	M0	3.9 x 4.	8	A	16
11	619/23	45	F	Left	7	UO	IDC	III	P	P	P	A	Lo	ID	25	T3	N2	M0	2.6 x 4.	7	A	17
12	641/23	53	F	Right	6	C	IDC	III	P	P	A	A	Lo	ID	20	T3	N0	M1	3.5 x 5.8	8	A	17
13	1009/2	65	F	Right	15	UO	IDC	I	P	A	A	A	Lo	WD	<1	T1	N0	M0	2.5 x 2.	1	A	16
14	2104/2	45	F	Left	13	LO	IDC	III	P	P	P	A	Lo	ID	3	T4	N2	M1	4.0 x 3.	8	A	29
15	2469/2	54	F	Right	17	LI	IDC	III	P	A	P	A	Lo	ID	90	T2	N2	M0	5.2 x 4.	8	A	18
16	2282/2	60	F	Left	5	UO	IDC	III	P	P	A	A	Mo	ID	20	T3	N0	M0	5.8 x 5.5	6	A	20
17	3278/2	47	F	Left	16	UO	IDC	II	P	A	P	A	Mo	ID	<1	T3	N1	M0	4.0 x 2.	1	A	23
18	3279/2	57	F	Right	24	LI	IDC	III	P	P	P	A	Lo	ID	90	T4	N2	M0	5.7 x 2.3	6	A	15
19	4115/23	45	F	Left	5	UO	IDC	III	A	A	A	A	Lo	ID	15	T3	N0	M0	4.2 x 2.	5	A	26
20	4901/2	52	F	Right	15	LI	IDC	II	A	A	P	A	Lo	ID	2	T3	N2	M0	4.5 x 2.	3	A	26
21	4967/2	33	F	Left	23	UO	IDC	I	P	A	P	A	Mo	WD	<1	T2	N2	M0	3.0 x 2.	1	A	24
22	5613/23	67	F	Left	12	UI	IDC	III	P	P	P	A	Mo	ID	12	T3	N2	M1	4.9 x 3.	9	A	26
23	6005/2	35	F	Left	6	LO	IDC	III	A	P	A	A	Mo	ID	15	T3	N0	M1	5.5 x 2.8	7	A	17
24	125/24	73	F	Left	14	UO	IDC	II	P	A	A	A	Mo	ID	<1	T2	N0	M0	3.0 x 4.	1	A	18
25	129/24	65	F	Left	2	UO	IDC	II	P	A	A	A	Mo	ID	<1	T4	N0	M0	4.5 x 3.	1	A	21
26	197/24	41	F	Left	8	LO	IDC	II	P	A	A	A	Mo	ID	<1	T2	N0	M0	5.9 x 2.	3	A	29
27	203/24	60	F	Left	14	C	IDC	II	A	A	A	A	Mo	ID	<1	T2	N0	M0	4.4 x 5.	2	A	17
28	290/24	63	F	Left	8	UO	IDC	I	P	A	P	A	Lo	WD	<1	T1	N1	M0	2.2 x 5.	3	A	29
29	319/24	39	F	Right	16	C	IDC	III	P	A	A	A	Lo	ID	15	T2	N0	M1	5.5 x 5.8	4	A	19
30	348/24	53	F	Right	16	LO	IDC	I	A	A	A	A	Mo	WD	<1	T1	N0	M0	6.0 x 2.	3	A	21
31	390/24	40	F	Left	12	UO	IDC	III	P	P	P	A	Lo	ID	15	T4	N2	M1	2.6 x 2.	4	A	15
32	477/24	35	F	Left	16	UO	IDC	II	P	A	P	A	Lo	ID	5	T3	N1	M0	5.1 x 4.7	2	A	21
33	495/24	65	F	Left	24	C	IDC	II	P	A	P	A	Lo	ID	<1	T2	N1	M0	4.6 x 5.5	2	A	21
34	714/24	47	F	Left	13	UO	IDC	III	P	P	A	A	Lo	ID	2	T3	N0	M0	3.3 x 4.	6	A	27
35	740/24	50	F	Left	18	C	IDC	I	P	A	A	A	Lo	WD	<1	T1	N0	M0	3.8 x 4.	1	A	18
36	835/24	66	F	Right	23	UO	IDC	I	P	A	A	A	Lo	WD	<1	T2	N0	M0	2.9 x 2.	2	A	23
37	848/24	52	F	Left	3	UO	IDC	III	P	P	P	A	Mo	ID	10	T4	N2	M1	5.6 x 3.4	4	A	18
38	849*/24	35	F	Left	19	C	IDC	III	P	A	P	A	Lo	ID	10	T3	N2	M1	4.0 x 6.	6	A	20
39	894/24	44	F	Right	14	UO	IDC	II	P	A	A	A	Mo	ID	<1	T2	N0	M0	3.5 x 4.	1	A	19
40	924/24	51	F	Left	7	UO	IDC	III	P	A	P	A	Lo	ID	15	T3	N2	M0	5.1 x 3.4	4	A	18
41	934/24	69	F	Left	22	UO	IDC	II	P	A	P	A	Mo	ID	<1	T2	N2	M0	2.8 x 2.	2	A	15
42	2296/2	55	F	Right	14	C	IDC	III	P	A	A	A	Lo	ID	90	T3	N0	M0	5.3 x 2.	8	A	21
43	2819/24	52	F	Right	17	LI	PLC	II	P	A	P	A	Mo	ID	<1	T4	N1	M0	2.5 x 5.2	3	A	20
44	3894/2	38	F	Left	19	UO	IDC	III	P	P	P	A	Lo	ID	70	T4	N1	M1	2.3 x 5.	5	A	19
45	4005/2	53	F	Left	12	C	IDC	III	A	P	A	A	Lo	ID	5	T3	N0	M1	3.6 x 2.	4	A	22
46	5544/24	43	F	Right	3	UO	IDC	I	A	A	A	A	Mo	WD	<1	T1	N0	M0	2.2 x 5.	2	A	28
47	5812/24	47	F	Right	18	LI	IDC	III	P	P	A	A	Lo	ID	15	T3	N0	M0	3.7 x 4.	8	A	21
48	5965/24	54	F	Left	17	UO	IDC	III	P	P	A	A	Mo	ID	2	T3	N0	M0	2.5 x 3.4	6	A	22
49	6218/24	63	F	Left	9	UI	IDC	III	A	A	A	A	Mo	ID	80	T3	N0	M1	2.8 x 4.	7	A	22
50	203/25	49	F	Right	14	UI	IDC	II	P	A	A	A	Mo	ID	<1	T4	N0	M0	5.4 x 3.	2	A	17