EXPRESSION OF PD L1, BCL-2, Ki-67 IN BREAST CANCER WITH CLINICOPATHOLOGICAL CORRELATION

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ABSTRACT

In India, breast cancer incidence is one of the most common cancers in women after cervical cancer. This has prompted intensive research into various risk factors from clinical parameters, morphological and biological markers, for creating a personalized regimen. The aim of the study is to evaluate the expression of Ki-67, Bcl-2, and PD-L1 in the archival blocks of invasive breast cancer patients, and determine possible correlation between PD-L1 marker expression and clinicopathological parameters, Bcl-2 and Ki-67 index. Immunohistochemistry was performed for Ki-67, Bcl-2, and p16 on archival blocks of all 50 cases of invasive breast cancer cases and their clinicopathological details obtained. Chi-square analyses were used to correlate with each marker and clinicopathological expression of breast cancer patients. Bcl 2 and Ki-67 were found to be significant with PD-L1 expression in the tumor cells with a p-value of <0.05. However, PD-L1 expression did not correlate with clinicopathological parameters like tumour size, lymph node status or morphological grade. The mean age of the participants is 50.4 years, female is 48 (96%) and males are 2 (4%). A maximum number of cases were Invasive carcinoma, No Specific Type (36) and Carcinoma with medullary like features were 9. PD-L1, a newer marker, would soon benefit breast cancer targeted immunotherapy, which now has become a common modality in the treatment of many other solid tumors.

Key Words: Immunohistochemistry, Tumors, Immunotherapy
INTRODUCTION

In India, breast cancer incidence in women is growing closer to that in the western world accounting for the second leading cause of death, the first being carcinoma of cervix.\textsuperscript{[1-3]} This has prompted an intensive study risk factors (morphological typing, clinical parameters, and biological markers) for assessment of prevention strategies, prognosis, and treatment modalities.\textsuperscript{[1]} The role of the pathologist in the evaluation of breast cancer now transcends that of determining the correct morphologic diagnosis, including the grading and staging of cancer. Breast oncologists increasingly make treatment decisions based upon the phenotypic and/or genotypic characteristics of the tumor, which is defined mainly based on biomarkers \textsuperscript{[4,5]}. The phenotype of primary breast tumors determines the type of medical or surgical treatment required for patient \textsuperscript{[6]}. Nowadays, there are varied diagnostic modalities further probing into genotype and also assessing the immune reaction towards the tumour cells. The immunohistochemical marker ER and HER2 are assessed in all tumors for determining the phenotype and treatment selection, whereas progesterone receptor (PR) and Ki67 for the assessment of prognosis \textsuperscript{[6]}. The expression of immunohistochemical markers in breast cancer has been proved to be a good predictive and prognostic indicator. The wide use of anti-apoptotic markers forms the basis of immunotherapy in tumours like melanoma and renal cell carcinoma. In our study, our aim is to evaluate whether anti-apoptotic markers like Bcl2 and PDL1 have any prognostic significance in breast carcinoma cases and also correlate it with ER, PR, HER2 neu and Ki67 status. Positivity of Bcl2 and PDL1 in the tumour cells would form part of immunotherapy.

The assessment of proliferation by Ki-67 marker is one of the major factors for deciding the treatment in breast cancer patients \textsuperscript{[7]} Ki-67 is a nuclear protein being associated with cellular proliferation and was originally identified by Gerdes et al. \textsuperscript{[8]} in the early 1980s. The Ki-67 index is used to assess the pathological Complete Response (pCR) in neoadjuvant chemotherapy-treated Triple Negative Breast Cancer (TNBC) cases.\textsuperscript{[9]} The Ki-67 positivity shows higher risk of recurrence and a worse prognosis in patients, especially with early breast cancer.\textsuperscript{[10]} Furthermore, in the 2013 St Gallen Consensus Conference, adding Ki-67 for the determination of proliferation and the differentiation of luminal A and B tumors was recommended.\textsuperscript{[11]} As TNBC includes NAC-sensitive(neo- adjuvant therapy) and NAC-resistant subgroups that have different survival outcomes, Ki-67 including Bcl-2 marker may serve as indicator for post-NAC assessment of pathological Complete Response(pCR).\textsuperscript{[12]}
The anti-apoptotic members of this family, such as Bcl-2 and Bcl-XL, prevent apoptosis either by sequestering proforms of death-driving cysteine proteases called caspases (a complex called the apoptosome) or by preventing the release of mitochondrial apoptogenic factors such as cytochrome c and AIF (apoptosis-inducing factor). The apoptotic marker Bcl-2 (B-cell lymphoma 2) expression in breast cancer has been associated with poor survival and reduced sensitivity to chemotherapy as it inhibits apoptosis in tumour cells induced by chemotherapeutic drugs. Assessing Bcl-2 along with other proliferative and newer markers involving the immune pathway would prove newer understanding in treating triple-negative breast cancer (TNBC), as it is highly invasive, has a high recurrence rate over 3 years, poor prognosis, and availability of only single treatment so far.

The interest of recent studies has been shifted to the expression of Programmed Death-Ligand 1 (PD-L1), which is a target of many cancer therapies involving the PD-1/PD-L1 pathway. The PD-1/PD-L1 pathway is one of many inhibitory immunological checkpoints. PD-1 and PD-L1 bind to each other to express an inhibitory signal that prevents the antitumor killing activity of T cells, which is an important mechanism of tumor cell immune escape. Therefore, blocking PD-1/PD-L1 provides a new strategy for tumor immunotherapy. This is because PD-L1 marker expression in Tumour Cells (TCs) and Tumour Infiltrating Lymphocytes (TILs) have been studied extensively in breast cancer after it was first identified in various other solid cancers like melanoma, Renal Cell Carcinomas (RCC), for which immunotherapeutic drugs have also been approved by FDA (Food and Drug Administration). Therefore, it is imperative that the identification of precise biomarkers of breast cancer and, potential therapeutic targets for the treatment of the disease, to improve the Overall Survival (OS). In this study a multivariate analysis is provided of the biomarkers and their clinicopathological correlation.

MATERIALS AND METHODS

This Cross-sectional observational study was conducted during the period of June 2013 till June 2018 (period of five years). This study was approved by the Institutional Ethics Committee of Chettinad Hospital and Research Institute. All the consecutive histology proven cases of Invasive Breast Carcinoma, which was diagnosed during the study period, were included in the study. The cases were retrieved from the files in the Department of Pathology, Chettinad Hospital, and Research Institute. A total of 63 cases were identified. Formalin-fixed and paraffin-embedded tissue blocks of these cases were retrieved from the archives of the Department of Pathology, Chettinad Hospital, and Research Institute. The clinical details of the cases including the clinicopathological parameters were obtained from the Medical Records Department (MRD) of the institute.
cases were excluded from the study due to lack of clinical details and non-availability of the paraffin blocks. Finally, a total of 50 cases were available for the study.

Methodology: Finally, a total of 50 cases were included in the study. Among them 41 was Modified Radical Mastectomy specimen with lymph node dissection and the rest was specimen from conservative surgery. 10 normal breast tissue blocks and control tissue blocks – tonsil for Ki-67 and Bcl-2 and salivary gland for PD-L1 were included for comparing them with the cases. The tissue blocks obtained were re-embedded and sections were cut at 3.5 microns. Hematoxylin and Eosin staining of the sections were done and analyzed to confirm the histological diagnosis and grade of tumor differentiation in carcinoma cases. Then appropriate areas for performing immunohistochemistry were marked. The clinicopathological characteristics including age, sex, menopausal status, side, site, duration of symptoms, family history, radiological grade, tumour size, TNM stage, lymph node involvement, Morphological and molecular subtype, Modified Scarff Bloom Richardson grade of tumour, ER, PR and Her 2/neu expression of the tumour along with recurrence or mortality status, were obtained wherever applicable from the medical records department.

Immunohistochemical Staining: Sections of 3.5 microns thick were cut in a semi-automated microtome (Leica, RM2245) using disposable blades. Positively charged hydrophobic slides were used. For each case, their sections, one for Ki-67, Bcl-2, and PD-L1 were taken. These cases were stained in 10 batches; each batch had 1 normal breast tissue, 1 positive control for each Ki-67, Bcl-2, and PD-L1.

Immunohistochemical markers: Ki-67-MIB-1: The marker is from Pathnsitu Biotechnologies Pvt. Ltd. A pre-diluted form. The clone is GM001 and a mouse monoclonal antibody.

Bcl-2: The marker is from Pathnsitu Biotechnologies and was in a pre-diluted form. The clone is EP36 and a Rabbit monoclonal antibody.

PD-L1 (CD274 Rabbit Polyclonal Antibody): The marker is from Elabscience Biotechnology Inc. The concentrated antibody was diluted at 1:100 with PBS (0.02% sodium azide, 100μg/mlBSA).

HRP polymertechnique: The prepared slides were processed through the following steps:

Overnight incubation (at 60 degrees Celsius), Xylene- 3 changes, 5 minutes each, Graded alcohol- 70% alcohol for 5 minutes followed by 90% alcohol for 5 minutes. Water wash for 10 minutes. Distilled water wash for 30 seconds. Antigen retrieval with a pressure cooker: Sections were subjected to antigen retrieval using pressure cooker technique using TRIS EDTA (pH 7.4) buffer solution for 15 – 20 minutes. Cool the pressure cooker...
under running tap water for 5-10 minutes. TRIS buffer wash for 5 minutes, 2 changes. Treatment with blocking agent – peroxidize block – for inhibiting endogenous peroxidases in the tissue for 10 minutes. TRIS buffer wash for 5 minutes, 2 changes. Application of appropriate primary antibody for 45 minutes. TRIS buffer wash for 5 minutes, 2 changes. Application of target binder (PolyExcel target binder, PathnSitu) for 10 minutes which enhances the final reaction product by increasing the sensitivity of the antigen-antibody reaction. TRIS buffer wash for 5 minutes, 2 changes. Application of secondary antibody (PolyExcel Poly HRP, PathnSitu) – horseradish peroxidase enzyme for 10 minutes. TRIS buffer wash for 5 minutes, 2 changes. DAB (Diaminobenzidine) (PolyExcel Stunn DAB Chromogen; PathnSitu) 50 micro liter and buffer (Poly Excel Stunn DAB-Buffer; PathnSitu) 1 ml are mixed and applied to the slides for 5 minutes. Wash in distilled water for 2 minutes. Slides were counterstained with Harris Hematoxylin. Water wash for 5 minutes. Air-dried and mounted with DPX (Distyrene plasticizer in Xylol).

**Scoring for Ki-67**

1 – No staining in tumour cells

2 – Nuclear staining in < 20% of tumour cells. (Low Index)

3 – Nuclear staining in ≥ 20% of tumour cells. (High Index)

**Scoring for Bcl-2**

0 - No staining or Faint membranous Staining observed in < 10% of tumour cells

1 – Faint or incomplete membranous staining ≥ 10% of tumour cells.

2 – Moderate complete membranous staining in ≥ 10% tumour cells.

3 – Strong Complete membranous staining in ≥ 10% tumour cells.

**Scoring for PD-L1 in tumour cells (TCs)**\(^{[14]}\)

1 – No or cytoplasmic staining (low) intensity.

2 – Cytoplasmic staining (intermediate) intensity with or without cytoplasmic granularity.

3 – Cytoplasmic staining with (high) intensity with or without cytoplasmic granularity.

**Scoring of PD-L1 in Tumour Infiltrating Lymphocytes (TILs)**\(^{[14]}\)
0 – Virtual absence of staining in TILs.

1 – Staining positive in <30% of TILs (LowInfiltrate).

2 - Staining positive in 31% - 60% of TILs (IntermediateInfiltrate).

3 - Staining positive in > 60% of TILs (HighInfiltrate).

**Statistical Analysis**

The frequencies and percentage analysis of various parameters were done using SPSS software version23 and R-Studio. Correlation of Ki-67, Bcl-2, and PD-L1 with histology and comparison of Ki-67 and Bcl-2 with PD-L1 was done using Chi-square analysis. Data visualization was done using SPSS software version25. For all tests, a two-sided p- value <0.05 was considered statistically significant.

**RESULTS AND ANALYSIS**

**PD-L1 IHC marker:** PD-L1 expression and its relationship with clinicopathological features were analyzed by chi-square test where, p-value <0.05 was considered statistically significant difference. PD-L1 protein was considered positive when expressed in the cytoplasm of the cell with few granules and was compared to staining in the control tissue. In this study the positive expression of PD-L1 in tumor cells and TILs was 62% and 24% respectively. In the <49 years age group 64% (16/25) and ≥49 years age group 60% (15/25) were PD-L1 positive. PD-L1 positivity was compared in postmenopausal and premenopausal women who were 56% and 64% respectively. Neither age nor menopausal status was significant. The PD-L1 expression was found to be insignificant in correlation with clinical parameters such as tumour size, tumour size grading, and lymph node status, and MSBR, NPI and TNM stage. The morphological and the molecular subtypes like TNBC were also not significantly correlating with PD-L1 expression in TC or TILs. The hormonal receptor expressions and HER2/neu expression were also not found to be significant. It was noted that Ki-67 and BCL2 were found significant in correlation with PD-L1 expression (p<0.05).

**Correlation between BCL2 and molecular subtypes:** The objective of the study to correlate between BCL2 and molecular subtypes such as Luminal A, Luminal B, TNBC, and HER2 was found to be insignificant with p-value = 0.108, which >0.05. BCL2 expression showed positive correlation in lower MSBR grades. Whereas it did not significantly correlate with TNM stage and so did the Ki-67 index.

DISCUSSION: Advances in the field of breast cancer over the last several decades have been remarkable and have clearly translated into better patient care as evidenced by earlier detection, better prognosis, and new targeted therapies. A failure with the one size fits all approaches to cancer therapy has been replaced by more personalized cancer therapy in the future. Hence, venturing into studying the newer markers for deciding certain therapeutic options makes this study relevant for the present era. [15]

In this study, a total of 50 invasive breast cancer cases were included. Out of the 50 cases, 36 (72%) were Invasive Breast Cancer, Not Otherwise Specified (NOS) and 9 (18%) cases showed medullary like features. The data is comparable to WHO classification, 2012 edition [63], comprising of 40% to 75% of invasive breast cancer, NOS. As the diagnosis of pure type of medullary carcinoma is very rare accounting for < 1%, high prevalence has still been reported, wherein it is mostly misdiagnosis of invasive carcinoma, NST with medullary like features or atypical Medullary Carcinoma. Notably only 1 case of Invasive papillary carcinoma and 1 case of Mucinous carcinoma was present. This was in accordance with a study conducted by Ramesh Chaven and colleague [16], in which 75% were invasive carcinoma of breast and single case of invasive papillary carcinoma and mucinous carcinoma was reported, because Special types of mammary carcinoma collectively constitute for only about 25% of invasive breast cancers. [17] While previous researches’ focused on IHC for diagnosis and for determining treatment response or pathological complete response, nowadays the trend had shifted to determining the immunoexpression for deciding targeted therapy. Even in the presence of various modalities for diagnosis of breast cancer like gene profile, always histopathological examination with IHC might lead to the diagnosis and is cost-effective, in many developing countries. In spite of many awareness programs and government policies, the burden of disease is on a constant rise. Hence, the need for this study becomes relevant.

In this study, the mean age of disease incidence was 50.4 years. The number of cases in the category < 49 years and > 49 years was observed to be equal to 50%. According, to recent data there has been constant rise in the incidence of breast cancer across all age groups, but largely involves the ‘less –than- 45 years age –group’ [18]. It is also observed that, the number of cases in the younger age group is more than the older age group, which can be explained by the fact that breast cancers in younger age group tend to be more aggressive and also because of lack of awareness in older patients seeking treatment for the disease [19]. It is also common in premenopausal women rather than in postmenopausal which is concordance with our data where the number of breast cancer occurring in premenopausal was 25 out of 48 cases. As a known fact that breast cancer is the leading cause of
cancer-associated death in women, has high incidence above all other cancers in females, our study also
emphasis that the majority of the participants were females (n=48, 96%) while only two were males (n=2,
4%), similar to a study by Chavan et al.[16].

It is also evident in our study that right side involvement (56%) is more common than the left side involvement,
with the commonest site of occurrence being upper outer quadrant about 42%. Another clinical parameter like
nipple retraction was seen in 18% and skin involvement was seen in only 8% of the cases. This data is consistent
with presence of occult nipple involvement and invasion of lymphatic vessels occurred in less than 10% of
invasive ductal carcinoma and the nipple involvement usually occurred in the setting of increased tumour size
and lymphovascular invasion elsewhere in the breast [20]. The mean tumor size in our study is 4.35 cm, and size
ruanged from 1 cm to 11 cm. The size of the tumor itself is identified as an independent predictive factor for
prognosis. Out of 50 cases, 10 (20%) cases were only < 2 cm signifying low grade, whereas more than half were
in the > 2 cm in size. The prognosis of breast cancer is largely influenced by the classical variables of tumour
size, lymph node status, histological grade and vascular invasion [21]. But on the contrary, in the current study the
lymph node metastasis and lymphovascular invasion were noted only in 24 (48%) and 9 (18%) cases out of 50,
respectively.

In view of surgical advances leading to breast-conserving therapy, it has become necessary to more accurately
classify patients based on the relative risk of recurrence or progression [22]. This made it a necessity to include
hormone receptor expression, Her 2 and Ki-67 index for classifying breast cancers, into so-called molecular
subtypes. The cases were classified based on classification of intrinsic molecular subtypes using the 13th St.
Gallen International Expert Consensus into (Fig. 1)

- luminal A: all ER and/or PR positive, HER2-negative, Ki-67 low(<14%);
- luminal B (HER2 negative): ER-positive, HER2 negative, and at least one of Ki-67 high (≥20%), PR
  negative or low(<20%);
- luminal B (HER2 positive): ER-positive, HER2 positive, any Ki-67, anyPR;
- HER2-overexpression: HER2 positive, ER and PR negative; and
- TNBC: ER and PR negative, HER2-negative.
- And recently Claudin low diagnosis has also been included.[23]
Figure 1: Molecular classification of breast cancer

But in our study none of the markers like Bcl-2 or PD-L1 came significant with molecular subtype.

**BCL-2 marker:** Luminal A breast cancer is the only molecular subtype in which the positive expression of BCL-2 has proved to be an independent favorable prognostic marker, which explains non-significant results against other subtypes. This can also be because the number of Luminal A cases in our study group was less in number when compared to TNBC type. It is known that estrogens can up-regulate BCL2 protein, though its role is still unsure. In our study, Luminal A cases, only 7/50 (14%) cases were positive for Bcl-2 expression. The highest numbers of cases negative for Bcl-2 were noted in TNBC (30%), whereas positive cases in TNBC and Her2 category were only 22% and 2%. This is inconcordance with one of study describing the importance of Bcl-2 expression in various subtypes. They stated that Bcl-2 was associated with low proliferative factors and positive ER and PR; therefore, this can be used for predicting good prognosis in patients with breast cancer such as Luminal A. Similarly, HER2-overexpression was associated with decreased BCL2 expression and tends to have good prognosis. TNBC also has low BCL2 expression. There was no statistically significant relationship between BCL2 expression and TNBC and HER2-overexpression breast cancer in our study too. Therefore, we can assume that BCL2 does not have a prognostic role in HR-negative breast cancer because this protein depends on estrogen and the ER pathway only. Whereas, Seong et al. and Chen et al. suggested that BCL2 expression is an independent, favorable prognostic factor in Hormone receptor and Her-2 negative breast cancers, predicting better outcomes in luminal cases predominantly. The BCL-2 expression also shows an inverse correlation with proliferative markers such as Ki-67, which denotes its antiproliferative role despite its antiapoptotic effect. This can be explained by the hypothesis stated by Vakkala et al. that positive estrogen...
and progesterone receptor status up-regulated Bcl2 expression which could decrease apoptosis and proliferation.\cite{29}

**PD-L1 marker:** All solid tumors show simultaneous PD-L1 positive expression in both TCs and ICs \cite{81}. PD-L1 expression by TCs was correlated significantly with objective response rate (ORR) in other solid tumours and also to clinical benefit to anti-PD-1 therapy, while the correlation of PD-L1 expression by Immune Cells/TILs with ORR does not reach the statistical significance in many solid cancers. \cite{30} TILs possess the possibility to predict the response of checkpoint blockades. Hence, in this study the authors compared PD-L1 expression in TCs with many clinicopathological characteristics such as age, tumour size, pathological T stage, lymph node status, NPI and TNM staging. But no significance was established among these parameters. Comparing PD-L1 with TNM stage was highly expressed in lower stage group (stage I, 26\%) and on comparing with MSBR Histological grade suggested high expression of PD-L1 TCs in intermediate group (grade 2) of the disease. However, type of expression in the study was contrary to an article stating significant correlation between clinical stage and higher histomorphological tumour grade \cite{31}.

[Diagram of PD-L1 inhibition and activation]
Figure 24: The mechanism of anti-PD-1 and anti-PD-L1 checkpoint blockades. PD-1 is expressed by T cells. PD-L1 is expressed in tumor cells and tumor infiltrating immune cells. Combination of PD-1 and PD-L1/PD-L2 contribute to the suppression of T-cell function. Inhibiting the interaction of PD-1 and its ligands can significantly enhance T cell function, resulting in antitumor activity.

Similarly, PD-L1 expression with negative Hormone receptor expression, negative Her 2 expression, and TNBC could not be correlated in our cases, whereas most of the study showed positive relation between TNBC subtypes. This can be explained that the predictive value of PD-L1 expression may not be uniformly applicable to all types of cancer. On the other hand, this may be associated with unresolved technical and biological issues that exclude an accurate detection of PD-L1 with a standardized criterion for the quantization of PD-L1 expression in samples. Moreover, it is well recognized that there are wide intra- and inter-tumoural variations in the expression of PD-L1, which indicates that sampling of tumour tissues may also impose on the outcome of PD-L1 detection. Another reason could be because of the small sample size, and it is a retrospective study could have its own limitations, such as selection bias, inter-observer variation and technical difficulty in retrieving the antibodies.

In current study is on comparing Bcl-2 with PD-L1 expression showed a positive correlation. This can be explained by the following study which stated that PD-L1 positive cells have markedly higher expression levels of Bcl-2 and it has been shown that knockdown of PD-L1 also downregulates anti-apoptotic proteins. PD-L1 was shown to inhibit the apoptosis of malignant melanoma initiating cells and could contribute to maintaining the stem cell- like properties of these cells. PD-L1 has also been shown to sustain stem cell-like features in breast cancer cells.

Similarly, PD-L1 expression in tumour cell was compared with proliferative marker Ki-67, which showed a significant correlation between the two with the p-value of 0.013 (significant p <0.05). This was in accordance with the study published by, Zhou et al. wherein the study suggests that the expression of PD-L1 in breast cancer cells with strong proliferative activity may have occurred because up-regulation of the proliferative marker by certain mechanisms, which would facilitate the immune escape of tumor cells. However, this mechanism should be investigated further.

Limitations of the study are small sample size, technical like standardized staining procedure and biological issues like loss of antigens during retrieval, or improper fixation and old archival blocks all could exclude an accurate detection of PD-L1 which is a newer marker in the market. It requires a standardized criterion for...
quantization of PD-L1 expression unique for each solid tumours. Moreover, it is well recognized that there are wide intra- and inter-tumoral variations in the expression of PD-L1, in their respective disease itself, which indicates that sampling of tumour tissues may also impose on the outcome of PD-L1 detection.

CONCLUSION: The research of potential predictive biomarkers is a key aspect of all anti-tumor treatment strategies [38]. Thus, this study was undertaken to show light upon certain upcoming markers for targeted immunotherapy, which could be the future breast cancer treatment, especially for refractive triple negative breast cancer cases. To improve the proportion of patients benefiting from therapy, the identification of predictive biomarkers should be addressed in the clinical trials. Despite the challenges for PD-L1 as a biomarker to predict response to PD-1/PD-L1 checkpoint blockades, the FDA granted accelerated approval of Atezolizumab for breast cancer immune therapy. However, PD-L1 alone may be not sufficient to predict the response to PD-1/PD-L1 blockades immunotherapy, hence PD-L1 expression, is compared with expression of certain approved biomarkers, like Ki-67 and BCL2 and also clinicopathological characteristics to establish any correlation that could possibly exist. These biomarkers along with others needs to be analyzed at the time of diagnosis itself and followed up after certain therapy. The cells or molecules in tumor microenvironment also should be further explored as the potential markers in future clinical trials. IHC-based PD-L1 expression on tumor cells or immune cells is an important, but not a definitive predictive biomarker for the response to PD-1/PD-L1 blockade. The following can be the limitations like variability in methods and antibodies may lead to different results. The clear definition for PD-L1 positivity is still not achieved. Standardization of staining and scoring methods should be warranted before PD-L1 can be widely used to predict response. Furthermore, the expression is dynamic and heterogeneous due to changes of microenvironment or therapy. The evaluation of PD-L1 at a single time point or single tumor may not predict the response to PD-1/PD-L1 pathway blockades.

REFERENCES


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