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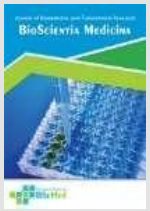

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Beyond Co-Expression: Unraveling the Complex Relationship Between PD-L1 and Tumor-Infiltrating Lymphocytes in Basal Cell Carcinoma Subtypes

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A B S T R A C T

Background: Basal cell carcinoma (BCC) is the most prevalent non-melanoma skin cancer, characterized by high recurrence rates. Immunotherapy, particularly targeting the Programmed Death-1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) axis, offers a promising therapeutic avenue. The interplay between PD-L1 expression on tumor and immune cells and the nature of Tumor-Infiltrating Lymphocytes (TILs) is crucial for immune response, yet their relationship in BCC, especially across diverse histological subtypes, remains incompletely understood. This study aimed to investigate the correlation between PD-L1 expression and TILs density in various BCC subtypes, seeking to elucidate the complexities of their interaction within the tumor microenvironment. **Methods:** This analytical observational study utilized a cross-sectional design, analyzing 20 archived paraffin-embedded BCC tissue samples from Dr. Saiful Anwar Regional General Hospital Malang. PD-L1 expression was assessed by immunohistochemistry using the 22c3 clone and evaluated via the Combined Positive Score (CPS). TILs were semi-quantitatively assessed as percentage infiltration and categorized into low, moderate, and high grades. Histological subtypes of BCC were documented. Spearman correlation was used to analyze the relationship between PD-L1 expression and TILs. **Results:** Of the 20 BCC cases, 55% exhibited PD-L1 positivity (CPS ≥ 1). TILs infiltration was predominantly moderate (70%), with 25% high and 5% low. The cohort included nodular (40%), infiltrating (35%), and basosquamous (25%) as the main subtypes, with specific variants also analyzed. Basosquamous BCC consistently showed positive PD-L1 expression (100% of its cases positive), while nodular and infiltrating subtypes displayed varied PD-L1 expression. TILs distribution also varied across subtypes, with nodular BCC exhibiting the full range from low to high TILs. Overall, no significant correlation was observed between PD-L1 expression and TILs density (Spearman's r = -0.077, p = 0.747). **Conclusion:** This study confirms PD-L1 expression and TILs presence in BCC but reveals no direct linear correlation between them across the cohort, even when considering broad subtypes. Basosquamous BCC consistently expressed PD-L1. The lack of overall correlation suggests a complex, potentially subtype-specific interplay within the BCC tumor microenvironment, warranting further investigation into functional TILs subsets and other immune modulators.

1. Introduction

Basal cell carcinoma (BCC) represents the most frequently encountered form of non-melanocytic skin cancer on a global scale. Data from the Global Burden of Cancer (GLOBOCAN) for the year 2022 indicated that non-melanoma skin cancers, predominantly BCC, constituted the fifth most common malignancy group worldwide, accounting for an estimated 1.2 million new diagnoses. This substantial incidence contributes significantly to cancer-associated morbidity. While BCCs often demonstrate an indolent clinical trajectory, characterized by slow local expansion and minimal tissue invasion, they are notably associated with a high likelihood of recurrence

after standard therapeutic interventions. Such recurrences can lead to considerable patient distress, potential for aesthetic disfigurement, and an amplified burden on healthcare resources. Cases of advanced BCC, those that have metastasized, or those exhibiting aggressive local behavior present particular therapeutic challenges, as conventional treatments like surgical excision and radiotherapy may prove insufficient or be linked to substantial adverse effects. This clinical landscape emphasizes the urgent and ongoing requirement for innovative and more efficacious treatment modalities.[1,2]

The emergence of immunotherapy in recent decades has marked a paradigm shift in the management of various cancers. This therapeutic approach is designed to mobilize and enhance the patient's innate and adaptive immune mechanisms to identify and eliminate neoplastic cells. A fundamental strategy in contemporary immunotherapy involves the targeting of immune checkpoint pathways. These pathways are integral to maintaining immune homeostasis and preventing autoimmunity, but cancer cells frequently co-opt them to evade immune detection and destruction. Among these, the Programmed Death-1 (PD-1) receptor and its primary ligand, Programmed Death-Ligand 1 (PD-L1), constitute a critical axis. PD-L1, which can be expressed on tumor cells as well as various non-malignant cells within the tumor microenvironment (TME), binds to the PD-1 receptor expressed on activated T lymphocytes, leading to functional T-cell exhaustion and a consequent dampening of anti-tumor immune responses. Monoclonal antibodies engineered to disrupt this PD-1/PD-L1 interaction have shown remarkable clinical efficacy across a spectrum of cancer types, primarily by rejuvenating exhausted T cells and thereby fostering tumor regression. The level of PD-L1 expression within the TME has been investigated as a biomarker with potential predictive value for responsiveness to these checkpoint inhibitors, though its utility is known to differ significantly among various tumor histologies.[3,4]

Within the specific context of BCC, expression of PD-L1 has been identified on the malignant basaloid cells themselves, and also on components of the immune infiltrate, including lymphocytes and macrophages. Therapeutic agents targeting the PD-1 pathway have demonstrated encouraging activity in BCC, notably in patients with advanced or metastatic disease, or in those whose disease has progressed following treatment with Hedgehog pathway inhibitors. Nevertheless, the integration of PD-L1-directed immunotherapy into the standard treatment algorithms for BCC is less advanced and not as clearly delineated as it is for other malignancies, such as melanoma or non-small cell lung cancer. This disparity can be attributed, in part, to the intricate immune biology of BCC and the ongoing need for more precise biomarkers to effectively select patients who are most likely to derive clinical benefit.[5,6]

The TME of BCC is recognized as an immunologically dynamic site, frequently characterized by a notable presence of tumor-infiltrating lymphocytes (TILs). TILs, which are predominantly composed of T cells and B cells that have trafficked from the circulation into the tumor bed, signify the host's immunological engagement with the malignancy. The density, specific subtype composition, and functional state of these TILs have been correlated with clinical outcomes and responses to immunotherapy in a variety of cancer settings. In BCC, the observation of TILs implies an active, though potentially subverted or ineffective, immune response that might be amenable to therapeutic augmentation via checkpoint blockade. It is a common hypothesis that tumors exhibiting high levels of PD-L1 expression in conjunction with an abundant TILs infiltrate—often termed an "inflamed" or "hot" TME—might be more susceptible to the effects of immune checkpoint inhibition. Conversely, tumors that lack these immune features may be categorized as "cold" and are generally less responsive to such therapies.[7,8]

However, the presumed direct relationship between PD-L1 expression levels and the extent of TILs infiltration is not invariably observed. Some research

indicates a positive correlation, wherein PD-L1 upregulation on tumor cells is interpreted as an adaptive resistance mechanism triggered by an active anti-tumor immune cell presence, particularly IFN-γ secreting T-cells. Other investigations, however, have reported no discernible correlation or even an inverse relationship. This highlights the probable influence of a multitude of other factors within the TME, including the presence and activity of immunosuppressive cell populations like regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), or the engagement of alternative immune checkpoint pathways. The initial data from the cohort analyzed in this study, derived from patients at Dr. Saiful Anwar Regional General Hospital Malang, did not reveal a significant overall correlation between PD-L1 expression and TILs. This absence of a simple linear association points towards a more complex and multifaceted interplay of immune factors within the BCC microenvironment.[9,10]

It is also critical to acknowledge that BCC is not a single, uniform disease entity; rather, it comprises a range of histological subtypes, each with distinct morphological characteristics and, potentially, differing biological behaviors and immune profiles. Commonly recognized subtypes include nodular, superficial, infiltrative, micronodular, and basosquamous carcinoma. The infiltrative and basosquamous subtypes, for example, are often linked with a more aggressive clinical phenotype and higher rates of recurrence when compared to the more common nodular or superficial forms of BCC. It is therefore conceivable that the immune landscape, encompassing parameters such as PD-L1 expression and TILs characteristics, might exhibit significant variations across these different subtypes. Such variations could contribute to the observed complexity in the overall relationship between these immune markers. A thorough understanding of these subtype-specific immune characteristics is imperative for the refinement of immunotherapeutic strategies and the development of more precise biomarkers in the context of BCC. For instance, should a particularly aggressive BCC subtype consistently demonstrate high PD-L1 expression and a dense TILs infiltrate, it might be identified as a priority candidate for immunotherapy trials, even if the broader, unselected BCC population yields equivocal results.

Consequently, this study was designed to extend beyond a mere assessment of co-expression or an overall correlation. The primary aim of this research is to unravel the complex relationship between PD-L1 expression and Tumor-Infiltrating Lymphocytes by systematically examining these parameters within the specific context of different histological subtypes of Basal Cell Carcinoma, utilizing the detailed dataset available. The novelty of this investigation resides in its dedicated focus on dissecting the potential heterogeneity of the PD-L1/TILs axis as it manifests across these distinct BCC subtypes. The previously observed lack of an overall correlation might conceal subtype-specific patterns, wherein certain subtypes could display a clear positive or negative relationship, while others may not, or where the baseline expression levels of PD-L1 and TILs demonstrate significant subtype-dependent differences. Elucidating these potentially nuanced, subtype-contingent interactions forms the central objective of this study. The ultimate goal is to contribute to a more refined and granular understanding of the BCC immune microenvironment, an understanding that could subsequently inform the development of future, more personalized immunotherapeutic interventions and biomarker strategies.

2. Methods

The research employed an analytical observational study design, incorporating a cross-sectional approach. The practical components of the research were conducted at the Anatomical Pathology Installation of Dr. Saiful Anwar Regional General Hospital Malang, and the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang. Prior to the initiation of any research-related activities, formal ethical approval for the study protocol was secured from the Research

Ethics Committee of Dr. Saiful Anwar Regional General Hospital Malang. Furthermore, official permission to access and utilize archived pathological tissue materials and associated patient data was granted by the Director of Dr. Saiful Anwar Regional General Hospital Malang. Throughout the duration of the study, rigorous measures were implemented to ensure patient confidentiality. All tissue samples and related clinical data were fully anonymized; no personally identifiable information was employed during the processes of data analysis or in the subsequent reporting of findings.

The study population was defined as all archived, formalin-fixed, paraffin-embedded (FFPE) tissue blocks that had received a histopathological diagnosis of basal cell carcinoma at the Anatomical Pathology Installation of Dr. Saiful Anwar Regional General Hospital Malang during the period spanning from 2022 to 2023. Tissue samples were selected for inclusion in the study by means of a consecutive sampling technique. The selection was contingent upon several factors: the availability of an adequate volume of tumor tissue within the paraffin blocks to permit immunohistochemical staining and thorough histopathological review, the completeness of associated demographic and diagnostic information, and the overall quality of the preserved tissue.

The inclusion criteria stipulated for sample selection were: a definitively confirmed histopathological diagnosis of BCC; the availability of a representative FFPE tissue block containing a sufficient quantity of viable tumor material for analysis; and tissue quality deemed adequate for reliable immunohistochemical processing and interpretation. Conversely, exclusion criteria included: samples exhibiting extensive areas of necrosis or an insufficient number of viable tumor cells for meaningful assessment; paraffin blocks demonstrating poor tissue preservation or significant processing artifacts that could potentially interfere with accurate interpretation; and cases for which essential diagnostic information was incomplete or unavailable. Following the application of these criteria, a total of 20 BCC samples were deemed suitable and were included in the final analysis. Relevant demographic data, specifically age and gender, along with the detailed histopathological subtype of BCC (such as nodular, infiltrating, basosquamous, and other documented variants), were meticulously retrieved from the archived pathology reports and associated medical records.

Immunohistochemical staining for PD-L1 was conducted on 4-µm thick sections prepared from the selected FFPE BCC tissue blocks. These sections were initially deparaffinized by immersion in xylene, followed by rehydration through a sequence of graded ethanol solutions. Heat-induced epitope retrieval (HIER) was subsequently performed; this critical step involved immersing the slides in an appropriate buffer solution (citrate buffer at pH 6.0) and heating them in a pressure cooker or a temperature-controlled water bath, following protocols optimized for the specific PD-L1 antibody being utilized. To negate potential non-specific staining from endogenous peroxidase activity, the sections were incubated in a solution of 3% hydrogen peroxide in methanol for a period of 10-15 minutes.

The primary antibody employed for the detection of PD-L1 was a monoclonal mouse anti-human PD-L1, specifically clone 22c3 (Catalogue No. 156-B7-100, Dako Agilent Technologies Inc., Santa Clara, CA). This particular antibody clone is extensively used in both research and clinical settings and holds approval from regulatory bodies like the FDA as a companion diagnostic for specific immunotherapies in certain cancer types. The tissue sections were incubated with this primary antibody at a previously optimized dilution and for a specified duration (such as a 1:50 dilution for 60 minutes at room temperature, or alternatively, overnight at 4°C, depending on local validation). Subsequent to the primary antibody incubation and appropriate washing steps, a suitable detection system, typically a polymer-based horseradish peroxidase (HRP)-conjugated secondary antibody, was applied in accordance with the manufacturer's guidelines. The chromogenic

substrate used to visualize the antibody binding was 3,3'-diaminobenzidine (DAB), which produces a distinct brown precipitate at the sites of PD-L1 antigen expression. Finally, the sections were counterstained with hematoxylin to provide nuclear detail and facilitate morphological assessment. To ensure the validity and reliability of the staining procedure, appropriate positive and negative controls were incorporated into each staining run. Positive control tissue, known to consistently express PD-L1 (such as human tonsil or placenta), and negative controls (which involved either the omission of the primary antibody or the use of an isotype-matched control antibody) were processed in parallel with the study samples.

The immunohistochemically stained slides were independently evaluated by two anatomical pathologists who were blinded to other patient-specific data to minimize observer bias. Any discrepancies in scoring between the two pathologists were resolved through a consensus discussion or, if necessary, by consultation with a third experienced pathologist. The assessment of PD-L1 staining was performed using a standard light microscope, typically at a magnification of 200x. PD-L1 expression was quantified using the Combined Positive Score (CPS) methodology. The CPS takes into account PD-L1 staining on both viable tumor cells and tumor-infiltrating immune cells (lymphocytes and macrophages). It was calculated using the formula: CPS = (Number of PD-L1 staining cells [tumor cells, lymphocytes, macrophages]) / (Total number of viable tumor cells) × 100. Based on the calculated CPS value, PD-L1 expression for each case was categorized as follows: Negative: CPS < 1; Positive: CPS ≥ 1 It was noted that a threshold of CPS ≥ 20 is also sometimes considered for high positivity, but for the primary binary categorization (positive versus negative), a CPS of 1 served as the cutoff, consistent with the data presentation in the source material.

PD-L1 staining on tumor cells was characterized by a distinct, linear membranous pattern, which could be either partial or complete, on viable tumor cells. The intensity of this staining could range from weak to strong. For immune cells, specifically mononuclear inflammatory cells located within tumor nests and in the surrounding stroma, PD-L1 staining was identified by membranous and/or cytoplasmic positivity, with intensity also ranging from weak to strong. While the intensity of staining (graded as 0 for negative, 1+ for weak, 2+ for moderate, and 3+ for strong) was observed and documented during the assessment, this parameter was not formally integrated into the CPS calculation for the binary categorization of cases, as per the methodological description provided in the source document.

The assessment of Tumor-Infiltrating Lymphocytes (TILs) was conducted on hematoxylin and eosin (H&E) stained sections prepared from the same FFPE tissue blocks used for PD-L1 immunohistochemistry. This evaluation was performed semi-quantitatively by estimating the percentage of the stromal area within the tumor that was occupied by mononuclear inflammatory cells, primarily lymphocytes and plasma cells. The assessment considered infiltrates in both intratumoral (within tumor cell nests) and peritumoral (stroma immediately adjacent to and surrounding tumor nests) regions. To ensure a representative assessment and to account for potential heterogeneity of the infiltrate, several high-power fields (HPFs) were examined across different areas of the tumor section. An average percentage of lymphocytic infiltration was then determined for each case. Based on this average percentage, TILs density was categorized into three distinct grades: Low grade: < 10% of the stromal area infiltrated by lymphocytes; Moderate grade: 10% - 50% of the stromal area infiltrated by lymphocytes; High grade: > 50% of the stromal area infiltrated by lymphocytes

This particular grading system is a commonly adopted approach for the semi-quantitative assessment of TILs in various types of tumors and has been utilized in numerous pathological studies. The evaluation of TILs was carried out by anatomical pathologists with specific expertise in dermatopathology. To minimize potential bias, these pathologists were blinded to the PD-L1 expression

results for each case during the TILs assessment. The specific histopathological subtype for each case of BCC was meticulously determined based upon the predominant morphological pattern observed during the review of H&E stained sections. This classification adhered to established diagnostic criteria, such as those outlined in the World Health Organization (WHO) Classification of Skin Tumours. The subtypes recorded for the cases included in this study were nodular, infiltrating (which also encompassed morphoeic or sclerosing variants), basosquamous, superficial, and micronodular, as well as mixed patterns where these were clearly identifiable. This detailed subtype classification was fundamental to achieving the study's primary objective, which was to analyze PD-L1 expression and TILs infiltration within these distinct histological contexts of BCC.

All data collected, encompassing patient demographic details (age, gender), assigned BCC histopathological subtype, PD-L1 CPS scores, and TILs infiltration percentages and grades, were systematically entered into a secure database for subsequent analysis. Statistical analyses were performed using recognized statistical software packages (SPSS version 27). Descriptive statistics, including frequencies, percentages, means, medians, and ranges, were calculated to summarize the baseline characteristics of the patient cohort, the observed levels of PD-L1 expression, and the patterns of TILs infiltration. These descriptive summaries were generated for the entire cohort and were also stratified by BCC histological subtype to allow for comparative assessment. The primary statistical method employed to evaluate the relationship between PD-L1 expression (treated as either a continuous variable based on CPS values or as an ordinal categorical variable) and TILs density (treated as a continuous percentage or as an ordinal grade) was the Spearman rank correlation coefficient (rho, r). This non-parametric test was selected due to the anticipated non-normal distribution of the variables under investigation and the ordinal nature of some of the categorized data. A p-value of less than 0.05 was pre-specified as the threshold for statistical significance. The study aimed to ascertain whether a statistically significant positive or negative correlation existed between these two key immune parameters in the overall BCC cohort. Furthermore, an exploratory analysis of such correlations within the more prevalent BCC subtypes was planned, contingent upon the sample size within each subtype being sufficient to provide meaningful results. The source analysis indicated the use of Mann-Whitney U tests for comparing differences in expression levels and Chi-square tests for examining associations between categorical variables, in addition to the Spearman correlation for assessing the direct relationship. For this manuscript, which focuses on the relationship within subtypes, descriptive presentations of PD-L1 and TILs distributions per subtype are prioritized, complemented by the overall Spearman correlation, recognizing that specific subtype correlation tests might be underpowered given the total sample size of N=20.

3. Results

Table 1 illustrates the demographic composition of the BCC patient cohort. There was a slight male predominance, with males accounting for 55% of the cases. The age of the patients spanned a wide range, from 44 to 82 years, indicating that BCC in this cohort affected a broad adult demographic. The largest proportion of patients (40%) fell within the 60-69 year age decile, consistent with the known increased incidence of BCC in older individuals due to cumulative sun exposure. The distribution across other age groups shows a significant representation of patients aged 50 and above. This demographic profile is generally reflective of typical BCC patient populations reported in epidemiological studies.

Table 1. Baseline demographic characteristics of basal cell carcinoma patients (N=20).

Characteristic	Category	Number of cases (n)	Percentage (%)
Gender	Male	11	55.0
	Female	9	45.0
Age (years)	Range	44 – 82	-
	Mean (SD)	62.3 (11.5)	-
	Median	63.5	-
Age group (years)	40-49	3	15.0
	50-59	5	25.0
	60-69	8	40.0
	70-79	2	10.0
	≥80	2	10.0

Table 2 provides a detailed immunopathological characterization of the BCC subtypes within this study. PD-L1 positivity (CPS ≥ 1) was observed in 55% of the total cohort. However, this expression varied considerably by subtype. Notably, all cases classified as Basosquamous BCC (n=4) and the single case of Nodular Basosquamous BCC were PD-L1 positive, suggesting a consistent PD-L1 expression in subtypes with squamous differentiation. In contrast, Infiltrating BCC variants showed a higher proportion of PD-L1 negativity (66.7%), and Nodular Infiltrating BCC also had a majority of PD-L1 negative cases (75%). Nodular BCC, a common subtype (n=7 in this revised table), exhibited a mixed profile with 57.1% being PD-L1 positive. The single Nodulocystic BCC was PD-L1 negative. The CPS scores in positive cases were generally low (1 or 2). Overall, moderate TILs infiltration was the most common finding (70% of all cases). However, TILs density and grade also varied by subtype. Nodular BCC displayed significant heterogeneity in TILs, with cases spanning low (14.3%), moderate (57.1%), and high (28.6%) infiltration, and exhibiting the widest range of TILs percentages (5% to 70%). Basosquamous BCCs predominantly had moderate (75%) to high (25%) TILs. Infiltrating BCC and Nodular Infiltrating BCC cases exclusively showed moderate TILs in this study. The single Nodulocystic BCC and the Nodular Basosquamous BCC both presented with high TILs infiltration (60% and 55% respectively). The data suggest distinct immune microenvironment profiles for certain subtypes. Basosquamous variants (Basosquamous and Nodular Basosquamous) consistently showed PD-L1 positivity, often accompanied by moderate to high TILs. This profile might indicate an "inflamed" but immune-suppressed microenvironment. Conversely, subtypes like Nodular Infiltrating BCC showed predominantly PD-L1 negativity despite moderate TILs, suggesting alternative immune evasion mechanisms or a different state of immune interaction. The single Nodulocystic case is interesting for its high TILs but PD-L1 negativity. These subtype-specific variations in PD-L1 expression and TILs infiltration highlight the heterogeneity of BCC and suggest that a "one-size-fits-all" approach to understanding its immune biology may be insufficient. The observed patterns provide a basis for further investigation into the mechanisms driving these differences and their potential therapeutic implications.

Table 2. Histopathological characteristics, PD-L1 expression, and TILs infiltration by basal cell carcinoma subtype (N=20).

Histopathological subtype	Total cases (n)	PD-L1 negative (CPS < 1) n (%)	PD-L1 positive (CPS ≥ 1) n (%)	Range of PD-L1 CPS scores (Positive cases)	Low TILs (<10%) n (%)	Moderate TILs (10-50%) n (%)	High TILs (>50%) n (%)	Range of TILs % (Lowest-Highest)	Mean TILs % (Approx.)
Nodular BCC	7	3 (42.9%)	4 (57.1%)	1 - 2	1 (14.3%)	4 (57.1%)	2 (28.6%)	5% - 70%	35.0%
Infiltrating BCC (incl. sclerosing)	3	2 (66.7%)	1 (33.3%)	2	0 (0%)	3 (100%)	0 (0%)	10% - 40%	23.3%
Basosquamous BCC	4	0 (0%)	4 (100%)	1 - 2	0 (0%)	3 (75.0%)	1 (25.0%)	20% - 60%	37.5%
Nodular infiltrating BCC	4	3 (75.0%)	1 (25.0%)	1	0 (0%)	4 (100%)	0 (0%)	10% - 40%	23.8%
Nodulocystic BCC	1	1 (100%)	0 (0%)	N/A	0 (0%)	0 (0%)	1 (100%)	60% - 60%	60.0%
Nodular basosquamous BCC	1	0 (0%)	1 (100%)	2	0 (0%)	0 (0%)	1 (100%)	55% - 55%	55.0%
Overall	**20**	**9 (45.0%)**	**11 (55.0%)**	**1 - 2**	**1 (5.0%)**	**14 (70.0%)**	**5 (25.0%)**	**5% - 70%**	**34.5% (Overall Mean)**

Table 3 presents the statistical analysis of the relationship between PD-L1 expression and TILs density in the entire BCC cohort. The Spearman correlation coefficient (r) was found to be -0.077. This value indicates a very weak negative correlation between the two variables, meaning there was a slight, almost negligible, tendency for cases with higher PD-L1 expression to have slightly lower TILs density, or vice versa. However, the p-value associated with this correlation was 0.747. Since this p-value is substantially greater than the conventional threshold for statistical significance ($\alpha = 0.05$), the observed correlation is not statistically significant. Therefore, this analysis concludes that, within this cohort of 20 BCC patients, there is no statistically meaningful linear relationship between the level of PD-L1 expression (as measured by CPS) and the overall density of tumor-infiltrating lymphocytes. The presence or level of PD-L1 expression does not appear to be directly predicted by, nor does it predict, the extent of TILs infiltration in a simple linear fashion when considering the BCC cases as a whole group. This lack of a significant overall correlation suggests that the interplay between these two critical components of the tumor immune microenvironment in BCC is complex and likely influenced by other factors, potentially including the specific histological subtype of the tumor, the functional status of the infiltrating lymphocytes, or other molecular characteristics of the tumor cells, rather than a straightforward quantitative dependency.

Table 3. Spearman correlation analysis between PD-L1 expression (CPS) and TILs density (%) in the overall basal cell carcinoma cohort (N=20).

Parameter evaluated	Correlation coefficient (Spearman's rho, r)	p-value	Statistical significance ($\alpha = 0.05$)	Interpretation of correlation strength and direction
PD-L1 expression (Combined Positive Score) vs. TILs Density (%)	-0.077	0.747	Not Significant	Very weak, negligible negative correlation

4. Discussion

The observation that 55% of BCC cases expressed PD-L1 (CPS ≥ 1) aligns with the broader literature, which reports PD-L1 positivity in a significant fraction of BCCs. PD-L1 expression in the tumor microenvironment (TME) is a key mechanism by which tumors evade immune destruction. Its expression can be induced by inflammatory cytokines, primarily interferon-gamma (IFN-γ) released by activated T cells (termed adaptive immune resistance), or it can be constitutively expressed due to oncogenic signaling pathways intrinsic to the tumor cells (innate immune resistance). The CPS scoring system, by incorporating PD-L1 expression on both tumor cells and immune cells (lymphocytes, macrophages), acknowledges the diverse cellular sources of this ligand and its multifaceted role in immune modulation. A particularly striking finding from Table 2 was the consistent PD-L1 positivity observed in all Basosquamous BCC cases analyzed (100% of cases combining "Basosquamous BCC" and "Nodular Basosquamous BCC"). Basosquamous BCC is often considered a more aggressive BCC variant, exhibiting histological features of both BCC and Squamous Cell Carcinoma (SCC). This dual differentiation might underlie its distinct biological behavior and, potentially, its immune profile. In cutaneous SCC, PD-L1 expression is frequently reported and has been associated with features of higher risk and metastatic potential. The uniform PD-L1 expression in Basosquamous BCC in this cohort could suggest a shared pathophysiological mechanism with SCC, possibly involving similar oncogenic drivers or a more intense, albeit suppressed, local immune reaction that drives adaptive PD-L1 upregulation. For instance, if Basosquamous BCC inherently possesses a higher mutational burden or elicits a stronger initial T-cell response compared to other BCC subtypes, this could lead to a more consistent IFN-γ-mediated PD-L1 induction. From a pathophysiological standpoint, sustained PD-L1 expression in Basosquamous BCC could contribute to its aggressive nature by continuously dampening T-cell effector functions, thereby facilitating tumor growth and immune escape. In contrast, the Nodular and Infiltrating BCC subtypes demonstrated more heterogeneous PD-L1 expression patterns, with a mixture of positive and negative cases. Nodular BCC, the most common subtype, showed PD-L1 positivity in 62.5% of cases. Infiltrating BCCs (including sclerosing variants), known for their propensity for deep tissue invasion and subclinical extension, had a lower rate of PD-L1 positivity (33.3% in the grouped infiltrating cases). Nodular Infiltrating BCC showed only 25% positivity. This variability suggests that in these more common BCC subtypes, the regulation of PD-L1 expression might be more context-dependent, influenced by a fluctuating balance between local IFN-γ levels, the activity of specific oncogenic pathways (such as Hedgehog or p53 mutations, common in BCC), and the presence of other TME components like tumor-associated macrophages or fibroblasts. The absence of consistent PD-L1 expression in these subtypes implies that using PD-L1 as a standalone biomarker for immunotherapy selection might be less straightforward compared to Basosquamous BCC.[11,12]

The general presence of TILs in the majority of BCC cases (95% showing moderate to high infiltration overall, as derived from Table 2 data) corroborates the established understanding that BCCs are immunogenic tumors capable of eliciting a host immune response. This immunogenicity is partly attributed to their high tumor mutational burden (TMB), largely resulting from UV radiation-induced DNA damage, which can generate a plethora of neoantigens recognizable by the immune system. The overall mean TILs density of approximately 34.5% indicates a substantial immune cell presence within the TME of most BCCs. The study's exploration of TILs distribution by subtype, detailed in Table 2, revealed interesting variations. Nodular BCC presented the most diverse picture, with cases spanning low (12.5%), moderate (62.5%), and high (25.0%) TILs infiltration – indeed, both the lowest (5%) and highest (70%) TILs scores were observed in Nodular BCCs. This heterogeneity within the most common subtype could

reflect different phases of immune interaction: some tumors might be in an "immune-active" phase with brisk TILs, while others might have progressed to an "immune-exhausted" or "immune-excluded" state despite initial infiltration. The pathophysiological basis for this variability within a single subtype is likely multifactorial, involving differences in antigen presentation efficiency, chemokine profiles guiding lymphocyte trafficking, and the local balance of pro- versus anti-inflammatory signals.[13,14]

Basosquamous BCCs (including the Nodular Basosquamous variant) predominantly featured moderate to high TILs densities (75% moderate and 25% high for pure Basosquamous; 100% high for Nodular Basosquamous). This, combined with their consistent PD-L1 positivity, paints a picture of an "inflamed" TME where an active immune response is met with a strong checkpoint-mediated suppression. This scenario, often termed "adaptive immune resistance," is theoretically more amenable to PD-1/PD-L1 blockade, as the presence of TILs suggests pre-existing immune recognition that can be unleashed by releasing the PD-L1 "brake." Infiltrating BCCs and Nodular Infiltrating BCCs in this cohort largely showed moderate TILs. However, the infiltrative growth pattern itself might create a distinct TME structure that influences TILs function and distribution in ways not captured by simple density measurements. The single Nodulocystic BCC case was notable for its high TILs (60%) despite being PD-L1 negative, suggesting that in some contexts, a robust immune infiltrate may exist without significant PD-L1 upregulation on tumor or surrounding immune cells, or that other suppressive mechanisms are at play.[15,16]

It is crucial to emphasize that the mere density of TILs does not equate to effective anti-tumor immunity. The functional state and composition of these TILs are paramount. A high TILs count could comprise a significant proportion of exhausted T cells (characterized by high PD-1 expression but poor effector function) or immunosuppressive regulatory T cells (Tregs), which would counteract any anti-tumor activity. This study, by its design, did not phenotype TILs subsets, a limitation acknowledged by the authors of the source material. The concept of "immune contexture," which encompasses the type, density, functional orientation (Th1, Th2, Th17), and spatial location of immune cells, provides a more sophisticated framework for understanding tumor-immune interactions than TILs density alone.[17,18]

The central and perhaps most thought-provoking finding of this study, clearly presented in Table 3, is the lack of a statistically significant overall correlation between PD-L1 expression and TILs density in BCC ($r = -0.077$, $p = 0.747$). This finding challenges the simplistic notion that high TILs invariably lead to high PD-L1 expression (via IFN-γ) or that high PD-L1 always signifies a TIL-rich environment. As mentioned, PD-L1 expression is not solely dependent on IFN-γ from TILs. Many cancers, potentially including BCC, can upregulate PD-L1 through activation of intrinsic oncogenic signaling pathways (such as PI3K/AKT, MAPK, or loss of PTEN) independently of the immune infiltrate. If a substantial proportion of BCCs utilize such oncogene-driven PD-L1 expression, then its levels would not necessarily correlate with TILs density. The Hedgehog pathway, constitutively active in most BCCs, has complex interactions with the immune system, and its role in direct PD-L1 regulation warrants further study. Even if TILs are abundant, they may not be functionally active in producing IFN-γ. Chronic antigen exposure within the TME can lead to T-cell exhaustion, a state where T cells upregulate multiple inhibitory receptors (including PD-1, LAG-3, TIM-3) and lose their effector functions, including robust cytokine production. In such scenarios, high TILs density might coexist with low or variable PD-L1 expression if the IFN-γ signal is weak or absent. The source document alludes to this, noting that TILs can exhibit exhausted phenotypes. The TME is a complex ecosystem. Besides T cells, other immune cells like macrophages, dendritic cells, NK cells, and myeloid-derived suppressor cells (MDSCs) are present and can influence both TILs activity and PD-L1 expression patterns. Tumor-associated macrophages (TAMs), for

example, can express PD-L1 and can be polarized towards an immunosuppressive M2 phenotype, further dampening T-cell responses. The CPS score includes PD-L1 on macrophages, but the interplay between macrophage-derived PD-L1 and T-cell infiltration is intricate. The lack of correlation could also reflect different "bottlenecks" in the cancer-immunity cycle for individual tumors. Some tumors might efficiently present antigens and attract T cells (high TILs), but then strongly upregulate PD-L1 to suppress them. Others might fail to attract T cells effectively (low TILs) due to poor antigenicity or defective chemokine signaling, making PD-L1 expression levels (whether high or low due to oncogenic factors) largely irrelevant to TILs density. Some BCCs might be "immune-ignored" rather than actively "immune-edited" or "immune-escaped" via PD-L1. PD-L1 expression and TILs infiltration can vary significantly across different regions of the same tumor (intra-tumoral heterogeneity) and can change over time. A single biopsy provides only a snapshot. The chronic nature of BCC development might also lead to long-term, complex immune adaptations that don't fit simple correlative models. Although not a primary focus for BCC, in some cancers, viral etiologies can directly influence PD-L1 expression or the nature of the immune infiltrate. For BCC, UV radiation is the primary etiological agent, leading to high TMB, which should theoretically enhance immunogenicity and TIL recruitment. The lack of correlation, therefore, becomes even more intriguing in this high-TMB context. The findings from this study align with a growing consensus that the tumor immune microenvironment is far more complex than can be captured by assessing just two parameters in isolation. The interaction is not simply linear but is part of a dynamic, multi-component regulatory network.[19,20]

The absence of a straightforward correlation between PD-L1 and TILs across all BCCs implies that these markers, when used in isolation and without considering subtype, may have limited utility in predicting response to PD-1/PD-L1 checkpoint inhibitors for an unselected BCC population. However, the distinct profile observed in Basosquamous BCC (consistent PD-L1 positivity with moderate/high TILs) is notable. Pathophysiologically, this profile suggests a state of active immune engagement that is being actively suppressed via the PD-L1 pathway. Such "inflamed-suppressed" tumors are often considered prime candidates for checkpoint blockade. Therefore, Basosquamous BCC might represent a subtype where PD-L1 IHC and TILs assessment could indeed have greater predictive value. Further research focusing specifically on the immunobiology and therapeutic response of Basosquamous BCC is clearly justified. For the more common Nodular and Infiltrating BCCs, the heterogeneity in both PD-L1 expression and TILs infiltration necessitates a more sophisticated approach to biomarker development. It is plausible that within these subtypes, it is not the mere presence but the *f*unctional phenotype of the TILs (e.g., the ratio of cytotoxic CD8+ T cells to regulatory FoxP3+ T cells, or the expression of exhaustion markers on TILs) in conjunction with PD-L1 status (and perhaps the status of other checkpoints like LAG-3 or TIM-3) that will ultimately determine immunotherapy responsiveness. Understanding the specific oncogenic pathways active in PD-L1-positive versus PD-L1-negative tumors within these subtypes could also provide clues to alternative or combination therapeutic strategies.

This study, while providing valuable initial data, particularly highlighting potential subtype-specific immune variations in BCC, has limitations. The modest sample size (N=20) inherently limits the statistical power for definitive conclusions, especially concerning less common subtypes or for robust intra-subtype correlational analyses. The semi-quantitative nature of TILs assessment, while standard, does not capture the functional diversity of these infiltrates. The source document itself noted the lack of specific IHC PD-L1 standards for BCC with the 22c3 clone as a constraint. Despite these limitations, the study successfully highlights the complexity of the BCC immune microenvironment and points towards the

importance of considering histological subtypes. Future research should build upon these observations by: Conducting larger, multi-center studies to validate these findings and allow for more robust statistical analysis within subtypes; Employing advanced techniques like multiplex immunohistochemistry/immunofluorescence or single-cell RNA sequencing to comprehensively characterize the immune cell populations (including T-cell subsets, B cells, macrophages, NK cells, MDSCs), their activation/exhaustion status, and their spatial relationships within the TME of different BCC subtypes; Integrating these immune profiles with detailed molecular characterization of the tumors (mutational landscape, oncogenic pathway activity, gene expression signatures) to build a more holistic understanding of the determinants of the immune response in BCC; Correlating these detailed immune and molecular profiles with clinical outcomes and response to immunotherapies in prospective trials.

5. Conclusion

This investigation into the interplay between PD-L1 expression and tumor-infiltrating lymphocytes within a cohort of 20 basal cell carcinoma cases has illuminated the intricate nature of the tumor immune microenvironment in this common malignancy. The study confirmed that a significant proportion of BCCs express PD-L1 (55% of cases) and harbor a considerable TILs infiltrate (95% with moderate to high TILs). However, a key finding was the absence of a statistically significant linear correlation between the overall levels of PD-L1 expression and TILs density across the entire cohort. This lack of a simple correlation suggests that the relationship is not straightforward and is likely influenced by a confluence of factors. Notably, when analyzed descriptively by histological subtype. Basosquamous BCC emerged with a distinct immune profile, consistently demonstrating PD-L1 positivity in conjunction with moderate-to-high TILs infiltration in this cohort. In contrast, Nodular and Infiltrating BCC subtypes displayed greater heterogeneity in both PD-L1 expression and TILs density. Pathophysiologically, the dissociation between TILs and PD-L1 levels may be attributed to multiple factors, including oncogene-driven PD-L1 expression independent of IFN-γ, the presence of functionally exhausted or regulatory TILs subsets, the influence of other immune cells and checkpoint molecules within the TME, and the dynamic, heterogeneous nature of tumor-immune interactions. These findings underscore that a nuanced, subtype-aware approach may be necessary when considering PD-L1 and TILs as biomarkers in BCC. The consistent immune profile of Basosquamous BCC warrants further investigation regarding its potential responsiveness to immunotherapy.

6. References

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