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Immunogenomic Characterization of Triple-Negative Breast Cancer in a Moroccan Cohort and Its Prognostic Implication

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ABSTRACT

Background: In North Africa, triple-negative breast cancer (TNBC) is a highly prevalent, aggressive, and diverse disease with unique clinical features. In this population, the prognostic significance of immune infiltration and genomic changes is still not fully understood. In order to investigate their relationships with relapse-free survival, this study sought to provide an integrated analysis of the tumor immune microenvironment and *BRCA1* and *BRCA2* variant landscape in Moroccan TNBC patients.

Methods: A retrospective cohort of Moroccan TNBC patients was evaluated using immunohistochemistry (IHC) to assess stromal tumor-infiltrating lymphocytes (TILs), programmed death-ligand 1 (PD-L1) expression, and immune cell subsets (CD3, CD4, CD20, and CD56). Targeted next-generation sequencing of *BRCA1* and *BRCA2* was performed on tumor tissue. Associations between immune biomarkers, genomic variants, and clinical outcomes were analyzed using appropriate statistical and bioinformatic approaches.

Results: The cohort exhibited significant heterogeneity in immune infiltration and advanced disease at diagnosis. Higher stromal TIL levels were observed in patients without relapse, although this association did not reach statistical significance in the present cohort. *BRCA1* and *BRCA2* variants were frequently detected and showed heterogeneous immune profiles across tumors. Limited redundancy among immune markers was found in correlation analyses, suggesting that these markers may capture complementary aspects of the immune microenvironment.

Conclusion: This integrated immunogenomic analysis suggests the potential prognostic relevance of the tumor immune microenvironment in Moroccan TNBC patients. The findings emphasize the value of multiparametric immune and genomic profiling for improved risk stratification and support further investigation of population-specific immunogenomic patterns in North African TNBC patients.

INTRODUCTION

Triple-negative breast cancer (TNBC), a unique and aggressive subtype of breast cancer, is defined by the lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).[1] Although its

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prevalence varies significantly across ethnic and geographic populations, TNBC accounts for 10% to 17% of all breast cancer cases in White women worldwide.[1] Breast cancer is a major cause of cancer-related death among women in North Africa, where its incidence has increased significantly.[2]

TNBC is associated with a poor prognosis, aggressive tumor behavior, and an increased risk of *BRCA1* alterations.[1] Premenopausal patients, sub-Saharan African women, and African American women have all been found to have a higher incidence of TNBC.[1] Although higher frequencies (20.0% to 26.4%) have been reported among African American women[1], reports from Europe, North America, and China show TNBC proportions ranging from 11.4% to 16.0%. This suggests that genetic susceptibility and environmental factors may play a role. In Malaysia, TNBC accounted for 12.4% of breast cancer cases in a cohort of 340 patients.[3]

Breast cancer accounts for approximately 36% of all female cancers in Morocco, making it the most common cancer among women.[2] Compared with populations in the West, the median age at diagnosis is significantly lower (median age, 48 years).[2] This early onset suggests a substantial contribution of genetic predisposition, including *BRCA1*-related susceptibility, to the breast cancer burden in the region. The reported prevalence of TNBC varies throughout North Africa. TNBC accounted for 19.8% of breast cancer cases in Algeria, a higher percentage than is typically found in cohorts from Europe and North America.[1] In Tunisia, the incidence of breast cancer continues to increase, with approximately 2000 new cases diagnosed annually.[4,5]

TNBC has an aggressive clinical course and a poor prognosis because it lacks clear therapeutic targets, in contrast to hormone receptor-positive and HER2-positive breast cancers.[1] Molecular studies have demonstrated significant overlap among TNBC, basal-like breast cancer, and *BRCA1*-associated tumors.[1] Approximately 70% of breast cancers observed in carriers of germline *BRCA1* mutations exhibit a triple-negative phenotype.[1]

Recent evidence indicates that TNBC tumors with impaired *BRCA1*-mediated homologous recombination repair may be particularly sensitive to platinum-based chemotherapy and poly(ADP-ribose) polymerase (PARP) inhibitors, which selectively target DNA repair-deficient cells.[1] These findings emphasize how crucial it is to describe *BRCA1* and *BRCA2* variants in TNBC in order to comprehend the biology of the disease and its prognosis.

The objective of this study was to characterize the clinical, immune, and genomic heterogeneity of TNBC and to evaluate the prognostic relevance of immune infiltration patterns and *BRCA1* and *BRCA2* variants in relation to relapse-free survival. Specifically, the study aimed to assess the association of stromal tumor-infiltrating lymphocytes (TILs), programmed death-ligand 1 (PD-L1) expression, and immune cell subsets with clinical relapse; to explore immunological profiles associated with distinct classes of *BRCA1* and *BRCA2* variants; and to investigate immunogenomic interactions that may contribute to heterogeneous relapse outcomes within this population.

METHODS

Study design and patient selection

Thirty retrospective analytical case series included 30 patients with histologically confirmed TNBC at Ibn Rochd University Hospital in Casablanca, Morocco. For each patient, clinicopathological variables, including age at diagnosis, tumor size, and tumor stage, were collected. Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples to assess immune infiltration across the cohort.

All consecutive TNBC cases diagnosed during the study period were screened for eligibility. Patients were included if adequate FFPE tumor material and complete clinicopathological data were available for analysis. Cases were excluded if tumor cellularity was insufficient, DNA quality was inadequate, or sequencing failed. This selection process aimed to minimize technical bias while maintaining a homogeneous triple-negative cohort.

For molecular analysis, FFPE tumor samples were subjected to targeted sequencing of the *BRCA1* and *BRCA2* genes. All 30 patients with interpretable sequencing results were included in the integrated immunogenomic analysis.

Immunohistochemistry and immune profiling

Immunohistochemical analyses were performed on 4-µm sections obtained from FFPE tumor tissue blocks using standard automated protocols.

Assessment of triple-negative status

ER and PR expression were evaluated using the EP1 and PgR636 clones, respectively, on the Dako Link 48 platform. Tumors were considered ER- and PR-negative if nuclear staining was observed in less than 1% of tumor cells, in accordance with American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. HER2 status was assessed using the 4B5 clone on the Ventana Benchmark GX platform. Tumors were classified as HER2-negative if the IHC score was 0 or 1+ according to ASCO/CAP guidelines. Cases with equivocal HER2 expression (IHC score 2+) were

excluded from the study to ensure a strict definition of triple-negative breast cancer.[6]

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes were evaluated on hematoxylin-eosin (H&E)-stained sections according to the recommendations of the International TILs Working Group. Stromal TILs were quantified as the percentage of the tumor stromal area occupied by mononuclear inflammatory cells. Based on predefined thresholds, cases were categorized into low (< 30%) and high (≥ 30%) TIL groups.[6,7]

TIL assessment was independently performed by 2 experienced pathologists who were blinded to clinical outcomes. In cases of discrepancy, a consensus review was performed.

PD-L1 expression

PD-L1 expression was assessed using the monoclonal antibody clone 22C3 with the Dako PD-L1 IHC 22C3 PharmDx kit on the Dako Link 48 Autostainer. Membranous staining in tumor cells and membranous or cytoplasmic staining in immune cells were considered positive. PD-L1 expression was quantified using the Combined Positive Score (CPS), defined as the number of PD-L1-positive tumor and immune cells divided by the total number of viable tumor cells, multiplied by 100. PD-L1 positivity was defined using a CPS threshold of 1 or greater. Although higher CPS cutoffs (eg, CPS ≥ 10) are commonly applied in clinical practice to identify patients eligible for immune checkpoint inhibitor therapy[8,9], the present study adopted a lower threshold to evaluate the prognostic significance of PD-L1 expression within the tumor microenvironment rather than its predictive value for immunotherapy response.

Sensitivity analyses using a CPS threshold of 10 or greater were considered exploratory due to the limited cohort size and were included to improve methodological comparability with clinical studies.

Immune cell subset analysis

The immune cell composition of the tumor microenvironment was further characterized by IHC using specific markers. CD3 (pan-T lymphocytes) was detected using a rabbit polyclonal antibody (FLEX Polyclonal Rabbit Anti-Human CD3 RTU), CD4 (helper T cells) using clone AB12, CD8 (cytotoxic T cells) using clone C8/144B, and CD56 (natural killer [NK] cells). These immune markers were analyzed to explore associations among immune cell subsets, relapse-free survival, and overall immune infiltration patterns.[10]

DNA Extraction and Library Preparation

Genomic DNA was extracted from 2 10-µm FFPE sections per case using the GeneJET DNA Purification Kit (Thermo Scientific), with proteinase K digestion and heat-mediated decrosslinking. DNA was quantified by Qubit fluorometry (Thermo Scientific) and diluted to 0.67 ng/µL. Libraries targeting *BRCA1* and *BRCA2* were prepared on the Ion Chef System (Thermo Scientific) using the Oncomine *BRCA* Research Assay (Thermo Scientific), with 132 to 133 primer pairs per pool and 22 amplification cycles.

Next-generation sequencing and bioinformatics pipeline

Targeted next-generation sequencing of *BRCA1* and *BRCA2* genes was carried out at the National Center for Scientific and Technical Research (CNRST) in Rabat, Morocco, using the Ion Torrent sequencing platform (Thermo Scientific).

Raw reads were trimmed of adapters and low-quality bases with Trimmomatic.[11] Clean reads were aligned to hg38 by BWA-MEM[12], and sorted with SAMtools.[13] PCR duplicates were marked with Picard. Variant calling was performed using FreeBayes.[14] Annotation and effect prediction employed SnpEff[15] and Ensembl VEP.[16]

Variants were classified according to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines into pathogenic, likely pathogenic, variants of uncertain significance (VUS), and benign or likely benign categories. Variants reported with conflicting interpretations of pathogenicity were analyzed cautiously and considered within the VUS spectrum for exploratory analyses.

Statistical analysis and data visualization

Data were imported and managed in Python (version 3.10) using the pandas and NumPy libraries. Continuous variables are presented as median (interquartile range [IQR]) due to the small sample size and the nonnormal distribution of the data, whereas categorical variables are reported as counts and percentages. Group comparisons (relapse vs no relapse) were performed using the Mann-Whitney *U* test for continuous variables and the χ^2 test or Fisher exact test for categorical variables via the SciPy library. Spearman rank correlation was used to assess associations among TIL levels, PD-L1 CPS, and CD marker percentages. Survival analyses, including Kaplan-Meier curves and log-rank tests, were performed using the lifelines software library. Given the limited sample size and the small number of relapse events, multivariable Cox proportional hazards modeling was not performed to avoid overfitting and unstable estimates.

RESULTS

Clinical and biological heterogeneity was observed within the cohort (Table 1). The median age

at diagnosis was 48 years (IQR, 42–54). Tumor size showed a right-skewed distribution, with most lesions measuring 2 to 6 cm and a minority extending up to 16.7 cm (Figure 1). The median tumor size was 4.2 cm (IQR, 2.6–6.8 cm). In addition, 33.3% of tumors were classified as T3, indicating the presence of relatively large primary tumors within the cohort.

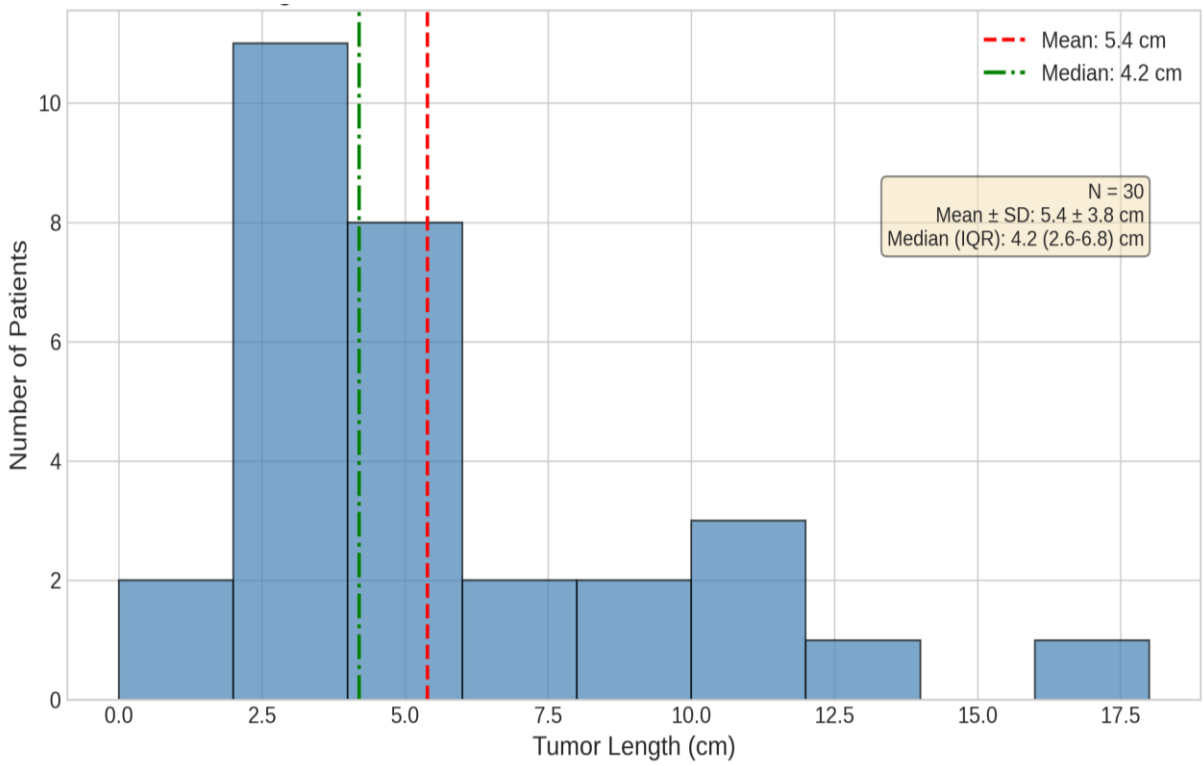

Figure 1. Distribution of Tumor Size in the Moroccan Triple-Negative Breast Cancer Cohort. Histogram shows the frequency distribution of primary tumor size (length) at diagnosis among the 30 patients in the cohort. The green dash-dotted vertical line represents the median tumor length (4.2 cm; interquartile range, 2.6-6.8 cm), and the red dashed vertical line represents the mean tumor length (5.4 cm; SD, 3.8 cm).

Table 1. Clinicopathological Characteristics of the Moroccan TNBC Cohort (N = 30)

Variable	Category	No.	%
Age, y	≤ 40	5	16.7
	> 40	25	83.3
	Median (IQR)	48	(42–54)
Tumor size, cm	Median (IQR)	4.2	(2.6–6.8)
T stage	T1	2	6.7
	T2	18	60.0
	T3	10	33.3
SBR grade[a]	SBR II	3	10.0
	SBR III	27	90.0
Ki-67	> 30%	26	86.7
Vascular emboli	Present	25	83.3
Family history of breast cancer	Yes	12	40.0
Lymph node involvement	Yes	21	70.0
Stromal TILs	Low (< 30%)	23	76.6
	High (≥ 30%)	7	23.3
PD-L1 expression	Positive (CPS ≥ 1)	9	30.0
Relapse	Yes	17	56.7
Follow-up, y	Median (IQR)	5.2	(3.8–6.1)

CPS, Combined Positive Score; IQR, interquartile range; SBR, Scarff-Bloom-Richardson; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer.
[a]SBR grade represents Scarff-Bloom-Richardson histological grade.

Considerable variability in stromal TIL levels was observed across the cohort (Figure 2). TIL levels ranged from 2% to 70%, with a median of 10%, indicating heterogeneous immune infiltration patterns among tumors.

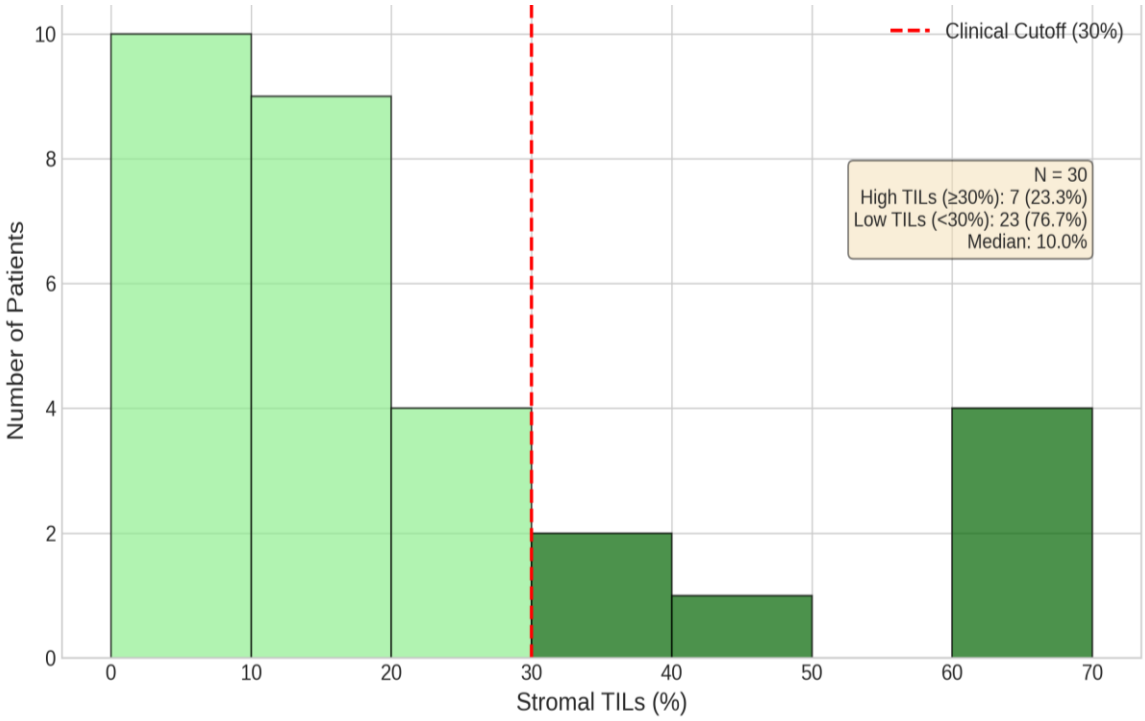

Figure 2. Distribution of Stromal Tumor-Infiltrating Lymphocyte (TIL) Percentages. Histogram illustrating the distribution of stromal TIL percentages across the cohort (N = 30). Light green bars indicate patients classified with low TIL levels (<30%; n = 23 [76.7%]), and dark green bars represent patients with high TIL levels (>=30%; n = 7 [23.3%]). The red dashed vertical line marks the clinical cutoff threshold of 30%. The median TIL level across the cohort is 10.0%. TIL indicates tumor-infiltrating lymphocyte.

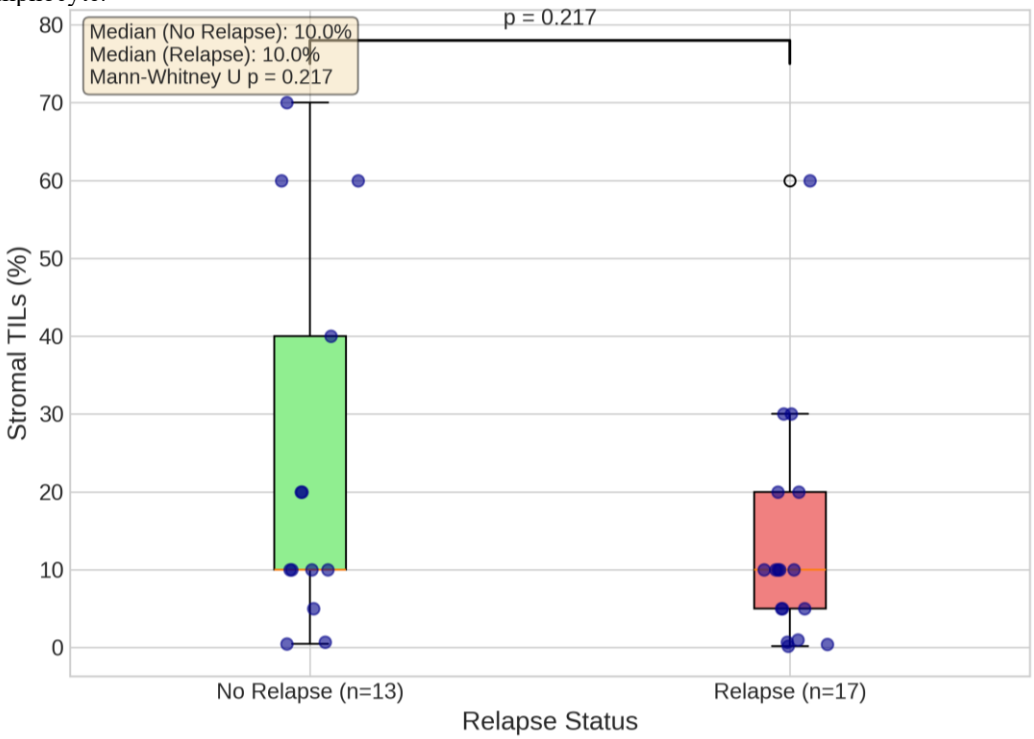

Figure 3. Comparison of Stromal Tumor-Infiltrating Lymphocyte (TIL) Levels by Patient Relapse Status. Boxplot with overlaid individual data points comparing stromal TIL percentages between patients without clinical relapse (n = 13, light green box) and those who relapsed (n = 17, light red box). The median TIL level is 10.0% in both patient subgroups. The difference in TIL levels between the groups was not statistically significant (P = .22; Mann-Whitney U test). TIL indicates tumor-infiltrating lymphocyte.

When stratified by relapse status, nonrelapsed patients tended to show slightly higher and more dispersed TIL levels compared with relapsed patients (Figure 3). However, this difference did not reach statistical significance (Mann-Whitney *U* test, *P* = 0.22; Table 2).

Kaplan-Meier survival analysis further demonstrated no statistically significant difference in relapse-free survival between patients with high vs low TIL levels (log-rank $P = 0.96$) (Figure 4). These findings should, therefore, be interpreted as exploratory and hypothesis-generating, given the limited cohort size.

Table 2. Immune Marker Expression by Relapse Status

Marker	No relapse (n = 13), median	Relapse (n = 17), median	*P* value[a]	Effect size (r)[b]
Stromal TILs, %	10.0	10.0	0.22	0.27
CD3, %	5.0	50.0	0.30	0.22
CD4, %	10.0	30.0	0.75	0.07
CD8, %	30.0	30.0	0.93	0.02
CD20, %	10.0	30.0	0.18	0.29
CD56, %	0.0	0.0	0.82	0.04
PD-L1 CPS	0.0	0.0	0.13	0.27

CPS, Combined Positive Score; TILs, tumor-infiltrating lymphocytes.
[a]Calculated using the Mann-Whitney *U* test. None of the comparisons reached statistical significance ($P \geq 0.05$).
[b]Effect size calculated as $r = Z / \sqrt{N}$.

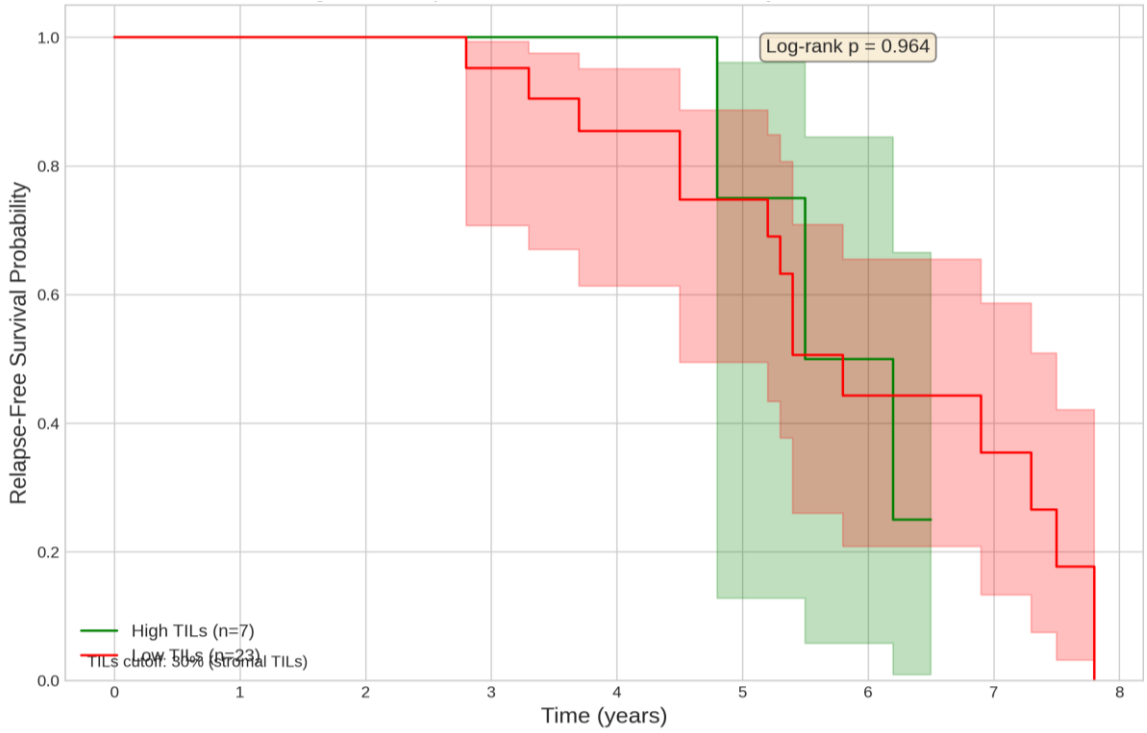

Figure 4. Kaplan-Meier Survival Curves for Relapse-Free Survival Stratified by Stromal Tumor-Infiltrating Lymphocyte (TIL) Levels. Relapse-free survival curves for patients with high stromal TIL levels (>=30%; n = 7, green line) vs low stromal TIL levels (<30%; n = 23, red line). Shaded bands represent the respective 95% CIs. No statistically significant difference in relapse-free survival was observed between the 2 groups (log-rank $P = 0.96$). TIL indicates tumor-infiltrating lymphocyte.

Spearman rank correlation analysis suggested predominantly weak associations among immune markers, consistent with largely independent expression patterns across the cohort. The highest correlation coefficient was observed between stromal TIL levels and CD3 expression ($r = -0.29$) (Figure 5). However, because the correlation coefficient was modest ($|r| < 0.30$), this finding should be interpreted cautiously within the context of the limited cohort size. The observed negative correlation between TIL levels and CD3 expression may reflect variability in semiquantitative immunohistochemical scoring or sampling heterogeneity rather than a true biological inverse relationship.

In addition, descriptive comparisons suggested a possible trend toward lower expression levels of CD56 (natural killer cell marker) and CD20 (B-cell marker) among patients who experienced relapse (Table 2); however, these differences did not reach statistical significance.

Variant analysis from targeted sequencing (Table 3) identified both germline and somatic *BRCA1* and *BRCA2* alterations in the cohort. A total of 30 tumor samples were successfully sequenced, revealing a

heterogeneous spectrum of variant types, including missense, frameshift, splice-site, and UTR variants. Immune marker expression varied across tumors carrying different variant categories, reflecting the biological diversity expected in triple-negative breast cancer.

Because multiple variants could be detected within the same tumor sample, the total number of variants exceeds the number of patients. Therefore, variant counts should not be interpreted as patient-level frequencies.

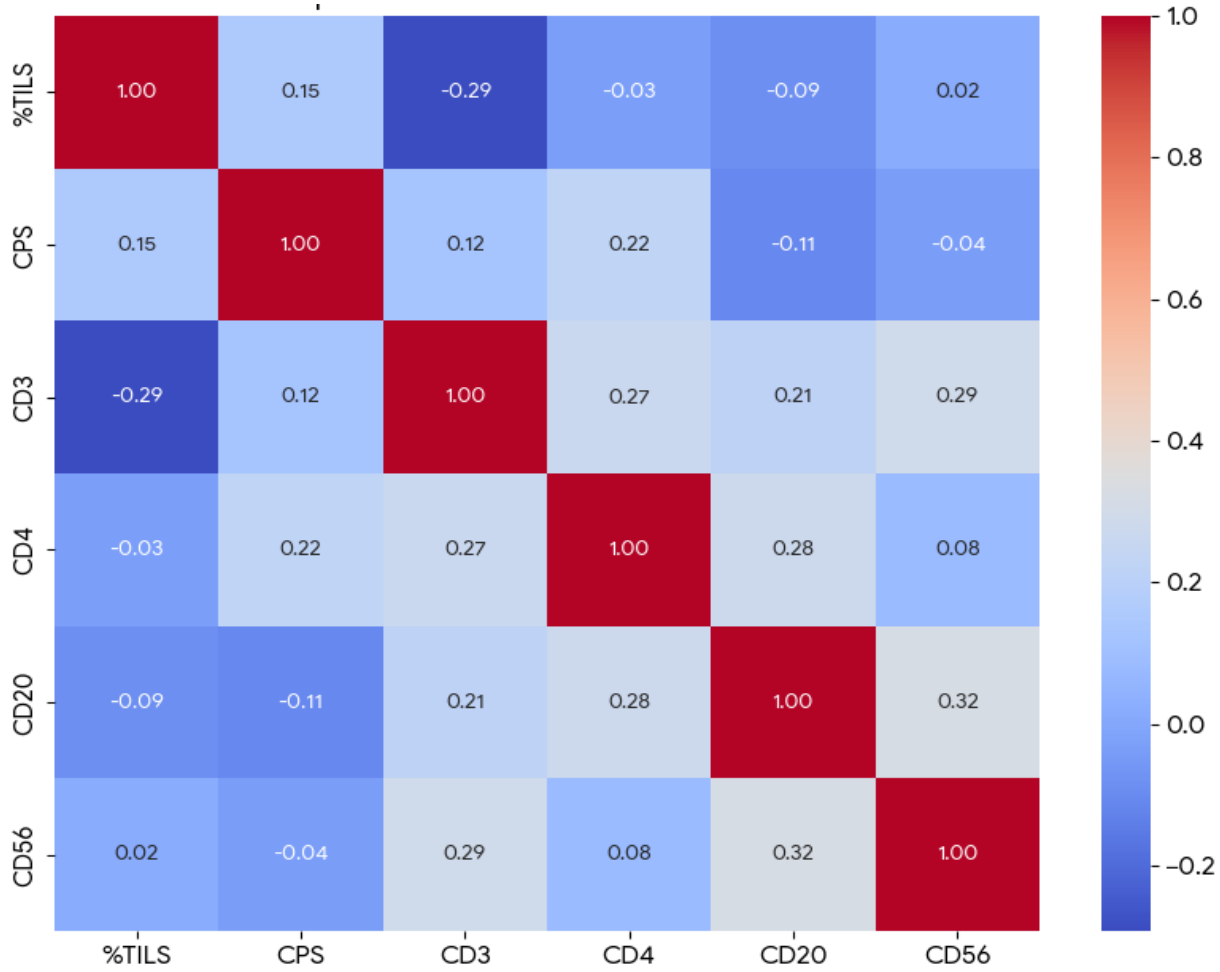

Figure 5. Spearman Correlation Matrix of Stromal Tumor-Infiltrating Lymphocyte (TIL) Levels, PD-L1 Combined Positive Score (CPS), and Immune Markers. Heatmap illustrating Spearman rank correlation coefficients (r) among stromal TIL levels (%TILS), PD-L1 CPS, and specific immune cell subset markers (CD3, CD4, CD20, and CD56) in the tumor microenvironment. The color bar on the right indicates the direction and strength of the correlation (red represents a positive correlation; blue, a negative correlation). CPS indicates Combined Positive Score; PD-L1, programmed death-ligand 1; TIL, tumor-infiltrating lymphocyte.

Table 3. Detected BRCA1 and BRCA2 Variants and Associated Immune Marker Expression in the Moroccan TNBC Cohort

Gene	Consequence	No. of variants[a]	Relapse, %	Median TILs, %	Median CD8, %
BRCA1	Frameshift	18	44.4%	15%	35%
BRCA1	Stop gained	12	50.0%	12%	35%
BRCA1	Splice site	9	33.3%	10%	30%
BRCA1	Missense	20	50.0%	15%	35%
BRCA2	Frameshift	19	47.4%	12%	35%
BRCA2	Missense	17	52.9%	15%	40%

TILs, tumor-infiltrating lymphocytes.
[a]A single tumor may harbor more than 1 variant; therefore, the total number of variants exceeds the number of patients.

Two relatively frequent 5'-UTR variants showed nonsignificant trends that may suggest a potential association with relapse. The *BRCA2* variant chr13:32 316 461:C>T was identified in 22 patients, including 10 relapse cases ($P = 0.09$), whereas the *BRCA1* variant chr17:41 276 045:T>C was detected in 19 patients, including 8 relapsed cases ($P = 0.06$). Because these associations did not reach statistical

significance, they should be interpreted cautiously and considered exploratory observations rather than definitive prognostic markers.

Exploratory analyses were also performed to investigate potential associations between genomic variants and CD56 expression, a marker of natural killer (NK) cell infiltration. Several genomic loci located within the *BRCA2* region on chromosome 13 and the *BRCA1* region on chromosome 17 showed nominal associations with CD56 expression (Supplementary Table S1). Some variants demonstrated low nominal *P* values, and a few remained statistically significant after Bonferroni correction; however, given the very small number of variant carriers, these findings should be interpreted with extreme caution and considered exploratory. These analyses are based on very small subgroups and are therefore not sufficient to support robust statistical conclusions. The observed findings may suggest potential associations, but they remain hypothesis-generating and require validation in larger independent cohorts.

Overall, although *BRCA1* and *BRCA2* variants were relatively common in this cohort, their associations with immune markers and relapse outcomes were heterogeneous and did not reach strong statistical significance. These findings highlight the complexity of the immunogenomic landscape of TNBC and underscore the need for larger studies integrating genomic and immune profiling in diverse populations.

DISCUSSION

This study provides an integrated description of clinical, immunological, and genomic characterization of Moroccan TNBC, emphasizing the population's marked biological heterogeneity as well as its aggressive clinical presentation. Given the retrospective analytical case-series design and the limited cohort size, the findings should be interpreted as exploratory and hypothesis-generating rather than confirmatory. These results provide region-specific insights that supplement current global knowledge on TNBC prognosis by integrating immune profiling with targeted genomic analysis.[1]

The case series was clinically characterized by advanced disease at diagnosis, with a median tumor size of 4.2 cm and 61% of patients presenting with stage III to IV tumors. These findings are in line with earlier reports from populations in North and West Africa[2–5,18], but they differ from current Western series that report smaller tumor sizes at diagnosis. Such patterns likely reflect a multifactorial process involving aggressive tumor biology as well as systemic delays in diagnosis related to socioeconomic factors, limited access to screening programs, and public health awareness. The comparatively young median age at diagnosis is also consistent with demographic patterns observed in African American populations with TNBC.[18]

Immune infiltration was explored as a potential prognostic factor in this retrospective case series. In line with the International TILs Working Group recommendations, higher stromal TIL levels were observed among patients without relapse, although this difference did not reach statistical significance in the present cohort. This observation appears to be consistent with previous reports suggesting a prognostic role of TILs in TNBC.[6]

However, the wide spectrum of TIL values observed—from immune-cold tumors to highly inflamed cases—underscores the marked heterogeneity of the tumor immune microenvironment.

Correlation analysis revealed predominantly weak associations among immune markers ($|r| < 0.30$). This may suggest that TIL levels, PD-L1, CD20, and CD56 capture complementary but largely independent aspects of the tumor immune microenvironment. The observed negative correlation between stromal TIL levels and CD3 expression should be interpreted with caution, as it may reflect methodological variability in semiquantitative immunohistochemical assessment, sampling heterogeneity, or the limited sample size. This finding therefore remains exploratory and requires validation in larger cohorts.

These findings are in line with the growing consensus that integrated immune profiling offers a more thorough evaluation of tumor-immune interactions and that depending solely on one immune biomarker may not be adequate for precise risk stratification.[1] This study also suggests the possible relevance of non-T-cell immune populations, moving beyond T-cell-centered analyses. Lower CD56 and CD20 expression levels were observed among relapsed patients in descriptive analyses, although these differences did not reach statistical significance, which may suggest that a more immunosuppressive tumor microenvironment could be associated with decreased NK cell and B-cell infiltration. These observations may extend previous findings to a TNBC cohort in North Africa and are in line with new evidence linking NK and B cells to effective antitumor immunity.[19] While therapeutic implications remain speculative, these results emphasize the importance of considering diverse immune compartments when evaluating prognosis.

Exploratory analyses suggested potential immunogenomic associations, particularly between certain *BRCA2* variants and CD56 expression.

Although some variants demonstrated low nominal *P* values, these observations were based on a small number of variant carriers and should therefore be interpreted cautiously. These findings may indicate a possible association between tumor genetic alterations and NK-cell infiltration patterns in TNBC, but remain hypothesis-generating and require validation in larger independent cohorts. The results of the variant-specific analyses (Supplementary Table S1) should be interpreted with caution due to the very limited number of carriers per variant, and should be considered exploratory. Functional studies will also be necessary to determine whether these variants are associated with or potentially involved in NK-cell recruitment, or whether they reflect indirect associations within the tumor microenvironment. At this stage, no causal relationship can be inferred from these findings.

The prevalence of *BRCA1* and *BRCA2* variants in this cohort (28%) is consistent with previous reports from North African and Arab populations[2–5], indicating a substantial hereditary contribution to TNBC in this region. Notably, immune profiling showed different trends based on *BRCA* status. Tumors harboring *BRCA1* variants tended to display a more homogeneous immune-inflamed profile within this limited cohort and were not associated with relapse during follow-up. This finding appears to be consistent with previous research suggesting that *BRCA1* deficiency may be associated with an increased tumor immunogenicity subtype of TNBC[10] and may contribute to the favorable prognostic profile observed in these cases.

High-frequency *BRCA2* 5′-UTR variants, on the other hand, showed nonsignificant trends toward association with relapse, underscoring the variable prognostic influence of *BRCA* mutations. These results may suggest that variant functional class could be important for prognostic interpretation and that *BRCA1* and *BRCA2* variants may have different biological and immunological effects. Furthermore, a complex interaction between inherited susceptibility and acquired tumor evolution shapes prognosis, as evidenced by the enrichment of somatic alterations affecting immune regulatory pathways among relapsed patients. Overall, these findings highlight the importance of integrated immunogenomic approaches for understanding TNBC heterogeneity, particularly in populations with different genetic backgrounds.[1,20]

Taken together, these findings highlight the complexity of TNBC immunogenomics and underscore the importance of integrating genomic and immune biomarkers when studying tumor heterogeneity.

Several limitations should be acknowledged. First, the small sample size (n = 30) limits the statistical power to detect associations and precludes multivariable modeling. Second, as a single-center study, findings may not be generalizable to other populations. Third, the retrospective design introduces potential selection bias. Fourth, interobserver agreement for TIL assessment was not formally evaluated. Fifth, functional validation for noncoding variants is lacking. Finally, treatment heterogeneity may confound some associations. Due to these limitations, all findings should be interpreted as hypothesis-generating and require validation in larger, prospective cohorts.

CONCLUSION

In conclusion, this integrated analysis suggests the potential prognostic relevance of the tumor immune microenvironment in Moroccan TNBC patients. Higher stromal TIL levels were observed among patients without relapse, along with descriptive patterns involving CD56 and CD20 expression and a possible *BRCA1*-associated immune-inflamed profile. These findings highlight the potential value of integrated immune profiling that combines global immune infiltration, specific immune cell subsets, and *BRCA1* and *BRCA2* variant status. Although these results require validation in larger cohorts, they provide preliminary insights into the immunogenomic landscape of TNBC in North African populations and may help refine future risk stratification strategies.

ETHICAL CONSIDERATIONS

This study was conducted in accordance with the ethical standards of the institutional and national research committees and with the Declaration of Helsinki (1964) and its later amendments. Ethical approval was obtained from the Ethics Committee of Ibn Rochd University Hospital, Casablanca, Morocco (approval number: 13/2022).

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

CONFLICT OF INTERESTS

The authors declare no competing interests.

FUNDING

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AI DISCLOSURE

Artificial intelligence tools were used solely for language editing and stylistic improvement. All scientific content was produced by the authors, and all data analysis, and interpretations were also performed by them.

AUTHOR CONTRIBUTION

AE: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft; AIA: Investigation, Software, Formal analysis; LA: Formal analysis, Methodology; OB: Investigation, Data curation; ZK: Investigation, Validation; KK: Investigation, Data curation; MK: Supervision, Writing – review & editing.

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