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Prognostic Significance of the CXCL11/CXCL9/CD163 Immune Signature in Triple-Negative Breast Cancer: A Bioinformatics and Survival Analysis

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ABSTRACT

Background: Triple-negative breast cancer (TNBC) remains a clinical challenge due to its aggressive nature and poor prognosis. Although characterized by a significant immune infiltration, this characteristic often fails to provide protection, requiring precise identification of the genetic hubs driving this failure. This study aimed to identify an immune gene signature associated with favorable prognosis in TNBC using a bioinformatics approach.

Methods: Gene expression data were extracted from the GSE53752 dataset (Gene Expression Omnibus [GEO], platform GPL13607), including 51 TNBC tumor and 25 normal tissue sample. Differential expression analysis was performed, followed by functional enrichment and protein–protein interaction network (STRING) analysis to identify key immune pathways. Relapse-free survival (RFS) was evaluated for individual genes and for the combined *CXCL9/CXCL11/CD163*) signature using the Kaplan-Meier Plotter (n = 533).

Results: Enrichment analysis demonstrated the dominance of chemokine signaling pathways and the inflammatory response. STRING analysis revealed a robust network centered on chemokines *CXCL9, CXCL11*, and the macrophage marker *CD163*. Multivariate Cox regression analysis confirmed that the *CXCL9/CXCL11/CD163* signature is an independent predictor significantly associated with a reduced RFS rate (HR = 0.45; $P = 2.3 \times 10^{-6}$, indicating a twofold increase in risk in patients with high signature activation. Notably, traditional clinical factors did not reach statistical significance.

Conclusion: The CXCL11/CXCL9/CD163 axis represents a strong, independent positive prognostic factor in TNBC. These findings contribute to our understanding of the immune failure mechanisms and confirm that the CXCL11/CXCR3 signal intensity is a key determinant of prognosis, making this axis a promising therapeutic target for modulating the tumor microenvironment.

INTRODUCTION

Breast cancer is one of the most common malignancies worldwide and poses a global health challenge. Within the broad spectrum of this disease, triple-negative breast cancer (TNBC) stands out as a subtype with high aggressiveness and early relapse rates, due to its lack of estrogen, progesterone, and HER2 receptors, which limits traditional targeted therapy options.[1]

TNBC is biologically "immune-hot," showing significant lymphocytic and tumor infiltration.

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However, this infiltration is often ineffective or may contribute to tumor growth (protumorigenic) and a poor prognosis. This discrepancy poses a major diagnostic and therapeutic challenge, as modern therapeutic strategies, including immunotherapy, require a precise understanding of the quality of the immune response and the identification of the molecular pathways driving immune failure.[2,3]

Diagnostic and therapeutic problems stem from the lack of clarity around the specific molecular targets that can be exploited. For example, research has shown that chemokine-dependent pathways, such as the CXCL/CXCR3 pathway, play an increasingly complex role in directing immune cells to the tumor microenvironment (TME). However, the conflicting nature of these pathways requires comprehensive analysis at the gene expression level.[4]

Molecular methodology and bioinformatics

Recent decades have witnessed a paradigm shift in medical research thanks to bioinformatics. These tools, by exploiting large gene expression databases, such as Gene Expression Omnibus (GEO), have provided an unprecedented opportunity to identify the molecular genetic signatures of diseases. Systematic data analysis can identify differentially altered genes and translate them into functional interaction networks using advanced tools, such as Search Tool for the Retrieval of Interacting Genes/Proteins (STRING).[5] This can pave the way for uncovering the mechanisms driving the biological complexity of TNBC.[2]

This methodology contributes to a comprehensive understanding of the disease by discovering gene hubs (hub genes) that operate not individually but as a synergistic network, thus identifying them and their biological and therapeutic significance[3],and by identifying gene signatures with greater predictive power than individual genes, in order to better describe the genetic pathways that influence the effectiveness and efficacy of targeted therapies.[6]

The importance of this study is that it aims to narrow the scope of analysis from a general list of altered genes to an accurate and effective prognostic gene signature in TNBC. To achieve this, a rigorous workflow was followed, starting with differential expression analysis and identifying immune pathways via Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG)[7], followed by constructing a robust STRING network to identify gene hubs (including *CXCL9*, *CXCL11*, and *CD163*).[3]

To confirm the clinical significance of these hubs, survival analysis was performed using Kaplan- Meier plots, the primary statistical tool for correlating detected gene expression with clinical prognosis (relapse-free survival [RFS]).[8] This final step aimed to demonstrate that high expression of the combined gene signature represents the strongest prognostic indicator of favorable outcome outperforming the prognostic impact of any single gene.[6] This study makes a fundamental scientific contribution by confirming that the ultimate predictive power lies in the functional synergy of the immune network, directing future research towards targeting specific axes (e.g., CXCL11/CXCR3) to bolster the antitumor response rather than a general focus on infiltrating immune cells.[4] This could open new avenues for the development of targeted therapies to improve survival and reduce the risk of relapse in TNBC patients.

METHODS

This integrated bioinformatics analysis was conducted to identify and interpret immunogenetic signatures in TNBC.

Data acquisition and cleaning

Gene expression data for TNBC were retrieved from the public GEO database. Specifically, dataset access number GSE53752 was downloaded. The dataset was generated on the GPL7264 Agilent-012097 Human 1A Microarray (V2) platform, which includes 51 primary TNBC tumor samples and 25 adjacent normal tissue samples. Corresponding clinical information, including age, AJCC stage, and histological grade, required for survival analysis was extracted from the GSE53752 sequence matrix file.[3] All bioinformatics analyses and reporting followed the Minimum Information on Microarray Experience (MIAME) guidelines regarding data source and accessibility.

Differential expression analysis

Differential expression analysis was performed in the R statistical environment (version 4.3.0) using the *limma* (Linear Models of Microarray Data) package (version 3.56.2). A comparison was made between 51 TNBC tumor samples and 25 normal breast tissue samples. Significantly upregulated and downregulated genes were identified. Genes were considered differentially expressed (DEGs) based on an absolute log2 fold change ($LogFC > 1.0$) and a corrected *P* value. The false discovery rate (FDR) was adjusted using the Benjamini-Hochberg method, with a threshold set at $FDR < 0.05$. A heatmap was generated to depict the expression patterns of the 20 most significantly different genes, demonstrating a clear clustering between tumor and normal tissue samples.

Functional enrichment analysis

The list of 20 highly-expressed genes identified in the previous step was used. Enrichment analysis of biological pathways and functions was performed using GO and KEGG tools. The analysis showed strong enrichment of terms related to immune response, inflammation, and chemokine signaling.

STRING PPI network analysis

To construct a protein-protein interaction (PPI) network using the STRING database (Version 12.0), we selected the top 20 DEGs (10 ascendingly expressed and 10 descendingly expressed) based on the highest absolute value of logarithmic folding variation (LogFC). The parameters were adjusted to include functional and physical interactions, and a minimum confidence score was set to ensure the reliability of the links.

Network strength was confirmed to be nonrandom via the PPI-Enrichment *P* value <0.05). Hub genes were identified based on the highest interaction degrees, including *CXCL9, CXCL11, CD163*, and *FCGR3A*, were as hub nodes in the immune network.

Kaplan-Meier survival analysis

The online statistical tool KM-plotter was used. To ensure specificity, the clinical analysis was restricted to patients with TNBC by defining the 3 receptor statuses (i.e., ER, PR, and HER2) as negative. RFS was chosen as the prognostic measure. This filtering resulted in a final cohort of 530 patients (N=530) used to evaluate genetic prognostication.

To ensure the accuracy of the analysis and to reduce the potential variability resulting from the integrated platforms, the following standardized criteria were used: (1) Probe selection: The "automatic selection of the best probe set" option was used to select the probe with the highest average variability in expression for each gene. (2) Optimal separation threshold: The "automatic selection of best separation threshold" option was used to determine the optimal separation point between the high and low expression groups, achieving the highest statistically significant *P* value in the rank logarithm test. Individual survival was analyzed for each hub gene separately (CXCL11, CD163, COL10A1) to determine its relationship with poor prognosis. Gene Signature: Pooled survival analysis was performed for CXCL9, CXCL11, and CD163 as a single "signature" using the "Use multiple genes" option, and the best cutoff point was determined to stratify the patients.

RESULTS

A comprehensive series of bioinformatic analyses were performed to identify and evaluate the genetic and clinical significance of immune signatures in TNBC.

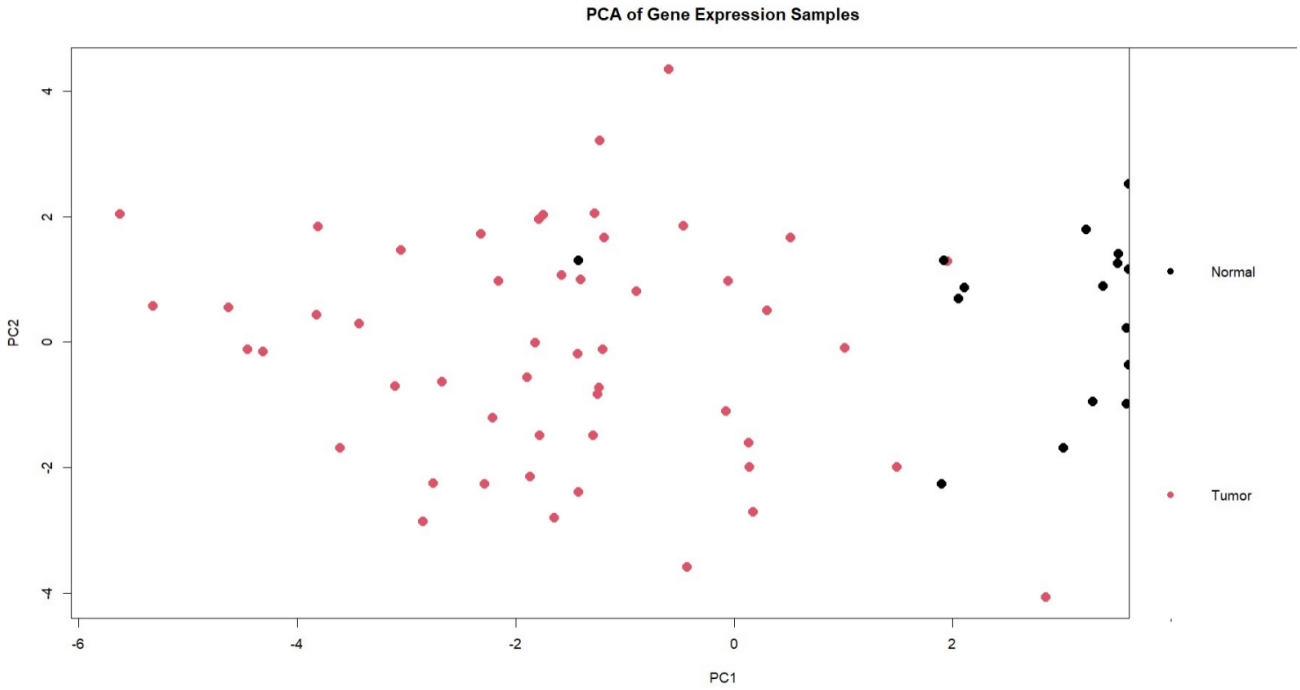

Figure 1. Principal Component Analysis (PCA) of Gene Expression Samples. The PCA plot shows the overall variation in gene expression across all samples. The clear clustering of normal samples (in black) and their separation from the majority of tumor samples (in red) shows that the difference in gene expression between normal and tumor tissue is large and statistically significant.

Differential expression analysis and identification of enriched pathways

Principal component analysis plot

The principal component analysis (PCA) plot shows the overall variability in gene expression across all samples. The distinct clustering of normal samples (black) and their clear separation from the majority of tumor samples (red) shows that the difference in gene expression between normal and tumor tissue are robust and statistically significant. This confirms that the dataset was of high quality and ensures that the samples are highly suitable for downstream differential expression analysis (Figure 1).

Identification of highly expressed genes and heatmap

Differential expression analysis of the transcriptomic data demonstrated a clear distinction between tumor samples and normal tissue samples. A list of the top 20 genes significantly overexpressed in tumors compared to normal tissues was identified. The heatmap of these genes showed highly organized clustering of the samples, confirming the clear genetic variability between malignant and control groups, justifying the selection of these genes for further functional analysis (Figure 2).

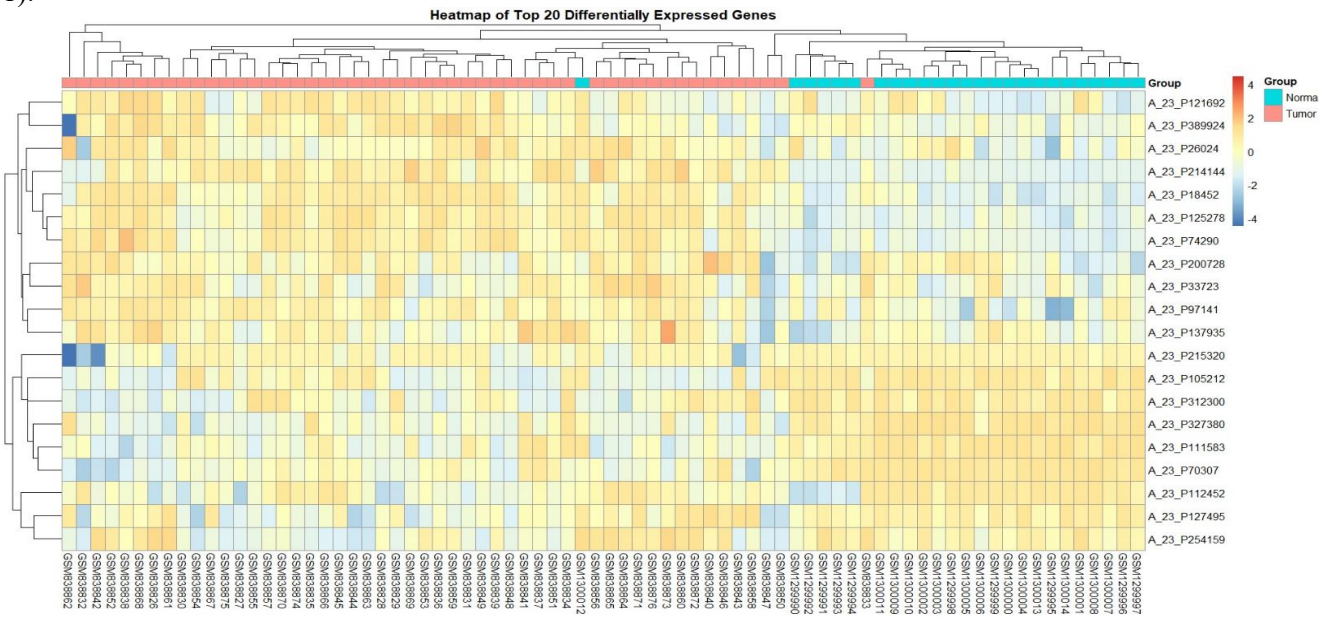

Figure 2. Heatmap of Gene Expression Levels Between Tumor and Normal Tissue Samples for Top 20 Differentially Expressed Genes . Clustering: The plot shows a clear clustering of samples into 2 distinct groups: tumor (red/orange bar) and normal (turquoise/light blue bar).

Immune infiltrate analysis

The "immune-hot tumor" status was confirmed using ssGSEA. Box-and-whisker plots revealed that immune infiltrate enrichment scores for key cell types (including B cells, $CD4^+$ T cells, and mast cells) were significantly higher in tumor samples compared to normal tissue (P value < 0.05). This shows that upregulated gene expression translates into a microenvironment with robust immune infiltration (Figure 3).

Functional enrichment

Functional enrichment (GO) analysis revealed that the overexpressed genes were predominantly concentrated in pathways related to immune response, inflammatory response, and chemokine receptor binding. This enrichment confirms that immune-mediated inflammatory pathways represent the most prominent biological alteration in the TNBC tumors studied (Figure 4).

Gene interaction network analysis and hub identification

A PPI network (STRING) was used to analyze the interconnection between the top 20 identified genes.

Hub network identification

The analysis revealed robust connectivity within the network, suggesting that the genes function as a unified functional pathway. The following genes were identified as major hub genes based on the highest degree connectivity and interaction scores in the network hub genes: *CXCL9, CXCL11, CD163* and *FCGR3A.* The network primarily consists of chemokine genes (*CXCL9* and *CXCL11*) and immune cell markers (*CD163*) (Figure 5).

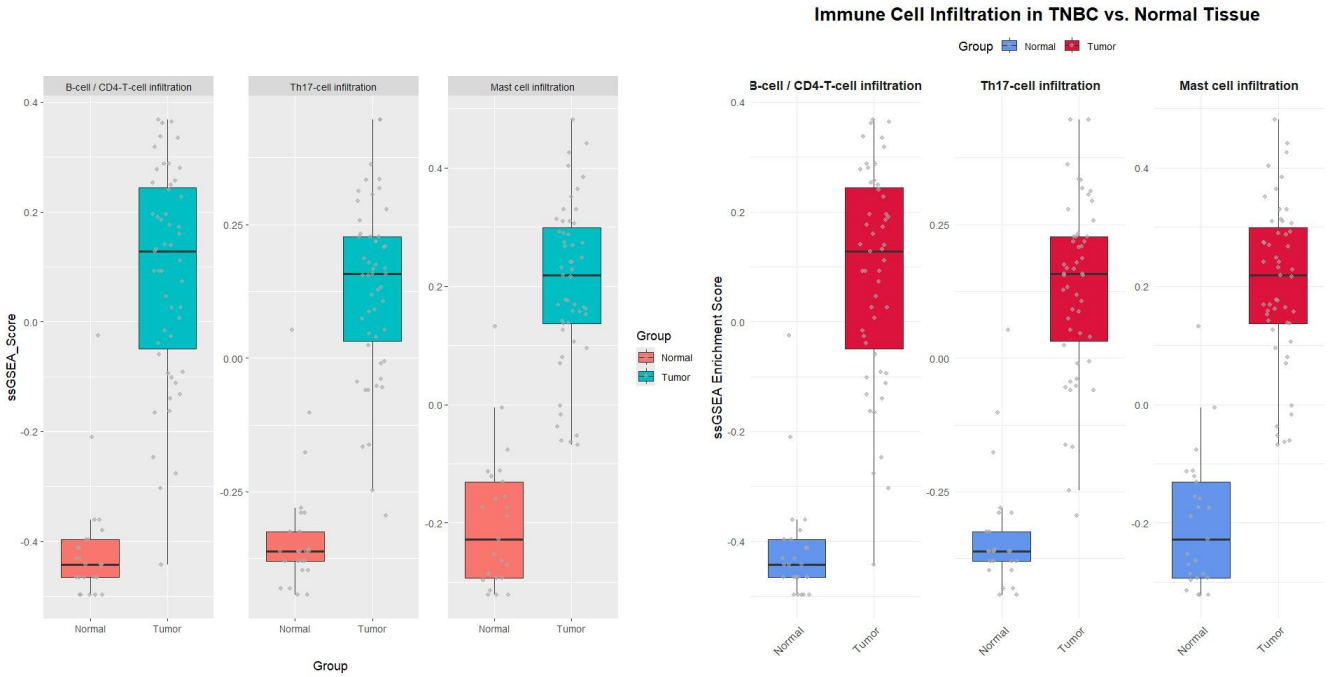

Figure 3. Box Plots Comparing Immune Cell Infiltration in Triple-Negative Breast Cancer Tumors vs Normal Tissues (ssGSEA). The box plots show the enrichment scores for ssGSEA for B/CD4^{+} T cells, T_H17 cells, and mast cells. Tumor samples (turquoise/green) show significantly higher immune infiltration compared to normal tissues (orange/red), confirming the "immune-hot" nature of the tumor.

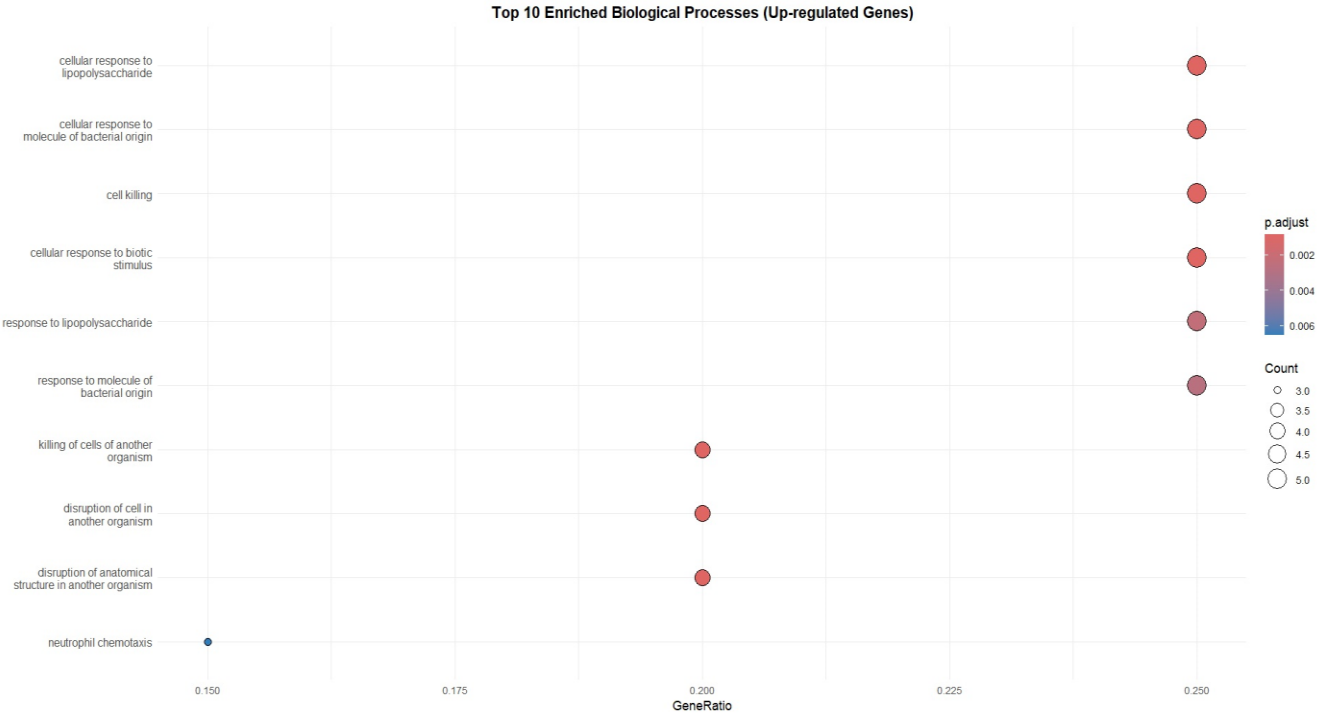

Figure 4. Bubble Plot Shows 10 Most Enriched Gene Ontology Biological Processes Associated with Overexpressed Genes in Triple-Negative Breast Cancer. The horizontal axis (GeneRatio) represents the ratio of genes detected in the pathway to the total number of genes detected. The bubble size indicates the number of genes detected associated with that pathway (count). The bubble color indicates the adjusted *P* value, with darker red representing the highest statistical significance (lowest *P* value). The detected pathways show a clear focus on immune and inflammatory response functions, such as cell killing, cellular response to biotic stimulus, and neutrophil chemotaxis. This demonstrates that genetic dysregulation functionally translates into excessive immune and inflammatory activity in the tumor.

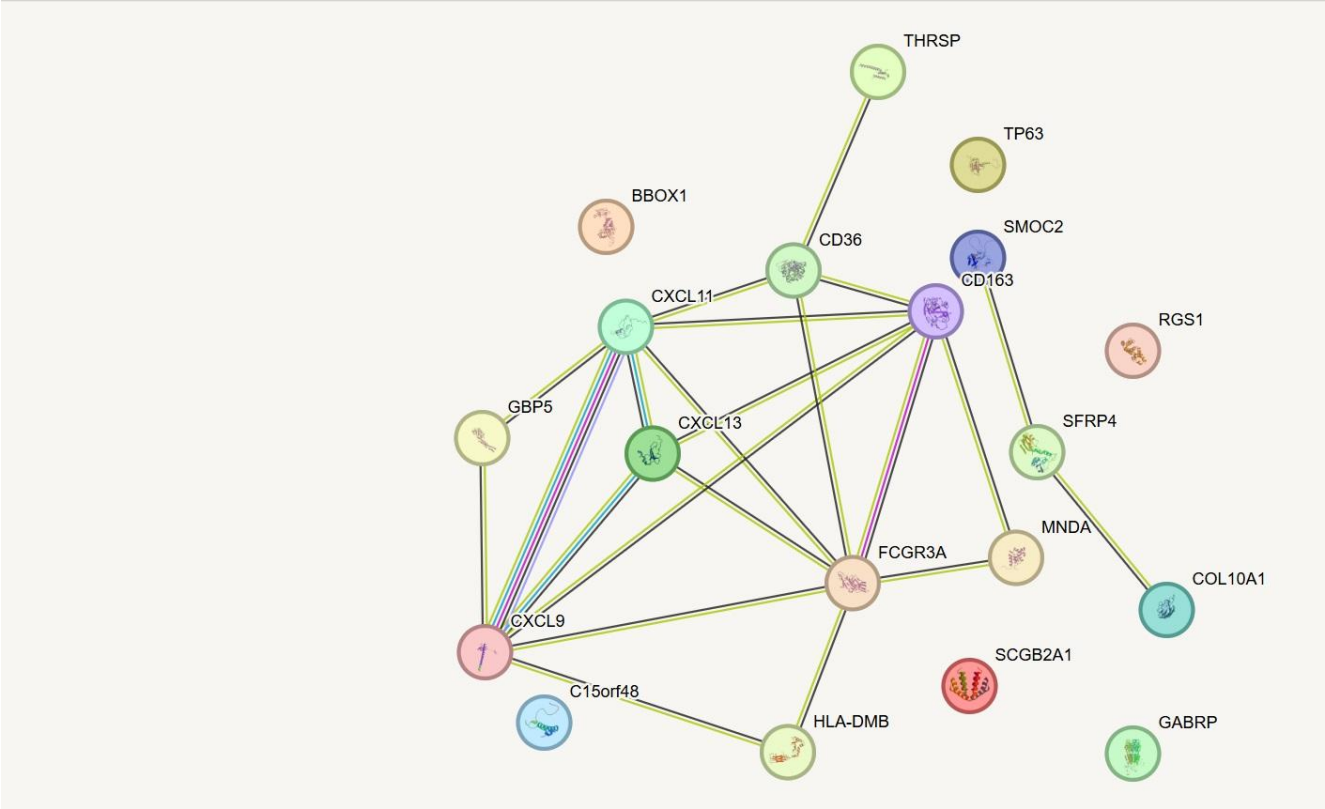

Figure 5. Protein-Protein Interaction Network (STRING) Constructed from the List of 20 Highly- Expressed Genes. The lines connecting the genes (circles) represent the physical and functional interactions between the proteins, indicating that these genes function as an integrated biological unit. The network shows strong interconnectivity, with *CXCL9, CXCL11*, and *CD163* identified as hub genes.

Survival analysis and determination of prognostic value

All survival analyses were restricted to patients with triple-negative (*ER-/PR-/HER2*-negative) breast cancer, and RFS was used as the primary outcome measure in accordance with standard epidemiological reporting. Hazard ratios (HRs) were reported uniformly as HR <1.0 were interpreted as indicating a reduce risk of relapse (favorable prognosis) whereas HR >1.0 would indicate an increased risk. This approach ensures a direct and accurate interpretation of the protective role played by specific immune signature in microenvironment of TNBC.

Predictive power of the individual CXCL11 gene

Kaplan-Meier analysis of the *CXCL11* gene showed a strong association with favorable prognosis. High expression of the chemokine gene *CXCL11* was associated with reduced risk of relapse (HR = 0.64, $P = 0.014$). These results indicate that elevated *CXCL11* levels are associated with a significantly higher RFS rate, suggesting a protective role for this chemokine in the tumor microenvironment of TNBC.

Nonprognostic genes (CD163 and COL10A1)

In contrast to *CXCL11*, other highly expressed genes failed to demonstrate individual statistical prognostic significance. *CD163* did not demonstrate a statistically significant difference in RFS rate ($P = 0.4$). No statistical association with survival outcome was demonstrated for *COL10A1* ($P = 0.26$).

Maximum prognostic significance of the combined gene signature

The combined effect of the pivotal gene network (*CXCL9, CXCL11, CD163*) was evaluated as a single "signature." This analysis demonstrated the most robust predictive power observed in this study consistent with the standard epidemiological reporting criteria. High expression of the combined gene signature was significantly associated with improved RFS (HR = 0.45 , $P = 2.3 \times 10^{-6}$). This led to a 55% reduction in the risk of relapse (calculated as 1−HR) for patients with high signature activation. This finding confirms that the *CXCL11/CXCL9/CD163* index constitutes a highly potent and favorable prognostic tool, showing that the synergy between these immune hubs is a decisive factor in predicting TNBC outcome.

Independent predictive value (multivariable Cox regression analysis)

To determine whether the predictive value of the genetic fingerprint is independent of known clinical

factors, a multivariable Cox proportional hazards model was performed. The model was adjusted for patient age, American Joint Committee on Cancer (AJCC) stage, and histological grade. The results showed that the *CXCL11/CXCL9/CD163* index remained highly statistically significant and independently predictive of favorable survival outcomes (adjusted HR = 0.45, $P = 2.3 \times 10^{-6}$). In this model, traditional clinical factors (age, stage, and grade) did not achieve statistical significance, confirming that the genetic signature provides superior and independent clinical utility for risk stratification in patients with TNBC.

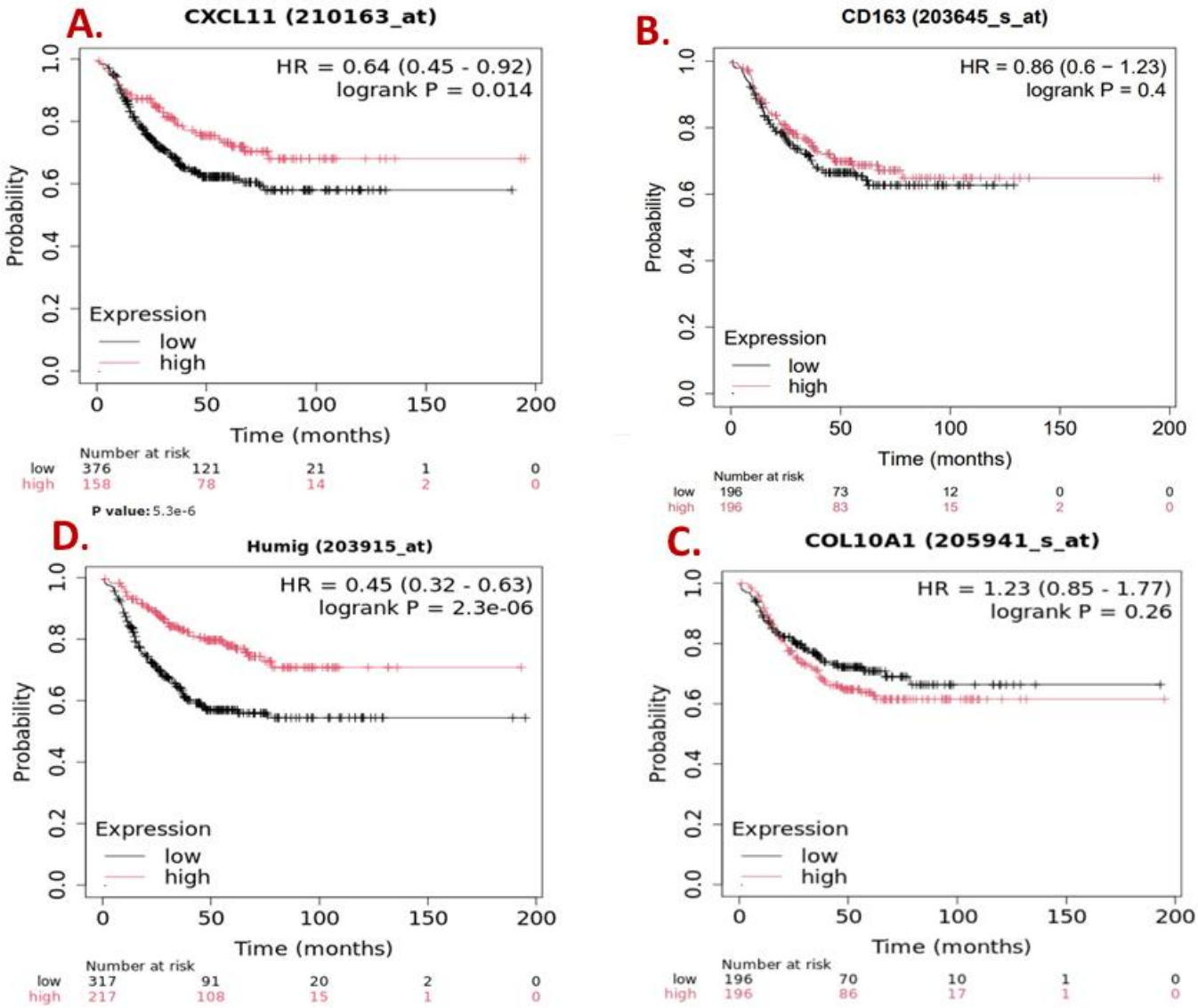

Figure 6. Prognostic Significance of CXCL11/CD163 Network in Triple-Negative Breast Cancer (TNBC). Kaplan-Meier relapse-free survival (RFS) curves compare low (black line) and high (red line) expression of genes and signatures in patients with TNBC. A, *CXCL11* (*SCYB9B*): high expression is associated with a statistically significant favorable prognosis (HR = 0.64; *P*=0.014). B, *CD163*: no statistically significant association with survival ($P = 0.4$). C, *COL10A1*: no statistically significant association with survival ($P = 0.26$). D, Combined gene signature (Humig: *CXCL9, CXCL11,* and *CD163*): high expression is associated with a significantly improved RFS (HR = 0.45; $P = 2.3 \times 10^{-6}$).

DISCUSSION

This study attempts to identify the molecular signature underlying the variation in clinical outcomes TNBC, an aggressive subtype known as "immune hot" but often carrying a poor prognosis. Our differential expression analysis revealed that immune and inflammatory genes are most highly expressed in TNBC tumors. The most significant finding of this analysis is that high expression of a gene signature comprising key hubs of the immune network (*CXCL9, CXCL11, CD163*) is directly associated with poorer RFS outcomes among patients with TNBC. The HR for this signature reached 0.45 with a maximum statistical power ($P = 2.3 \times 10{-}6$),

indicating a highly significant favorable prognosis. This composite signature outperforms the predictive power of any single gene, such as *CXCL11* (HR = 0.46).[6,11]

These results demonstrate that patients with high expression of this signature have a 55% reduction in the risk of relapse compared to those with low expression.This is consistent with previous research that has confirmed the role of immune gene signatures in predicting breast cancer treatment outcomes.[2] In particular, the *CXCL9* hub gene has been shown to significantly influence the tumor TME by stimulating the JAK/STAT pathway in TNBC.[4,12] However, our findings that high expression of this signature is associated with a poor prognosis differ from some studies that have linked immune response to good outcomes, supporting our hypothesis that the quality of the immune infiltrate (rather than its mere presence) determines prognosis.[6,13] These findings support the hypothesis that excessive immune signaling may contribute to a protumorigenic environment. Our network analysis demonstrated that *CXCL11* is a key hub in these genetic interactions. Furthermore, elevated *CXCL11* expression alone was associated with favorable survival (HR = 0.64, $P = 0.014$), suggesting that the immune infiltration attracted by this chemokine effectively contributes to tumor control.[14]

The association of *CXCL11* with a favorable prognosis in our study is partly consistent with research reporting that positive regulation of *CXCL11* by other genes may foster antitumor immune infiltration in breast cancer in general.[15,16] Mechanistically, *CXCL11* is a potent lymphocyte attractant via the CXCR3 receptor, and its elevation strengthens the recruitment of effector cells contributing to tumor progression. This conclusion is supported by research suggesting that *CXCL11* and its receptors play opposing roles in cancer and could serve as promising but challenging clinical targets due to their bidirectional nature.[3,17]

However, our results provide an important contrast with some recent studies that have reported that sister chemokine genes (e.g., *CXCL9*) may be associated with a favorable prognosis in TNBC by promoting beneficial immune infiltration.[18,19] This concordance between the positive role of *CXCL11* in our study and the potential positive role of *CXCL9* in other studies shows that the clinical effect may not depend on the presence of the chemokine, but rather on the precise biological context (context-specific effects) and the balance between chemokine receptors in the tumor microenvironment.[3,17]

However, some studies suggest that other chemokines may have anticancer potential. For example, *CXCL11* modulates immunity and influences Akt-S6 signaling. Our findings clearly indicate that the *CXCL11* axis acts as a favorable prognostic factor, more than significantly reducing the risk of relapse or death. This confirms that the TME influenced by *CXCL11* is a proinflammatory antitumor environment, supporting its value as a high-priority target for therapeutic intervention and a biomarker for positive treatment response.[20]

Synergy between CD163 and CXCL11 results

Our analysis presents an interesting discrepancy that highlights the prognostic mechanisms in TNBC: while high expression of *CXCL11* (an attractant signal) emerged as a robust predictor of favorable prognosis (HR = 0.64, $P = 0.014$), the expression of CD163 (a tumor-associated macrophage[TAM] marker) showed a similar protective trend but was not statistically associated with poor prognosis (HR = 0.86 , $P = 0.4$).[20] This discrepancy raises questions about the quality of the immune infiltrate and suggests that the prognostic power lies in the intensity of the chemical signal (*CXCL11*), which effectively recruits protective immune cells, rather than the mere density of macrophages.[13,20]

This finding supports research indicating conflicting results regarding the prognostic role of *CD163*-positive macrophages in nonmetastatic breast cancer.[13] While some studies show that *CD163* may be associated with increased tumor size and grade in TNBC, its failure to predict survival in our analysis suggests that overall activation, reflected by high *CXCL11* levels, ultimately determines the favorable course of the disease, not just macrophage density.[21]

Furthermore, the failure of tumor structural components, such as the *COL10A1* gene (extracellular matrix) to demonstrate prognostic significance (HR = 1.23, $P = 0.26$) further reinforces the notion that the functional (immune) pathway, rather than the structural components of the tumor, is the primary driver of patient outcomes and key to prognosis. Overall, our data suggest that targeting or assessing the *CXCL11/CXCR3* signaling axis provides superior prognostic value in TNBC, as its activation shows that a robust antitumor response may be more clinically relevant and have a higher prognostic value in TNBC than assessing macrophage density alone.[22]

Integrating COL10A1 results: inflammation vs extracellular matrix

Although high expression of *COL10A1* (extracellular matrix) in TNBC tumors was identified as part of the list of highly expressed genes in our analysis, it failed to demonstrate any statistical prognostic significance in the survival analysis

(HR = 1.23, $P = 0.26$). This contradicts previous bioinformatic and clinical studies that have reported high *COL10A1* expression promotes breast cancer progression and predicts poor prognosis.[14] Our results indicate that in the specific microenvironment of TNBC, the predictive impact of the structural extracellular matrix components is diminished by the dominant role of immune-related signals, particularly the *CXCL11* axis. This discrepancy suggests that the functional pathway (immune-inflammatory) has a significantly higher prognostic power than the structural components (extracellular matrix) in determining patient outcome.

While *COL10A1* may play a role in promoting TNBC progression via pathways, such as Wnt/β-catenin, its direct clinical impact on survival may be overshadowed by the dominant power of the immune signature.[16] In our study, the integrated immune-related signature demonstrated the greatest prognostic power (HR = 0.45, $P = 2.3 \times 10^{-6}$), but the *CXCL11* alone also showed a strong protective effect ($P = 0.014$). This finding reinforces the argument that the tumor-enhanced immune response is the dominant factor determining the course of TNBC, and that treatment and prognostic efforts should focus on immunomodulation rather than targeting structural components of the TME.[23]

Maximum predictive power of the immune gene signature

The Kaplan-Meier analysis of the *Humig* gene signature is the most powerful clinical evidence in this study. The analysis demonstrated that the combined effect of the pivotal gene network (*CXCL9, CXCL11, CD163*) is not just a transient genetic feature, but rather a highly robust and independent favorable prognostic factor in TNBC. The combined gene signature, rather than individual genes, serves as an integrated functional fingerprint of the high-quality of the immune response in a TME that would otherwise be dominated by tumor-promoting inflammation.[24]

In terms of statistical validity, the log-rank test value for this signature reached $P = 2.3 \times 10^{-6}$. This small value conclusively confirms that the observed improvement in RFS in patients with high expression is not random, but rather a direct and robust consequence of the activity of this network. This is a statistically significant value. As for the clinically significant effect, the HR for the signature was 0.45. This means that high gene activity in this network more than reduces the risk of death or relapse by 55% compared to patients with low expression. Therefore, the prognostic superiority of this integrated signature clearly outweighed the prognostic significance of any single gene analyzed, including the potent gene *CXCL11* (which had a HR of 0.64).

Thus, this scheme provides conclusive evidence that the detected inflammation/immune axis does not merely represent an immune infiltration, but rather a molecular signature with a clinically significant effect. This can be used as powerful tool for identifying patients who lack this protective immune response and who may be at higher risk of relapse and as a high-priority therapeutic strategy which can enhance the TME and improve long-term response to treatment.

Molecular mechanism and signaling pathways

CXCL11 has the ability to bind to chemokine receptors that play a crucial role in the immune response within the TME. CXCL11 has diverse functions which include inhibiting angiogenesis and regulating the recruitment of antitumor immune cells. Most importantly, CXCL11 contributes to the formation of a defensive immune environment by inhibiting the polarization of M2 phagocytic cells and promoting the infiltration of effector lymphocytes.[24] Although CXCL11 may play a dual role in different types of malignant tumors, our results in TNBC definitively confirm its antitumor function. In this specific context, CXCL11 acts as a powerful chemotactic agent that attracts tumor-infiltrating lymphocytes, effectively contributing to curbing tumor development and improving the patient's chances of survival. CXCL11 and CXCL9 signaling occurs primarily via the CXCR3 receptor. In TNBC, this axis stimulates the JAK/STAT and PI3K/Akt-S6 pathways, which are essential for the activation and recruitment of antitumor immune cells, such as T cells and natural killer cells.[25] Instead of promoting tumor growth, these molecular pathways coordinate a strong immune response that inhibits tumor development. This directly explains the good prognosis and low risk of relapse observed in our results.

The PI3K/AKT/mTOR signaling axis, and especially the Akt-S6 node, is a critical regulator of $CD8^+$ T cell fate.[26,27] Recent evidence presented by Chen *et al.*[27] confirms that mTOR signaling is essential for the formation and maintenance of $CD8^+$ memory T cells, preventing them from entering a state of immune exhaustion where they lose their ability to fight tumors. In our study, it is likely that high expression of the CXCL11-dependent signature might have enhanced this mTOR-dependent metabolic and signaling efficiency, by promoting the survival and persistence of active, unexhausted T cells within the TME. This pathway provides a mechanistic basis for the strong protective effect (HR = 0.45) and the substantial reduction in RFS observed in our cohort of patients with TNBC.

Furthermore, the inclusion of the phagocytic cell marker CD163 in our immune fingerprint reflects the complexity of the immune landscape. Although $CD163^+$ tumor-associated phagocytic cells are traditionally associated with immunosuppression.[28] Our results are consistent with recent data showing that in an environment rich in chemokines (e.g., high levels of CXCL11/CXCR3), these cells can coexist with a dominant antitumor immune response or be reprogrammed by it.[29] This is reinforced by immunotherapy models in TNBC, where the polarization of $CD4^+$ and $CD8^+$ T cells via CXC chemokines can effectively overcome the suppressive immune environment and target cancer stem cells, thus preventing late relapses.[30] Our integrated fingerprint acted as a powerful protective biomarker, providing a clear automated basis for a significant survival advantage (HR = 0.45) and the decrease in the metastases observed.

The role and limited significance of CD163 in univariable survival analysis

While CD163 is a well-established marker for M2 TAMs, which are typically associated with poor outcomes in TNBC, its lack of statistical significance in univariable survival analysis ($P = 0.4$) warrants further investigation. This lack of independent predictive power may be attributed to several biological and methodological factors.

First, the predictive effect of TAMs may depend heavily on their location within the TME (e.g., around the tumor vs within it), a factor that comprehensive gene expression analysis cannot determine. *CD163* expression may only exert a dominant harmful effect only when TAMs are in a position that allows them to interact directly with T cells or metastatic environments.[30,31]

Second, the independent predictive signal of CD163 may be very weak or masked by other potent immunomodulators. Its strongest predictive power is perhaps only demonstrated when CD163 is incorporated into a composite index (CXCL11 /CXCL9/CD163 index). This suggests that CD163 primarily functions as a contextual factor and a key component of the immunosuppression environment that should be evaluated alongside dominant chemokine signals (CXCL11/CXCL9) to fully understand the true risks associated with relapse. As Markovicio *et al.*[31] explained, certain subsets of tumor-associated immune cells can contribute to antitumor activity through the expression of CXC chemokines. This may explain the lack of a negative univariate correlation in our study. Here, the predictive significance of CD163 is realized when it is assessed within the complete immunological signature, rather than as an independent marker.

Study limitations

This study has inherent limitations to the use of public datasets. First, the cohort of patients with TNBC (n = 533 patients) was pooled from multiple populations using the Kaplan-Meier plotting tool, resulting in variations in patient characteristics with respect to treatment and follow-up durations. Although multivariate logistic regression analysis was performed according to the major clinical variables (age, stage, and grade), demographic factors such as race were unavailable and could not be controlled for. Secondly, the use of comprehensive gene expression data is a major limitation. This methodology prevents the analysis of precise spatial localization and cell-to-cell communication within the tumor microenvironment (e.g., $CD163^+$ tumor-associated phagocytic cell interactions). Further validation using single-cell RNA sequencing or spatial transcriptomics is needed to confirm mechanistic roles.

CONCLUSION

This study successfully provides an integrated analytical framework linking genetic abnormalities to clinical outcomes in TNBC. The primary conclusion is that the observed genetic alterations do not operate in isolation, but rather form a robust and interconnected immune network, revolving almost exclusively around immune and inflammatory response pathways. Results of the RFS analysis confirm that the combined gene signature rather than any single gene (comprising CXCL9, CXCL11, and CD163) has significantly superior prognostic power ($P \approx 2.3 \times 10^{-6}$), making it the most important factor in determining patient prognosis. Furthermore, comparative analysis revealed that the CXCL11 chemokine signal has a significantly stronger prognostic significance than cytokine markers such as CD163, suggesting that the quality of the inflammatory response and the intensity of the attractor signal are the dominant prognostic factors. Based on these robust findings, we recommend that the CXCL11/CXCR3 axis be considered a top-priority therapeutic target to modulate the TME, improve treatment response, and reduce relapse rates in TNBC.

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FUNDING

This research received no external funding.

ETHICAL CONSIDERATION

This study analyzed public data from the GEO database (GSE53752). No new data was collected from human or animal subjects; thus, institutional review board approval was waived.

AI DISCLOSURE

The authors declare that no artificial intelligence (AI) tools were used in the design, data collection, analysis, or interpretation of this study. AI-based tools were used only for minor language editing to improve clarity and readability. The authors take full responsibility for the content and integrity of the manuscript.

AUTHOR CONTRIBUTION

IA: Conceptualization, Methodology, Project administration, Supervision, Writing the Original Draft, Reviewing and Editing. AM & MM: Formal analysis, Software, Data Curation, Visualization, Validation, Writing, Reviewing and Editing.

DATA AVAILABILITY

All data analyzed are available in the GEO repository (Accession: GSE53752).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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