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ORIGINAL ARTICLE

Subtype-specific genetic drivers of immune evasion in breast cancer

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Background: Immune evasion is a hallmark of cancer and a driver of therapeutic resistance. Although immunotherapy is effective in highly immunogenic cancers, its efficacy in breast cancer (BC) remains limited. We aimed to determine the prognostic relevance of immune-related gene signatures across distinct BC subtypes.

Materials and methods: We used transcriptomic and clinical data from three independent cohorts [Gene Expression Omnibus (GEO), The Cancer Genome Atlas, and GSE96058]. We analyzed 106 genes associated with the evasion of immune destruction (EID) and 182 genes involved in the evasion of killing by cytotoxic T lymphocytes (ECTL). Expression of signatures was stratified by BC subtypes. Cox regression and Kaplan−Meier curves were used to assess survival, with false discovery rate (FDR) correction ensuring statistical robustness.

Results: High expression of the ECTL signature was significantly associated with improved overall survival (OS) in basal BC patients [hazard ratio (HR) 0.25, 95% confidence interval (CI) 0.16-0.4, $P = 8.6e{-}10$, FDR <1%], and similar trends appeared in human epidermal growth factor receptor 2 (HER2)-positive BC. For EID genes, high expression also correlated with favorable OS in basal BC (HR 0.3, 95% CI 0.18-0.49, $P = 3.4e{-}7$). Gene-level analyses revealed *CXCL10*, *CXCL9*, *CXCR4*, and *JAK3* as robust predictors of OS in basal BC, validated across independent datasets. This four-gene signature demonstrated strong predictive power for response to immune checkpoint inhibitors (ICIs) (area under the curve = 0.722, $P = 1.3E{-}07$). In HER2-positive BC, a three-gene signature (*IL2RG*, *CD3E*, *CD3G*) was consistently prognostic (HR 0.41, 95% CI 0.24-0.71, $P = 0.0011$, GEO) across independent datasets.

Conclusions: We identified subtype-specific signatures that predict survival and immunotherapy response, providing clinically actionable biomarkers.

Key words: immune evasion, immune checkpoint inhibitors, tumor microenvironment, prognostic biomarker, multigene signature, TIL

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed malignancy in women, with an estimated 2.3 million new cases and >665 000 deaths reported per year.[1] It remains the leading cause of cancer-related mortality in females, reflecting profound disease heterogeneity and therapeutic resistance despite substantial advances in detection and treatment.[2] Based on estrogen receptor-alpha (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2 (HER2) expression levels, BC is classified into four principal molecular subtypes with distinct biological behaviors, immune profiles, and therapeutic responses. Luminal A BC (ER-positive and/or PR-positive, HER2-negative) constitutes ∼60% of cases and is associated with a favorable prognosis.[3] Luminal B (ER-positive and/or PR-positive, HER2-positive-positive) accounts for 30% of cases, exhibits high Ki-67 expression (>14%), and has a poorer prognosis.[4] HER2-positive BC (ER-negative, PR-negative, HER2-positive), representing 10% of cases, is highly aggressive with poor outcomes.[5] Triple-negative breast cancer (TNBC) (ER-negative, PR-negative, HER2-negative) comprises 15%-20% of cases, is the most aggressive subtype, and frequently occurs in younger women.[6] BC subtypes guide treatment decisions for anti-hormonal therapies, anti-HER2 treatments, and cyclin-dependent kinase 4/6 inhibitors, along with other standard therapies, such as surgery, radiotherapy, chemotherapy, or a combination of the above.[7] Nevertheless, available treatment strategies often cause severe adverse effects and can lead to therapy resistance: in ER-positive BC, half or more of the recurrences can occur late, even

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up to 20 years after the initial diagnosis,[8] and in HER2-positive BC resistance to available Food and Drug Administration (FDA)-approved drugs is a significant issue,[9] underscoring the need for additional strategies to improve long-term outcomes.

Evading immune destruction is a hallmark of cancer,[10] enabling tumor cells to escape immune surveillance, proliferate, and metastasize.[11] Cancer cell immune suppression and evasion mechanisms are under intense scrutiny to advance the efficacy of immunotherapy strategies, including immune checkpoint inhibitors [ICIs, e.g. anti-programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1), anti-cytotoxic T-lymphocyte-associated protein-4], cancer vaccines, chimeric antigen receptor-T cells, bispecific antibodies, and oncolytic viruses.[12] ICIs alone or in combination with conventional treatments, such as radiotherapy and chemotherapy, have shown particular success in tumors with high immunogenicity and abundant tumor-infiltrating lymphocytes (TILs) in several cancers, including melanomas, non-small-cell lung cancers, and renal cell carcinomas.[13,14]

BC has historically been considered poorly immunogenic and was initially not a primary focus of immunotherapy research.[15] Nevertheless, compared with normal breast tissue, breast tumors and their adjacent stroma exhibit increased immune cell infiltration, involving cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, which recognize and eliminate malignant cells.[16] In BC, the extent, composition, and activity of TILs vary significantly among and within subtypes.[17] TILs are more prevalent in TNBC and HER2-positive BCs, correlating with increased immunogenicity and better responses to immunotherapies.[18-22] Based on the results of the KEYNOTE-355 trial, the FDA granted accelerated approval in 2020 for patients with PD-L1-positive metastatic TNBC for the anti-PD-1 antibody pembrolizumab plus chemotherapy, since the trial showed significantly improved progression-free survival compared with treatment with chemotherapy alone.[23] The final overall survival (OS) analysis of KEYNOTE-355 data further confirmed clinical benefit: among patients with a PD-L1 combined positive score of $\geq$10, the median OS improved from 16.1 months with chemotherapy alone to 23.0 months with pembrolizumab—chemotherapy.[24]

Additionally, the KEYNOTE-522 trial demonstrated substantial benefits of pembrolizumab combined with chemotherapy (paclitaxel with carboplatin followed by anthracycline with cyclophosphamide every 3 weeks) in high-risk, early-stage TNBC, regardless of PD-L1 status, leading to FDA approval in 2021 for neoadjuvant and adjuvant use.[25] The final analysis of the KEYNOTE-522 trial demonstrated that adding pembrolizumab to neoadjuvant chemotherapy followed by adjuvant pembrolizumab significantly improved 5-year OS in early-stage TNBC, with OS rates of 86.6% versus 81.7% ($P = 0.002$).[26]

Despite TNBC's higher immunogenicity and the successful trials, only 5%-20% of patients respond to ICI-based immunotherapy.[12] TNBC frequently develops mechanisms of acquired immune privilege, including checkpoint hijacking, T-cell exclusion, and reduction of expression of major histocompatibility complex (MHC)-I, that blunt immune attack and limit the efficacy of ICIs.[27] Ongoing clinical trials are exploring additional immunotherapy combinations in TNBC, underscoring the ongoing interest in enhancing treatment options for this aggressive BC subtype.[12,28] Currently, in BC, PD-L1 expression remains the only biomarker implemented in clinical practice; however, its limitations as a standalone marker highlight the need for approaches that combine PD-L1 assessment with other immune-related factors to improve patient selection.[29]

HER2-positive tumors, while immunologically less active than TNBC, often exhibit TILs and PD-L1 expression. Significant cross-talk between the HER2 and PD-L1 pathways can also influence response to anti-HER2 therapy.[30] Combining PD-1/PD-L1 inhibitors with anti-HER2 therapies has demonstrated synergistic effects in preclinical studies by reversing immune suppression and enhancing the efficacy of trastuzumab. However, further clinical trials are needed to optimize patient and biomarker selection and confirm long-term benefits in HER2-positive BC.[31]

In contrast, ER-positive/HER2-negative BCs are considered immunologically 'cold' subtypes, characterized by lower levels of TILs and reduced responsiveness to immune-mediated killing.[18,19] This immunosuppressive tumor microenvironment is also reflected in the limited success of clinical trials with ICIs, which have shown modest efficacy and minimal improvements in patient outcomes.[12]

The molecular mechanisms by which BC cells escape immune destruction, particularly across different subtypes, remain poorly understood. The steadily growing number of clinical trials evaluating various immunotherapeutic strategies across all subtypes of BC also underscores the increasing need for more practical predictive biomarkers.[12] In this context, gene signatures specific to immune evasion offer a critical framework for understanding the genetic and pathway-level determinants of cancer-intrinsic immune escape.[32,33] One such signature is associated with the cancer hallmark evasion of immune destruction (EID) from a unified database of cancer hallmark genes. The 106-gene signature represents a conservative set of immune-evasion genes compiled from comprehensive omics studies.[33] Another immune function-related collection is a 182-gene signature associated with the evasion of killing by cytotoxic T lymphocytes (ECTLs) and identified through genome-wide CRISPR screens.[32] The ECTL gene set encompasses critical regulators of immune-evasion pathways, including interferon (IFN) signaling, tumor necrosis factor-induced cytotoxicity, antigen presentation, and autophagy, which enable cancer cells to resist immune-mediated destruction. Several genes in this set, such as *PTPN2*, *SOCS1*, and *ADAR1*, have been implicated in immune suppression and resistance to checkpoint immunotherapies.[32] The ECTL gene signature offers a valuable resource for investigating subtype-specific mechanisms of

CTL evasion and identifying predictive biomarkers that may guide the development of more effective immunotherapeutic strategies.[34]

Several prior studies have developed immune-related prognostic signatures in BC,[35-39] typically based on large immune gene panels selected through data-driven statistical filtering. In contrast, we adopted a biologically grounded approach by interrogating two mechanistically defined, complementary gene sets (the EID and ECTL signatures) with direct relevance to immune evasion, stratified by molecular subtype. Analyzing predefined gene signatures offers several advantages over genome-wide differential gene expression analysis. Instead of scanning thousands of genes individually, it narrows the focus to biologically coherent sets with established functional relevance, thereby capturing coordinated pathway activity. Several prognostic and predictive tools are built on such curated panels, particularly those linked to processes like senescence, proliferation, or hypoxia.[40-43]

We also examined the correlation between individual markers and disease outcomes to gain insights into subtype-specific mechanisms of evading immune surveillance. Together, these analyses aim to reveal how distinct immune-evasion programs operate across BC subtypes and identify robust biomarkers that could enhance patient stratification for immunotherapy.

MATERIALS AND METHODS

Database assembly

Firstly, to establish an integrated gene array cohort, we conducted a comprehensive search within the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) and the European Genome-phenome Archive (https://ega-archive.org/) repositories to locate datasets containing transcriptome-level gene expression profiles with associated clinical data. Only datasets comprising at least 30 samples were included. Further, we focused on those generated using GEO platforms GPL96, GPL570, and GPL571. These platforms share a common set of 22 277 genes assessed using identical probe sequences. This standardization ensures consistent sensitivity, specificity, and dynamic range across datasets.

Each microarray was normalized using Microarray Suite 5.0 (MAS5). We chose MAS5 because it allows per-sample normalization, facilitating integration of multiple cohorts, and it has been benchmarked against RT—PCR as highly reliable.[44] A second scaling normalization was applied to mitigate batch effects by setting the mean expression of the overlapping 22 277 probes to 1000 for each array.[45]

Secondly, in addition to the integrated gene array cohort, we utilized two additional datasets to validate the analysis results. These include the RNA sequencing (RNAseq)-based The Cancer Genome Atlas (TCGA) cohort of BC patients[46] and the GSE96058 dataset.[47] The preprocessed and normalized data from the original repositories were used to analyze these datasets.

Molecular subtypes were identified based on the PAM50 classification.[48] Due to the low number of cases, tumors with normal-like characteristics were not analyzed in our study. In the RNAseq patient cohort, the initially reported receptor statuses were used to define the receptor status for each patient.

The evasion of immune destruction (EID) hallmark signature

A previously established 106-gene immune-evasion signature was evaluated across BC subtypes.[33] This gene signature was constructed by compiling genes associated with the 10 cancer hallmarks, including 'EID', as defined by Hanahan and Weinberg in their landmark 2011 publication.[10] The corresponding genes were identified through comprehensive omics studies for each cancer hallmark and integrated to form a unified dataset of genes associated with cancer hallmarks. The integrated dataset encompasses 6763 individual genes linked to various cancer hallmarks, of which 749 are specifically associated with the hallmark of immune evasion. The 106-gene signature represents a refined, conservative core set of immune-evasion genes derived from multiple overlapping resources, as detailed in Menyhart et al.[33] For each BC subtype, the impact of the immune-evasion signature, represented by the weighted mean of the 106 genes, was assessed independently on survival outcomes to account for the distinct tumor microenvironment of each subtype.

Another immune function-related gene collection is the 182-gene signature, linked to ECTL, which was identified through comprehensive genome-wide CRISPR screens.[32] The impact of the 182-gene signature related to ECTL was investigated as the weighted mean across the different BC subtypes.

Statistical analysis

OS served as the primary endpoint due to the limited availability of BC-specific survival data. For the EID and ECTL multigene signatures, we calculated the weighted mean expression of the member genes to obtain a continuous signature score. Associations between the signature score and OS were assessed using univariate Cox proportional hazards regression.

To define 'high' versus 'low' expression groups for Kaplan—Meier analysis, we systematically tested all possible cut-offs within the interquartile range (25th-75th percentiles) of expression values. The best cut-off giving the smallest log-rank *P* value was selected. If two cut-offs produced the same *P* value, we chose the one with the hazard ratio (HR) furthest from 1 (i.e. the strongest separation). This approach is equivalent to the widely used 'maximally selected rank statistic' method. To control for multiple testing during cut-off selection, we applied the Benjamini—Hochberg false discovery rate (FDR) procedure.[49] Kaplan—Meier curves were generated to visualize OS differences between low- and high-expression groups. To further depict expression patterns across BC subtypes, we

used beeswarm plots, classifying patients into high- and low-expression groups according to the optimal cut-off determined by the best cut-off method.

Signature-based survival analysis was followed by gene-level evaluation. Genes showing consistent, significant associations with OS across datasets were shortlisted. We then iteratively combined the top-performing individual genes into minimal multigene signatures, retaining a combination only if it provided a stronger association with survival (i.e. lower HR and more significant *P* value) than any constituent gene alone. Final subtype-specific signatures were selected based on optimal prognostic value and reproducibility across the independent datasets.

The prognostic relevance of the minimal gene signatures was analyzed in a cohort of BC patients treated with ICIs.[50] Expression differences between responders and non-responders were compared using the Mann–Whitney *U* test, and predictive accuracy was quantified using receiver operating characteristic analysis [area under the curve (AUC) values]. All statistical analyses were conducted in R (v4.2) using Bioconductor libraries (https://www.bioconductor.org/), including survival, survminer, and pROC. A *P* value <0.05 was considered statistically significant unless FDR correction was applied.

RESULTS

Database setup

The clinical and pathological characteristics of the study population, comprising 1880 patients, are summarized in Table 1. The median age of patients was 51 years (SD ± 13 years), and the median follow-up duration was 73.2 months (SD ± 46 months). Among the cohort, 69.7% of patients were ER-positive, while 22.3% were ERBB2-positive. Node positivity was observed in 38.4% of patients ($n = 1178$). Tumor size, available for 946 patients, had a mean of 2.2 cm (SD ± 1.3 cm). Tumor grade distribution showed that 14.5% were classified as grade I, 36.8% as grade II, and 48.7% as grade III. Regarding molecular subtypes, 22.9% of patients were categorized as basal-like, 31.7% as luminal A, 23.4% as luminal B, 19.3% as HER2-enriched, and 2.7% as normal-like (Figure 1A). These data provide a comprehensive overview of the patient cohort's demographic, clinical, and molecular features.

Table 1. Characteristics of the BC patient population from the gene array dataset

	Integrated database
Total *n*	1880
Clinical and pathological parameters	
Patients age (median + SD)	51 ± 13 years
Follow-up (median + SD)	73.2 ± 46 months
ER-positive	69.7%
ERBB2-positive	22.3%
Node-positive ($n = 1178$)	38.4%
Tumor size ($n = 946$)	2.2 ± 1.3 cm
Grade I	14.5% ($n = 175$)
Grade II	36.8% ($n = 443$)
Grade III	48.7% ($n = 586$)
Molecular subtype distribution	
Basal	22.9% ($n = 431$)
Luminal A	31.7% ($n = 596$)
Luminal B	23.4% ($n = 440$)
HER2-enriched	19.3% ($n = 362$)
Normal-like	2.7% ($n = 51$)

BC, breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; SD, standard deviation.

The prognostic value of the evasion of immune destruction (EID) gene signature is subtype-specific

Of the 106 EID-related genes, 105 were identified in the gene array probe sets; hence, all relevant analyses were based on the 105-core gene set. To ensure the robustness and reproducibility of the results, we evaluated the prognostic power of the EID-related gene signature across two additional independent BC datasets with sufficient follow-up data, the TCGA and the GSE96058 datasets (Figure 1A). Of the 106 EID-related genes, 104 were present in the latter two datasets. EID signature expression was consistently detectable across all BC subtypes, with patients clearly separated into high- and low-expression groups according to the best cut-off method (Supplementary Figure S1, available at https://orcid.org/10.1016/j.iotech.2025.101075).

The survival analysis revealed notable differences in the prognostic impact of the EID gene set, calculated as the mean expression of the available 105 genes across the BC subtypes identified by the PAM50 gene signature.

In basal BC patients, the mean EID expression signature was significantly associated with improved OS in the gene array cohort [HR 0.3, 95% confidence interval (CI) 0.18-0.49, $P = 3.4e{-}7$, OS, Figure 2A], with a FDR <1%. The signature demonstrated significant correlations with survival outcomes in the two additional datasets (GSE96058: HR 0.38, 95% CI 0.23-0.61, $P = 5e{-}5$; TCGA: HR 0.33, 95% CI 0.14-0.76, $P = 0.0061$, Figure 2B), with a consistent association between the higher expression levels of the weighted mean signature and improved survival. These findings highlight the reproducibility and robustness of the signature in predicting survival, independent of the dataset used, underscoring its potential as a reliable prognostic tool in basal BC.

Similar tendencies were observed for the mean EID signature and OS in the HER2-positive BC subtype. In the gene array dataset, the mean of the 105-gene signature demonstrated a significant association with OS with an HR of 0.39 (95% CI 0.22-0.69, $P = 0.00086$, Figure 3A) at FDR = 10%. Nevertheless, in the GSE96058 dataset, the association was only a marginal tendency (HR 0.53, 95% CI 0.25-1.14, $P = 0.097$), while the correlation was not significant in the TCGA dataset ($P > 0.05$).

Additionally, the trends between the mean expression of the EID signature and OS in the luminal A and luminal B subtypes did not remain significant in any of the datasets after controlling for the FDR ($P > 0.05$) (Supplementary Figure S2, available at https://orcid.org/10.1016/j.iotech.2025.101075). The lack of significant associations underscores the subtype specificity of the signature.

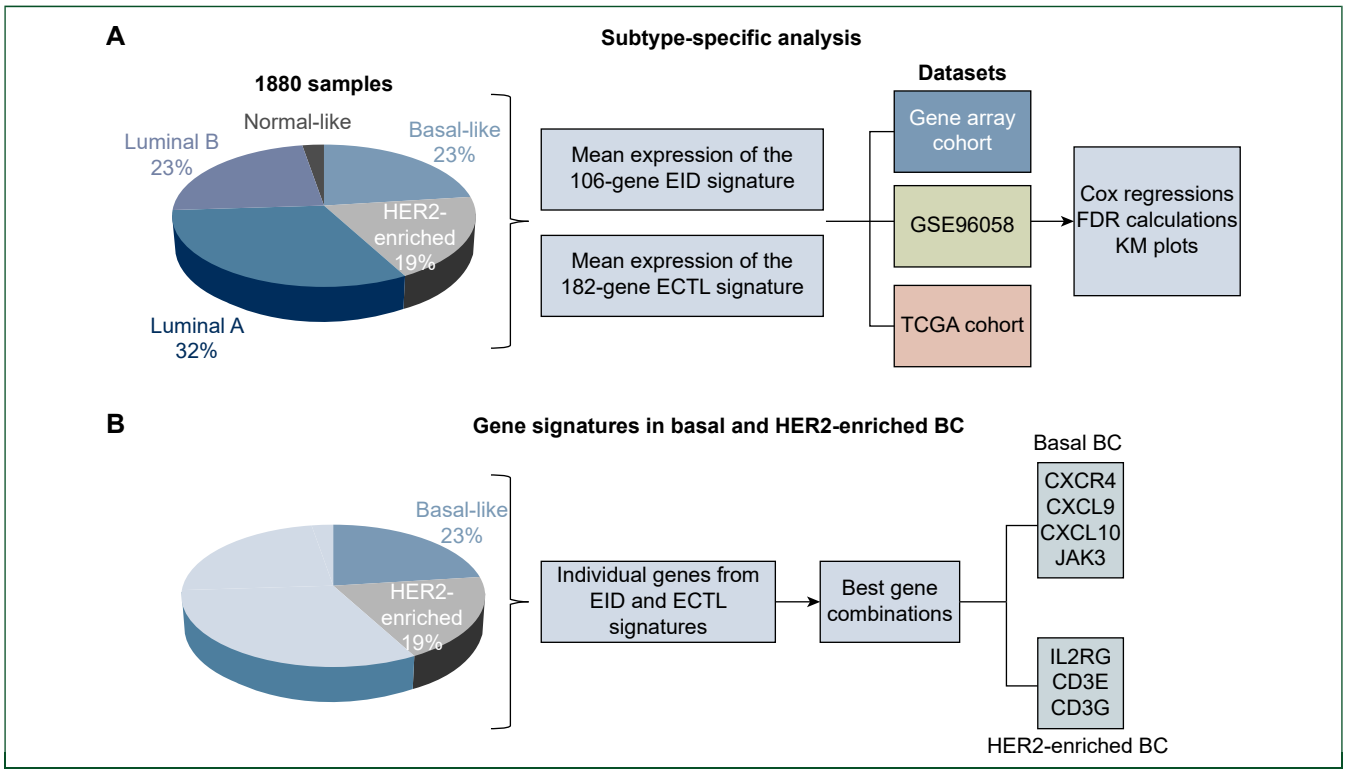

Figure 1. Workflow for evaluating the association between immune-evasion signatures and overall survival across breast cancer subtypes. (A) A total of 1880 breast cancer samples (gene array cohort) were classified into four subtypes: luminal A (32%), luminal B (23%), HER2-enriched (19%), and basal-like (23%). Subtype-specific overall survival was analyzed using the mean expression of the 106-gene EID and 182-gene ECTL signatures. Additional datasets (GSE96058 and TCGA) were used to validate the findings. Statistical methods, including Cox regression, FDR calculations, and Kaplan–Meier survival plots, were applied to identify survival patterns. (B) The contribution of individual genes from the two signatures was evaluated, and optimal combinations were used to create gene signatures for basal and HER2-enriched breast cancers.
BC, breast cancer; ECTL, evasion of killing by cytotoxic T lymphocytes; EID, evasion of immune destruction; FDR, false discovery rate; HER2, human epidermal growth factor receptor 2; KM, Kaplan–Meier; TCGA, The Cancer Genome Atlas.

The subtype-specific prognostic significance of the evasion of killing by cytotoxic T lymphocytes (ECTL) gene signature

Of the 182 lymphocyte evasion (ECTL)-related genes, 168 were detected among the probes in the gene array cohort. Consequently, all subsequent analyses focused on this 168-gene core set. Expression levels of the ECTL signature were consistently detectable across all BC subtypes, with clear separation into high- and low-expression groups as defined by the best cut-off method (Supplementary Figure S3, available at https://orcid.org/10.1016/j.iotech.2025.101075).

A univariate survival analysis revealed significant variation in the prognostic relevance of the ECTL signature, calculated as the mean expression of the identified 168 genes across BC subtypes classified by the PAM50 gene signature. In basal BC patients, the mean expression of the ECTL genes was strongly associated with improved OS, with an HR of 0.25 (95% CI 0.16-0.4, $P = 8.6e{-}10$, Figure 2C) at FDR <1%. Out of the 182 lymphocyte evasion-related genes, 146 were identified in the GSE96058 and TCGA datasets. In the GSE96058 dataset, the high mean expression of the 146 genes was correlated with significantly improved OS with an HR of 0.44 (95% CI 0.23-0.81, $P = 0.0075$) among basal BC patients and was also associated with a marginally improved outcome in the TCGA dataset (HR 0.4, 95% CI 0.15-1.09, $P = 0.063$) (Figure 2D).

Similar, albeit weaker, tendencies were observed for the mean ECTL signature and OS in the HER2-positive BC subtype. In the gene array dataset, the mean of the 168-gene signature demonstrated an HR of 0.48 (95% CI 0.27-0.84, $P = 0.0081$, Figure 3B), and in the TCGA dataset, an HR of 0.31 (95% CI 0.11-0.9, $P = 0.023$). However, none remained significant after correcting for multiple hypothesis testing (FDR $\geq$ 50%). The association was also not significant in the GSE96058 dataset ($P > 0.05$).

Additionally, we identified weak associations between the mean expression of the ECTL signature and OS in luminal A and luminal B BC in the GSE96058 dataset (luminal A: HR 0.59, 95% CI 0.4-0.87, $P = 0.0071$; luminal B: HR 0.62, 95% CI 0.4-0.98, $P = 0.039$), but not in the other two datasets (TCGA and gene array cohorts; $P > 0.05$) (Supplementary Figure S4, available at https://orcid.org/10.1016/j.iotech.2025.101075).

Our findings highlight the differential prognostic impact of the ECTL gene set across distinct BC subtypes, with relevance mainly in the basal and, to some extent, in the HER2-positive BC subtypes.

Gene signatures associated with survival in basal and HER2-positive BC

Translating complex gene signatures into clinical practice remains challenging. To address this, we decomposed the

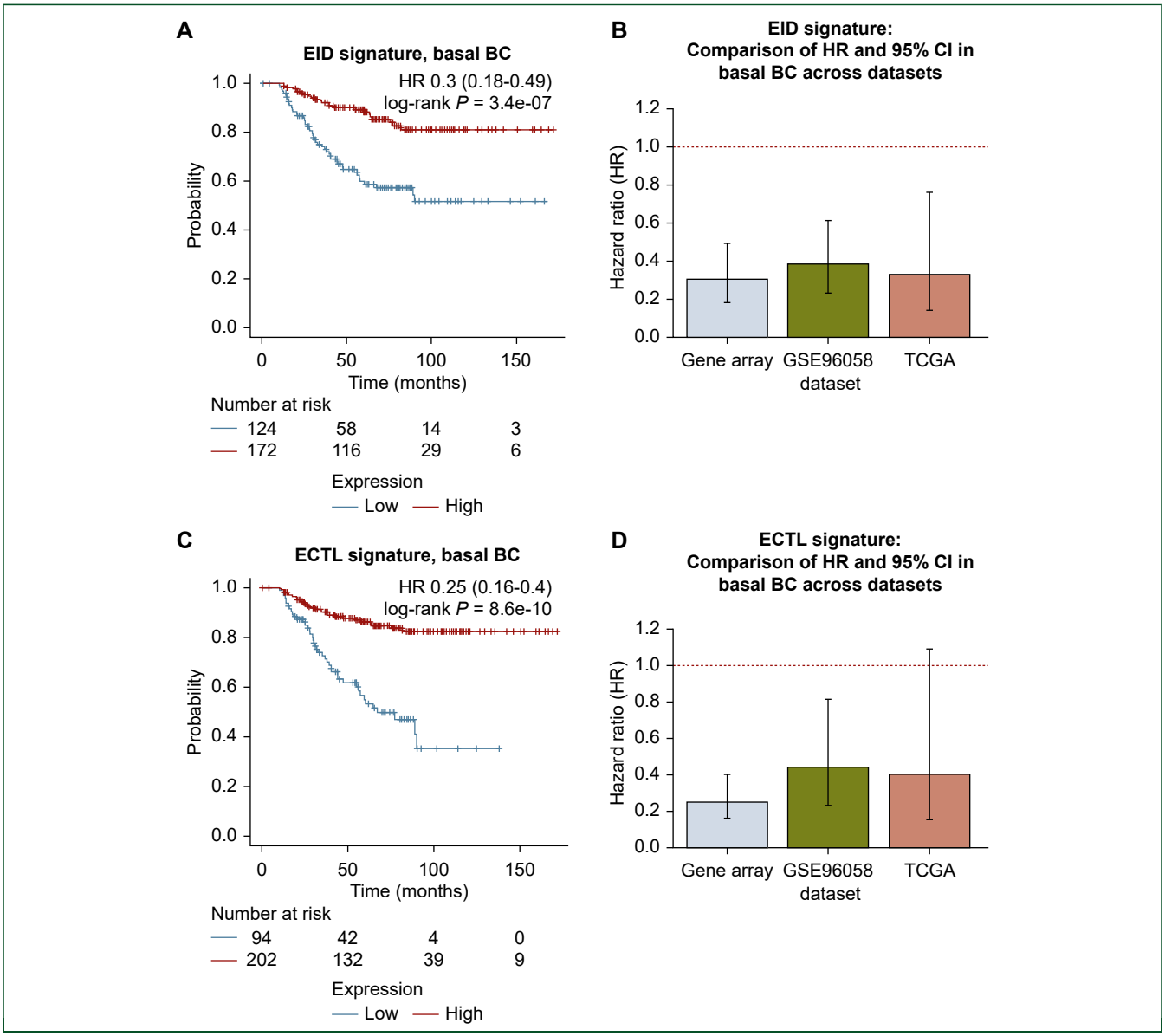

Figure 2. Prognostic impact of the evading immune destruction (EID) and evasion of killing by cytotoxic T lymphocytes (ECTL) gene signatures in basal breast cancer (BC). (A) Patients with high mean expression of the EID signature (red curves) consistently demonstrated significantly improved OS compared with those with low expression (black curves) in the gene array cohort. (B) The association between high mean EID signature and OS in basal BC samples was also significant in the GEO96058 and TCGA datasets. The colored bar charts illustrate the dataset-specific hazard ratio (HR) values, the whiskers the 95% confidence intervals (CIs), and the red dotted line represents HR = 1. (C) The high mean expression of the 182-gene ECTL signature correlated with better OS in basal BC patients in the gene array cohort (D), and the ECTL signature was also associated with improved survival in the GEO96058 and TCGA datasets. The colored bar charts illustrate the dataset-specific HR values, the whiskers the 95% CIs, and the red dotted line represents HR = 1.
OS, overall survival; TCGA, The Cancer Genome Atlas.

two immune-evasion signatures into individual genes and tested each by univariate Cox regression to quantify its contribution to survival. Top-performing genes were then iteratively combined into minimal multigene signatures and re-evaluated by Cox regression; combinations were retained only when they provided a stronger association with survival than any constituent gene alone. Final subtype-specific panels were selected based on optimal prognostic performance and reproducibility across independent datasets. Because prognostic effects were most evident in the basal and HER2-positive subtypes, we focused mechanistic interpretation on these groups (Figure 1B).

Clinically relevant gene signature in basal BC

In the basal BC subtype, the combined mean expression of four genes (*CXCL10*, *CXCL9*, *CXCR4*, and *JAK3*) provided a more robust predictive value than any genes separately in the gene array cohort (HR 0.2, 95% CI 0.12-0.32, $P = 1.2E-13$, Figure 4A). The combined gene signature showed similar predictive power in the GSE96058 dataset (HR 0.36, 95% CI 0.21-0.62, $P = 0.00011$) and the TCGA dataset (HR 0.31, 95% CI 0.11-0.83, $P = 0.014$), validating the robustness and generalizability of this signature. The four-gene signature had a similarly strong prognostic value both in the cohort of younger (<50 years of age, HR 0.14, 95% CI 0.06-0.32, $P = 6.1E-08$) and older patients (>50 years of

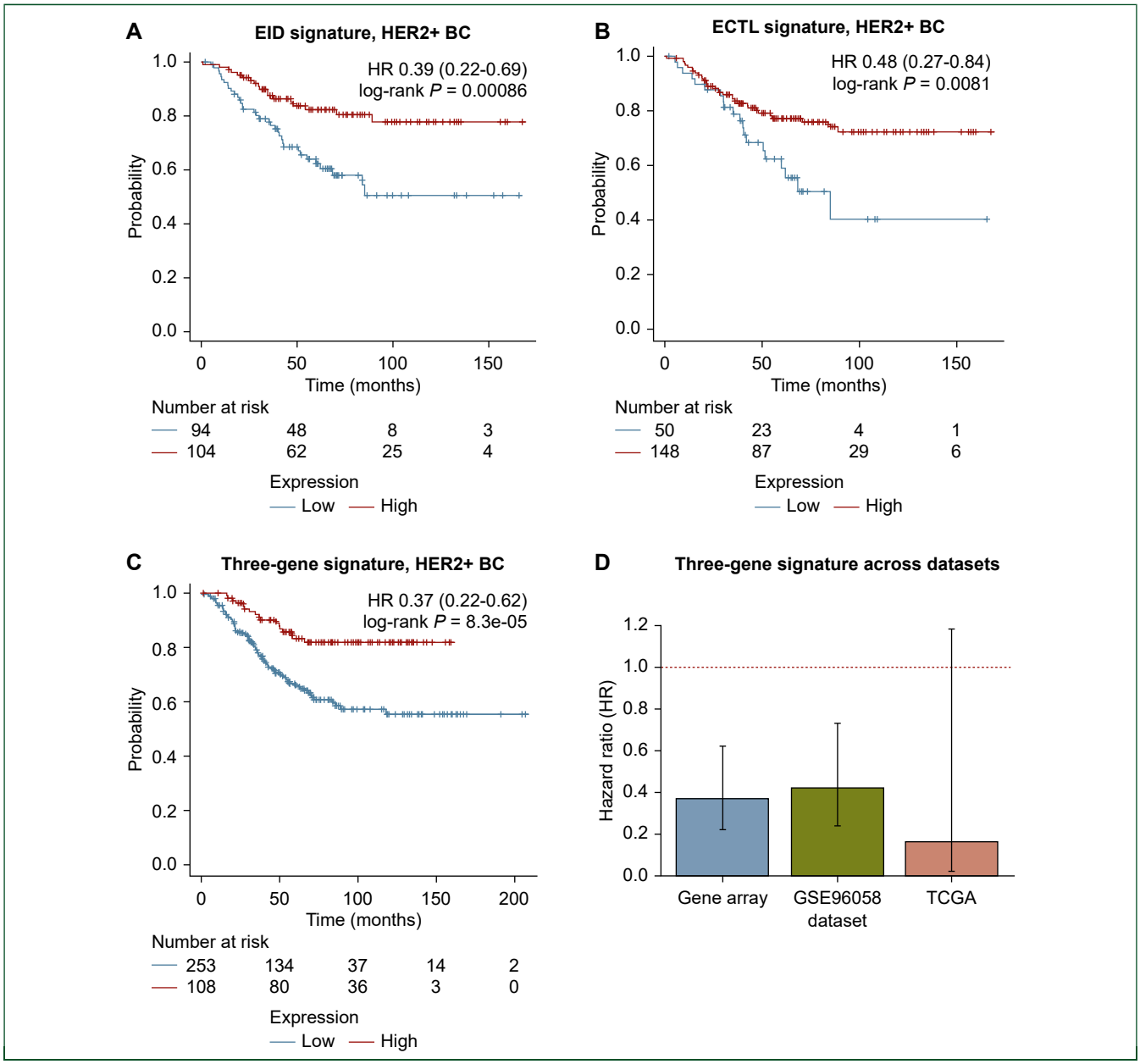

Figure 3. Prognostic impact of the evading immune destruction (EID) and evasion of killing by cytotoxic T lymphocytes (ECTL) gene signatures in HER2-positive breast cancer (BC). (A) Kaplan–Meier survival curves showing the association between high (red curves) and low (black curves) expression of the EID gene signature with overall survival (OS) in HER2-positive BC patients in the gene array cohort. (B) The high mean expression of the 182-gene ECTL signature correlates with better OS in basal BC patients in the gene array patient cohort. (C) Prognostic impact of the three-gene signature (*IL2RG*, *CD3E*, and *CD3G*) in HER2-positive breast cancer in the gene array cohort. (D) The three-gene signature was also associated with improved survival in the GEO96058 and TCGA datasets. The colored bar charts illustrate the dataset-specific hazard ratio (HR) values, the whiskers the 95% confidence intervals, and the red dotted line represents HR = 1.
HER2, human epidermal growth factor receptor 2; TCGA, The Cancer Genome Atlas.

age, HR 0.19, 95% CI 0.1-0.36, $P = 2E\text{-}08$) at an FDR of 1%, underlying its utility across different demographics. Unfortunately, there were not enough patient samples to assess the prognostic value of the four-gene signature across disease stages. Nevertheless, the high expression of the four-gene signature showed a strong association with improved survival in the basal BC patient cohort treated with neoadjuvant therapy, with an HR of 0.05 (95% CI 0.01-0.39, $P = 6.3E\text{-}05$), at an FDR of 1%.

Moreover, we also assessed the prognostic relevance of the combined expression of the four genes in a dataset of BC patients treated with ICI therapy. In a multigene analysis, we found a significantly increased mean expression of the four-gene signature among responders to ICI compared with non-responders ($P = 7.1E\text{-}06$). The mean gene expression significantly differentiated between responders versus non-responders to ICI therapy (AUC 0.722, $P = 1.3E\text{-}07$) (Figure 4B).

In basal BC, a single gene, *ATG9A*, was associated with worse OS with similar prognostic effectiveness across two independent datasets at FDR $\leq$ 5%, validating its robustness and generalizability [gene array cohort: HR 2.22, 95%

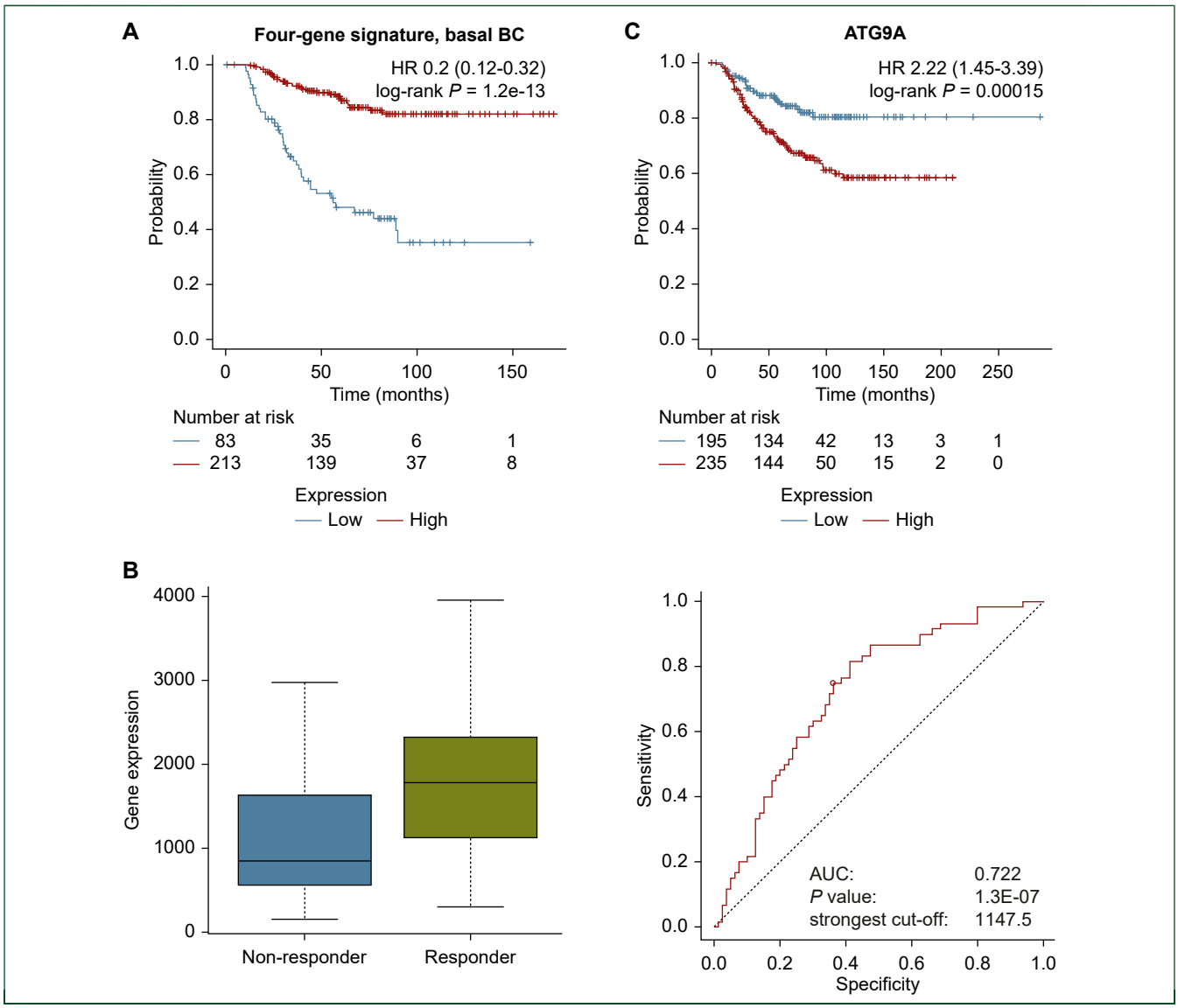

Figure 4. Prognostic impact of the four-gene signature (*CXCR4*, *CXCL9*, *CXCL10*, *JAK3*) in basal breast cancer (BC). (A) Based on gene array cohort data, the four-gene signature was associated with improved overall survival in basal BC patients. (B) The high expression of the four-gene signature was linked to an improved response to immune checkpoint inhibitor (ICI) therapy in basal BC patients. The receiver operating characteristic (ROC) curve demonstrates robust predictive performance, with an area under the curve (AUC) of 0.722, effectively distinguishing responders from non-responders. (C) High expression of the *ATG9A* gene was associated with worse overall survival in basal BC patients.
HR, hazard ratio.

CI 1.45-3.39, $P = 0.00015$ (Figure 4C); GSE96058 dataset: HR 2.67, 95% CI 1.48-4.83, $P = 0.00068$]. The trend was similar in the TCGA dataset, but the association was not significant, likely due to the small sample size ($P > 0.05$).

Clinically relevant gene signature in HER2-positive BC

In HER2-positive BC, the combined mean expression of three genes (*IL2RG, CD3E*, and *CD3G*) provided a signature linked to a good prognosis in the gene array cohort (HR 0.37, 95% CI 0.22-0.62, $P = 8.3E-5$) (Figure 3C), with similar, albeit weaker results in the two additional independent BC datasets (GSE96058 dataset: HR 0.42, 95% CI 0.24-0.73, $P = 0.0017$; TCGA: HR 0.16, 95% CI 0.02-1.18, $P = 0.0389$) (Figure 3D).

Functional exploration of the minimal prognostic signatures

To investigate the biological coherence of the minimal prognostic signatures, we carried out enrichment analyses using Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome resources by the search engine Enrichr.[51] For the basal-specific four-gene signature (*CXCL9*, *CXCL10*, *CXCR4*, and *JAK3*), the top GO terms were centered on cytokine-mediated signaling and inflammatory response, consistent with a role in immune cell recruitment and activation. KEGG analysis highlighted chemokine and cytokine receptor signaling as well as Janus kinase (JAK)—signal transducer and activator of transcription proteins (STAT) signaling, reflecting IFN-driven immune

activation. Reactome pathways converged on signaling cascades associated with interleukin-mediated responses and chemokine receptor activity, confirming that this signature represents a coordinated axis of T-cell trafficking and effector signaling (Supplementary Figure S5, available at https://orcid.org/10.1016/j.iotech.2025.101075). In summary, the basal four-gene signature reflects an immune-inflamed program, with CXCL9/10 recruiting T cells, CXCR4 coordinating chemokine-driven trafficking, and JAK3 activating downstream γ-chain cytokine/JAK—STAT signaling.

The HER2-positive three-gene signature (*IL2RG*, *CD3E*, and *CD3G*) was strongly enriched for T-cell activation processes in GO, including thymic selection, interleukin (IL)-2/IL-15 signaling, and regulation of T-cell proliferation. KEGG analysis identified T-cell receptor signaling, Th1/Th2 and Th17 differentiation, and primary immunodeficiency pathways, pointing to essential roles in adaptive immune function. Reactome analysis reinforced these findings by highlighting T-cell receptor (TCR) signaling, cytokine receptor interactions, and downstream signaling modules involved in lymphocyte activation (Supplementary Figure S6, available at https://orcid.org/10.1016/j.iotech.2025.101075). Thus, the HER2-positive three-gene signature captures a core T-cell activation module, with IL2RG mediating γ-chain cytokine signaling and CD3E/CD3G forming part of the TCR/CD3 complex essential for adaptive immune activation. Together, these results demonstrate that both minimal gene sets form biologically coherent immune modules.

DISCUSSION

Individual biomarkers of cancer survival often fail to account for the interactions driving tumor progression. In contrast, multigene signatures offer a more robust and clinically relevant tool by capturing the complexity of tumor biology. In this study, we applied two mechanistically defined immune-evasion gene sets—EID (106 genes) and ECTL (182 genes)—to interrogate prognostic patterns across PAM50 BC subtypes. Unlike previous approaches that relied on larger, data-driven gene panels,[52,53] our strategy was hypothesis-driven, focusing on biologically coherent gene sets with direct relevance to immune evasion. Importantly, we further reduced these large panels into concise minimal signatures that retained prognostic power while improving feasibility for translational applications.

In basal BC, both immune-evasion signatures were consistently associated with improved OS across independent datasets, while in HER2-positive tumors the associations were weaker and less consistent after FDR correction. The immunologically 'cold' nature of luminal A and luminal B tumors was reflected in the absence of robust prognostic signals in these subtypes. This subtype specificity underscores the value of stratifying analyses by molecular class rather than aggregating all BCs together. A limitation of our study is that by focusing exclusively on the predefined EID (106 genes) and ECTL (182 genes) sets, we may have overlooked other genes or pathways with stronger prognostic or predictive potential.

The seemingly paradoxical finding that higher expression of immune-evasion signatures correlated with favorable survival in basal and HER2-positive tumors can be explained by their immune contexture. Both subtypes are characterized by high levels of TILs and inflamed microenvironments, suggesting active immune surveillance. In such settings, expression of immune-regulatory genes may reflect not only evasion mechanisms but also the presence of vigorous cytotoxic T-cell activity. Thus, immune-evasion signatures may serve as proxies for inflamed tumor microenvironments that remain sensitive to immunogenic chemotherapy and immune checkpoint blockade.

The basal subtype is highly heterogeneous in prognosis, and chemotherapy was historically the only systemic treatment. The advent of ICIs marked a therapeutic shift, though current single-agent ICIs benefit only ∼20% of patients and are associated with significant toxicity.[54] Implementing extended multigene panels in clinical practice is limited by cost and complexity. We therefore derived a concise and clinically practical basal signature comprising four genes (*CXCL9*, *CXCL10*, *CXCR4*, and *JAK3*), which demonstrated robust prognostic value across independent datasets, in both younger and older patients, and in those receiving neoadjuvant therapy. High expression of this panel was also associated with improved ICI response, supporting its predictive utility.

The basal four-gene signature reflects the interplay of immune activation and evasion within the tumor microenvironment. CXCL9 and CXCL10, IFN-γ-inducible chemokines and ligands for CXCR3, recruit CD8+ T cells and NK cells, promoting an immune-active phenotype linked to improved survival.[55,56] CXCL9, predominantly expressed in M1 macrophages, activates the JAK—STAT signaling pathway and enhances MHC-II pathway activity, underscoring its potential as a prognostic biomarker and a marker of immunotherapy efficacy in TNBC.[57] CXCR4, a chemokine receptor for CXCL12, promotes tumor cell migration and metastasis, a hallmark of basal BC aggressiveness[58] and is implicated in cancer-associated fibroblast-mediated immune exclusion and myeloid-derived suppressor cell recruitment.[27] The expression of *CXCL9* and *CXCL10* is frequently reduced in TNBC via BIRC2-mediated NF-κB suppression and Chi3l1-induced neutrophil recruitment.[27] A prior bioinformatics study of TNBC and normal tissue identified *CXCR4* and *CXCL10* as hub genes whose overexpression correlated with favorable recurrence-free survival, reinforcing their prognostic and therapeutic potential.[59] However, the effects of CXCR4 inhibition remain inconclusive: while POL5551 reduced migration and metastasis in TNBC models,[60] other inhibitors increased metastatic spread in xenografts.[61]

JAK3, a central kinase in the JAK—STAT pathway, is essential for cytokine receptor signaling and lymphoid cell

development.[62,63] In BC, JAK3 supports IFN-γ-mediated MHC-I up-regulation and immune activation but is often expressed at low levels, which correlates with poor survival outcomes in basal-like tumors.[64] Bioinformatics analyses further link *JAK3* expression with infiltration of tumor-associated macrophages, dendritic cells, CD8+ T cells, NK cells, and T-cell exhaustion markers, emphasizing its role in shaping the immune microenvironment.[64] Together, these findings highlight the duality of the basal four-gene panel, capturing both immune-activating and immune-suppressive processes that influence patient outcomes.

In our datasets, *ATG9A* emerged as a poor prognostic marker in basal tumors. *ATG9A*, a key regulator of autophagy, was previously shown to be overexpressed in TNBC and to drive proliferation and invasion.[65] Elevated *ATG9A* levels are associated with worse outcomes in oral squamous carcinoma[66] and with chemotherapy resistance via protection from mitochondrial damage.[67] Inhibiting ATG9A suppresses cancer phenotypes in TNBC cells,[65] underscoring autophagy as a therapeutic vulnerability in aggressive basal tumors.[68]

In HER2-positive BC, a minimal three-gene signature (*IL2RG*, *CD3E*, and *CD3G*) was consistently associated with favorable OS. Similar findings in ovarian cancer linked these genes to γδ T-cell infiltration and improved survival.[69,70] *IL2RG* encodes the common γ-chain receptor essential for T, B, and NK cell activation, with mutations causing severe immunodeficiency.[71-73] IL-2 signaling via *IL2RG* promotes T-cell proliferation and NK cell-mediated antibody-dependent cellular cytotoxicity against HER2-positive cells,[74-76] and *IL2RG* up-regulation has been described in early-stage TNBC.[77] CD3E and CD3G, integral components of the TCR/CD3 complex, are critical for T-cell activation,[78] and their expression correlates with TILs and response to neoadjuvant anti-HER2 therapy.[79] Collectively, this three-gene panel encapsulates a core T-cell activation module central to adaptive immunity in HER2-positive tumors.

Additionally, enrichment analyses confirmed that both minimal signatures are biologically coherent immune modules rather than statistical artifacts. The basal panel converged on chemokine-mediated recruitment and JAK–STAT effector signaling, consistent with an inflamed tumor microenvironment, whereas the HER2-positive panel highlighted TCR and cytokine signaling central to adaptive activation.

In summary, our findings identify minimal, mechanistically grounded immune signatures with prognostic and predictive value in basal and HER2-positive BC. These compact panels not only reflect key immune dynamics but also provide clinically feasible biomarkers. This work's primary strength lies is the identification of actionable targets for further exploration in experimental models and clinical cohorts, ultimately clarifying their clinical relevance.

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DISCLOSURE

The authors have declared no conflicts of interest.

REFERENCES

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229-263.
2. Burguin A, Diorio C, Durocher F. Breast cancer treatments: updates and new challenges. *J Pers Med*. 2021;11(8):808.
3. Gao JJ, Swain SM. Luminal A breast cancer and molecular assays: a review. *Oncologist*. 2018;23(5):556-565.
4. Ades F, Zardavas D, Bozovic-Spasojevic I, et al. Luminal B breast cancer: molecular characterization, clinical management, and future perspectives. *J Clin Oncol*. 2014;32(25):2794-2803.
5. Loibl S, Gianni L. HER2-positive breast cancer. *Lancet*. 2017;389 (10087):2415-2429.
6. Bergin ART, Loi S. Triple-negative breast cancer: recent treatment advances. *F1000Res*. 2019;8:F1000.
7. Joshi H, Press MF. 22 — Molecular oncology of breast cancer. In: Bland KI, Copeland EM, Klimberg VS, Gradishar WJ, editors. *The Breast*. Fifth Edition Amsterdam, The Netherlands: Elsevier; 2018282-307.e5.
8. Richman J, Dowsett M. Beyond 5 years: enduring risk of recurrence in oestrogen receptor-positive breast cancer. *Nat Rev Clin Oncol*. 2019;16(5):296-311.
9. Padmanabhan R, Kheraldine HS, Meskin N, Vranic S, Al Moustafa AE. Crosstalk between HER2 and PD-1/PD-L1 in breast cancer: from clinical applications to mathematical models. *Cancers (Basel)*. 2020;12 (3):636.
10. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
11. Ghorani E, Swanton C, Quezada SA. Cancer cell-intrinsic mechanisms driving acquired immune tolerance. *Immunity*. 2023;56(10):2270-2295.
12. Debien V, De Caluwé A, Wang X, et al. Immunotherapy in breast cancer: an overview of current strategies and perspectives. *NPJ Breast Cancer*. 2023;9(1):7.
13. Barbari C, Fontaine T, Parajuli P, et al. Immunotherapies and combination strategies for immuno-oncology. *Int J Mol Sci*. 2020;21(14): 5009.
14. Shiravand Y, Khodadadi F, Kashani SMA, et al. Immune checkpoint inhibitors in cancer therapy. *Curr Oncol*. 2022;29(5):3044-3060.
15. Emens LA. Breast cancer immunotherapy: facts and hopes. *Clin Cancer Res*. 2018;24(3):511-520.
16. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. *Proc Natl Acad Sci U S A*. 2012;109(8):2796-2801.
17. Bareche Y, Buisseret L, Gruosso T, et al. Unraveling triple-negative breast cancer tumor microenvironment heterogeneity: towards an optimized treatment approach. *J Natl Cancer Inst*. 2020;112(7):708-719.
18. Ali HR, Provenzano E, Dawson SJ, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. *Ann Oncol*. 2014;25(8):1536-1543.
19. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a

pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol*. 2018;19(1):40-50.
20. Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol*. 2013;31(7):860-867.
21. Savas P, Salgado R, Denkert C, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat Rev Clin Oncol*. 2016;13(4):228-241.
22. Stanton SE, Adams S, Disis ML. Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: a systematic review. *JAMA Oncol*. 2016;2(10):1354-1360.
23. Cortes J, Cescon DW, Rugo HS, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet*. 2020;396(10265):1817-1828.
24. Cortes J, Rugo HS, Cescon DW, et al. Pembrolizumab plus chemotherapy in advanced triple-negative breast cancer. *N Engl J Med*. 2022;387(3):217-226.
25. Schmid P, Cortes J, Dent R, et al. VP7-2021: KEYNOTE-522: phase III study of neoadjuvant pembrolizumab + chemotherapy vs. placebo + chemotherapy, followed by adjuvant pembrolizumab vs. placebo for early-stage TNBC. *Ann Oncol*. 2021;32(9):1198-1200.
26. Schmid P, Cortes J, Dent R, et al. Overall survival with pembrolizumab in early-stage triple-negative breast cancer. *N Engl J Med*. 2024;391(21):1981-1991.
27. Ababneh E, Velez S, Zhao J. Immune evasion and resistance in breast cancer. *Am J Cancer Res*. 2025;15(4):1517-1539.
28. Serrano García L, Jávega B, Llombart Cussac A, et al. Patterns of immune evasion in triple-negative breast cancer and new potential therapeutic targets: a review. *Front Immunol*. 2024;15:1513421.
29. Tarantino P, Barroso-Sousa R, Garrido-Castro AC, et al. Understanding resistance to immune checkpoint inhibitors in advanced breast cancer. *Expert Rev Anticancer Ther*. 2022;22(2):141-153.
30. Polk A, Svane IM, Andersson M, Nielsen D. Checkpoint inhibitors in breast cancer — current status. *Cancer Treat Rev*. 2018;63:122-134.
31. Yang T, Kang L, Li D, Song Y. Immunotherapy for HER-2 positive breast cancer. *Front Oncol*. 2023;13:1097983.
32. Lawson KA, Sousa CM, Zhang X, et al. Functional genomic landscape of cancer-intrinsic evasion of killing by T cells. *Nature*. 2020;586(7827):120-126.
33. Menyhart O, Kothalawala WJ, Győrffy B. A gene set enrichment analysis for cancer hallmarks. *J Pharm Anal*. 2025;15(5):101065.
34. Zhang H, Hong Z, Li P, Jiang H, Wu P, Chen J. Identification and validation of an immune evasion molecular subgroup of patients with colon cancer for implications of immunotherapy. *Front Genet*. 2022;13:811660.
35. Dugo M, Huang C-S, Egle D, et al. The immune-related 27-gene signature DetermaIO predicts response to neoadjuvant atezolizumab plus chemotherapy in triple-negative breast cancer. *Clin Cancer Res*. 2024;30(21):4900-4909.
36. Ensenyat-Mendez M, Orozco JIJ, Llinàs-Arias P, et al. Construction and validation of a gene expression classifier to predict immunotherapy response in primary triple-negative breast cancer. *Commun Med*. 2023;3(1):93.
37. Iwase T, Blenman KRM, Li X, et al. A novel immunomodulatory 27-gene signature to predict response to neoadjuvant immunochemotherapy for primary triple-negative breast cancer. *Cancers (Basel)*. 2021;13(19):4839.
38. Liu Y, Fu W, Chen W, Lin Y, Zhang J, Song C. Correlation of immune infiltration with clinical outcomes in breast cancer patients: the 25-gene prognostic signatures model. *Cancer Med*. 2021;10(6):2112-2124.
39. Xu H, Wang G, Zhu L, Liu H, Li B. Eight immune-related genes predict survival outcomes and immune characteristics in breast cancer. *Aging (Albany NY)*. 2020;12(16):16491-16513.
40. Di Giovannantonio M, Hartley F, Elshenawy B, et al. Defining hypoxia in cancer: a landmark evaluation of hypoxia gene expression signatures. *Cell Genom*. 2025;5(2):100764.
41. Lehoczki A, Menyhart O, Andrikovics H, et al. Prognostic impact of a senescence gene signature in multiple myeloma. *GeroScience*. 2025;47(3):5025-5037.
42. Saul D, Kosinsky RL, Atkinson EJ, et al. A new gene set identifies senescent cells and predicts senescence-associated pathways across tissues. *Nat Commun*. 2022;13(1):4827.
43. Stone L. Hypoxia-related gene panel is prognostic. *Nat Rev Urol*. 2018;15(7). 398-398.
44. Gyorffy B, Molnar B, Lage H, Szallasi Z, Eklund AC. Evaluation of microarray preprocessing algorithms based on concordance with RT-PCR in clinical samples. *PLoS One*. 2009;4(5):e5645.
45. Győrffy B. Discovery and ranking of the most robust prognostic biomarkers in serous ovarian cancer. *Geroscience*. 2023;45(3):1889-1898.
46. Koboldt DC, Fulton RS, McLellan MD, et al. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70.
47. Brueffer C, Gladchuk S, Winter C, et al. The mutational landscape of the SCAN-B real-world primary breast cancer transcriptome. *EMBO Mol Med*. 2020;12(10):e12118.
48. Parker JS, Mullins M, Cheang MCU, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167.
49. Menyhart O, Weltz B, Győrffy B. MultipleTesting.com: a tool for life science researchers for multiple hypothesis testing correction. *PLoS One*. 2021;16(6):e0245824.
50. Kovács SA, Fekete JT, Győrffy B. Predictive biomarkers of immunotherapy response with pharmacological applications in solid tumors. *Acta Pharmacol Sin*. 2023;44(9):1879-1889.
51. Xie Z, Bailey A, Kuleshov MV, et al. Gene set knowledge discovery with enrichr. *Curr Protoc*. 2021;1(3):e90.
52. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750-2767.
53. Ring BZ, Hout DR, Morris SW, et al. Generation of an algorithm based on minimal gene sets to clinically subtype triple negative breast cancer patients. *BMC Cancer*. 2016;16:143.
54. Kwa MJ, Adams S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): where to go from here. *Cancer*. 2018;124(10):2086-2103.
55. Han X, Wang Y, Sun J, et al. Role of CXCR3 signaling in response to anti-PD-1 therapy. *EBioMedicine*. 2019;48:169-177.
56. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation — a target for novel cancer therapy. *Cancer Treat Rev*. 2018;63:40-47.
57. Wu L, Sun S, Qu F, et al. CXCL9 influences the tumor immune microenvironment by stimulating JAK/STAT pathway in triple-negative breast cancer. *Cancer Immunol Immunother*. 2023;72(6):1479-1492.
58. Gupta N, Mohan CD, Shanmugam MK, et al. CXCR4 expression is elevated in TNBC patient derived samples and Z-guggulsterone abrogates tumor progression by targeting CXCL12/CXCR4 signaling axis in preclinical breast cancer model. *Environ Res*. 2023;232:116335.
59. Chuan T, Li T, Yi C. Identification of CXCR4 and CXCL10 as potential predictive biomarkers in triple negative breast cancer (TNBC). *Med Sci Monit*. 2020;26:e918281.
60. Xiang J, Hurchla MA, Fontana F, et al. CXCR4 protein epitope mimetic antagonist POL5551 disrupts metastasis and enhances chemotherapy effect in triple-negative breast cancer. *Mol Cancer Ther*. 2015;14(11):2473-2485.
61. Lefort S, Thuleau A, Kieffer Y, et al. CXCR4 inhibitors could benefit to HER2 but not to triple-negative breast cancer patients. *Oncogene*. 2017;36(9):1211-1222.
62. Johnston JA, Kawamura M, Kirken RA, et al. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature*. 1994;370(6485):151-153.
63. Liongue C, Ratnayake T, Basheer F, Ward AC. Janus kinase 3 (JAK3): a critical conserved node in immunity disrupted in immune cell cancer and immunodeficiency. *Int J Mol Sci*. 2024;25(5):2977.

64. Liu F, Wu H. Identification of prognostic biomarkers and molecular targets among JAK family in breast cancer. *J Inflamm Res*. 2021;14:97-114.
65. Claude-Taupin A, Fonderflick L, Gauthier T, et al. *ATG9A* is overexpressed in triple negative breast cancer and its in vitro extinction leads to the inhibition of pro-cancer phenotypes. *Cells*. 2018;7(12):248.
66. Tang JY, his E, Huang YC, et al. ATG9A overexpression is associated with disease recurrence and poor survival in patients with oral squamous cell carcinoma. *Virchows Arch*. 2013;463(6):737-742.
67. Wen J, Yeo S, Wang C, et al. Autophagy inhibition re-sensitizes pulse stimulation-selected paclitaxel-resistant triple negative breast cancer cells to chemotherapy-induced apoptosis. *Breast Cancer Res Treat*. 2015;149(3):619-629.
68. Mailler E, Guardia CM, Bai X, et al. The autophagy protein ATG9A enables lipid mobilization from lipid droplets. *Nat Commun*. 2021;12(1):6750.
69. Li Q, Yang Z, Ling X, et al. Correlation analysis of prognostic gene expression, tumor microenvironment, and tumor-infiltrating immune cells in ovarian cancer. *Dis Markers*. 2023;2023:9672158.
70. Zou C, Zhao P, Xiao Z, Han X, Fu F, Fu L. γδ T cells in cancer immunotherapy. *Oncotarget*. 2017;8(5):8900-8909.
71. Ren J, Yu D, Fu R, et al. IL2RG-deficient minipigs generated via CRISPR/Cas9 technology support the growth of human melanoma-derived tumours. *Cell Prolif*. 2020;53(10):e12863.
72. Walsh NC, Kenney LL, Jangalwe S, et al. Humanized mouse models of clinical disease. *Annu Rev Pathol*. 2017;12:187-215.
73. Zheng X, Huang C, Lin Y, et al. Generation of inactivated IL2RG and RAG1 monkeys with severe combined immunodeficiency using base editing. *Sig Transduct Target Ther*. 2023;8(1):327.
74. Carson WE, Parihar R, Lindemann MJ, et al. Interleukin-2 enhances the natural killer cell response to Herceptin-coated Her2/neu-positive breast cancer cells. *Eur J Immunol*. 2001;31(10):3016-3025.
75. Kumari B, Sakode C, Lakshminarayanan R, Roy PK. Computational systems biology approach for permanent tumor elimination and normal tissue protection using negative biasing: experimental validation in malignant melanoma as case study. *Math Biosci Eng*. 2023;20(5):9572-9606.
76. Yuan Y, Kolios AGA, Liu Y, et al. Therapeutic potential of interleukin-2 in autoimmune diseases. *Trends Mol Med*. 2022;28(7):596-612.
77. Li Y, Huang F, Deng R, Jiang D. Delivery of IL2RG mRNA via nanoparticles to enhance $CD8^+$ T cell promotes anti-tumor effects against late-stage triple-negative breast cancer. *Cancer Nano*. 2024;15(1):54.
78. Garcillán B, Fuentes P, Marin AV, et al. *CD3G* or *CD3D* knockdown in mature, but not immature, T lymphocytes similarly cripples the human TCR$\alpha\beta$ complex. *Front Cell Dev Biol*. 2021;9:608490.
79. Griguolo G, Serna G, Pascual T, et al. Immune microenvironment characterisation and dynamics during anti-HER2-based neoadjuvant treatment in HER2-positive breast cancer. *NPJ Precis Oncol*. 2021;5(1):23.