

# Prognostic immune-related gene models for breast cancer: a pooled analysis

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**Abstract:** Breast cancer, the most common cancer among women, is a clinically and biologically heterogeneous disease. Numerous prognostic tools have been proposed, including gene signatures. Unlike proliferation-related prognostic gene signatures, many immune-related gene signatures have emerged as principal biology-driven predictors of breast cancer. Diverse statistical methods and data sets were used for building these immune-related prognostic models, making it difficult to compare or use them in clinically meaningful ways. This study evaluated successfully published immune-related prognostic gene signatures through systematic validations of publicly available data sets. Eight prognostic models that were built upon immune-related gene signatures were evaluated. The performances of these models were compared and ranked in ten publicly available data sets, comprising a total of 2,449 breast cancer cases. Predictive accuracies were measured as concordance indices (C-indices). All tests of statistical significance were two-sided. Immune-related gene models performed better in estrogen receptor-negative (ER-) and lymph node-positive (LN+) breast cancer subtypes. The three top-ranked ER- breast cancer models achieved overall C-indices of 0.62–0.63. Two models predicted better than chance for ER+ breast cancer, with C-indices of 0.53 and 0.59, respectively. For LN+ breast cancer, four models showed predictive advantage, with C-indices between 0.56 and 0.61. Predicted prognostic values were positively correlated with ER status when evaluated using univariate analyses in most of the models under investigation. Multivariate analyses indicated that prognostic values of the three models were independent of known clinical prognostic factors. Collectively, these analyses provided a comprehensive evaluation of immune-related prognostic gene signatures. By synthesizing C-indices in multiple independent data sets, immune-related gene signatures were ranked for ER+, ER-, LN+, and LN- breast cancer subtypes. Taken together, these data showed that immune-related gene signatures have good prognostic values in breast cancer, especially for ER- and LN+ tumors.

**Keywords:** breast cancer, prognostic models, immune-related gene

## Introduction

Breast cancer is the most common cancer among women. As a clinically and biologically heterogeneous disease, accurate prognostic predictions are very important for breast cancer treatment planning. In the past, the pathological Tumor-Node-Metastasis Classification of Malignant Tumors staging system of the American Joint Committee on Cancer was the predominant prognostic prediction tool. In recent decades, molecular breast cancer subtypes have been identified based on pathological markers including estrogen receptor (ER), progesterone receptor, human epidermal growth factor 2 (HER2), and Ki67. Measured expression levels of these biomarkers are routinely used in clinical practices to stratify patients for prognostic predictions and to select treatments.<sup>1-4</sup> In particular, four distinct subtypes of breast cancers have been defined: luminal A-like,

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luminal B-like, HER2-positive, and triple negative. These subtypes were first adopted by the St Gallen International Expert Consensus panel in 2013 as a basis for selecting adjuvant systemic therapy regimens (ie, endocrine therapy, chemotherapy, or anti-HER2 therapy).<sup>2</sup> Nevertheless, diagnosis and treatment decision-making for breast cancer still relies on classical histopathological and immunohistochemical techniques. More quantitative diagnostic approaches and individualized treatment plans remain unmet needs. To meet these needs, multigene prognostic models have emerged, including 21-gene recurrence score,<sup>5–7</sup> PAM50,<sup>8–13</sup> MammaPrint<sup>®</sup>,<sup>14–16</sup> and Breast Cancer Index<sup>™</sup>.<sup>17–20</sup>

Historically, prognostic multigene predictors strictly consisted of proliferation-associated genes.<sup>21,22</sup> The tests were highly effective at classifying recurrence risks for ER-positive (ER+) and lymph node-negative (LN-) breast cancer subtypes; prognoses for these subtypes were well predicted by Ki67 indices or levels of DNA synthesis or cell cycle regulatory genes. Although most ER-negative (ER-) or LN-positive (LN+) breast cancers are highly proliferative, multigene predictors do not have discriminative power for prognoses within either subtype. Therefore, alternative prognostic signatures must be proposed for these subtypes that are not limited to proliferation-related genes.<sup>23,24</sup> Recent preclinical studies have underscored inflammation and the stromal immune landscape as important drivers of breast cancer. There has been growing interest in establishing prognostic multigene signatures focused on immune-related genes, such as the *STAT-1* gene, interferons, and immune response genes.<sup>25–32</sup>

Currently, no immune-related signature has been widely accepted in clinical practice; it is unknown whether one test is the most robust. In this study, all published immune-related prognostic gene models were systematically reviewed to evaluate performances across independent data sets. The aims of this study were two-fold: first, to investigate the prognostic effects of immune-related gene signatures and, second, to identify the most robust signature for the prognostication of nonmetastatic breast cancer.

## Methods

The design of this study had two major sections. First, published literature describing prognostic immune-related gene signatures and publicly available genomic data sets were systematically reviewed. Second, a multistudy evaluation of immune-related prognostic models that met specific inclusion criteria was performed. The literature search strategies as well as inclusion criteria of prognostic models and data sets are detailed in the following subsections.

## Literature search strategies

Systematic literature searches of articles published between July 2006 and March 2017 were performed independently by two authors (KC and JZ). In both cases, Internet-based searches of the PubMed and Web of Science platforms were performed without language or regional restrictions, using combinations of the following keywords: “breast cancer,” “breast neoplasm,” “breast carcinoma,” “immune-related gene,” “immune response gene,” “prognostic model,” and “immune-related predictor.” Reference lists of retrieved articles were manually screened to identify relevant articles. If a single study generated multiple publications, then either the highest quality publication or the most recent publication was chosen for subsequent analyses.

## Inclusion criteria of prognostic models

Published prognostic models of immune-related gene signatures were evaluated for inclusion in the current study. Eligible studies included the following: 1) a claim of providing prognostic value for nonmetastatic breast cancer cases; 2) a model that was based on mRNA expression levels, as quantified by microarray technologies; 3) a model that was developed using a training data set and then validated with a test data set; 4) a training data set of at least 30 subjects; 5) defined outcome measurements of either overall survival, disease-free survival (DFS), or recurrence-free survival (RFS); 6) a model that was fully specified or could be rederived from the specified data set; and 7) a model for which  $\geq 80\%$  of genes could also be identified in the validation data set.

## Inclusion criteria of data sets and samples

Publicly available microarray data sets were selected as validation data sets for this study. Eligible data sets included the following: 1) gene expression data gathered from human nonmetastatic breast cancer sources; 2) continuous time-to-event survival data; 3) at least 40 samples with 15 deaths; 4) measurements of gene expression profiles using U133A GeneChip technologies (Affymetrix/Thermo Fisher Scientific, Santa Clara, CA, USA); and 5) clinical characteristics of the enrolled subjects, including ER levels and LN status.

## Evaluation of published prognostic models on independent data

Immune-related gene prognostic models that met the inclusion criteria were evaluated on the included data sets. CEL files containing pixel intensity calculations of the validation data sets were downloaded from public databases (such as

the Gene Expression Omnibus). Gene expression levels were normalized based upon background corrections. For each prognostic model, the risk was calculated for each sample in the validation data set. The risk score calculation was defined as follows:  $\text{score} = \frac{\sum w_i x_i}{\sum |w_i|}$ , where  $x_i$  was the expression level of the involved gene in the model, and  $w_i$  was either +1 or -1, depending on the correlation between the gene and the prognosis of the paired subject described in the original publication.<sup>23,24,33</sup>

Concordance indices (C-indices) were calculated as previously described.<sup>34</sup> For each model tested on each validation data set, the C-index was defined as the probability that a patient was predicted to have a lower risk score when compared to another patient who survived longer than the first patient. A C-index of 0.5 indicated that the model did not predict risk better than random guess. A score of 1 indicated perfect prediction of risk. C-indices generated across all of the validation data sets were synthesized into a summary value using random effects modeling. All computations were completed using R (version 3.3.2).

## Stratification by ER status and LN status

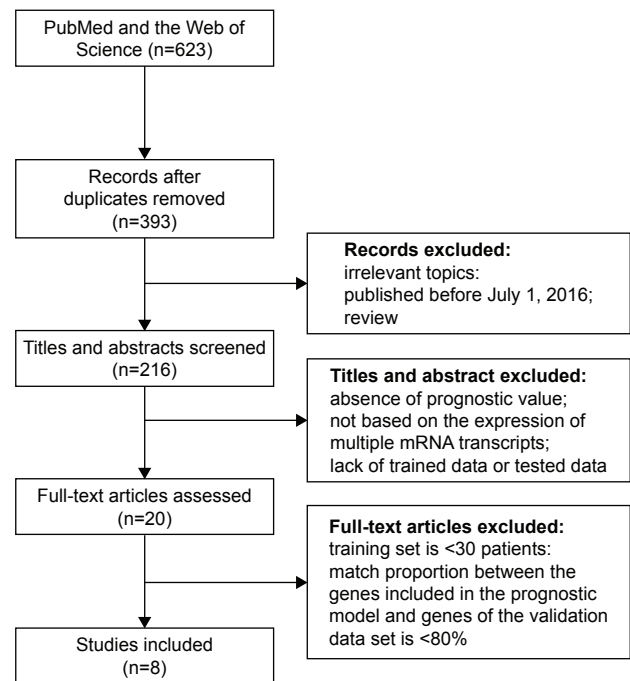
The discriminative power of each immune-related prognostic model for predicting breast cancer prognosis was evaluated in patients depending on ER and LN status. Patients were stratified into four subgroups (ER+ versus ER- and LN+ versus LN-). C-indices were computed in each of these four subgroups for every data set and then synthesized into a final assessment.

## Statistical analyses

All outcomes were reported with 95% CIs. Statistical heterogeneities between data sets were assessed using  $\chi^2$  tests, with significance levels set to  $P < 0.05$ . Statistical tests were two-sided, and  $P$ -values  $< 0.05$  were considered statistically significant. Random effects modeling was used if there was significant heterogeneity; otherwise, fixed effects modeling was reported.

## Results

A systematic review of prognostic immune-related gene signatures for breast cancer was performed. After excluding redundant literature, a total of 393 papers were investigated. From these papers, eight models emerged as candidates that passed the study inclusion criteria and were selected for further analyses (Figure 1). Table 1 summarizes the features of the included models. The performance of each model was evaluated in each of ten data sets that were selected based on above-stated inclusion criteria (Table 2).



**Figure 1** Flow chart for model selection process.

## Heterogeneity analyses

Heterogeneities across data sets were assessed using each immune-related gene model. Based on the results, C-indices of summary values were synthesized using either random effects or fixed effects models. Both methodologies produced similar summary values, resulting in similar ranking patterns for the immune-related gene models. Given that significant heterogeneity was identified in several models (Table 3), the random effects modeling methodology was chosen to synthesize C-indices across the prognostic models.

## Evaluation of prognostic models on independent data sets

Prognostic accuracy of each model was evaluated in every data set, with the exception of the training data set used by the original authors. Evaluations were quantified by C-indices (Figure 2A), and the models were ranked by summary values, from high to low. The top-ranked model was authored by Oh et al,<sup>27</sup> with a summary C-index of 0.61. Two other models had better than chance predictive values, with summary C-indices of 0.54 and 0.53, respectively.<sup>25,32</sup>

## Stratification by known prognostic factors

Further analyses were performed within subgroups that were stratified by ER status (ER+ versus ER-), or LN status (LN+ versus LN-). Only a subset of data sets, those in which these factors were recorded, could be assessed.

**Table 1** Features of eight published models that prognosticate breast cancer outcomes

First author; publication year	Clinical subtype	Training data (n)	Validation data (n)	Gene signature
Teschendorff et al <sup>25</sup> 2007	ER–	713	343	7-gene panel: <i>CIQA, IGIC2, LY9, TNFRSF17, SPPI, XCL2, HLA-F</i>
Teschendorff et al <sup>26</sup> 2010	All	1,223	830	<i>IL2, IL12, IFNG, IL4, IL13, TGFβ</i>
Oh et al <sup>27</sup> 2012	LN–	684	616	<i>UBE2C, PRC1, CCNB2, CDC20, CD3D, CD52, CCL19, TRBV20-1</i>
Bianchini et al <sup>28</sup> 2010	ER+, HER2–, HER2+	684	233	15-gene panel: <i>LYN, STK17A, CSF1R, PIM2, LCK, STK17B, BTK, HCK, PRKCB1, PRKCQ, FYN, ITK, ZAP70, MAP4K1, STK10</i>
Nagalla et al <sup>29</sup> 2013	All	977	977	B/P metagenes, M/D metagenes, T/INK metagenes
Yau et al <sup>30</sup> 2010	ER–	199	75	14-gene panel: <i>RG54, CXCL13, HAPLN1, HRBL, MATN1, PRTN3, SSX3, RPS28, EXOC7, ABO, CLIC5, RFXDC2, ZNF3, PRRG3</i>
Ursini-Siegel et al <sup>31</sup> 2010	HER2+, basal	179	2,481	43-gene SRIS: <i>CD247, CD3E, CD4, CD28, CTLA4, CXCL11, CXCL13, CXCL9, CXCR3, HLA-DQA1, IL1B, ITK, LAG3, LCK, NFATC2, PPIA, PTPRC, SLAMF6, SLAMF7, SLAMF9, TCRA, RELT, CD27, VTCN1, ATRN, CD69, ICOS, IL7R, B2M, CD8B, GZMB, IFNG, IL12B, LILRB3, TIA1, CD72, IGHG1, IGHM, IGHG2, IGJ, IL4, C1S, C3</i>
Cheng et al <sup>32</sup> 2013	ER–	1,981	184	Lymphocyte-specific immune recruitment panel: <i>PTPRC, CD53, LCP2, LAPTM5, DOCK2, IL10RA, CYBB, CD48, ITGB2, EVI2B</i>

**Notes:** <sup>a</sup>43 gene ShcA-regulated immune signature (SRIS). See supplementary material for additional information.

**Abbreviations:** ER, estrogen receptor; HER2, human epidermal growth factor 2; LN, lymph node.

Most immune-related gene models performed better in ER– data sets. The top-ranked three models for ER– breast cancer were authored by Nagalla et al,<sup>29</sup> Teschendorff et al,<sup>25</sup> and Oh et al.<sup>27</sup> These models achieved summary C-indices between 0.62 and 0.63. The remaining five models<sup>26,28,30–32</sup> also provided predictions that were better than chance, with summary C-indices varying from 0.56 to 0.59 (Figure 2B). Interestingly, summary C-indices in data from ER+ subjects were lower than in data from ER– subjects. The two top-ranking models for ER+ subjects were those authored by Oh et al<sup>27</sup> and Cheng et al<sup>32</sup> with summary C-indices of 0.59 and 0.53, respectively. The other six models showed no predictive advantage above chance in ER+ subjects (Figure 2C).

**Table 2** Features of ten publicly available microarray data sets used to validate published risk scores

Data sets	Microarray platform	Sample size (n)	Information about ER	Information about LN
ETABM158	UI33A	130	Yes	Yes
GSE11121	UI33A	200	Yes	Yes
GSE2034	UI33A	286	Yes	Yes
GSE25066	UI33A	508	Yes	Yes
GSE2603	UI33A	121	Yes	Yes
GSE3494-GPL96	UI33A	251	Yes	Yes
GSE45255	UI33A	139	Yes	Yes
GSE4922-GPL96	UI33A	289	Yes	Yes
GSE6532-GPL96	UI33A	327	Yes	Yes
GSE7390	UI33A	198	Yes	Yes
Total		2,449	10	10

**Abbreviations:** ER, estrogen receptor; LN, lymph node.

Data were also stratified by the status of invasion to the LNs. The prognostic models performed better in LN+ data sets. The four top-ranking models for LN+ were designed by Oh et al,<sup>27</sup> Nagalla et al,<sup>29</sup> Ursini-Siegel et al,<sup>31</sup> and Cheng et al.<sup>32</sup> Summary C-indices in these models ranged from 0.56 to 0.61. In the remaining four models,<sup>25,26,28,30</sup> no predictive advantages above chance were observed (Figure 2D). Synthesized C-indices for LN– subjects were lower than those calculated for LN+ subjects. Only two models, originally published by Oh et al<sup>27</sup> and Teschendorff et al,<sup>25</sup> predicted outcomes better than chance (Figure 2E).

### Univariate and multivariate analyses

Univariate analyses were used to investigate correlations between risk scores and the presence of ERs or LN invasion.

**Table 3** Heterogeneity analyses for eight publicly available models

First author; publication year	All data (P)	ER– (P)	ER+ (P)	LN+ (P)	LN– (P)
Nagalla et al <sup>29</sup> 2013	0.13	0.01	0.08	0.63	0.25
Teschendorff et al <sup>25</sup> 2007	0.01	0.05	0.07	0.91	0.14
Oh et al <sup>27</sup> 2012	0.12	0.02	0.07	0.68	0.01
Ursini-Siegel et al <sup>31</sup> 2010	0.18	0.13	0.30	0.47	0.01
Teschendorff et al <sup>26</sup> 2010	0.01	0.38	0.03	0.47	0.08
Bianchini et al <sup>28</sup> 2010	0.07	0.01	0.04	0.02	0.01
Cheng et al <sup>32</sup> 2013	0.01	0.08	0.33	0.67	0.97
Yau et al <sup>30</sup> 2010	0.15	0.29	0.31	0.99	0.16

**Note:** P-values of heterogeneity analyses in validation data sets are presented when analyzing all the data, ER–, ER+, LN+, and LN– cohorts, respectively.

**Abbreviations:** ER, estrogen receptor; LN, lymph node.

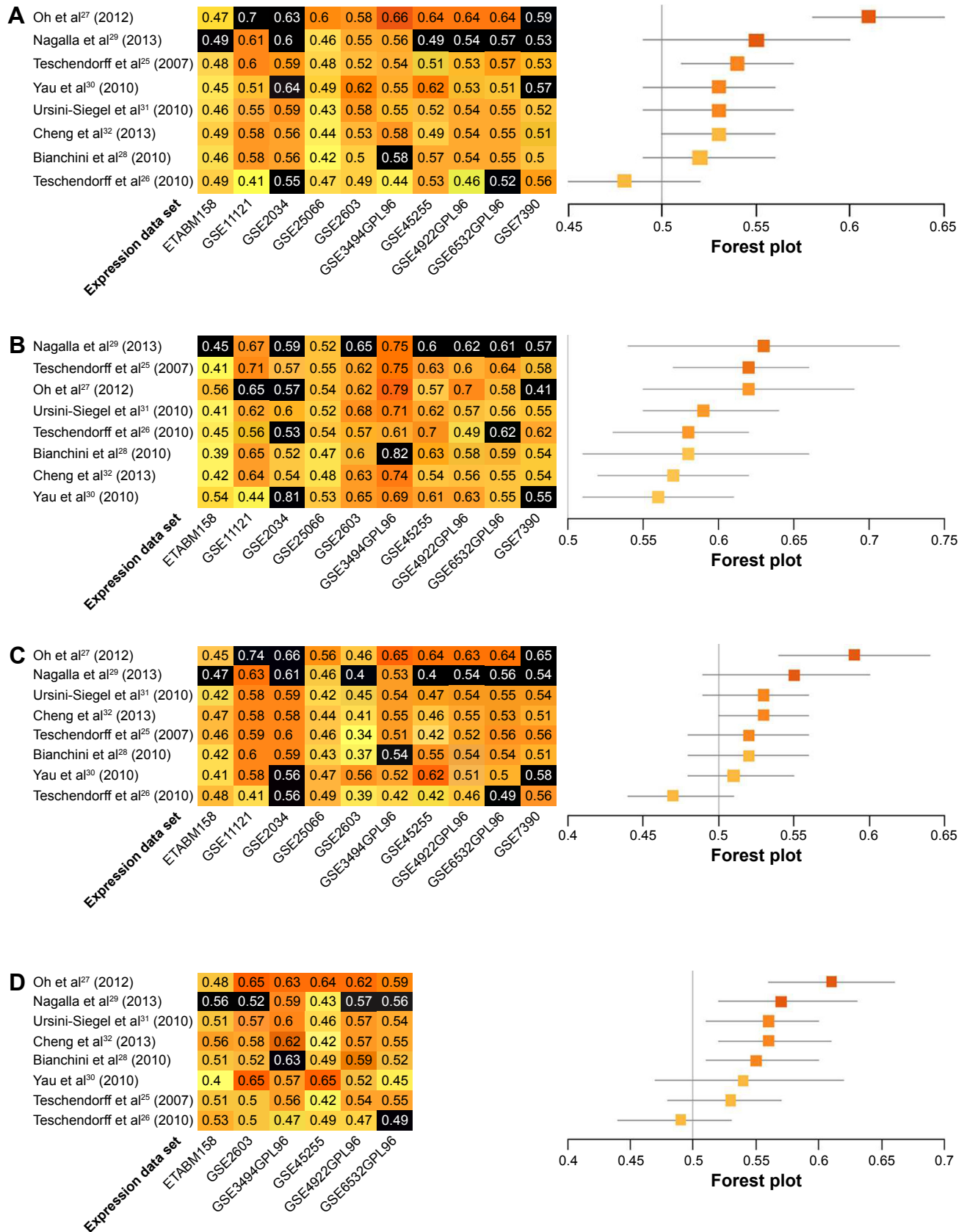
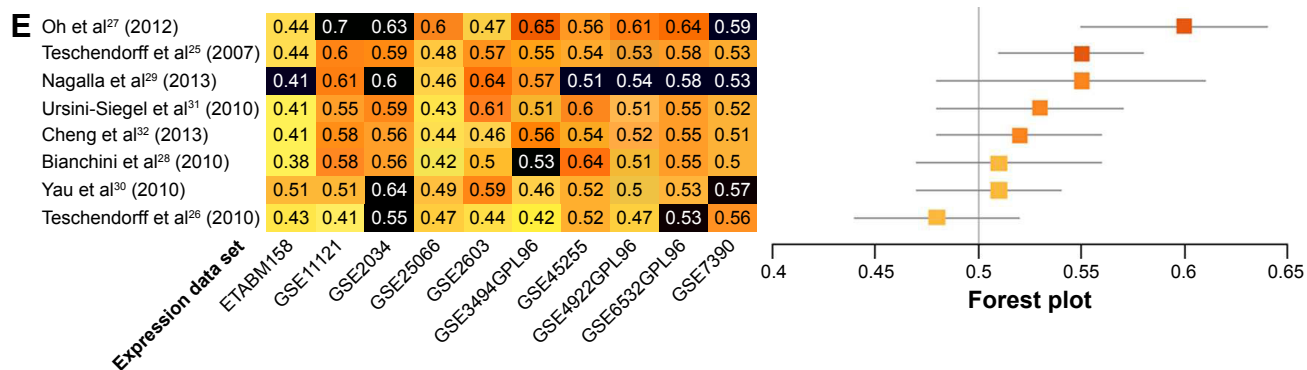


Figure 2 (Continued)



**Figure 2** Performance assessment of published immune-related risk scores. **Notes:** (A) C-indices are given for predictions of overall survival for each of eight models in each of ten microarray data sets (left panel). Data sets used as training data sets during model development are shown in black. Darker shades of orange corresponded to higher prediction levels; 0.5= random risk score, 1.0= perfect prediction. Models are ordered from the highest to the lowest summary C-index (top to bottom). Summary C-indices are given for each model with training data sets excluded (orange boxes; right panel); 95% CIs (gray lines) were obtained by resampling cases. C-indices are presented for predictions of overall survival in ER-, ER+, LN+, and LN- data sets (left panels of B-E, respectively). Summary C-indices in ER-, ER+, LN+, and LN- data sets are given for each model with training data sets excluded (orange boxes; right panels of B-E, respectively). **Abbreviations:** C-indices, concordance indices; ER, estrogen receptor; LN, lymph node.

Risk scores from the prognostic models designed by Nagalla et al,<sup>29</sup> Teschendorff et al,<sup>25</sup> Cheng et al,<sup>32</sup> Bianchini et al,<sup>28</sup> Yau et al,<sup>30</sup> and Ursini-Siegel et al<sup>31</sup> significantly correlated with ER status (range of odds ratios: 1.01–1.06;  $P < 0.05$ ). Risk scores from the models of Oh et al<sup>27</sup> and Yau et al<sup>30</sup> significantly correlated with LN status (odds ratios: 1.02 and 1.22, respectively;  $P < 0.05$ ). In multivariate analyses that controlled for the status of ER, LN, HER2, and age, DFS was associated with risk scores in the models of Oh et al<sup>27</sup> ( $P = 0.0443$ ), Cheng et al<sup>32</sup> ( $P = 0.0391$ ), and Bianchini et al<sup>28</sup> ( $P = 0.0343$ ). These findings demonstrated that modeling immune-related gene signatures may provide unique information to prognosticate breast cancer cases.

## Discussion

Gene expression profile studies on breast cancer have uncovered new insights into the genetic pathways that contribute to tumorigenesis. This research has inspired the development of gene expression signatures to predict patient outcomes. It is generally understood that proliferation-associated genes account for the majority of predictive power of many previously reported prognostic genetic tools, such as the 21-gene Oncotype DX test (Genomic Health, Inc., Redwood City, CA, USA).<sup>5,7</sup> However, these prognostic models are only applicable for low proliferation tumors, as found in ER+ and LN- breast cancer subtypes. Therefore, developing new prognostic signatures, independent of proliferation phenotypes, is a challenging unmet need.<sup>23,24</sup>

In recent years, research revealed that immune-related gene signatures were associated with both clinical outcomes and pathological complete response rates in breast

cancer.<sup>25,35–37</sup> A whole-transcriptome analysis of the NCCTG-N9831 adjuvant trastuzumab trial in early-stage HER2+ breast cancer revealed that tumors enriched for immune-functioning genes had increased RFS rates. Conversely, trastuzumab did not increase RFS in patients whose tumors lacked elevated immune-functioning gene expression scores. These data suggested that the increased expression of a subset of immune-functioning genes predicted benefit from adjuvant trastuzumab.<sup>38</sup> Additionally, the Finland Herceptin trial reported that higher levels of tumor-infiltrating lymphocytes at diagnosis had a significant inverse association with distant recurrence rates in primary ER-/HER2- breast cancers.<sup>39</sup>

Previously, investigators pooled genomic data to identify predictors of clinical outcomes in breast cancer.<sup>25–32</sup> The current study is the largest pooled analysis of immune-related prognostic gene models for breast cancer; all published immune-related prognostic gene models were evaluated. For ER- breast cancer, the top-ranking risk score was achieved by the model of Nagalla et al.<sup>29</sup> This model comprised three distinct immunity-related metagenes indicative of tumor-immune cell interactions. The first metagene, the B/P cluster, consisted primarily of immunoglobulin-encoding genes that were expressed in B-cell and plasma cell populations. The second metagene was the T/NK cluster, which contained components of the T-cell receptor-cluster of differentiation 3 complex and granzymes. This was largely restricted to T cells and natural killer cells. The third metagene was the M/D cluster, which included the major histocompatibility complex class II and myeloid-specific markers. These were expressed at the highest levels in monocytes and dendritic cells. This prognostic model covered the most comprehensive

array of immune-related genes in the cohort studied here. This may explain why the model of Nagalla et al had the highest prognostic value.

In the current study, seven of ten validation data sets were training data sets used to develop the model of Nagalla et al.<sup>29</sup> This reduced the statistical power for the validation of this model. Therefore, the model of Teschendorff et al,<sup>25</sup> the second best prognostic model for ER– breast cancer, may have the most clinical value. This model employed a methodology called profile analysis using clustering and kurtosis (PACK). PACK was a semi-supervised algorithm, consisting of two main steps. First, a feature selection criterion was performed. Second, a supervised step correlated the selected features with specific phenotypes.<sup>40</sup> Despite being a conservative procedure that removed true positives, PACK efficiently removed false positives, providing more reliable identifications of prognostic markers. Another advantage of PACK was that it tested prognostic genes by applying two statistical tools: singular value decomposition<sup>41</sup> and the shrunken centroids classifier.<sup>42</sup> Two of the potential prognostic genes identified by the model of Teschendorff et al<sup>25</sup> were associated with breast cancer clinical outcomes. Specifically, *CIQA*, a gene involved in the classical complement pathway, harbored a single nucleotide polymorphism that correlated with distant breast cancer metastases.<sup>43</sup> Two other studies also found prognostic value for *OPN* in metastatic breast cancer.<sup>44,45</sup>

In the current study, the prognostic model by Oh et al<sup>27</sup> ranked first in ER+ breast cancer and third in ER– breast cancer. This was the only model that integrated proliferation- and immunity-related genes into a single prognostic model for nonmetastatic breast cancer patients. Additionally, this model used a large data set, which reduced size-based sampling problems. Prognostic genes were selected based upon both statistical significance and biological relevance. Together, these factors explain the success of the model of Oh et al in prognosticating breast cancer outcomes.

The current study also revealed that immune-related gene models performed best in ER– and LN+ breast cancer subtypes. This finding directly opposed current proliferation-related gene models. ER– and LN+ breast cancers tend to present as high-grade diseases that frequently harbor *p53* mutations, leading to worsen prognoses. These molecular and pathological tendencies may explain why differences in clinical outcome within the ER– subgroup cannot be explained by the differential activation of cell cycle pathways. Thus, proliferation-related gene models do not have prognostic power in this breast cancer subtype. Unlike

proliferation-related functional gene signatures, immune-related gene expression profiles have more individualized properties. Immune-related models have achieved statistical significance in both high and low proliferation breast neoplasms.<sup>29</sup> Therefore, immune-related gene signature-based tools may be advantageous for the prognostication of ER– and LN+ breast cancers. Future use of these tools may complement more established proliferation-based gene expression panels.

It is of high clinical relevance to identify patients who will experience poor outcomes prior to the initiation of therapy. Patients with lower immune-related gene expression scores may require increased follow-up frequency and systemic management after traditional adjuvant therapy (eg, metronomic chemotherapy for triple-negative breast cancer or ovary functional suppression for ER+ breast cancer). Alternatively, these patients might be candidates for future clinical trials evaluating therapeutic approaches to enhance immune activity within breast tumors, such as by targeting checkpoint proteins.<sup>46</sup>

The coverage of immune-related gene signatures included many critical and well-studied immunomodulatory genes, such as *TGFβ*, *CD28*, and *CTLA4*. Members of the immunomodulatory *Siglec* gene family were not included in these models. Most of the *Siglec* family members contain immunoreceptor tyrosine-based inhibitory motifs in regions exposed to cytosolic compartments. *Siglec* proteins are inhibitory receptors for both innate and adaptive immune cells and may attenuate reductions in tumor cell immunity. CD169/*Siglec-1* is bound to cell surface-associated (MUC1) receptors that were expressed on breast cancer cells.<sup>47</sup> High densities of CD169+ macrophages in regional LNs surrounding colorectal tumors, endometrial carcinomas, malignant melanomas, and breast neoplasms were associated with improved clinical prognoses. These findings may be due to increases in cytotoxic immune cell infiltrates in these tumors.<sup>48–50</sup> Therefore, expression levels of *Siglec* immunomodulatory gene family members could affect prognostic breast cancer outcomes. Functions of *Siglec* gene products have been well studied in oncogenic model systems. Further investigations should concentrate on clinical oncology. Future immune-related prognostic gene models for breast cancer should consider assessing *Siglec* gene expression levels.

The study reported here had several limitations. First, the selected models included only intracellular immune-related genes. Therefore, it remains unknown whether interstitial genes in the microenvironment, such as *CCL-18*,<sup>51</sup> can

prognosticate breast cancer patient outcomes. Second, useful gene expression profiles that could be included in this study were limited by incomplete clinical information. This caveat was an unavoidable inadequacy found in all publicly available data sets. Third, evaluations of these gene models were limited to the breast cancer subtypes discussed above. Few authors have investigated the impact of immune-related prognostic gene models on conventional breast cancer subtypes. Few studies have published validation data sets containing clinical information that specifies patient molecular subtypes. Rather, previous studies published information specifying ER status and regional LN involvement. This allowed, in the current study, for the stratification of summary C-indices by these characteristics. The model by Nagalla et al<sup>29</sup> was the only tool in which subgroup analyses were performed in subtypes. In those studies, basal-like breast tumors were positively associated with T/NK, M/D, and B/P metagenes. Similarly, luminal B tumors trended toward association with the T/NK metagene and were significantly associated with the M/D metagene. None of the metagenes were significantly associated with the luminal A, HER2-enriched, or claudin-low tumor subtypes.

Despite these limitations, the current analysis revealed the top-ranked immune-related prognostic gene models for ER+, ER-, LN+, and LN- breast cancer subtypes. Moreover, these data showed that immune-related gene signatures had good prognostic value in breast cancer, especially for ER- and LN+ tumors. Therefore, immune-related gene models may be clinically relevant and complementary to current proliferation-related gene models. The use of immune-related gene models may fulfill an unmet need in breast cancer prognostication.

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## Disclosure

The authors report no conflicts of interest in this work.

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## Supplementary material

### Full forms of genes provided in Table 1

STAT1: Signal transducer and activator of transcription 1

IFN: Interferons

IR: Immune response gene

C1QA: Complement C1q subcomponent subunit A

LY9: T-lymphocyte surface antigen Ly-9

TNFRSF17: Tumor necrosis factor receptor superfamily member 17

SPP1: Bone sialoprotein I

XCL2: Chemokine (C motif) ligand 2

HLA-F: HLA class I histocompatibility antigen, alpha chain F

IL2: Interleukin 2

IL12: Interleukin 12

IFNG: Interferon gamma

IL4: Interleukin 4

IL13: Interleukin 13

TGFB: Transforming growth factor beta

UBE2C: Ubiquitin-conjugating enzyme E2 C

PRC1: Protein regulator of cytokinesis 1

CCNB2: G2/mitotic-specific cyclin-B2

CDC20: The cell-division cycle protein 20

CD3D: T-cell surface glycoprotein CD3 delta chain

CD52: Cluster of differentiation 52

CCL19: Chemokine (C-C motif) ligand 19

TRBV20-1: T-cell receptor beta variable 20-1

LYN: LYN Proto-oncogene, Src family tyrosine kinase

STK17A: Serine/threonine kinase 17a

CSF1R: Colony-stimulating factor 1 receptor

PIM2: Pim-2 proto-oncogene, serine/threonine kinase

LCK: LCK proto-oncogene, Src family tyrosine kinase

STK17B: Serine/threonine kinase 17b

BTK: Bruton tyrosine kinase

HCK: HCK proto-oncogene, Src family tyrosine kinase

PRKCB1: Protein kinase C beta

PRKCQ: Protein kinase C theta

FYN: FYN proto-oncogene, Src family tyrosine kinase

ITK: IL2 Inducible T-cell kinase

ZAP70: Zeta chain of T-cell receptor associated protein kinase 70

MAP4K1: Mitogen-activated protein kinase 1

STK10: Serine/threonine kinase 10

B/P metagenes: B/P cluster consisted of genes (mostly immunoglobulin-encoding genes)

M/D metagenes: Genes of M/D cluster (including major histocompatibility complex class II and myeloid-specific markers)

T/NK metagenes: Expression of genes in T/NK cluster (such as components of the T cell receptor–cluster of differentiation 3 complex and granzymes)

CXCL13: C-X-C motif chemokine ligand 13

HAPLN1: Hyaluronan and proteoglycan link protein 1

HRBL: Arf-GAP domain and FG repeats-containing protein 2

MATN1: Matrilin 1, cartilage matrix protein

PRTN3: Proteinase 3

SSX3: SSX family member 3

RPS28: Ribosomal protein S28

EXOC7: Exocyst complex component 7

ABO: Alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase

CLIC5: Chloride intracellular channel 5

RFXDC2: Regulatory factor X domain containing 2

ZNF3: Zinc finger protein 3

PRRG3: Proline rich and Gla domain 3

CD247: CD247 molecule

CD3E: CD3e molecule

CD4: CD4 molecule

CD28: CD28 molecule

CTLA4: Cytotoxic T-lymphocyte associated protein 4

CXCL11: C-X-C motif chemokine ligand 11

CXCL13: C-X-C motif chemokine ligand 13

CXCL9: C-X-C motif chemokine ligand 9

CXCR3: C-X-C motif chemokine receptor 3

HLA-DQA1: Major histocompatibility complex, Class II, DQ alpha 1

IL1B: Interleukin 1 beta

ITK: IL2 Inducible T-cell kinase

LAG3: Lymphocyte activating 3

LCK: LCK Proto-oncogene, Src family tyrosine kinase

NFATC2: Nuclear factor of activated T-cells 2

PPIA: Peptidylprolyl isomerase A

PTPRC: Protein tyrosine phosphatase, receptor type C

SLAMF6: SLAM family member 6

SLAMF7: SLAM family member 7

SLAMF9: SLAM family member 9

TCRA: T-cell receptor alpha locus

RELT: RELT, TNF receptor

CD27: CD27 molecule

VTCN1: V-set domain containing T-cell activation inhibitor 1

ATRN: Attractin

CD69: CD69 molecule

ICOS: Inducible T-cell costimulator

IL7R: Interleukin 7 receptor

B2M: Beta-2-microglobulin

CD8B: CD8b molecule  
GZMB: Granzyme B  
IL12B: Interleukin 12B  
LILRB3: Leukocyte immunoglobulin like receptor B3  
TIA1: TIA1 Cytotoxic granule associated RNA binding protein  
CD72: CD72 molecule  
IGHG1: Immunoglobulin heavy constant gamma 1 (G1m marker)  
IGHM: Immunoglobulin heavy constant Mu  
IGHG2: Immunoglobulin heavy constant gamma 2 (G2m marker)  
IGJ: A J chain is a protein component of the antibodies IgM and IgA.

C1S: Complement C1s subcomponent  
C3: Complement C3  
PTPRC: Protein tyrosine phosphatase, receptor type C  
CD53: CD53 molecule  
LCP2: Lymphocyte cytosolic protein 2  
LAPTM5: Lysosomal protein transmembrane 5  
DOCK2: Deducator of cytokinesis 2  
IL10RA: Interleukin 10 receptor subunit alpha  
CYBB: Cytochrome B-245 beta chain  
CD48: CD48 molecule  
ITGB2: Integrin subunit beta 2  
EVI2B: Ecotropic viral integration site 2B

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