



REVIEW

# Emerging immune gene signatures as prognostic or predictive biomarkers in breast cancer

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**Abstract** Several multigene assays have been developed to predict the risk of distant recurrence and response to adjuvant therapy in early breast cancer. However, the prognostic or predictive value of current proliferation gene signature-based assays are limited to hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2-) early breast cancer. Considerable discordance between the different assays in classifying patients into risk groups has also been reported, thus raising questions about the clinical utility of these assays for individual patients. Therefore, there still remains a need for better prognostic or predictive biomarkers for breast cancer. The role of immune cells comprising tumor microenvironment in tumor progression has been recognized. Accumulating evidences have shown that immune gene signatures and tumor-infiltrating lymphocytes (TILs) can be prognostic or predictive factors in breast cancer, particularly with regard to HER2+ and triple-negative breast cancer. In this review, I summarize current multigene assays for breast cancer and discuss recent progress in identifying novel breast cancer biomarkers, focusing on the emerging importance of immune gene signatures and TILs as prognostic or predictive biomarkers.

**Keywords** Breast cancer · Prognostic biomarker · Predictive biomarker · Multigene assay · Immune gene signatures · Tumor-infiltrating lymphocytes

## Introduction

For optimal treatment decision-making in patients with early breast cancer, it is important to accurately predict the risk of recurrence and response to therapy. Clinicopathological variables, such as tumor size, lymph node (LN) status, and tumor grade are traditional prognostic factors, whereas estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status are both prognostic and predictive factors for breast cancer (Donegan 1997; Cianfrocca and Goldstein 2004). ER status predicts responses to endocrine therapy and HER2 status is useful for identifying patients who are likely to benefit from anti-HER2 therapy (Cianfrocca and Goldstein 2004; Nicolini et al. 2018).

However, these traditional factors alone are not sufficient for optimal treatment decisions, and, consequently, several molecular assays based on multiple gene expression signatures have been developed to better predict the prognosis and treatment responses among breast cancer patients. Since the introduction of the first-generation multigene assays, including MammaPrint (van 't Veer et al. 2002) and Oncotype DX (Paik et al. 2004), several prognostic assays for early breast cancer, such as Prosigna (Parker et al. 2009) and EndoPredict (Filipits et al. 2011) have been subsequently developed. These assays have been shown to provide additional prognostic value beyond that provided by clinicopathological factors. Two assays—Oncotype DX (Paik et al. 2006; Albain et al. 2010; Sparano et al. 2018) and MammaPrint (Cardoso et al. 2016)—have also been shown to be predictive for adjuvant chemotherapy in hormone receptor-positive and HER2-negative (HR+/HER2-) early breast cancer.

Although substantial progress has been made with regard to molecular breast cancer assays, several major challenges remain. These assays are strong prognostic

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factors for early recurrence, but they are suboptimal for predicting late recurrence (> 5 years after diagnosis) in HR+/HER2- early breast cancer (Partridge and Carey 2017). Considerable discordance between the different assays in classifying patients into risk groups has also been reported, raising questions about the accuracy of these assays for individual patients (Bartlett et al. 2016; Varga et al. 2019). Moreover, the prognostic or predictive values of these assays are limited to HR+/HER2- breast cancer but not other subtypes of breast cancer (Varga et al. 2019). Therefore, there is a need for better predictors for late recurrence and chemotherapy benefit in HR+/HER2- early breast cancer and novel prognostic or predictive markers for other subtypes of breast cancer, including HR-/HER2+ and triple-negative breast cancer (TNBC, HR-/HER2-), in which it is difficult to accurately predict the response to first-line chemotherapy or HER2-targeted therapy. More recently, with the development of immunotherapies in breast cancer, there is an emerging need to identify reliable biomarkers to predict which patients will benefit from immune checkpoint inhibitors.

Tumors comprise not only tumor cells but also surrounding stroma, including extracellular matrix (ECM) and various stromal cells, such as endothelial cells, fibroblasts and infiltrating immune cells (Joyce 2005). ECM and stromal cells constituting the tumor microenvironment are known to contribute to cancer progression through interactions with malignant cells (Hanahan and Weinberg 2011). Tumor-associated stromal cells, such as cancer-associated fibroblasts and macrophages, have been reported to play roles in breast cancer progression (Mao et al. 2013). There is accumulating evidence supporting the clinical significance of stromal and immune gene signatures as prognostic or predictive markers for breast cancer. However, these gene signatures have not been incorporated into the most current multigene assays, and to date, there is no commercial assay mainly based on immune gene signatures. Given that most of currently available assays rely on the expression of HR or proliferation-related genes in tumor cells and that their utility is limited to early HR+/HER2- breast cancer (Varga et al. 2019), stromal or immune gene expression reflecting the tumor microenvironment may contribute to the development of better prognostic or predictive markers for breast cancer. In this review, I summarize the current multigene prognostic assays for breast cancer and discuss recent progress in identifying novel prognostic or predictive biomarkers, with a focus on immune gene signatures. I also discuss how stromal or immune gene signatures can be incorporated into current multigene assays to improve their prognostic or predictive ability and can be used to develop novel assays for breast cancer.

## Multigene signature-based prognostic assays for HR+/HER2- early breast cancer

The commonly used commercial molecular assays for breast cancer include Oncotype DX, MammaPrint, Prosigna, EndoPredict, and Breast Cancer Index (BCI). These assays are available for clinical use. The details of the assays are provided in Table 1.

Oncotype DX, the most commonly used test, is a 21-gene signature assay based on quantitative real-time reverse transcription-PCR (qRT-PCR). Patients are classified into low-, intermediate-, and high-risk categories according to the recurrence score (RS) calculated from the expression of 21 genes, comprising 16 cancer-related and five reference genes (Paik et al. 2004). The prognostic and predictive value of the Oncotype DX RS for patients with pathologic N0 or N1 status (pN0-N1), ER+ breast cancer was validated in several retrospective trials (Paik et al. 2004; Paik et al. 2006; Albain et al. 2010; Dowsett et al. 2010; Mamounas et al. 2017). The TAILORx trial prospectively validated the clinical utility of Oncotype DX in HR+/HER2-, lymph node-negative (LN-) breast cancer. Low-risk patients (RS 0–10) who were treated with endocrine therapy alone had very low recurrence rates at 5 years (Sparano et al. 2015). A further study conducted to assess the predictive value of RS in patients with intermediate risk (RS 11–25) revealed no benefits of chemotherapy in patients aged > 50 years but showed chemotherapeutic benefits in those aged ≤ 50 years who had a RS 16–25 (Sparano et al. 2018). Taken together, TAILORx results demonstrated that patients with low or intermediate RS do not benefit from adjuvant chemotherapy. More recently, a secondary analysis of the TAILORx data demonstrated that clinical risk stratification provided additional prognostic information for patients aged ≤ 50 years with a RS of 16–25 (Sparano et al. 2019). Importantly, this study showed that there was no benefit from chemotherapy for women aged ≤ 50 years with a RS of 16–20 and low clinical risk. However, patients with a RS of 16–25 and high clinical risk benefitted from chemotherapy.

MammaPrint, a microarray-based 70-gene assay was the first molecular assay to receive approval by the Food and Drug Administration (FDA) in the United States (Ross et al. 2008). This assay classifies patients into low- and high-risk groups based on the risk of distant recurrence within 5 years and 10 years (van 't Veer et al. 2002). The prognostic ability of this assay in early breast cancer was independently validated by a study conducted by the TRANSBIG consortium (Buyse et al. 2006). The prospective MINDACT trial assessed the clinical utility of the addition of MammaPrint to standard clinicopathological factors for selecting patients for adjuvant chemotherapy. It demonstrated a clinical rationale behind excluding chemotherapy based on low MammaPrint risk scores in early breast cancer (Cardoso et al.

**Table 1** Comparison of the commercially available multigene tests for early breast cancer

Multigene assays	MammaPrint	Oncotype DX	Breast Cancer Index	Prosigna	Endopredict	GenesWell BCT
Company	Agendia	Genomic Health	Biotheranostics	Nanostring	Sividon	Gencurix
Year first validated	2002 (Van 't Veer et al. 2002)	2004 (Paik et al. 2004)	2008 (Ma et al. 2008)	2009 (Parker et al. 2009)	2011 (Filipits et al. 2011)	2017 (Gong et al. 2017)
Current indications for testing	pN0-N1	HR+/HER2-, pN0-N1	HR+/HER2-, pN0	HR+, pN0-N1, postmenopausal status	HR+/HER2-, pN0-N1, postmenopausal status	HR+/HER2-, pN0-N1
No of genes	70	21 (16+5)	11 ([5+2]+4)	58 (50+8)	12 (8+4[3+1])	9 (6+3)
Combination with clinical parameters in score calculation	Prognostic and predictive	Prognostic and predictive	No	Yes (tumor size and LN status)	Yes (tumor size and LN status [EPclin])	Yes (tumor size and LN status)
Major retrospective validation studies	TRANBIG (Buyse et al. 2006)	NSABP B14 (Paik et al. 2004), TransATAC (Dowsett et al. 2010), SWOG8814 (prognostic and predictive) (Albain et al. 2010), NSABP B28 (Mamounas et al. 2017), NSABP20 predictive (Paik et al. 2006)	TransATAC (Sgroi et al. 2013), Zhang et al. (2013), Sgroi et al. (2016)	ABCSG8 (Gnant et al. 2014), TransATAC (Sestak et al. 2015) and DBCG 77B (Laenkholm et al. 2018)	ABCSG06 (Filipits et al. 2011) and ABCSG08 (Fitzal et al. 2015)	Gong et al. (2017), Kwon et al. (2018)
Prospective validation study (prediction of chemotherapy benefit)	MINDACT (Cardoso et al. 2016)	TAILORx (Sparano et al. 2018)	No	No	No	No
Tissue type used	FFPE (2011) (Mittmpergher et al. 2011), Frozen/Fresh	FFPE	FFPE	FFPE	FFPE	FFPE
Methodology	Microarray	qRT-PCR	qRT-PCR	Nanostring nCounter	qRT-PCR	qRT-PCR
Genomic scores	MammaPrint Index (MPI -1 to +1)	Recurrence score (RS 0-100)	Risk score (0-10)	ROR score (0-100)	EP score (0-15), EPclin score	BCT score (0-10)
Risk categories (risk of recurrence)	low, high	low, intermediate, high	low, high	low, intermediate, high	low, high	low, high

FFPE formalin-fixed, paraffin-embedded, HER2 human epidermal growth factor receptor 2, HR hormone receptor, LN lymph node, pN0-N1 pathologic N0 or N1, qRT-PCR quantitative real-time reverse transcription-PCR

2016). Recently, the PROMIS trial—conducted to determine whether MammaPrint could inform treatment decisions for patients with intermediate Oncotype DX RS (RS 18–30)—showed that, in 33.6% of cases, treatment decisions were changed based on MammaPrint results (Tsai et al. 2018).

The Prosigna assay, which was approved by the FDA in 2013 (Nielsen et al. 2014), calculates a risk of recurrence (ROR) score based on the expression of 50 genes, molecular subtypes, tumor size, LN status, and proliferation score (Parker et al. 2009). The Prosigna assay classifies postmenopausal HR+ breast cancer patients into low-, intermediate-, and high-risk categories according to the risk of 10-year recurrence (Parker et al. 2009; Dowsett et al. 2013). The prognostic value of ROR scores was validated in the ABCSG8 (Gnant et al. 2014) and TransATAC trials (Sestak et al. 2015), showing that low ROR is associated with a very low 10-year risk of distant recurrence. ROR score was also shown to predict 10-year distant recurrence in chemotherapy-treated patients in the DBCG77B trial (Laenkholm et al. 2018). The ability of the Prosigna to predict who might benefit from chemotherapy is currently under investigation in the OPTIMA trial (Bartlett et al. 2013), and some studies have reported its possible predictive value. Prosigna ROR and intrinsic subtype have been demonstrated as significant predictors of response to neoadjuvant chemotherapy in HR+/HER2– breast cancer (Prat et al. 2016). A recent study also showed that ROR is prognostic for high-risk premenopausal patients and the basal-like or luminal B subtypes predict the benefit of adjuvant cyclophosphamide-based chemotherapy in high-risk patients (Jensen et al. 2018).

The EndoPredict assay provides an EPclin score based on a combination of 12 gene signatures (EP score), tumor size, and LN status (Filipits et al. 2011). This assay was validated using the ABCSG06 (Filipits et al. 2011) and ABCSG08 cohorts (Fitzal et al. 2015) of ER+ postmenopausal patients and was demonstrated to be prognostic for 10-year risk of distant recurrence in ER+/HER2– breast cancer treated with endocrine therapy. EPclin score was also shown to be an independent prognostic factor for node-positive (LN+), ER+/HER2– breast cancer patients treated with adjuvant chemotherapy followed by hormone therapy (Martin et al. 2014). Recently, a modified EPclin test (mEPclin), which was a combination of EP score, postneoadjuvant pathologic tumor size, and LN status, was developed; it independently predicted the risk of distant recurrence after neoadjuvant chemotherapy (Loibl et al. 2018).

The BCI is a seven-gene signature, combining the molecular grade index (five genes) and the HOXB13:IL17BR ratio (two genes) (Ma et al. 2008). This index was validated to be prognostic for late distant recurrence as well as early recurrence in ER+/HER2–, LN– breast cancer (Sgroi et al. 2013; Zhang et al. 2013). BCI was also shown to be prognostic in both LN– and LN+ breast cancer (Sgroi et al. 2016).

The GenesWell BCT is a recently developed prognostic assay. The BCT score calculated by this assay was validated to predict the risk of late distant metastasis as well as early recurrence in patients with pN0–N1, HR+/HER2– breast cancer (Gong et al. 2017). BCT score is calculated based on the combination of expression of six prognostic genes normalized by three reference genes with two clinical variables (LN status and tumor size) (Gong et al. 2017). The six prognostic genes consist of five proliferation-related genes (*UBE2C*, *TOP2A*, *RRM2*, *FOXM1*, and *MK167*) and one immune response gene (*BTN3A2*). BCT score was also shown to be predictive of chemotherapy benefit among Asian patients with HR+/HER2–, LN– breast cancer (Kwon et al. 2018). Although further prospective studies for assessing the clinical utility of this assay in large populations are warranted, existing evidence suggests that the inclusion of immune-related genes may be useful for predicting late distant recurrence and chemotherapy benefit in HR+/HER2– early breast cancer. Notably, the prognostic value of this assay was validated in Asian populations including more young patients, whereas most other assays were validated in populations of Western postmenopausal women.

### Comparisons among the current multigene assays for breast cancer

The clinical utility of these multigene breast cancer assays has been evaluated by several international guidelines. Based on the increasing evidences supporting the prognostic value of commonly used multigene assays, such as Oncotype DX, MammaPrint, EndoPredict, Prosigna, and BCI, the St. Gallen International Breast Cancer Conference Panel suggested that the results of these assays can help decide whether adjuvant chemotherapy should be included to treat luminal breast cancer (Coates et al. 2015). The American Society of Clinical Oncology Breast Cancer Guidelines Advisory Group and Clinical Practice Guidelines Committee also recently found sufficient clinical utility of the multigene assays Oncotype DX, Prosigna, and BCI, to guide decisions on the need for adjuvant systemic therapy in early breast cancer; however, there is no biomarker to guide the choice of specific regimens (Harris et al. 2016). Importantly, the Oncotype DX RS along with biological factors, such as tumor grade, proliferation rate, ER/PR expression, and HER2 expression, were incorporated into the eighth edition of the American Joint Committee on Cancer staging system for breast cancer (Giuliano et al. 2017). These results demonstrate that the multigene assays provide additional prognostic or predictive information beyond traditional factors and can be used to assist decisions about adjuvant therapy.

Several studies have compared the prognostic values of multigene signatures. ROR (Dowsett et al. 2013) and EPclin



(Buus et al. 2016) scores were shown to be more accurate for predicting the risk of distant recurrence than RS among endocrine-treated postmenopausal women with ER+ breast cancer. Similarly, a recent study, which included 774 postmenopausal patients with ER+/HER2- breast cancer from the ATAC trial and compared the prognostic values of six multigene signatures, demonstrated that ROR, BCI, and EPclin are more prognostic for overall and late distant recurrence than RS in patients with LN- breast cancer (Sestak et al. 2018). All six signatures provided less information for distant recurrence in patients with one to three positive nodes. However, it was noteworthy that BCI and EPclin, which are based on the combination of gene expression and clinical information, showed a better prognostic ability than the other signatures in patients with LN+ disease. These results suggest that clinical factors are important for predicting late recurrence and prognosis in LN+ breast cancer.

A recent study reviewed 14 available studies and found discordances among the five commercially available assays for early breast cancer in terms of patient risk stratifications (Varga et al. 2019). Overall discordance between the Oncotype DX and the other assays (MammaPrint, BCI, Prosigna, and EndoPredict) ranged from 42 to 66%. The proportion of patients classified as high risk was the smallest with Oncotype DX RS (11.5%) when patients with a RS  $\geq 31$  were defined as high risk, whereas 63% and 48.4% of patients were classified as high risk according to the EndoPredict assay's EP and EPclin scores, respectively. These results showed that there are considerable differences in risk stratification among the five most common multigene assays and that these assays should not be used interchangeably, thereby suggesting that the clinical utility of each assay should be validated by adequate prospective clinical trials.

Most current multigene assays for breast cancer are based on the expression of HR or proliferation-related genes. Oncotype DX relies on the genes that define the ER status, HER2 status, tumor proliferation, and tumor invasion (Paik et al. 2004). EndoPredict is also based on three proliferation genes and five HR-related genes (Filipits et al. 2011). For MammaPrint and Prosigna assays, genes associated with various pathways—such as adhesion and angiogenesis—as well as proliferation and HR-related genes are included (Tian et al. 2010; Wallden et al. 2015). However, proliferation-based gene signatures are known to be strongly prognostic for early recurrence in ER+/HER2- breast cancer but less prognostic for late recurrence (Gingras et al. 2015). Recent studies have reported that the expression of genes associated with epithelial-mesenchymal plasticity and with stromal activation and immune responses can be better predictors for late recurrence (Mittempergher et al. 2013; Cheng et al. 2014). Moreover, it has been suggested that molecular analysis of circulating tumor cells and circulating tumor DNA after adjuvant therapy—rather than transcriptome analysis

of primary tumor—may be better for identifying patients at high risk of late recurrence (Ignatiadis and Dawson 2014).

### Immune gene signatures as prognostic or predictive biomarkers in breast cancer

Proliferation-related gene signatures from tumor cells have limitations in predicting prognosis of or response to therapies in breast cancer. Given that both tumor cells and the surrounding stromal cells are prognostic, analysis of both the surrounding stromal and tumor tissue may facilitate the identification of better gene signatures associated with patient outcomes and responses to therapy in breast cancer. Accordingly, attempts have been made previously to identify prognostic or predictive stromal gene signatures for breast cancer using gene expression microarray data.

Finak et al. (2008), found a new 26-gene stroma-derived prognostic predictor (SDPP) associated with clinical outcome of breast cancer patients. Importantly, 26-gene SDPP derived from tumor stroma predicted clinical outcomes in several published whole tumor-derived expression datasets. Five biological categories, including matrix remodeling, hypoxia, fibroblast signaling, ER signaling, and immune response, were identified in the 26-gene SDPP. Good clinical outcome clusters overexpressed a set of immune-related genes, including T cells and natural killer (NK) cell markers representing the T-helper type 1 (Th1) immune response. In contrast, poor clinical clusters showed the markers of an increased hypoxic and angiogenic response, tumor-associated macrophage responses, as well as a decrease in chemokines that stimulate NK cell migration and mediate pro-survival signals in T lymphocytes. Of note, SDPP predicted clinical outcome of patients from multiple clinical subtypes, and its prognostic power improved on combining it with other signatures. This suggested that integrating tumor microenvironment factors into prognostic prediction may contribute to the development of novel assays beyond the currently available molecular assays. Another study revealed the importance of the molecular composition of matrix-producing cells—rather than the extent of matrix production—in breast cancer prognosis. (Winslow et al. 2016). Stromal gene signature was also shown to predict resistance to neoadjuvant chemotherapy with 5-fluorouracil, epirubicin, and cyclophosphamide among patients with ER- tumors (Farmer et al. 2009); in that study, a 50-gene signature that predicts poor responses to anthracycline-based neoadjuvant chemotherapy was developed.

A meta-analysis analyzing biological processes in different breast cancer subtypes revealed that tumor invasion and immune response-related genes were associated with the prognosis in HER2+ and ER-/HER2- breast cancer, whereas the prognostic value of proliferation-related

genes was limited to the ER+/HER2- subtype (Desmedt et al. 2008). In line with this, a number of studies based on pooled gene expression microarray analyses have identified immune gene signatures associated with clinical outcome of HR- breast cancer patients (Table 2). Generally, high expression of immune response-related genes was associated with favorable prognosis of patients with HR- breast cancer. Downregulation of seven genes related to immune/defense response (*CIQA*, *IGLC2*, *LY9*, *TNFRSF17*, *SPP1*, *XCL2*, and *HLA-F*) was associated with a greater risk of distant metastasis (Teschendorff et al. 2007), and the classifier based on the expression of these seven genes was further developed and validated to identify ER- patients with a good prognosis independently of LN status (Teschendorff and Caldas 2008). T cell surrogate marker (lymphocyte-specific kinase metagene) had a strong positive prognostic significance in all ER- tumors and ER+/HER2+ breast cancer, whereas B cell surrogate markers (IgG metagenes) had no prognostic significance (Rody et al. 2009). Moreover, 14 novel prognostic genes (*CXCL13*, *CLIC5*, *RGS4*, *RPS28*, *RFX7*, *EXOC7*, *HAPLN1*, *ZNF3*, *SSX3*, *HRBL*, *PRRG3*, *ABO*, *PRTN3*, *MATN1*), including eight genes linked to immune/inflammatory chemokine regulation, were identified for HR- breast cancer, including TNBC, and this HR-/TNBC gene signature index was shown to be superior to other gene signatures with regard to the prediction of metastasis outcome of patients with early-stage HR- breast cancer/TNBC (Yau et al. 2010).

Moreover, using two HR-/TNBC gene signatures derived from previous studies, seven gene signatures identified by Treschendorff et al. (Teschendorff et al. 2007; Teschendorff and Caldas 2008), and 14 prognostic gene candidates identified by Yau et al. (Yau et al. 2010), an optimized five-gene (*TNFRSF17*, *CLIC5*, *HLA-F*, *CXCL13*, *XCL2*) predictor based on a functional pathway through interferon-gamma (IFN- $\gamma$ ) and IL-10 was further developed. Integrated cytokine score calculated from the expression of this five-gene panel was validated as a significant predictor for distant recurrence in formalin-fixed, paraffin-embedded (FFPE) samples of HR- breast cancer/TNBC (Yau et al. 2013). A ratio of high B-cell and low IL-8 metagenes was reported to be associated with good prognosis in TNBC (Rody et al. 2011). A recent study also found that elevated expression of T cell immune response-related genes (*BTN3A2* and *CD2*) was associated with a lower risk of distant metastasis in FFPE tissue samples of HR-/HER2+ breast cancer, but not in other subtypes (Han et al. 2017). Moreover, risk scores based on the expression of *MMP11* and *CD2* were prognostic for distant metastasis in HR-/HER2+ breast cancer, demonstrating the prognostic significance of immune signatures for identifying HR-/HER2+ breast cancer patients with good prognoses.

However, some studies have shown that immune gene signatures, such as B cell metagene can be prognostic for HR+ breast cancer—particularly highly proliferative breast cancer—regardless of ER status, whereas most immune gene-based signatures have been shown to be associated with prognosis of patients with HR- breast cancer (Table 2). B cell metagene, including immunoglobulin genes (*IGKC*, *IGHG*, and *IGHM*), was associated with longer metastasis-free survival in highly proliferative LN- breast cancer regardless of *ESR1* and *ERBB2* status (Schmidt et al. 2008). These results suggest that humoral immune responses may be associated with favorable prognosis in highly proliferative early breast cancer. Similarly, another study (Bianchini et al. 2010) showed that a B cell/plasma cell metagene dominated by immunoglobulin genes (*IGKC*, *IGHG3*, *IGL*, *IGHA1*, and *IGHG3*) has a strong prognostic value for ER+ highly proliferative cancer but a lesser prognostic value for ER- cancer and no prognostic value for ER+/low proliferation, indicating that this B cell/plasma cell metagene can identify patients with favorable prognosis among highly proliferative ER- and ER+ cancers. These results are also consistent with a study which revealed that high proliferative activity was associated with an increased immune responses in both ER- and ER+ breast cancer and that a positive prognostic effect of immune response genes was found in LN- breast cancer regardless of ER status (Oh et al. 2012). A five-gene signature including B cell response genes (*IGK@*, *GBP1*, *STAT1*, *IGLL5*, and *OCLN*) was associated with relapse-free survival in breast cancer (Ascierto et al. 2012).

More recently, 17 immunity genes (*APOBEC3G*, *CCL5*, *CCR2*, *CD2*, *CD27*, *CD3D*, *CD52*, *CORO1A*, *CXCL9*, *GZMA*, *GZMK*, *HLA-DMA*, *IL2RG*, *LCK*, *PRKCB*, *PTPRC*, and *SH2D1A*) were derived from the Affymetrix gene expression microarray dataset that included 1951 patients (Yang et al. 2018). This 17-immunity gene signature was prognostic for distant metastasis-free survival only among patients with ER- and highly proliferative breast cancers. Patients with high expression of these immunity genes had significantly better outcomes. A Cox model to predict the risk of distant metastasis was built using a 72-gene panel incorporating breast cancer subtypes, proliferation score, and immunity score. The prognostic significance of this model was validated in independent gene expression datasets. This study suggested that immunity gene expression is an important prognostic factor, which needs to be incorporated into current multigene assays for improving the prediction of distant metastasis risk in breast cancer.

Immune gene signatures or the expression of immune-related genes have also been reported to be associated with response to treatment in breast cancer. Higher expression of immune-related genes including cytotoxic molecules, T cell receptor signaling pathway components, Th1-related cytokines, and B cell markers was correlated with

**Table 2** Immune gene signature-based prognostic models for breast cancer

No	Gene signatures or prognostic model	Gene lists	Association with clinical outcome	References
<b>HR– breast cancer</b>				
1	Seven-gene expression classifier	Immune response ( <i>CIQA</i> , <i>IGLC2</i> , <i>LY9</i> , <i>TNFRSF17</i> , <i>SPPI</i> , <i>XCL2</i> , and <i>HLA-F</i> )	High immune response module → favorable prognosis in ER– breast cancer	Teschendorff and Caldas (2008)
2	14-gene HR–/TNBC prognostic signature index	14 genes including 8 genes associated with immune/inflammatory chemokine regulation ( <i>CXCL13</i> , <i>CLIC5</i> , <i>RG54</i> , <i>RPS28</i> , <i>RFY7</i> , <i>EXOC7</i> , <i>HAPLN1</i> , <i>ZNF3</i> , <i>SSX3</i> , <i>HRBL</i> , <i>PRRG3</i> , <i>ABO</i> , <i>PRTN3</i> , <i>MATN1</i> )	High HR–/TNBC index → poor prognosis in HR– breast cancer	Yau et al. (2010)
3	Ratio of high B cell and low IL-8 metagenes	B cell metagene including IgG IL-8 metagene including <i>IL-8</i> , <i>CXCL1</i> , <i>CXCL2</i>	High B-cell/low IL-8 metagenes → favorable prognosis in TNBC	Rody et al. (2011)
4	Optimized five-gene integrated cytokine score	Interferon gamma and IL-10 related pathway ( <i>TNFRSF17</i> , <i>CLIC5</i> , <i>HLA-F</i> , <i>CXCL13</i> , <i>XCL2</i> )	High integrated cytokine score → poor prognosis in HR–/TNBC	Yau et al. (2013)
5	Prognostic model for HR–/HER2+ breast cancer	Proliferation-related ( <i>MMP11</i> ) and T-cell immune response ( <i>CD2</i> )	High risk score → poor prognosis in HR–/HER2+ breast cancer (high <i>CD2</i> expression → favorable prognosis)	Han et al. (2017)
<b>HR+ and HR– breast cancer</b>				
6	B cell metagene	Immunoglobulin genes ( <i>IGKC</i> , <i>IGHG</i> , and <i>IGHM</i> etc.)	High B cell metagene → favorable prognosis in highly proliferative tumors	Schmidt et al. (2008)
7	B cell/plasma metagene	Immunoglobulin genes ( <i>IGKC</i> , <i>IGHG3</i> , <i>IGL</i> , <i>IGHA1</i> , and <i>IGHG3</i> )	High B-cell/plasma cell metagene → favorable prognosis in highly proliferative cancers	Bianchini et al. (2010)
8	Prognostic index for LN– breast cancer	4 proliferation-related genes ( <i>UBE2C</i> , <i>PRCI</i> , <i>CCNB2</i> , <i>CDC20</i> ) and 4 immunity genes ( <i>CD3D</i> , <i>CD52</i> , <i>CCL19</i> , <i>TRBV20-1</i> )	High prognostic index → favorable prognosis in fast proliferating lymph node-negative breast cancer regardless of ER status)	Oh et al. (2012)
9	Five-gene signature	B cell response ( <i>IGK@IGHKC</i> ], <i>GBPI</i> , <i>STAT1</i> , <i>IGLL5</i> , and <i>OCLN</i> )	High five-gene signature → favorable prognosis in breast cancer	Ascierto et al. (2012)
10	Immunity-stratified risk of distance metastasis (iRDM) prediction model	72 genes (17 immunity genes + 19 proliferation genes + 11 basal genes + 14 ER genes + 3 HER2 genes + 2 invasion genes + 6 housekeeper genes)	High iRDM → poor prognosis in all subtypes of breast cancer (17 immunity genes → favorable prognosis only in patients with ER– and highly proliferative breast cancers)	Yang et al. (2018)

ER estrogen receptor, HER2 human epidermal growth factor receptor 2, HR hormone receptor, LN lymph node, TNBC triple-negative breast cancer

a pathological complete response in TNBC patients treated with neoadjuvant chemotherapy (Lee et al. 2015). A recent study showed that combining radiosensitivity and immune gene signatures may be potential to predict the response to adjuvant radiotherapy (Cui et al. 2018). These results suggested that immune gene signatures may be useful to select patients who are mostly like to benefit from neoadjuvant chemotherapy or adjuvant radiotherapy in breast cancer.

### Tumor-infiltrating lymphocytes as prognostic or predictive biomarkers in breast cancer

Various immune cells present in the tumor microenvironment, including cytotoxic T cells, Th cells, dendritic cells, NK cells, and macrophages, are usually called tumor-infiltrating lymphocytes (TILs) (Fridman et al. 2012; Savas et al. 2016). However, the role of TILs on tumor progression may be different depending on the type of TILs. Some infiltrating immune cells (M2 macrophages, myeloid-derived suppressor cells, regulatory T cells [Treg], and Th2 cells) are known to contribute to pro-tumor activity, whereas tumor-infiltrating dendritic cells, M1 macrophages, Th1 cells, CD8+ T cells, and NK cells suppress tumor progression (Salgado et al. 2015b). TILs are reportedly associated with prognosis and response to therapy in human cancer (Fridman et al. 2012; Luen et al. 2017).

Consistent with these findings, a growing number of studies are showing the significance of TILs as prognostic or predictive markers in breast cancer. In particular, a high TIL level has been observed in TNBC and HER2+ breast cancer and have been associated with a favorable prognosis (Loi et al. 2013; Adams et al. 2014; Loi et al. 2014; Salgado et al. 2015a; Hida et al. 2016; Ingold Heppner et al. 2016; Althobiti et al. 2018). TILs have also been shown to predict improved responses to neoadjuvant chemotherapy, particularly in TNBC (Ono et al. 2012), as well as to trastuzumab in HER2+ breast cancer (Loi et al. 2014; Ingold Heppner et al. 2016), and to neoadjuvant anti-HER2 agents (trastuzumab or lapatinib) in HER2+ breast cancer (Salgado et al. 2015a).

However, the prognostic significance of TILs in breast cancer differs according to the molecular subtype of breast cancer, based on ER/PR or HER2 expression status. In contrast to HR– breast cancer, high TIL levels have been associated with a poor prognosis in ER+ breast cancer (Tsang et al. 2014; Denkert et al. 2018). Tsang et al. (2014), showed that in ER+ breast cancer, high TIL levels were associated with poor prognostic features, including high tumor grade and lymphovascular invasion, and they have a strong positive correlation with intratumoral accumulation of Tregs. However, high TIL levels were associated with favorable prognosis features in ER–/HER2+ breast cancer. Similarly, a recent study including 3771 patients treated with

neoadjuvant therapy was conducted for assessing the prognostic and predictive significance of stromal TILs in different subtypes of breast cancer; it demonstrated that high TIL levels were associated with shorter overall survival in HR+/HER2– breast cancer, whereas there was a correlation between favorable prognosis and high TIL levels among patients with TNBC (Denkert et al. 2018). In this study, a high TIL concentration also predicted a response to neoadjuvant chemotherapy in all molecular subtypes. This study suggested that the difference in the prognostic value of TILs between HR+/HER2– and HR– breast cancer may be explained by different immune cell composition and prognostic effect of each type of immune cell in each subtype of breast cancer. Higher infiltration levels of most immune cell types were observed in TNBC than in HR+/HER2– breast cancer, and most immune cells—including T cells, B cells, and macrophages—were associated with a favorable prognosis in TNBC. In contrast, the presence of T cells was not prognostic, and macrophages were associated with a poor prognosis in HR+/HER2– breast cancer, furthermore, the only cell types associated with improved outcome in HR+ breast cancer were B cells and myeloid dendritic cells. Further studies to investigate the difference in interactions between immune cells and tumor cells according to breast cancer subtypes will be required.

With the emerging importance of TILs as a prognostic or predictive biomarker for breast cancer, there have been efforts to standardize the methods for evaluating TILs. Methodological recommendations on the tumor area for evaluation, the type of immune cell, TIL location (stroma or intratumoral TILs), and scoring methods have been made and updated by an international working group (Salgado et al. 2015b; Dieci et al. 2018). However, further efforts are required to incorporate TILs into diagnostic assays and standard practice as current methods are still experimental.

### Emerging biomarkers predicting the response to immunotherapies in breast cancer

Breast cancer, particularly HR+ breast cancer (the most common form), is considered a weakly immunogenic cancer because of the low level of TILs and low mutation rates (Kandoth et al. 2013). However, recent studies have shown that immunogenicity is important in subtypes of breast cancer, including TNBC and HER2+ breast cancer and that TNBC is the most immunogenic subtype associated with the highest TIL levels, which in turn suggests that TNBC is an emerging area of immune-oncology therapeutics (Esteva et al. 2019). Although numerous immunotherapies, such as immune checkpoint inhibitors (anti-CTLA4, PD-1, PD-L1 antibodies) have been approved for other cancers, there were no FDA-approved immunotherapies for breast



cancer (Vonderheide et al. 2017) until 2019, when the FDA approved the first immunotherapy for patients with locally advanced or metastatic TNBC expressing PD-L1: a combination of atezolizumab (anti-PD-L1 antibody) and nab-paclitaxel (2019). The number of clinical trials of immunotherapies for breast cancer has increased rapidly in the past 6 years, and 285 clinical trials (as of September 2018) of monotherapies or combination breast cancer therapies are ongoing, in which the agents targeting PD-1 or PD-L1 are predominant (Esteva et al. 2019). Although the number of clinical trials of immunotherapies for TNBC is the largest, ongoing clinical trials are testing the efficacy of immunotherapies in all subtypes of breast cancer (Esteva et al. 2019). To maximize the benefits and minimize the toxicities of cancer immunotherapies, it is important to identify effective biomarkers that predict responses to immunotherapies. With the advances in the development of immunotherapies for breast cancer, there is an accompanying need for reliable predictive biomarkers for immunotherapies in breast cancer.

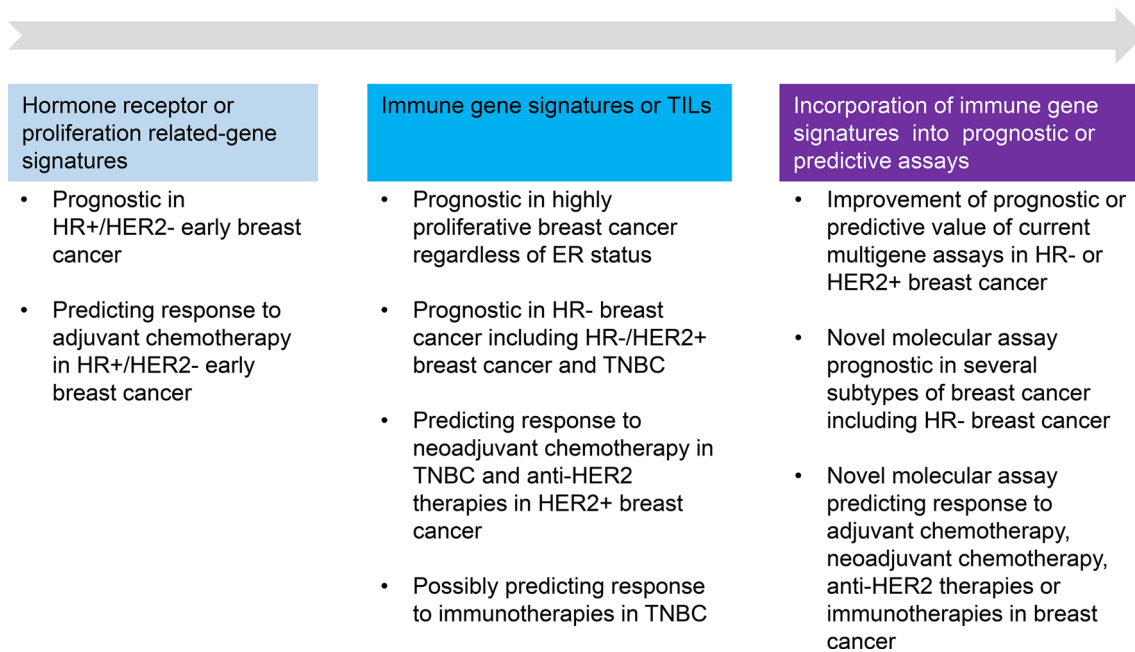
Currently, PD-L1 expression by immunohistochemistry (IHC) is the most widely used biomarker for selecting patients for immunotherapies and is FDA approved as a companion diagnostic test for anti-PD-1 therapy in some cancer types, including non-small cell lung cancer (Chan et al. 2019; Havel et al. 2019). PD-L1 expression varied widely across cancer types. The percentage of tumors with positive PD-L1 expression by IHC was relatively low in breast cancer compared with other cancer types, such as thymic and nasopharyngeal cancers (Yarchoan et al. 2019). PD-L1 expression by IHC or gene expression was observed in approximately 20–40% of breast cancer and higher PD-L1 expression (up to 60% of PD-L1 expression) has been reported in TNBC than other subtypes (Miglietta et al. 2019). As in other cancers, PD-L1 has been suggested as a potential predictive biomarker for immune checkpoint inhibitors in breast cancer and its predictive value has been evaluated in clinical studies. Some studies showed that PD-L1 positivity is associated with greater responses to anti-PD-1/PD-L1 therapies in breast cancer (Dirix et al. 2018; Adams et al. 2019a, b). Importantly, a Phase III trial randomized patients with TNBC to receive atezolizumab+ nab-paclitaxel versus placebo+ nab-paclitaxel demonstrated that there was a greater survival benefit for atezolizumab arm in PD-L1-positive patients than PD-L1-negative patients, suggesting the predictive value of PD-L1 for anti-PD-L1 therapy in TNBC (Schmid et al. 2018). However, many technical and biological issues including the standardization of PD-L1 testing need to be addressed to implement PD-L1 as a reliable marker for identifying patients that will benefit from immunotherapies. Moreover, because PD-L1 expression alone is insufficient for properly selecting patients for immunotherapies, further efforts are needed to identify additional predictive markers to improve patient selection for

immunotherapies. Another emerging predictive biomarker is a tumor mutation burden (TMB) and a higher TMB has been shown to be correlated with clinical benefit from immune checkpoint inhibitors across multiple cancer types (Chan et al. 2019). However, the association between TMB and response to immune checkpoint inhibitors in breast cancer is unclear and further investigation is required.

Of note, tumor immune microenvironment features, such as TILs and interferon-inflammatory immune gene signatures, are also known to be associated with the response to immune checkpoint inhibitors (Gibney et al. 2016; Havel et al. 2019). CD8+ T cell density at the invasive margin of a tumor, as measured by IHC, was strongly associated with the pembrolizumab (anti-PD-1 antibody) response in melanoma (Tumeh et al. 2014). Ayers et al. (2017), showed that IFN- $\gamma$ -related gene expression predicts clinical responses to anti-PD-1 therapy. Another recent study reported that a tumor inflammation signature based on 18 genes that measure an adaptive immune response within tumors is associated with sensitivity to PD-1-targeting therapy (Danaher et al. 2018); these 18 genes comprised the genes associated with cytotoxic cells, antigen presentation, and IFN- $\gamma$  activity. However, these markers have not yet been clinically validated in several cancers, including breast cancer, and further validation will be required for them to be used clinically.

## Conclusion

Over the past two decades, several multigene assays have been developed to predict breast cancer prognosis and responses to therapy beyond what can be accomplished by traditional clinical factors. However, there still remains a need for better prognostic or predictive biomarkers for breast cancer. The clinical significance of stroma, including ECM and immune cells, as well as of tumor cells has been recognized, and several studies have shown that immune gene signatures or TILs can predict prognosis and responses to neoadjuvant chemotherapy or anti-HER2 therapies in HER2+ breast cancer or TNBC. Furthermore, high TIL levels or immunogenicity of HR- breast cancer, particularly TNBC, suggest the needs for appropriate predictive biomarkers for immunotherapies. The integration of immune gene signatures reflecting the tumor microenvironment, in combination with other molecular gene signatures, is expected to improve the prognostic and predictive value of currently available assays (Fig. 1). For example, the incorporation of immune gene signatures into current proliferation gene-based multigene assays may improve their prognostic ability or their ability to predict the response to neoadjuvant chemotherapy or anti-HER2 agents in HR- or HER2+ breast cancer. Furthermore, given the prognostic value of immune gene signatures in highly proliferative tumors regardless of ER status, it



**Fig. 1** Strategies for the development of novel prognostic or predictive biomarkers in breast cancer. Incorporation of immune gene signatures or tumor-infiltrating lymphocytes (TILs) into the prediction of prognosis and response to treatment may improve the prognostic or predictive ability of current multigene assays or may contribute to the development of novel multigene assays beyond the currently available commercial assays. *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *HR* hormone receptor, *TNBC* triple-negative breast cancer

will be possible to develop the novel assay identifying low-risk patients from high-risk patients across all subtypes of breast cancer. Immune gene signatures or TILs can also be used to develop the predictive biomarkers to select patients that will benefit from immunotherapies. Therefore, it is necessary to incorporate immune gene signatures into the prognostic and predictive signatures in the future development of novel multigene assays for breast cancer.

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**Compliance with ethical standards**

**Conflict of interest** The author declares no conflict of interest.

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