Signatures of tumor–immune interactions as biomarkers for breast cancer prognosis

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Abstract

Breast cancer mortality is usually due to distant recurrence of cancer at an advanced stage of the disease rather than from primary cancer. Therefore, prediction of breast cancer recurrence at the time of diagnosis could lead to advances in personalized treatment of cancer patients in order to prevent risk of recurrence. Two prognostic biomarkers that are currently being used in clinical practice are a 70-gene MammaPrint® signature and a 21-gene Oncotype DX® panel. These assays generate relative risks of recurrence, but they do not provide a 'yes' or 'no' answer about recurrence in a given patient. These tests include genes that are involved in the cell cycle, invasion, metastasis and angiogenesis related to breast cancer. Emerging evidence suggests that a signature of genes involved in tumor-immune interactions may provide a more accurate prognostic tool. This paper reviews recent advances in the discovery of prognostic biomarkers for breast cancer patients.

Keywords

breast cancer recurrence; cancer testis antigens; HER2; immune function genes; MammaPrint®; myeloid-derived suppressor cells; Oncotype DX®; prognostic biomarkers

Patients with early-stage breast cancer die from tumor relapse rather than from primary cancer. Therefore, prognostic biomarkers and predictors of response to therapy are urgently needed to reduce the incidence of tumor relapse and advanced breast cancer. This review highlights the current developments in the discovery of biomarkers and predictors of response to therapy for breast cancer patients. This will include different subtypes of breast

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cancer based on the status of hormone receptors, HER2 status, nodal status and status of proliferation and invasion, as well as immune cell infiltrates.

Breast cancer biological subtypes & histological grading can predict risk of tumor recurrence

HER2, estrogen receptor & progesterone receptor

It has been reported that amplification of the *HER2* oncogene in 25% of breast cancers is associated with a shorter disease-free and overall survival. Carr *et al*. conducted retrospective studies on 190 breast cancer patients and showed that HER2 was an independent prognostic biomarker, regardless of tumor grade, estrogen receptor (ER)/ progesterone receptor (PR) status and lymph node metastasis [1]. They showed that patients with HER2-positive (HER2+) tumor had a shorter median disease-free interval (22 months) compared with controls (40 months). However, more frequent expression of HER2 in high-grade ductal carcinoma *in situ* (DCIS) lesions compared with invasive breast cancer raised questions as to the validity of *HER2* amplification as an independent prognostic biomarker. Such discordance was addressed by showing that HER2 overexpression in DCIS was associated with an increased risk of rapid progression to invasive disease (2). In fact, invasive foci were detected in HER2+ DCIS at a higher frequency than in HER2-negative (HER2−) DCIS. Such discordance was also addressed by testing a hypothesis that HER2+ DCIS is at a greater risk of progression to HER2− invasive carcinoma under immune pressure. For instance, it was reported that relapse of neu− invasive mammary carcinoma can occur following T-cell-mediated rejection of the neu+ mammary carcinoma (3,4). In addition, presence of the HER2-specific T-cell responses was detected in patients with HER2− breast cancer, and such immune responses were associated with nuclear translocation of IFN-γRα. in the tumor lesions [5]. The authors suggested that presence of HER2-reactive T cells in the absence of the antigen was indicative of HER2+ DCIS in the past and its progression to HER2− breast cancer due to T-cell-mediated HER2 loss. This hypothesis was also supported by the observations showing that HER2-targeted vaccination of DCIS patients resulted in the regression of DCIS lesions as well as HER2 loss following the induction of HER2-specific T-cell responses [6]. Similar observations were made in breast cancer patients whose tumor lost HER2 expression following trastuzumab-based neoadjuvant therapy [7]. However, these reports brought the immune cells into the picture, which further challenged the status of HER2 as an independent prognostic biomarker. In addition, patients with HER2+ but node-negative (node−) breast cancer had a low risk of recurrence at 5-year follow-up whereas those with ER-positive (ER+)/PR-positive breast cancer and HER2 overexpression had a worse disease-free survival [8]. Overall, the prognostic impact of HER2-positivity was lower in node− compared with node-positive (node+) patients. These findings led to the use of multiple tumor biomarkers for breast cancer prognosis, ER/PR status in particular. For instance, patients with triple-negative (ER-negative [ER−]/PR-negative/HER2−) breast cancer are at greater risk of relapse with a one-to three-times greater risk following initial treatment compared with patients who had other subtypes of breast cancer, including patients with HER2+ tumors [9].

Genomic grade index

Histological grading of the tumor is another factor that contributes to predicting risk of breast cancer recurrence. In order to determine whether gene expression profiling can grade tumors more accurately than the conventional histological grade, the genomic grade index (GGI) was developed using microarray analysis [10]. The GGI includes 97 genes that are associated with tumor grade and breast cancer subtypes, and can define patient prognosis and predict chemotherapy sensitivity [11–15]. According to this assay, luminal A and normal-like breast cancer subtypes are categorized as low GGI, whereas HER2, basal-like and luminal B breast cancer subtypes and the subgroup of tumors previously unclassified are categorized as high GGI [16]. In fact, the prognostic value of the genes involved in tumor cell proliferation is better evaluated with GGI than with classic histological grade.

PAM50™

The PAM50 is a quantitative reverse transcriptase-PCR-based assay that measures the expression of 50 genes and five control genes to identify breast cancer subtypes known as luminal A, luminal B, HER2-enriched and basal-like tumors. A comparative analysis of Oncotype DX® and PAM50 showed that high-risk specimens identified by Oncotype DX corresponded to luminal B or basal-like tumors identified by PAM50. In addition, 83% of low-risk specimens were found to be luminal A tumors. In the intermediate-score group, 53 specimens (approximately half) were classified as luminal A, a lower risk of recurrence compared with luminal B, 13 were classified luminal B and another nine fell into two other groups, HER2-enriched and normal-like breast cancers [17]. Although PAM50 gives more prognostic information than clinical factors and immunohistochemistry (IHC), overdependence of PAM50 on proliferation-related genes reduces its accuracy as a standalone prognostic biomarker. Normal tissue or benign tumor tissue sampled concurrently with a malignant tumor is a significant source of bias in the PAM50 genomic predictor [18–21].

IHC4

The IHC4 test provides a prognostic score based on the expression of four IHC markers (*ER*, *PR*, *HER2* and *Ki-67*) in tumor biopsy specimens. This assay was validated in ER^+ breast cancer patients [22] to identify patients with low risk of relapse who will not benefit from chemotherapy. However, variations in IHC scores reduce the reproducibility of this assay.

A complex of genes expressed by tumor cells & stromal cells can predict breast cancer relapse

An advance in our understanding of the tumor microenvironment led to the development of molecular biomarkers of breast cancer prognosis by focusing on genes that are involved in cell cycle, invasion, metastasis and angiogenesis of breast cancers. This approach stems from the observations showing that intratumor genetic heterogeneity is a key mechanism involved in tumor progression and response to treatment [23]. In addition, clinical studies suggested that a stromal gene expression profile may also predict breast cancer outcome. This knowledge led to the development of two prognostic biomarkers, a 70-gene

MammaPrint® signature and a 21-gene Oncotype DX panel, which are currently being used in clinical practice.

70-gene MammaPrint signature

The 70-gene MammaPrint signature (Agendia BV, Amsterdam, The Netherlands) is a fully commercialized microarray-based assay for breast cancer prognosis that requires either fresh-frozen tumor specimens or tissues collected into an RNA preservative solution. This assay has been US FDA-cleared for use in lymph node⁻ and ER⁺ or ER⁻ breast cancer patients with tumors smaller than 5 cm. It is also being extended to node⁺ patients [24,25]. In 2007, Agendia received a second clearance from the FDA for a sampling and room temperature shipping procedure using a RNA preserving solution (RNARetain®). The MammaPrint signature is focused on genes associated with cell proliferation, invasion, metastasis, stromal integrity and angiogenesis. The test measures the level of expression of 70 genes in tumor specimens and then uses a specific formula or algorithm to produce a score that determines high risk or low risk of recurrence, without an intermediate-risk group. Each microarray contains three identical sets of the 70 genes to be analyzed. Normalization genes and negative-control genes are also present on each microarray. A low-risk MammaPrint result means a 10% risk of tumor relapse within 10 years without any additional adjuvant treatment. A high-risk MammaPrint result means a 29% chance of tumor relapse within 10 years without any additional adjuvant treatment [26]. Comparative analysis of data obtained from patients with node⁺ and node⁻ breast cancer showed a similar prognostic value for the MammaPrint test [25].

The MammaPrint assay is largely a prognostic, rather than predictive, assay. However, recent reports suggest a predictive value for the MammaPrint signature [27]. Patients who were identified as high risk by the MammaPrint test and received adjuvant chemotherapy showed an improved relapse-free survival such that only 6% of patients developed tumor relapse within a 5-year follow-up [28]. In fact, adjuvant chemotherapy improved relapse-free survival in 20.7% of the high-risk group. The MINDACT trial is underway to assess the predictive capability of the MammaPrint assay [27]. Node⁻ or node⁺ patients are eligible for the trial regardless of ER status. Patients who are classified as high risk by using standard clinicopathological factors and MammaPrint receive chemotherapy, while those identified as low risk by both methods receive hormonal therapy as appropriate. Any patients with discordance between standard criteria and the MammaPrint assay will randomly receive either adjuvant chemotherapy or hormonal therapy as clinically appropriate.

The main advantage of MammaPrint is in its ability to identify patients who could be spared unnecessary adjuvant therapy in the low-risk group showing a less-than-10% risk of recurrence within a minimum of 5 years. The 70-gene signature also provides additional information that can help refine the prognostic value of traditional markers. For example, the MammaPrint assay has identified a subgroup of early HER2⁺ breast cancer with a favorable long-term outcome [29].

21-gene Oncotype DX panel

Oncotype DX is an assay that employs quantitative reverse transcriptase-PCR, using formalin-fixed paraffin-embedded tumor specimens, to determine the expression of a panel of 21 genes, primarily in ER^+, lymph node$^−$ patients. The Oncotype DX panel includes 16 cancer-related genes, such as groups related to hormone receptors (*ER*, *PR*, *BCL2* and *SCUBE2*), proliferation (*Ki-67*, *STK15*, *SURVIVIN*, *CYCLIN B1* and *MYBL2*), invasion (*STRMELYSIN3* and *CATHEPSIN L2*), HER2 (*HER2* and *GRB7*), the macrophage marker CD68, the antiapoptosis gene *BAG1* and *GSTM1*, as well as five reference 'housekeeping' genes. A scoring algorithm is used to weigh them relatively with the heaviest weighting for ER- and proliferation-related genes in order to develop a recurrence score. The method applies a Kaplan–Meier estimate to show that the proportion of patients in the low-risk group free of a distant recurrence at 10 years was significantly greater than the proportion of patients identified as high-risk. Multivariate Cox proportional analysis of age, tumor size and recurrence score in relation to the likelihood of distant recurrence were also performed. There are three risk levels based on the recurrence score on a scale of 0–100, which are low risk (<18), intermediate risk (18–30) and high risk (31). Oncotype DX was tested prospectively in the NSABP Trial B-14, comprising 2644 patients with ER^+, histological node$^−$ tumors [30]. Patients were randomized to receive either tamoxifen or placebo. In tamoxifen-treated patients, the 21-gene signature revealed a 5-year recurrence rate of 22.1% for patients with the high-risk score, compared with 2.1% for those with the low-risk score, and 30.5 and 6.8% at 10 years, respectively. Patients with intermediate risk showed a recurrence rate of 14.3% at 10 years.

A retrospective case–control study [31] identified Oncotype DX as a predictive biomarker for hormonal therapy in the NSABP B-14 trial as well as for chemotherapy in the NSABP trial B-20 [32]. The trial showed a large benefit of chemotherapy for patients with a high recurrence score, and minimal benefit for those with a low score. There was no clear-cut benefit for patients with an intermediate recurrence score. The 10-year relapse-free survival was improved from 60 to 88% by adding chemotherapy to hormone therapy in the high-risk group [33]. In fact, adjuvant chemotherapy improved relapse-free survival in 30% of the high-risk group. Further analysis revealed that the 21-gene signature was better than standard clinicopathological variables at predicting recurrence [34].

Oncotype DX is best suited for identifying breast cancers that are less likely to relapse (low-risk group). Despite being prognostic and predictive to chemotherapy, Oncotype DX still returns 40–66% of cases as intermediate risk [35] with no clear data to suggest a benefit of chemotherapy. However, preliminary data suggest that addition of standard clinicopathological variables to the Oncotype DX recurrence score can help reduce the number of cases that fall into the intermediate-risk group.

Both MammaPrint and Oncotype DX panels determine probability of tumor relapse in a given patient. For example, a patient in the high-risk group has approximately a 30% risk of recurrence (29% risk by MammaPrint) or approximately a 70% chance of relapse-free survival, and a patient in the intermediate-risk group has approximately a 14.3% risk of recurrence within 5–10 years. Development of a prognostic biomarker that can identify

high-risk and low-risk groups by providing 'yes' or 'no' answers about tumor relapse would significantly improve management of the disease. Genetic instability and plasticity of tumor cells facilitates constant changes in the tumor cells in response to the host defense system, making it difficult to develop a gold-standard prognostic biomarker that can accurately determine whether a patient would relapse following initial treatment. However, understanding a network of genes that are involved in the tumor–host interactions may dramatically improve our ability to provide 'yes' or 'no' answers about recurrence.

Recent reports show that manipulation of patient selection resulted in the generation of entirely different signatures that included only 20% of the MammaPrint genes in more than 50% of the patients and appearance of ten new genes that were absent in the MammaPrint gene signature [36]. In addition, 50% of patients who were grouped as intermediate risk by the Oncotype DX were classified as high risk by the 70-gene MammaPrint signature [36]. A comparative analysis of five published breast cancer gene signatures on the same dataset also revealed an outcome prediction agreement of 80% between Oncotype DX and MammaPrint signatures [37].

Stromal gene expression

Both MammaPrint and Oncotype DX panels have been derived from whole tissue consisting of tumor cells and the surrounding stroma. Samples with insufficient tumor cell content are generally excluded from such analyses. This would result in filtered analyses in which key components of the tumor microenvironment, such as tumor-associated stromal cells and infiltrating immune cells, are ignored. Under normal conditions stroma provides a barrier to epithelial cell transformation and growth. During transformation of epithelial cells, stroma undergoes changes that further facilitate cancer progression [38–40]. Tumor stroma is comprised of extracellular matrix and various cell types including fibroblasts, endothelial cells and infiltrating immune cells, which interact with malignant cells via paracrine, physical and hormonal pathways. Early studies showed that the normal mammary microenvironment is capable of converting the malignant cells by triggering differentiation of the cells [41,42]. These data suggest that malignant cells can only thrive in an abnormal microenvironment. To determine the prognostic value of stromal cells, Park and colleagues performed gene expression analysis on stromal cells isolated from breast tissues by laser capture microdissection. Such analysis showed that gene expression signatures derived from whole tumors were different from the signatures derived from the tumor stroma, with the latter showing a strong link to clinical outcome [43]. Tumor stroma from patients with good outcome showed overexpression of immune-related genes such as T-cell and natural killer cell markers with a skewed Th1 profile. By contrast, a diminished expression of the immune-related genes was evident in the tumor stroma from patients with poor outcome. These tumors, instead, showed expression of genes that were involved in hypoxic and angiogenic responses [43]. It was also reported that gene signatures of CD10$^+$ stromal cells are associated with poor prognosis, particularly in HER2$^+$ tumors of patients who were not treated [44]. The signature was also important in differentiating DCIS and associated invasive samples. Although this analysis requires laser capture microdissection of CD10$^+$ cells, which is not a simple procedure for prognosis, it would suggest that tumor stroma should also be targeted in order to improve prognosis in patients with CD10$^+$ tumor stromal

signature. More recently, gene expression analyses were performed on stroma-poor fine-needle and stroma-rich core-needle biopsies from breast cancer patients to identify the prognostic value of stroma-associated genes [45]. The study showed that, among highly proliferative cancers (ER[−] or ER[+]), a subset of tumors with high expression of a B-cell/plasma cell metagene had a favorable prognosis. This immune function gene signature showed no prognostic value in poorly proliferating cancers. These findings suggest that infiltrating immune cells may have prognostic value in a subset of cancer patients with highly proliferating tumor cells who are at risk of tumor relapse following conventional therapies.

Mammostrat®

The Mammostrat test is a protein-based IHC assay of tumor specimens that employs a set of five biomarkers (*SLC7A5*, *HTF9C*, *P53*, *NDRG1* and *CEACAM5*). These markers are independent of one another and do not directly measure proliferation or ER/PR expression. Therefore, histopathological information about the proliferation and ER/PR status of a tumor can be used for interpretation of Mammostrat results [46]. Increased Mammostrat scores are associated with poor prognosis in ER[+] breast cancer patients.

Status of tumor-infiltrating immune cells can predict breast cancer relapse

Breast cancers often express 'self' antigens such as HER2 and MUC-1, to which cells of the immune system were tolerized during the thymic selection. However, numerous lines of evidence suggest that cells of the immune system are capable of recognizing and reacting with tumor cells [47]. Breast carcinomas are often infiltrated by inflammatory cells including macrophages and T cells. It was shown that tumor-infiltrating lymphocytes are an invariable feature of breast cancers, such that immune cells were present in all breast cancers examined [48]. In fact, the transition from normal breast to ductal carcinoma is associated with an increased infiltration of mononuclear cells at the parenchyma [49]. Immune-cell infiltrates in the tumor bed or tumor stroma have been studied in the context of protumorigenic inflammation and tumor immunosurveillance. It is now becoming clear that specific infiltrating immune cells have distinct prognostic and predictive value for breast cancer patients. Whereas tumor-infiltrating dendritic cells, M1 macrophages, Th1 cells, CD8^{+} T cells and natural killer cells are associated with a favorable prognosis, the presence of M2 macrophages, myeloid-derived suppressor cells, Tregs and Th2 cells is associated with poor prognosis in cancer patients. An analysis of three major microarray breast cancer datasets revealed that overexpression of immune response genes in ER[−] breast cancers was associated with a favorable prognosis [50]. A meta-analysis of 1044 hybridizations showed that tumor infiltration of lymphocytes in ER[+] breast cancers was associated with poor prognosis, whereas that in ER[−] breast cancer was associated with a favorable outcome [51]. Analysis of 1781 primary invasive breast cancers using microarray datasets showed a positive correlation between the expression of T-cell metagene, LCK and a favorable outcome in ER as well as in ER[+]/HER2^{+} breast cancers [52]. In a recent study, 1334 breast cancer patients were randomized into training and validation sets in order to determine the prognostic value of CD8^{+} T-cell infiltrates [53]. The study showed that high total and distant stromal CD8^{+} T-cell counts were associated with a favorable prognosis.

Emerging evidence suggests that the success of chemotherapy depends on the status of antitumor immune responses. It was recently reported that neoadjuvant chemotherapy altered the immune cell infiltrates by increasing the CD8:CD4 T-cell ratio associated with a favorable outcome [54]. A retrospective study of tumor tissues from 152 breast cancer patients revealed that the CD8^{+} T-cell infiltrate was an independent predictive factor of response to neoadjuvant chemotherapy [55]. Analysis of a total of 1058 pretherapeutic breast cancer core biopsies from two neoadjuvant studies also showed that the percentage of tumor-associated lymphocytes in breast cancer was an independent predictor of response to neoadjuvant chemotherapy [56].

A signature of genes involved in tumor–immune interactions can predict relapse or no relapse

Given that both tumor cells and infiltrating immune cells have shown prognostic values, understanding a cross-talk between tumor cells and infiltrating immune cells may result in the development of a global prognostic biomarker for breast cancer patients. Currently, there is no prognostic biomarker in clinical practice that can provide 'yes' or 'no' answers about relapse in a given patient at an early stage of breast cancer, based on clinopathological criteria (including tumor size and invasiveness, and spread to the lymph nodes and distant sites), nor is there any biomarker that can predict who may benefit from active immunotherapy. Several lines of evidence suggest that signatures of the immune response can predict breast cancer outcome. Teschendorff *et al.* reported that downregulation of a seven-gene signature related to the immune response significantly increased the risk of distant metastasis, independent of lymph node status and lymphocytic infiltration, in ER[−] breast cancer patients [50]. In addition, microarray data from a number of different carcinomas, including 132 breast cancer patients, showed a prognostic value of immune-related genes, particularly the Th1-associated genes [57]. Rody *et al.* also reported that T-cell metagenes can predict a favorable prognosis in ER[−]/HER2^{+} breast cancer patients [52]. Sabatier *et al.* used public gene expression and histoclinical data of 2145 early-stage breast cancers and determined that immune function genes – Th1 type in particular – were associated with a good prognosis in basal breast cancers [58]. The immune function signature was different from two other published immune function signatures [50,52], although they still highlight the prognostic value of the immune response genes. The authors have recently identified a network of immune function genes (a five-gene signature) associated with relapse-free survival in the tumor tissue of breast cancer patients, independent of ER and lymph node status, or lymphocytic infiltration [59]. The five-gene prognostic signature was distinct from the 70-gene MammaPrint signature and from the Oncotype DX recurrence score assay panel. Among these genes, *IGLL* and *IGKC* had a favorable prognosis. These findings were consistent with previous studies on tumor stroma showing that a B-cell/plasma cell metagene had a favorable prognosis [45]. T-cell-associated genes were also detected in the tumor lesion of patients with relapse-free survival, which is consistent with other reports [50,51,57]. Presence of such immune function genes would suggest the possibility for the expression of highly immunogenic tumor antigens in the tumors of patients who remained relapse free. Among the known tumor antigens, cancer testis antigens (CTAs) hold promise because of their ability to induce

strong immune responses. CTAs were first detected in a highly immunogenic cancer, melanoma, where spontaneous regression of cancer was reported. Interestingly, expression of MAGE-4 antigen was reported to be associated with a reduced risk of breast cancer recurrence [60]. The authors recently observed that expression of a panel of CTAs along with a five-gene signature of immune function was restricted to patients who remained relapse-free [PAYNE K, MANJILI MH, UNPUBLISHED DATA]. This observation suggests that patients who are identified as high risk may benefit from an active immunotherapy for the induction of CTA, as well as the triggering of CTA-reactive immune responses.

Conclusion

Characterization of biomarkers for breast cancer prognosis or response to therapy are listed in **Table 1**. Breast cancer is a multifaceted process and none of the present biomarkers can reliably predict a binary outcome, such as recurrence or no recurrence. Emerging evidence suggests that combined use of the available biomarkers cannot improve the accuracy of prognosis or prediction of response to therapy. This is because each biomarker represents certain sets of genes with specialized function rather than representing a network of genes that are involved in host–tumor interaction. There are also very few common genes between the published gene signatures. For example, *SCUBE2*, an estrogen-regulated gene, is the only common gene in the 21 genes of the Oncotype DX and the 70 genes of the MammaPrint panel. These findings suggest the need for a more comprehensive and novel biomarker that can improve the accuracy of outcome prediction. Considering the role of the immune response in a favorable prognosis, there is also a need for developing a biomarker that can predict responses to active immunotherapy.

Future perspective

Progress in our understanding of the tumor microenvironment resulted in the development of biomarkers for breast cancer prognosis. Early markers focused on receptors expressed on tumor cells as well as tumor grade. This approach was improved by including an expanded list of genes that were involved in tumor invasion and apoptosis as well as angiogenic factors. A different approach was also developed by focusing on tumor stroma and tumor-infiltrating immune cells. Emerging evidence suggests that identification of genes that are involved in a cross-talk between tumor cells and tumor stroma – particularly host immune responses – may result in the development of a global prognostic biomarker that could also predict responses to therapy **(Figure 1)**.

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Executive summary

Breast cancer biological subtypes & histological grading can predict risk of tumor recurrence

■ The prognostic impact of HER2 positivity is lower in node-negative compared with node-positive patients. Such limitations led to the use of multiple tumor biomarkers and approaches for breast cancer prognosis, including genomic grade index, PAM50™ and IHC4.

A complex of genes expressed by tumor cells & stromal cells can predict breast cancer relapse

■ The MammaPrint® assay has been US FDA-cleared for use in lymph node-negative and ER-positive or ER-negative breast cancer patients with tumors smaller than 5 cm. It is also being extended to node-positive patients. It is largely a prognostic, rather than predictive, assay. However, recent reports suggest a predictive value for the MammaPrint signature.

■ The main advantage of MammaPrint is in its ability to identify patients who could be spared unnecessary adjuvant therapy in the low-risk group, who show a less than 10% risk of recurrence within a minimum of 5 years. The 70-gene signature also provides additional information that can help refine the prognostic value of traditional markers such as HER2.

■ The Oncotype DX® panel includes 16 cancer-related genes as well as five reference 'housekeeping' genes. It serves as a prognostic as well as a predictive biomarker primarily in ER-positive, lymph node-negative patients.

■ Oncotype DX returns 40–66% of cases as intermediate risk with no clear data to suggest a benefit of chemotherapy. However, preliminary data suggest that addition of standard clinicopathological variables to the Oncotype DX recurrence score can help reduce the number of cases that fall into the intermediate-risk group.

■ Gene expression signatures derived from whole tumors are different from the signatures derived from the tumor stroma, with the latter showing a strong link to clinical outcome. Tumor stroma from patients with a good outcome showed overexpression of immune-related genes such as T-cell and natural killer cell markers with a skewed Th1 profile.

The status of tumor-infiltrating immune cells can predict breast cancer relapse

■ Whereas tumor-infiltrating dendritic cells, M1 macrophages, Th1 cells, CD8+ T cells and natural killer cells are associated with a favorable prognosis, presence of M2 macrophages, myeloid-derived suppressor cells, Tregs and Th2 cells is associated with poor prognosis in cancer patients.

■ Emerging evidence suggests that success of chemotherapy depends on the status of the antitumor immune responses.

A signature of genes involved in tumor–immune interactions can predict relapse or no relapse

■ Given that both tumor cells and infiltrating immune cells have shown to have prognostic values, understanding a cross-talk between tumor cells and infiltrating immune cells may result in the development of an improved prognostic biomarker for breast cancer patients.

■ We have recently identified a network of the immune function genes (a five-gene signature) and a panel of cancer testis antigens in the tumor tissue of breast cancer patients who were relapse-free, independent of ER and lymph node status, or lymphocytic infiltration.
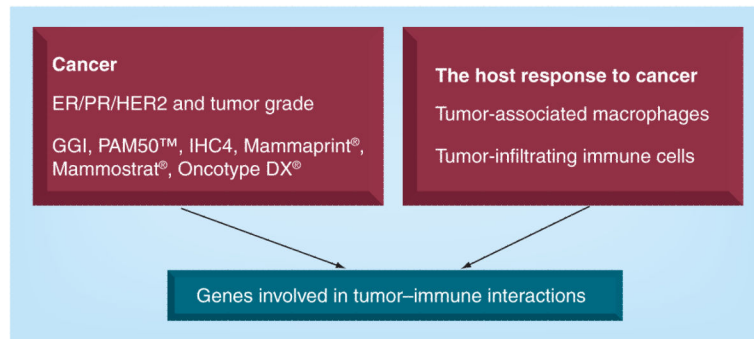
Figure 1. Development of breast cancer biomarkers
ER: Estrogen receptor; GGI: Genomic grade index; PR: Progesterone receptor.

Table 1

Biomarkers for breast cancer prognosis and prediction of response to therapy.

Assay	Method	Marker	Prognosis	Predictor	Patient eligibility
GGI	Microarray	97 genes	Yes	Yes	Node$^-$/ER$^+$
PAM50™	qRT-PCR	55 genes	Yes	Yes	Node$^-$/ER$^+$
IHC4	IHC	4 genes	Yes	ND	Node$^-$/ER$^+$
Mammostrat®	IHC	5 genes	Yes	ND	Node$^-$/ER$^+$
MammaPrint®	Microarray	70 genes	Yes	Yes	Node$^\pm$/ER$^\pm$
Oncotype DX®	qRT-PCR	21 genes	Yes	Yes	Node$^-$/ER$^+$
Immune function genes	qRT-PCR	5 immune function genes and CTAs	In trial	In trial	Node$^\pm$/ER$^\pm$

±: Positive and negative; −: Negative; +: Postitive; CTA: Cancer testis antigen; IHC: Immunohistochemistry; ND: Not determined; qRT: Quantitative reverse transcriptase.