

Molecular Subtypes and Local-Regional Control of Breast Cancer



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KEYWORDS

- Breast cancer • Molecular subtypes • Local-regional recurrence • Gene signatures
- Immunohistochemical surrogates

KEY POINTS

- The analysis of cancer gene expression patterns expands the understanding of breast cancer as a heterogeneous group of diseases.
- The presence or absence of estrogen, progesterone, and HER2/neu receptor (ER/PR/HER2) expression is key to molecular subtype stratification.
- Immunohistochemical techniques are widely applied to identify the markers of the different molecular subtypes.
- Gene expression profiling techniques, including commercially available tools such as Onco-type DX and MammaPrint, are considered complementary to the known prognostic factors.
- According to the currently available data, the different molecular subtypes are associated with different patterns of local-regional recurrence and response to treatment.

INTRODUCTION

Breast cancer is a heterogeneous disease that affects one anatomic site, yet is phenotypically variable.^{1,2} The identification of different biological subtypes occurs primarily through the use of techniques including immunohistochemistry³ and gene expression profiling.¹ To date, several studies have shown that the different biological subtypes are associated with variations in treatment response and disease-specific

Conflicts of Interest: The authors have nothing to disclose.

Funded by National Institutes of Health grant R01GM097220.

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Surg Oncol Clin N Am 27 (2018) 95–120
<http://dx.doi.org/10.1016/j.soc.2017.08.005>

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outcomes.^{4–10} Currently, decision-making for individual patients is based on several factors, including tumor morphology and grade classification, tumor size, presence of lymph node metastases, and expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)/neu (HER2). Although knowledge of these factors aids in treatment planning, there is a clear need to enhance the understanding of both prognostic and predictive markers that will facilitate customized treatment. The advent of novel technologies to aid in the identification of new markers also will be critical.

Through molecular analysis of breast cancers with gene expression profiling, both Perou and colleagues¹ and Sorlie and colleagues² showed that breast cancer could be subclassified into different subtypes. Broadly, these subtypes include luminal ER-positive (luminal A and luminal B), HER2 enriched, and basal-like (Table 1). Gene expression profiling can be costly, time-consuming, and, depending on the platform, may require a fresh tumor biopsy sample that has not been fixed in formalin.^{11,12} Given these constraints, gene expression profiling can be difficult to implement on a broad scale. Nevertheless, several groups including the American Society of Clinical Oncology (ASCO), the National Comprehensive Cancer Network (NCCN), and the St Gallen Group have issued guidelines and recommendations supporting the implementation of molecular analysis as useful for risk stratification and for treatment planning.^{13–15}

To facilitate the implementation of breast cancer subtype classification, efforts have been made to use immunohistochemical analysis to create approximated subtypes. Although taking this approach may enable stratification of breast cancers into subgroups that have outcomes comparable to those defined by gene expression profiling, there is not precise overlap.¹⁶ Furthermore, in addition to the evaluation of standard biomarkers that can be assessed with immunohistochemistry (ER, PR, and HER2), the contribution of other factors, such as proliferative rate and expression of cytokeratins, also may be important. For example, the St Gallen 2013 classification included the evaluation of Ki67 (a marker of cell proliferation)¹⁷ and a cutoff of PR of less than 20% as factors associated with the luminal B, HER2-negative subtype.¹⁵

The different molecular subtypes reflect the biological diversity of breast cancer. In a time in which medicine is moving toward a personalized approach, a critical goal is the correlation of the different disease subtypes with clinical outcomes and targeted therapeutics. Several studies have evaluated the variance in systemic disease recurrence and survival among the intrinsic subtypes.^{18–21} To this end, additional work has pointed toward the growing significance of molecular subtypes in the risk of local-regional recurrence along with clinical and pathologic features.⁵

Table 1 Classification of molecular subtypes and correlation with biomarker staining on immunohistochemistry				
Molecular Subtype	ER		PR	HER 2
Luminal A	Positive	and/or	Positive	Negative
Luminal B	Positive	and/or	Positive or negative ^a	Negative
Luminal B	Positive	and/or	Positive or negative ^b	Positive
HER2	Negative		Negative	Positive
Triple negative or basal-like	Negative		Negative	Negative

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.
^a (PR <20% + Ki 67 >14%).
^b (Any PR + any Ki 67).

Local-regional recurrence is described as ipsilateral, in-breast recurrence after lumpectomy, chest wall recurrence after mastectomy, or recurrence in the ipsilateral axillary, or supraclavicular lymph nodes (less commonly infraclavicular and/or internal mammary nodes).²² Overall, among patients with stage I-II breast cancer, approximately 10% to 15% will develop a local recurrence after breast-conserving surgery (BCS) and radiation therapy, and 10% to 20% of patients with stage I-IIIa disease will experience a chest wall recurrence after mastectomy.²² To date, several factors have been associated with aggressive cancer biology and the increased risk for local-regional recurrence. These factors include the following:

- Lymphovascular invasion²³
- Young age²⁴
- Increasing tumor size²⁵
- Close or involved margin status^{26,27}
- Positive nodal status^{28,29}
- High tumor grade^{23,30}
- Extensive intraductal component³¹
- Multifocal/multicentric disease³²
- Negative hormone receptor status^{33,34}
- Lack of adjuvant systemic therapy^{35–37}

It follows that local-regional recurrence may be associated with a more aggressive tumor biology. The importance of adequate local disease control has been highlighted by findings from randomized studies showing the impact of local-regional recurrence on survival.^{38–40} Data from the Early Breast Cancer Trialists' Collaborative Group overview analysis has demonstrated that for every 4 local recurrences prevented, approximately 1 death may be avoided.⁴¹

Defining the biological characteristics of breast cancer facilitates (1) characterization of different tumor histologic types, (2) understanding of disease prognosis, and (3) systemic treatment planning. Typically, immunohistochemistry is used to characterize intracellular or cell surface cancer-related protein expression. Among several factors, the most frequently evaluated immunohistochemical breast cancer prognostic and predictive markers include ER, PR, HER2, and Ki67.

ESTROGEN RECEPTOR/PROGESTERONE RECEPTOR

It has been known for some time that estrogen plays an important stimulatory role in the normal breast and in the development and progression of breast cancer. Moreover, ER is one of the most important prognostic biomarkers in breast cancer. ER belongs to a group of nuclear hormone receptors that act as transcription factors. There are 2 isoforms: ER α (the clinically measured isoform) and ER β . Patients whose tumors are ER-positive benefit from endocrine therapy targeting ER (such as tamoxifen and aromatase inhibitors), and treatment can reduce local and distant recurrence and mortality.^{42,43} However, ER-positive breast cancers do not respond as well to cytotoxic chemotherapy and are less likely to achieve a pathologic complete response (pCR) when compared with patients with ER-negative breast cancer who receive neoadjuvant chemotherapy.^{44,45} PR also manifests as 2 major isoforms (PR-A and PR-B) and plays a role in downstream ER signaling.⁴⁶ It is likely that PR acts as a driver for the development of breast cancer that may be most impactful in postmenopausal women.⁴⁷ The ER is thought to regulate PR expression, and the presence of PR expression is considered indicative of a functional estrogen-ER axis.⁴⁸ In most cases, PR expression correlates with ER expression, and from a practical standpoint, robust

PR expression in the absence of ER may necessitate repeat testing. The presence of PR expression carries prognostic significance in early breast cancer with ER-positive and PR-positive cases having the best outcome.⁴⁹ Additionally, PR expression correlates with tumor responsiveness to endocrine therapy even when PR expression is low (ie, $\geq 1\%$ of tumor cell nuclei).⁵⁰ However, in the setting of an ER-positive breast cancer, PR assessment may not add significant predictive information. That being said, breast cancers that are both ER-positive and PR-positive may derive greater benefit from endocrine therapy than ER-positive/PR-negative tumors.⁵¹

Currently, the evaluation of ER and PR receptor expression is standard practice and most often performed using immunohistochemistry. ER and PR immunoexpression manifests as nuclear staining, and heterogeneous (“physiologic”) expression is typically observed in normal breast ductal epithelium. Up to 80% of breast cancers are ER-positive⁵² and 55% to 65% are positive for PR expression.⁵³ ASCO and the College of American Pathologists (CAP) have provided recommendations regarding ER/PR measurement and reporting.⁵⁴ Per these recommendations, pathology reports for ER and PR results should include the following details: percentage and/or proportion of tumor cells staining positively in a tissue section or cytology preparation, the overall intensity of staining (weak, moderate, strong), and an interpretation as to hormone receptor status. As to the latter, ER and PR expression is considered positive when $\geq 1\%$ of tumor cells stain positively, negative when less than 1%, and interpretable when preanalytic variables preclude an accurate assessment. These include the use of fixatives other than 10% neutral buffered formalin during tissue processing, duration of fixation less than 6 hours or more than 72 hours, delay in fixation/cold ischemic time more than 1 hour, decalcification of specimens using acids, and inappropriate intrinsic/extrinsic assay controls. Some institutions provide a composite score combining percentage and intensity of tumor cell hormone receptor expression.

Most pathology laboratories use immunohistochemical methods to determine ER and PR status. Testing is often performed on biopsy material before surgery, as there is excellent agreement in hormone receptor expression between biopsy and resection specimens.⁵⁵ This enables clinicians, oncologists, surgeons, and patients to possess vital prognostic and therapeutic information before establishing a treatment plan. However, there is institutional variability in the manner by which ER and PR immunoexpression is evaluated. At some centers, pathologists use digital image analysis (DIA) in which slides are scanned and converted into high-resolution computer images so that quantitative immunohistochemistry can be performed.⁵⁶ DIA is being used with greater frequency owing to established intraobserver and interobserver variability in scoring results by pathologists.⁵⁷ Finally, repeat testing of ER and PR on excision specimens in cases in which prior core biopsy showed weak or equivocal expression is also undertaken at some institutions.

ANDROGEN RECEPTOR

Another hormone receptor currently being studied in breast cancer research is the androgen receptor (AR). Like ER and PR, immunohistochemical AR expression is nuclear and usually strong and diffuse when present (ie, present in $>80\%$ of tumor cell nuclei). Expression is seen in most ER-positive breast cancers, as well as subsets of triple-negative breast cancer (TNBC) and HER2-positive carcinomas. In the spectrum of TNBCs, tumors with robust AR expression often manifest as apocrine carcinoma (“carcinoma with apocrine differentiation”), a ductal tumor in which the invasive cells exhibit extensive apocrine cell change ($>90\%$ of tumor cells exhibiting apocrine features).⁵⁸ Most pathologists view AR immunoexpression in 1% of tumor

cell nuclei as AR-positive. Molecular studies using RNA expression profiling have revealed several potentially clinically relevant breast cancer subtypes. One of these is known as the molecular apocrine group, defined as tumors that are ER-negative but AR-positive.⁵⁹ These tumors often exhibit an expression profile that overlaps with luminal, basal-like, and occasionally HER2-positive groups. Interestingly, many of these tumors, but not all, show some degree of apocrine differentiation on histopathology.

From a clinical perspective, AR-positive breast cancers appear to behave favorably when compared with AR-negative tumors, regardless of ER status.⁶⁰ Among TNBCs, the significance of AR expression on prognosis and clinical course continues to be investigated. In some studies, AR-positive TNBC has a better prognosis when compared with AR-negative TNBC; however, others have found a decreased survival for patients with early-stage disease.⁶¹ Further confounding the prognostic significance of AR expression is the presence of HER2 overexpression in molecular apocrine cases. The favorable clinical course of AR-positive TNBC may be related to the genetic overlap with luminal tumors. Because the AR can be targeted via aromatase inhibition, there has been considerable clinical interest in interrogating TNBC for AR expression; however, there are no guidelines regarding routine testing for AR for all patients with breast cancer.

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2

HER2 (erbB-2) is a transmembrane tyrosine kinase receptor that regulates cell growth, proliferation, and survival through several different signaling pathways, such as the mammalian target of rapamycin and RAS/RAF/MEK/extracellular signal-regulated kinase (ERK) pathways.⁶² *HER2* gene amplification is observed in 15% to 30% of breast cancers and is a strong prognostic biomarker for an aggressive clinical course.⁶³

Importantly, *HER2* gene overexpression is also a strong predictive marker of response to anti-HER2 therapy, which includes humanized monoclonal antibodies that bind to the extracellular domain of the HER2 receptor (trastuzumab and pertuzumab), small molecular receptor tyrosine kinase inhibitors (lapatinib), and an antibody drug conjugate of trastuzumab (ado-trastuzumab emtansine; T-DM1). Immunohistochemistry for HER2 protein overexpression has been developed, and testing for this protein is standard for invasive breast carcinomas whether primary or metastatic. Overexpression of the HER2 protein typically occurs secondary to *HER2/neu* gene amplification.⁶⁴ As per the most recent 2013 ASCO/CAP guideline recommendations⁶⁵ regarding HER2 testing in breast carcinoma, the threshold for a positive test on immunohistochemistry is strong circumferential staining in more than 10% of invasive tumor cells (score 3+), whereas criteria for a negative result includes (A) no staining observed or incomplete, faint/barely perceptible membrane staining in $\leq 10\%$ of invasive tumor cells (score 0), or (B) incomplete, faint/barely perceptible membrane staining in greater than 10% of tumor cells (score 1+). An equivocal result (score 2+), is rendered when there is either incomplete and/or weak-to-moderate circumferential membrane staining in more than 10% of invasive tumor cells or there is complete, intense, circumferential membrane staining in $\leq 10\%$ of invasive tumor cells. Breast cancers with an equivocal result for HER2 overexpression are reflexed to either fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), or silver-enhanced in situ hybridization (SISH) assays to determine *HER2* gene amplification. Immunohistochemistry and gene amplification assays usually yield similar results, as gene amplification induces immunohistochemically detected protein overexpression in 95% of cases. Most institutions use FISH, however CISH and SISH

techniques are increasingly being used, as they may be implemented using light microscopy. Moreover, probes for *HER2* as well as centromere 17 (centromere enumeration probe, CEP17) are used so that the ratio of *HER2* signals to CEP17 can be calculated. A positive (amplified) test result is rendered when the average *HER2* copy number ≥ 6.0 signals per cell or *HER2*/CEP17 ratio ≥ 2.0 , whereas a negative (not amplified) result is found when the average *HER2* copy number is < 4.0 and the *HER2*/CEP17 ratio is < 2.0 . As for *HER2* immunohistochemistry, an equivocal result is observed with an *HER2*/CEP17 ratio less than 2.0 and the average *HER2* copy number is ≥ 4.0 but less than 6.0.

Ki67

Ki-67 is a nuclear proliferation marker expressed in all phases of the cell cycle except G_0 .⁶⁶ In general, breast cancers expressing high levels of Ki67 correlate with worse outcomes.^{67,68} Meta-analyses by de Azambuja and colleagues⁶⁸ in 2007 and Stuart-Harris and colleagues⁶⁹ in 2008, showed that Ki67 was associated with prognosis, disease-free survival, and overall survival. In 2009, the St Gallen Consensus stratified tumors according to the Ki67 value as low (less than 15%), intermediate (16%–30%), and highly proliferative (greater than 30%), to help identify patients who could potentially benefit from treatment with chemotherapy or endocrine therapy. Additional studies reported a Ki67 level higher than 10% to 14%^{70,71} and 20%,¹⁵ as characteristic of high risk for poor outcomes. The implementation of Ki67 has been complex, as some studies have used 10%, 14%, or 20% cutpoints for treatment recommendations.^{72,73} Accordingly, Cheang and colleagues⁷⁴ defined a Ki67 cutoff to distinguish luminal A and B subtypes using immunohistochemistry. This work defined the luminal A subtype as ER-positive and/or PR-positive, *HER2*-negative, and Ki67 low (ie, a Ki67 index of $< 14\%$), and the luminal B subtype as ER-positive and/or PR-positive, *HER2*-negative, and Ki67 high (ie, a Ki67 index of $\geq 14\%$) and showed that stratification using the immunohistochemical panel of 4 biomarkers (ie, ER, PR, *HER2*, and Ki67) was statistically significant.⁷⁴ ASCO guidelines have not included Ki67 in the list of routinely assessed markers for breast cancer prognosis.⁷⁵ In Europe, the St Gallen International Expert Consensus recommended the use of proliferation markers, such as Ki67, in addition to standard parameters (stage, grade, hormone receptor status) when choosing systemic treatment regimens.¹⁵ A role for Ki67 was also found in the neoadjuvant setting, in particular for patients undergoing preoperative endocrine therapy,⁷⁶ indicating how a variation in Ki67 expression may predict long-term outcomes. Nishimura and colleagues,⁷⁷ in 2010, investigated the role of Ki67 in neoadjuvant systemic treatment and found that marker values before starting treatment could help to predict response, whereas those measured after chemotherapy could help predict disease-free survival. Additionally, measurement of Ki67 is cost-effective when compared with multigene assays. At some centers, Ki67 is included in surgical pathology reports along with other standard markers, and can aid in the identification of luminal B tumors not identified by ER, PR, and *HER2*.

MOLECULAR SUBTYPES

Luminal A

Frequency: 30% to 40% of all invasive breast cancers. *Gene expression profiling (GEP):* PIK3CA mutations, MAP3KI mutations, ESR1 high expression, XBP1 high expression, GATA3 mutations, FOXA1 mutations; gain of 1q, 8q, loss of 8p, 16q. *Morphology:* Grading 1 or 2; most are well-differentiated carcinomas of no special type (NST), classic lobular carcinomas, tubular, mucinous, neuroendocrine and

cribriform carcinomas.⁷⁸ *Immunohistochemical profile (IHC):* ER-positive, PR high expression ($\geq 20\%$), HER2-negative, and low Ki-67.^{79,80} Several studies showed that a Ki-67 of 14% was the cutoff point to separate luminal A from luminal B subtypes.⁷⁴ More recently, this cutoff was changed to 20%.⁸¹

Luminal B (Human Epidermal Growth Factor Receptor 2-Negative)

Frequency: 20% to 30% of all invasive breast cancer. *GEP:* TP53 mutations, PIK3CA mutations, Cyclin D1 amplification, MDM2 amplification, ATM loss, enhanced genomic instability, focal amplifications (eg, 8p12, 11q13). *Morphology:* Grading 2 or 3; less well-differentiated cancers, mostly invasive ductal carcinomas NST and also some invasive micropapillary carcinomas.⁷⁸ *IHC profile:* ER-positive, lower PR expression ($< 20\%$),¹⁵ HER2-negative and higher level of Ki67 labeling index ($> 14\%$ or 20%).^{52,74} *Correlation to oncotype results:* high recurrence score is suggestive of luminal B like subtype.¹⁵ Furthermore, on a molecular basis, it seems that luminal A and luminal B breast cancers have specific gene profiles that lead to oncogenic proliferation.

Human Epidermal Growth Factor Receptor 2

Frequency: 12% to 20% of all invasive breast cancers. *GEP:* HER2 amplification, TP53 mutations, PIK3CA mutations, FGFR4 high expression, epidermal growth factor receptor (EGFR) high expression, APOBEC mutations, cyclin D1 amplification, high genomic instability.^{1,2,82-85} *Morphology:* grading 2 or 3; infiltrating carcinoma NST, apocrine and pleomorphic lobular carcinomas. *Classification:* in the study from Fountzilas and colleagues,⁸⁶ hormone receptor status was shown to affect survival, metastatic spread, and treatment response. Therefore, on IHC, HER2-positive tumors can be divided into 2 subtypes: HER2-enriched subtype (ER-negative and/or PR-negative/HER2-positive)⁸⁶⁻⁸⁸ and luminal HER2 subtype (ER-positive and/or PR-positive, HER2-positive); and further divided into 2 phenotypes based on PR expression: ER-positive, PR-positive, HER2-positive and ER-positive, PR-negative, HER2-positive. There is preclinical evidence for crosstalk between the HER2 and ER signaling pathways with a negative effect on the response to endocrine therapy.⁸⁹⁻⁹³

Triple-Negative Breast Cancer

Representing 15% to 20% of all invasive breast cancers, TNBC is defined by the absence of expression of ER, PR, and HER2. Despite its simple definition, it is a morphologically, genetically, and clinically heterogeneous category of breast cancer. Most TNBCs manifest as invasive ductal carcinoma NST; however, the category also includes variants such as metaplastic carcinoma, carcinoma with medullary features (formerly known as medullary carcinoma), carcinoma with apocrine features (formerly known as apocrine carcinoma), secretory carcinoma (formerly known as juvenile breast cancer), and adenoid cystic carcinoma.⁵⁸ Gene expression profiling has enabled the subdivision of these cancers into different, prognostically significant subtypes that, in some cases, correlate with a particular pathologic variant. The most common, and best-characterized, molecular subtypes of TNBC are basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stemlike (MSL), and luminal androgen receptor (LAR).⁹⁴ BL1 breast cancers usually manifest clinically and pathologically as invasive ductal carcinoma NST and have high Ki67 proliferative indices, and their expression profile is enriched in genes associated with basal cytokeratin genes (including *KRT5*), cell cycle and DNA replication, and DNA damage response pathways. BL2 tumors also manifest as invasive ductal carcinoma NST and also show increased basal cytokeratin gene expression, *TP63*, and growth factor signaling (eg, EGF and insulinlike growth factor 1 receptor

pathways). In general, the immunomodulatory subtype of TNBC overlaps with carcinoma with medullary features, as both often contain a brisk lymphocytic infiltrate. Mesenchymal and MSL subtypes may present clinically as metaplastic carcinoma, defined as breast cancer with epithelial and mesenchymal differentiation, sometimes taking the form of heterologous elements like bone and cartilage. These tumors are enriched in genes important for cell differentiation and growth factor signaling pathways. Finally, the LAR subtype often presents as carcinoma with apocrine differentiation and correlates with the molecular apocrine type. As expected, LAR shows increased expression of luminal cytokeratin genes (including *KRT7* and *KRT1*) and enriched in AR mRNA and protein expression, possibly explaining their responsiveness to hormone therapies.

Gene Expression Testing

The information obtained from microarray-based gene expression profiling has revealed further information about breast cancer as a group of different diseases with different biological characteristics and behaviors.^{95,96} A key finding, in addition to the validation of ER-positive and ER-negative cancers as different diseases from a molecular point of view, was that the expression of proliferation-related genes was also associated with prognosis of ER-positive tumors.^{12,97} Through the advent of methods for gene expression testing, a prognostic multigene assessment has also been developed and validated in clinical trials, and integrated into clinical practice.⁹⁸ Through the work of Perou and colleagues¹ and Sorlie and colleagues,² a group of “intrinsic genes” was identified, revealing 4 molecular subtypes of breast cancer¹: luminal A and B, HER2-enriched, and basal-like. The groups of genes primarily responsible for the segregation of molecular subtypes are genes related to expression of ER, regulation of proliferation, HER2, and genes mapping to chromosome 17.^{1,2} The prognostic signatures currently available provide clinicians with data complementary to factors with known prognostic significance, including tumor size and nodal status (Table 2). To date, these gene signatures are more informative for patients with ER-positive disease, but have a more limited role for patients with ER-negative tumors. One of the main objectives of molecular subtyping was the ability to identify factors to aid in the discrimination between patients with a favorable versus an unfavorable prognosis to guide therapeutic decision-making.⁹⁶ In recent years, several prognostic signatures have been identified⁹⁶ that revealed overlapping groups of patients with a poor prognosis,⁹⁹ primarily characterized by cancers with high expression of proliferation-related genes.^{95,99} Given the significance of proliferation-related gene expression for prognosis for both ER-positive and ER-negative disease, these factors are active targets for cytotoxic chemotherapies.^{95,100} In terms of outcome prediction generated from molecular subtyping, the insights obtained appear to extend beyond the short-term time frame (eg, 10 years instead of 5),^{101,102} serving as complementary to the parameters routinely used in clinical practice.^{96,99}

70 Gene Signature

MammaPrint (Agendia, Amsterdam, Netherlands) is a microarray-based test, primarily implemented in Europe, that can be used for risk stratification (ie, low or high) for patients with ER-positive or ER-negative breast cancer. It was based on data derived from stage I and II patients younger than 55 years, with node-negative tumors smaller than 5 cm. The test can be performed with fresh or formalin-fixed tumor samples. Much of the data related to this test were obtained from retrospective studies, and the findings were then confirmed prospectively by the RASTER study.¹⁰³ The results

Table 2
Gene signatures

Test	Method	Result	Clinical Application	Level of Evidence
MammaPrint	• Microarray • 70 gene • Fresh tissue, frozen material and alternatively formalin fixed and paraffin embedded (FFPE)	Distant metastasis at 5 y (without adjuvant treatment): • Low risk 13% • High risk 56%	Prognosis of N0, <5 cm diameter, stage I/II disease, age <61 y	II
Oncotype DX	• qRT – PCR • 21-gene • Fresh frozen tissue and/or FFPE	Risk of 10 y distant recurrence: • Low (<18) • Intermediate (18–30) • High (≥ 31)	Prediction of recurrence risk in ER+ and N0 disease treated with tamoxifen	I
Prosigna/PAM50	• qRT – PCR • 50-gene • Routinely processed tissue (e.g. FFPE)	Risk of distant recurrence from 5 to 10 y after diagnosis: • Node-negative cancers - Low (0–40) - Intermediate (41–60) - High (61–100) • Node-positive cancers - Low (0–40) - High (41–100)	Prediction of recurrence risk in hormone receptor positive breast cancer after 5 y of hormonal therapy in postmenopausal women	I

of the prospective MINDACT trial recently published showed survival rates of approximately 95% at 5 years for low-risk patients who did not receive chemotherapy.¹⁰⁴

21 Gene Recurrence Score Assay

In the United States, implementation of the Oncotype DX assay has been widespread for patients with node-negative hormone receptor-positive breast cancer.¹⁰⁵ It is performed using a reverse-transcription polymerase chain reaction on RNA isolated from paraffin-embedded breast cancer tissue, measuring the expression of 21 genes (16 cancer-related genes and 5 reference genes). The Oncotype DX 21 gene recurrence score (RS) assay has been defined as a continuous variable (ranging from 0 to 100). This assay can be considered as an independent prognostic factor in node-negative, ER-positive breast cancer, measuring the risk of distant relapse at 10 years. With values ranging from 0 to 100, patients are stratified into 3 groups: (1) low risk (RS <18), (2) intermediate risk (RS 18–31), and (3) high risk (RS ≥ 31). These groups are associated with 10-year relapse rates of 7%, 14%, and 30%, respectively. For patients with ER-positive tumors, those who fall into the high-risk group may have greater benefits from the use of chemotherapy.¹⁰⁵ Appropriate management of patients in the intermediate-risk group will be revealed through results of the TAILORx study (NCT00310180). Patients (ER-positive and node-negative) enrolled in TAILORx were assigned to low-risk (RS <11), intermediate-risk (RS 11–25), and high-risk groups (RS >25). The main aim of the study was to evaluate the risk of relapse after surgery for

the intermediate-risk group. Patients in this risk category were randomly assigned to hormone treatment alone or in combination with chemotherapy. The trial completed enrollment and results are pending. Emerging data from several studies highlight how Oncotype DX may also play a role in different patient scenarios, including those with positive lymph nodes, ER-positive and HER2-positive disease, and patients treated with aromatase inhibitors instead of tamoxifen.^{106,107}

Based on the available evidence, guidelines have been developed for the use of Oncotype DX clinically. NCCN guidelines suggest that Oncotype DX can be used as a predictor of recurrence and can be used as a guide for treatment decision-making in ER-positive, node-negative breast cancer based on level I evidence.¹⁴

ASCO guidelines suggest it can be used as a tumor marker for risk of recurrence.¹⁰⁸

In Europe, the latest St Gallen International Expert Consensus recommended that the validated assay should be used to clarify the indication for chemotherapy and added to other known prognostic and predictive factors.¹⁵ Along with the ability of the RS to aid in predicting the response to adjuvant chemotherapy,^{107,109} additional studies have shown how the RS can be predictive of local-regional and distant recurrence.^{105,110} Although evidence suggests that the RS obtained with the Oncotype DX correlates with traditional clinicopathologic data, the information obtained regarding prognosis from tumor size, lymph node status, and histologic grade remain as independent variables.^{109,111}

Clinical Outcomes: Local-Regional Recurrence

Significant progress has been made in terms of systemic therapy and targeted treatments, improving outcomes for patients with breast cancer. Optimal local-regional control continues to be relevant in terms of clinical outcomes. The risk of a local-regional recurrence (LRR) has been correlated to different parameters (both pathologic and clinical) with the aim to guide treatment decisions.

RISK FACTORS FOR LOCAL-REGIONAL RECURRENCE

Breast-Conserving Therapy

Age

Studies have found that age is a risk factor for LRR after breast-conserving therapy, with 10-year local recurrence rates reportedly higher for younger patients.^{112,113} One relevant study showing the impact of age was the EORTC 22881 to 10882 trial.¹¹⁴ A tumor bed boost used in addition to standard whole breast irradiation resulted in a reduction in local recurrence from 23.9% to 13.5% in patients aged 40 years or younger when compared with a decrease from 7.3% to 3.8% in patients older than 60 years.¹¹⁴ Accordingly, clinicians should consider patient age when considering treatment planning in patients undergoing BCS.

Margins

Positive margins (defined as ink on invasive carcinoma or ductal carcinoma in situ) are associated with a twofold increase in the risk of local recurrence when compared with negative margins (>2 mm). This was an independent risk factor, not mitigated by radiation boost or other characteristics. There is no evidence that more widely free margins reduced this risk even in cases with other risk factors, including young age, unfavorable biology, lobular cancers, or cancers with an extensive intraductal component (EIC).¹¹⁵

Extensive intraductal component

EIC is defined as an infiltrating ductal cancer in which more than 25% of the tumor volume is ductal cancer in situ (DCIS) and DCIS extends beyond the invasive cancer into

surrounding normal breast parenchyma. Invasive breast carcinoma is accompanied by EIC in 15% to 30% of patients.¹⁴ It can be considered as a risk factor for local recurrence.^{114,116,117}

Mastectomy

Several studies have identified risk factors for local recurrence after mastectomy (Jagsi and colleagues¹¹⁸ and the Breast Cancer Cooperative Group (DBCG82) b and c studies).¹¹⁹ Jagsi and colleagues¹¹⁸ demonstrated how tumor size larger than 2 cm, margins less than 2 mm, premenopausal status, and lymphovascular invasion can be considered as independent prognostic factors for LRR. Ten-year LRR was 1.2% for those patients with no risk factors, and as high as 40.6% for those with 3 risk factors. This retrospective analysis suggested the benefit of postmastectomy radiation therapy (PMRT) to reduce the risk of recurrence for patients with node-negative disease and high-risk factors. In the Breast Cancer Cooperative Group (DBCG82) b and c studies, tumor size, histology positive for ductal carcinoma, high tumor grade, invasion of the pectoralis major fascia, several positive nodes, and extracapsular spread were identified as risk factors for LRR.^{119,120} Abdulkarim and colleagues¹²¹ reported an increased risk of local-regional recurrence in women with node-negative, TNBC with tumors ≤ 5 cm when mastectomy was performed without radiation. This prospective study further identified that risk factors for LRR include node-positive disease, tumors larger than 2 cm, and lymphovascular invasion.

Additional Risk Factors

Large tumor size, poor tumor differentiation, nodal status, multifocal/multicentric disease, and adjuvant treatment have all been found to be relevant to the assessment of the risk for LRR.¹²²

Lymphovascular invasion

Lymphovascular invasion is a factor associated with a higher risk of recurrence. In patients treated with mastectomy, this parameter is one of the risk factors to be considered when offering postmastectomy radiation therapy.^{14,123}

Receptor status

ER, PR, and HER2 status, known as predictors of response to targeted treatment, also have a prognostic role after breast-conserving therapy and mastectomy.⁴² As described in the analysis of the Danish 82 b and c trials, triple-negative disease was associated with higher local recurrence rates after mastectomy.⁶

BRCA1 and 2 mutation carriers

Patients with sporadic breast cancer treated with BCS have an approximate 10% risk of ipsilateral breast tumor recurrence. Although some studies have reported a risk reduction of contralateral breast cancers in BRCA mutation carriers who take tamoxifen or undergo oophorectomy, contralateral prophylactic mastectomy can decrease the risk to less than 10%.¹²⁴ Risk reduction strategies in BRCA1/2 mutation carriers, including bilateral prophylactic mastectomy, oophorectomy, and/or the use of tamoxifen are being implemented in many countries.^{124,125} BRCA1 mutation cancers are more likely to have ER-negative breast cancer and are also more likely to develop tumors with a higher histologic grade.

There are several studies showing that the different intrinsic subtypes have differences in overall prognosis² and different rates of local-regional disease recurrence (Table 3).

Table 3 Local-regional recurrence by molecular subtype	
Molecular Subtype	Frequency, %
Luminal A	0.8–8
Luminal B	1.5–8.7
HER2 ^a	1.7–9.4
Triple negative	3–17

Abbreviation: HER2, human epidermal growth factor receptor 2.
Patients treated with upfront surgery.
^a Luminal HER2-positive and HER2-enriched.

INTRINSIC SUBTYPES, WHAT ROLE?
Luminal A

The luminal A molecular subtype is generally associated with a highly favorable prognosis¹²⁶ and typically shows less frequent and less extensive lymph nodal involvement.^{127,128} This subtype also tends to have a more indolent course with a slower evolution over time when compared with the other molecular subtypes.¹²⁹ Additionally, a positive hormone receptor status is both a favorable prognostic factor and also predictive of response to endocrine therapy.^{42,130,131} In terms of LRR, several retrospective studies have shown similar outcomes with percentages ranging between 0.8% and 8.0%.^{4,5,9,132,133}

Luminal B

The luminal B molecular subtype is associated with a more intermediate prognosis when compared with the luminal A molecular subtype.¹²⁶ The risk of LRR for luminal B tumors, as described in literature, ranges from 1.5% and 8.7% with a peak of incidence in the first 5 years after diagnosis.^{4,5,9,132,133}

Luminal Tumor Considerations

The luminal molecular subtypes have lower local-regional recurrence rates, with both conservative surgery and mastectomy.¹³⁴ In the study by Lowery and colleagues,¹³⁴ it was estimated that the rate of LRR for both luminal A and B subtypes after BCS and radiation was approximately 5%, even in the setting of noncompliance with endocrine therapy. It is important for clinicians to reinforce that endocrine therapy reduces the LRR and mortality rates by more than 50% in patients with ER-positive breast cancer between 5 and 10 years from diagnosis.⁴²

HER2-Positive Disease

Historically, HER2 overexpression has been associated with a higher frequency of LRR based on studies in which patients were not treated with HER2-targeted therapy, ranging between 4% and 15%.^{4,5,9,132,133} In the meta-analysis from Lowery and colleagues,¹³⁴ 12,592 patients were evaluated for LRR after BCS with radiation therapy and mastectomy. The results showed increased rates of LRR for patients with HER2-positive tumors, with higher rates of recurrence observed after BCS versus mastectomy. In this study, fewer than 6% of the HER2-positive patients were treated with trastuzumab. A subgroup analysis of 9306 patients from 9 studies showed a lower rate of LRR after mastectomy for luminal HER2-negative tumors, when compared with luminal HER2-positive cancers (7.5% vs 9.4%), although in this analysis the use of trastuzumab was also low. In this subgroup analysis, no difference was observed in

the risk of LRR after BCS for luminal HER2-negative tumors compared with luminal HER2-positive (Relative Risk (RR) 0.8%–95% confidence interval) tumors. Overall, both luminal HER2-negative and luminal HER2-positive cancers showed a positive trend toward lower LRR rates when compared with ER-negative/HER2-positive and TNBC subtypes.¹³⁴ More recently, patients with HER2-positive tumors receive targeted therapies, including trastuzumab and pertuzumab, and this has modified the natural course of this disease subtype, resulting in improved outcomes.¹²⁶ HER2-positivity is a predictive factor for response to trastuzumab¹³⁵ and also a prognostic factor for LRR.^{131,136–138} In a study by Panoff and colleagues,¹³⁹ patients with HER2-positive tumors who underwent mastectomy and received trastuzumab, had an LRR of 1.7%. This finding was supported by an analysis of 6 studies from Yin and colleagues,¹⁴⁰ who also showed trastuzumab treatment resulted in a decrease in LRR by 50%. It has been suggested that breast cancer cells with radiation damage could be more vulnerable to injury when deprived of the mitogenic cell signaling provided by the HER2 pathway activation.¹⁴¹ Further to this point, a study conducted in patients with T1 (a or b) HER2-positive breast cancer showed a risk of LRR of 2.0% to 5.7% for those who did not receive trastuzumab, and 0% for those patients treated with trastuzumab $\pm$ chemotherapy at 5 years after diagnosis.¹⁴²

Triple-Negative Breast Cancer

TNBC is associated with an unfavorable prognosis when compared with the other breast cancer subtypes secondary to a higher risk of disease recurrence.^{126,143,144} For TNBC, the involvement of regional lymph nodes is associated with a poor prognosis, without a direct relationship to the number of involved nodes.¹³⁵ The TNBC subtype has been associated with a higher risk of local-regional relapse¹⁰ and contralateral disease, as shown by Bessonova and colleagues¹⁴⁵ and Malone and colleagues,¹⁴³ and also systemic relapse.¹²⁶ Peak incidence for recurrence in TNBC has been reported in the first 1 to 3 years, as reported in studies by Jatoi and colleagues¹²⁹ and Kumar and Aggarwal.¹⁴⁶ In the meta-analysis by Lowery and colleagues,¹³⁴ the TNBC subtype was associated with increased LRR rates, after BCS and mastectomy, ranging from 3% to 17%. Other studies have reported that TNBC is not associated with higher LRR risk.^{147,148} Abdulkarim and colleagues¹²¹ reported no difference in local recurrence events, whereas Wang and colleagues¹⁴⁷ found patients with TNBC undergoing mastectomy to have inferior outcomes. Several other studies have described the TNBC subtype as having the highest risk for LRR.^{4,5,9,10,22,132,133} Moreover, after the diagnosis of a local-regional recurrence, this molecular subtype is associated with a high incidence of distant metastases and cancer-related mortality.¹⁴⁶

Another topic of debate is the apparent radiation resistance of this molecular subtype, which may be impacted by ER negativity, shorter cell cycle duration, and less time for DNA damage repair. Kyndi and colleagues⁶ reported a smaller reduction in LRR rates after PMRT in TNBC, and no overall survival benefit, thought to be secondary to radio-resistance-associated hormone receptor negativity.¹⁴⁶ Some studies are evaluating ways to improve radio-sensitivity through use of cisplatin-based chemoradiation in the setting of TNBC.¹²⁶

LOCAL-REGIONAL RECURRENCE AND MOLECULAR SUBTYPES IN THE NEOADJUVANT SETTING

Huang and colleagues¹⁴⁹ reported in the neoadjuvant setting that multifocal/multicentric disease, number of positive axillary nodes, axillary dissection with fewer

than 10 nodes, lymphovascular invasion, extracapsular extension, skin or nipple involvement, and ER-negative disease were significantly associated with LRR. Patients who achieved a pCR had a lower rate of LRR (2% vs 12%). The study by Levy and colleagues¹⁵⁰ evaluated the long-term local-regional control rates after breast-conserving therapy for patients undergoing surgery before or after chemotherapy. In this study, ER-negative and/or PR-negative disease were associated with higher rates of LRR, and the 10-year LRR rate was not related to surgical treatment approach.¹⁵⁰ Mamounas and colleagues¹⁵¹ also reported that after neoadjuvant treatment, surgical approach was not related to a different incidence in LRR. Thus, for patients treated with neoadjuvant therapy, as for patients treated with surgery first, clinical stage, nodal involvement, and histology can be considered prognostic factors for LRR. Two large trials, NSABP B-18 and B-27, have also shown that a pCR (defined in these studies as the absence of invasive tumor in the breast), after Neoadjuvant Chemotherapy can be considered as a prognostic factor for LRR.¹⁵¹ von Minckwitz and colleagues¹⁵² studied 6377 patients after neoadjuvant treatment, and found pCR was associated with better outcomes in luminal B/HER2-negative, HER2-positive (nonluminal), and TNBC subtypes, but not for patients with luminal B/HER2-positive or luminal A cancers. Liedtke and colleagues¹⁵³ found that patients experiencing a pCR had excellent outcomes regardless of receptor status, but, when comparing patients with TNBC who did not achieve a pCR with other subtypes, TNBC was associated with a lower overall and postrecurrence survival rate, with a risk of relapse and death found to be higher in the first 3 years after diagnosis. Despite this, when patients with TNBC achieve a pCR, they have improved survival. In a series published by Swisher and colleagues¹⁵⁴ at the MD Anderson Cancer Center, the LRR-free survival rate for patients with TNBC with residual disease post-treatment was estimated at approximately 89.9% versus 98.6% for patients with a pCR. This study concluded that TNBC was an independent predictive factor for LRR.¹⁵⁴ The role of pCR on LRR was not observed for patients with HER2-positive subtypes.¹⁵⁴ Similar findings were reported in the studies of Peterson and colleagues¹⁵⁵ and Kiess and colleagues.¹⁵⁶ Taken together, these findings point toward the role of molecular subtypes in the locoregional recurrence and could help to facilitate patient selection for optimal treatment planning.

LOCAL-REGIONAL RECURRENCE AND AXILLARY DISEASE

Many changes have occurred in the management of the axilla over the past several years. Two trials that have proposed treatment changes include the AMAROS trial and ACOSOG Z0011. The AMAROS trial was a multicenter phase 3 trial in which patients were enrolled with T1 and T2 breast cancers (both unifocal and multifocal), a clinically negative axilla, and positive sentinel nodes. Patients were treated with BCS or mastectomy and were assigned to axillary node dissection versus radiation treatment (breast tangential fields, axillary and supraclavicular nodes). The study endpoints were to evaluate the axillary recurrence, disease-free survival (DFS), overall survival, and quality of life in terms of arm lymphedema and motility. The investigators showed additional metastases were found in 33% of cases and no differences were identified in terms of DFS and overall survival between the 2 treatment groups, with less morbidity reported in the axillary radiation therapy group (lymphedema 11% vs 23%).¹⁵⁷

The American College of Surgeons (ACOSOG) Z0011 trial enrolled clinically node-negative women with T1 and T2 breast cancer with 1 to 2 positive sentinel nodes who underwent BCS and breast irradiation (breast tangential fields). This study

showed that 27% of patients in the axillary dissection arm had additional positive non-sentinel nodes. There was no difference in regional recurrence and no difference in overall survival and DFS between those who had completion axillary dissection versus those undergoing sentinel node dissection alone. Nodal recurrences were reported in 1% of patients.^{158,159} These results suggest that regional control can be achieved by radiation and systemic therapy, regardless of cancer subtypes.¹⁶⁰

ONGOING TRIALS AND FUTURE PERSPECTIVES

Currently, PMRT is not recommended for patients at low risk for LRR (<10%).¹⁶¹ The St Gallen recommendations indicate PMRT should be considered in cases in which the 10-year LRR rate is 20% or higher.^{162,163} Therefore, the need to quantify the LRR rate is essential, and molecular subtyping could be a key part of this evaluation.

Currently there are several trials examining the need for radiation therapy based on disease subtype.

IDEA Study (Individualized Decisions for Endocrine Therapy Alone)

In this prospective multicenter study, after the confirmation of a stage 1 invasive breast cancer, after BCS, hormone receptor–positive, HER2-negative patients with an Onco-type DX RS ≤ 18 could be included. The enrolled patients receive endocrine therapy alone without radiotherapy.¹⁶⁴

PRECISION Trial (Profiling Early Breast Cancer for Radiotherapy Omission)

This trial is a prospective study performed on unifocal T1 (≤ 2 CM), ER-positive ($\geq 10\%$), PR-positive, HER2-negative, grade 1 or 2, pN0 or pN0(i+) associated with a PAM 50 (Prosigna, Seattle, WA) low-risk score. All the participants undergo omission of radiation therapy and will receive adjuvant endocrine treatment.¹⁶⁵

LUMINA

This prospective single-arm study aims to evaluate the risk of ipsilateral breast cancer recurrence after breast-conserving therapy without radiation. Patients included in the study are 55 years of age; tumors ≤ 2 cm; ductal, tubular, or mucinous disease; ER-positive and PR-positive; HER2-negative; node-negative; with negative margins.¹⁶⁶

SUPREMO Trial

A randomized phase III trial assessing the role of chest wall irradiation in women with intermediate-risk breast cancer following mastectomy. This is a trial from the Medical Research Council/European Organization for Research and Treatment of Cancer not yet recruiting.¹⁶⁷

SUMMARY

Luminal A cancers are associated with a better prognosis and an LRR that occurs more frequently after 5 or 10 years from diagnosis. Although the expression of ER and PR receptors overlaps between luminal A and luminal B cancers, it is critical to identify luminal B breast cancers because this subgroup is associated with a worse prognosis and could benefit from additional local and systemic treatment. HER2-positive cancers have improved outcomes in both local and systemic control with targeted therapies. TNBC remains a clinical challenge, and several clinical trials are ongoing in attempts to identify mechanisms to help improve outcomes for this patient subgroup. The increasing prognostic and predictive information obtained

from both prospective and retrospective studies on molecular subtypes will allow clinicians to customize treatment in terms of surgery, regional and systemic therapy, and follow-up. Implementing knowledge about tumor biology allows clinicians to distinguish high-risk versus favorable, low-risk disease subtypes through several different criteria. Further investigations will be necessary to potentially change guidelines regarding treatments offered for the local-regional control of breast cancer.

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