Luminal B Breast Cancer: Molecular Characterization, Clinical Management, and Future Perspectives
Felipe Ades, Dimitrios Zardavas, Ivana Bozovic-Spasojevic, Lina Pugliano, Debora Fumagalli, Evandro de Azambuja, Giuseppe Viale, Christos Sotiriou, and Martine Piccart

ABSTRACT
Gene expression profiling has reshaped our understanding of breast cancer by defining and characterizing four main intrinsic molecular subtypes: human epidermal growth factor receptor 2–enriched, basal-like, luminal A, and luminal B subtypes. Luminal B breast cancer has been reported to have lower expression of hormone receptors, higher expression of proliferation markers, and higher histologic grade than luminal A. It also exhibits worse prognosis and has a distinct profile of response to hormone therapy and chemotherapy. Although luminal cancers share similarities, the studies conducted in recent years using next-generation sequencing technology show that luminal A and B breast cancers should be perceived as distinct entities, with specific oncogenic drivers, rather than more proliferative varieties of luminal tumors. This review discusses the definition and molecular characterization of luminal B breast cancer and presents the available clinical evidence for chemotherapy and endocrine therapy patterns of response. It also provides an overview of ongoing research on molecularly targeted agents for this disease.

INTRODUCTION
In the early 2000s, high-throughput technology enabled the interrogation of the expression of thousands of genes in a single experiment. This led to the determination that breast cancer comprises at least four molecularly distinct diseases with different features, clinical behaviors, and treatment response profiles. These intrinsic molecular subtypes were defined as: basal-like, human epidermal growth factor receptor 2 (HER2)–enriched, and luminal A and B subtypes. Additional studies separated luminal breast cancers into at least two subgroups: luminal A and luminal B. Luminal A tumors were characterized as having the highest expression of estrogen-related and low expression of proliferation-related genes, whereas luminal B cancers showed lower expression of estrogen receptor (ER) as well as low expression of progesterone receptor (PgR) genes and higher expression of proliferation cluster genes (MKI67) and cell cycle–associated genes (CCNB1 and MYBL2). Luminal B tumors showed increased expression of growth factor receptor genes, with approximately 20% being HER2 positive by mRNA levels and immunohistochemistry (IHC). Table 1 lists proposed surrogate IHC definitions for the classification of luminal B breast cancer.
IntClusts. IntClusts 1, 6, and 9 were enriched for luminal B cancers characterized by 17q23/20q cis-acting aberrations, 8p12 cis-acting aberrations, and 8q cis-acting aberrations/20q amplifications, respectively. IntClust 2, which exhibited a high mortality rate, was enriched for luminal A cancers, a significant pathophysiologic molecular alteration for this subtype.20

Next-generation sequencing (NGS) studies have helped to elucidate the molecular uniqueness of luminal B breast cancer. The Cancer Genome Atlas (TCGA) Network initiative characterized a high number of primary breast cancers using a wide variety of platforms. This analysis provided an abundance of molecular characteristics distinguishing luminal B cancer from the other subtypes. Luminal A and luminal B breast cancers are distinct entities, albeit with overlap. An unsupervised clustering analysis of methylation data showed that the group with a hypermethylated profile was enriched for luminal B cancers. This group had also a low number of MAP3K1 and MAP2K4 mutations. It was shown that luminal B cancers had lower frequency of PIK3CA mutations (29% v 45%) and higher frequency of TP53 mutations (29% v 12%) than luminal A cancers (Fig 1).14 Another gene found to be mutated to a significant degree in both luminal A and B cancers was GATA3, with a frequency of 14% and 15%, respectively. However, different types of mutations were also identified for the two subtypes, with hotspot CA intron 4 deletions associated with luminal A cancer and exon 5 frame shifts associated with luminal B cancer.21 Of note, GATA3 mutations were shown to confer endocrine sensitivity in the neoadjuvant setting and are positively correlated with Ki67 decline on aromatase inhibition \( P = .01 \).22

RUNX1 was identified through genome-wide analysis as a transcription factor involved in mediating ER genomic recruitment and regulating tethering genes.21-24 Loss-of-function mutations affecting this gene, as well as its dimerization partner CBFB, could lead to a phenotype of endocrine resistance. RUNX1 deletion was associated with poorly differentiated breast cancer25; interestingly, pathway representation and analysis by direct reference on graphical models (PARADIGM) –inferred pathway analysis associated RUNX1 loss-of-function mutations with the luminal B subtype, as well as features of endocrine resistance.22

<table>
<thead>
<tr>
<th>Study</th>
<th>ER Status</th>
<th>PgR Status (%)</th>
<th>HER2 Status</th>
<th>Ki67 Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blows et al18*</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>13.25</td>
</tr>
<tr>
<td>Cheang et al11*</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Prat et al16*</td>
<td>Positive</td>
<td>&gt; 20</td>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Goldhirsch et al12*</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Harbeck et al1*</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>20 to 25</td>
</tr>
</tbody>
</table>

**Abbreviations:** ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PgR, progesterone receptor.

ER positive and/or PgR positive.

Fig 1. Percentage of somatic mutations and molecular alterations across different intrinsic subtypes of breast cancer. (A) Reported copy number alteration across different breast cancer intrinsic subtypes. (B) Frequencies of the most commonly mutated cancer-related genes across different breast cancer intrinsic subtypes. HER2, human epidermal growth factor receptor 2.
LUMINAL B DEFINITION IN CLINICAL PRACTICE

Subtype Definition Using Gene Expression Classifiers

Several molecular signatures have been developed in an effort to predict the molecular subtype in clinical practice, but their performance is controversial, and their use in daily practice is limited because of the relatively high cost and technical complexity. One of these molecular signatures, prediction of microarray (PAM) 50, is generated from an expanded intrinsic set of 1,906 genes derived from four microarray studies.\(^2\,^3\,^8\,^26\) After applying several minimization methods, 50 genes involved in proliferation, or related to ER, HER2, myoepithelial, and basal characteristics, were selected. The PAM method was used to generate the algorithm. The original signature has been implemented in a clinical assay (Prosima) based on NanoString (Seattle, WA) technology. Because this technology allows the use of material extracted from formalin-fixed paraffin-embedded samples, the test can be widely applied in the clinical setting. This assay is able to identify the same intrinsic subtypes as the original work,\(^27\) and its diagnostic value has been validated in two studies.\(^28\,^29\)

Surrogate Histologic and IHC Markers

In clinical practice, luminal B cancers are differentiated from luminal A cancers based on proliferation markers.\(^9\,^11\,^30\) ER and HER2 expression show a bimodal distribution with clear cutoff points for assessment.\(^31\) However, proliferation is determined by several genes with a continuous distribution; thus, its status cannot be identified dichotomously. Therefore, establishing a cutoff to determine high and low proliferative tumors in the clinical setting remains a challenge.

Proliferation status has been assessed in clinical practice by different techniques, with tumor grade being the most widely used.\(^30\) Nonetheless, technical issues limit its use as a surrogate marker. In one study of 675 breast cancer samples, the concordance rate for grade classification among three different pathologists was as low as 43%.\(^32\)

Ki67 is a nuclear marker expressed in all cell-cycle phases except G0.\(^33\) It is evaluated by IHC, with results expressed in a quantitative way, meaning the percentage of stained cells. Important intratumor variation occurs, depending on the tumor section analyzed and the pathologist evaluation.\(^34\) In a meta-analysis including 46 studies and 12,155 patients, high Ki67 labeling was correlated with an increased risk of relapse and worse survival. The threshold for Ki67 in these studies varied between 3.5% and 35%, and the criteria to define the threshold were diverse.\(^35\) In a study aimed at defining a Ki67 cutoff, tumors of 357 patients were profiled by PAM50 and compared with Ki67 results; 101 breast cancers were classified as luminal A and 69 as luminal B. The optimal Ki67 level discriminating luminal A from B was 13.25%. This cutoff point was rounded up to 14%, and recommended as a surrogate marker for luminal cancer classification. The high rate of misclassification, occurring in 25% of the cases,\(^31\) is worth noting. The 14% level was endorsed by the 2011 St Gallen Early Breast Conference consensus panel.\(^12\) In 2013, major disagreements emerged among the panels; the majority voted for a cutoff of ≥20%, whereas others recommended a lower, local laboratory-specific cutoff or the use of multigene expression assay.\(^33\)

PgR status has also been used to define luminal B cancer. PgR negativity is a marker of worse prognosis in luminal cancers\(^36\,^37\); in breast tumors assessed as ER positive and having Ki67 > 14%, loss of PgR was identified as an adverse prognostic factor (relapse: hazard ratio [HR], 1.96; 95% CI, 1.44 to 2.68).\(^38\) In another study, luminal B cancers classified as ER positive, HER2 negative, Ki67 > 14%, and PgR < 20% had worse prognosis than tumors with PgR ≥ 20%.\(^6\)

Despite the multiple attempts to establish a clinically useful, IHC-based luminal B surrogate marker, efforts so far have failed to accurately reproduce the PAM50 classification\(^39\) or to define a gold standard, which in large part stems from the controversies about the most reliable way to quantify proliferation.

Prognostic Signatures Within Intrinsic Subtypes

Gene expression signatures are used to provide prognostic information beyond that provided by the clinicopathologic parameters and are particularly useful in the discrimination of luminal breast cancers. Most luminal A tumors are classified at low genomic risk, whereas luminal B cancers are often classified at high genomic risk\(^40\,^42\) (Table 2). Several gene expression profiling (GEP) prognostic signatures have been developed with little overlap between their constituent genes. Despite this, a significant overlap in their performance is observed.\(^40\) In a meta-analysis of publicly available breast cancer gene expression and clinical data, gene coexpression modules related to proliferation, ER, and HER2 were used to evaluate the role of constituent genes. The study identified 524 genes associated with survival; of these, 71% were strongly coexpressed with proliferation, 26% with ER, and 2.2% with HER2,\(^9\) revealing the importance of proliferation genes as a common driving force behind signature performance.

Actually, these multigene prognosticators do not really identify different risk groups within intrinsic subtypes; they rather divide breast tumors according to their proliferation pattern. Because HER2-enriched and basal-like cancers are almost all high proliferative cancers, GEP prognostic signatures add little information in these subtypes. However, in luminal breast cancers, the signatures are able to identify low and high proliferative groups, roughly correlated with the luminal A and luminal B subtypes.\(^9\,^40\,^43\)

Table 2. Correlation Between Intrinsic Subtype Classification by PAM50 and OncotypeDX and MammaPrint Prognostic Signatures

<table>
<thead>
<tr>
<th>Subtype</th>
<th>PAM50 Classification</th>
<th>OncotypeDX Classification</th>
<th>MammaPrint Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>Low</td>
<td>Good</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Poor</td>
<td>34</td>
</tr>
<tr>
<td>Luminal B</td>
<td>Low</td>
<td>Good</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Poor</td>
<td>46</td>
</tr>
<tr>
<td>HER2 positive/ ER negative</td>
<td>Low</td>
<td>Good</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Poor</td>
<td>16</td>
</tr>
<tr>
<td>Basal like</td>
<td>Low</td>
<td>Good</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Poor</td>
<td>7</td>
</tr>
</tbody>
</table>

NOTE. Data adopted.\(^40\)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PAM, prediction of microarray.
A revealing study compared 47 published breast cancer signatures with 1,000 randomly generated signatures composed of the same number of genes. Not only did > 90% of the random signatures predict outcome, but 60% of the random signatures also performed better than the original ones. This strengthens the notion that prognostic signatures do not identify specific mechanisms of cancer aggressiveness. Instead, because a large number of transcripts deregulated in breast cancer correlate with proliferation genes, random signatures are also proliferation related and able to discriminate prognosis.

### CLINICAL BEHAVIOR

Luminal B breast cancer is recognized as having an aggressive clinical behavior, with prognosis similar to that of HER2-enriched and basal-like groups, whereas luminal A breast cancer is identified as having a more favorable clinical outcome. Luminal B cancers show increased relapse rate in the first 5 years after diagnosis, decreasing over time, and a metastatic dissemination time pattern similar to basal-like and HER2-enriched cancers. In a series of 831 untreated node-negative patients, the HR for early metastasis (< 5 years) was 2.86; for late metastasis (> 5 years), it was 0.65 in comparison with luminal A. Moreover, luminal B cancers have a distinctive pattern of site dissemination, with predilection for bone and to a lesser degree for lung. This is in contrast with luminal A breast cancer, for which bone is also the principal metastatic site, although it shows a low frequency of metastasis to other sites.

### LUMINAL B AND BENEFIT OF CHEMOTHERAPY

Most luminal B cancers are classified as having a high OncotypeDX recurrence score (RS). The NSABP (National Surgical Adjuvant Breast and Bowel Project) B-20 study assessed node-negative ER-positive patients randomly assigned to tamoxifen versus tamoxifen plus chemotherapy. Patients with high RS (≥ 31) derived large benefit from chemotherapy (recurrence: HR, 0.26; 95% CI, 0.13 to 0.53), with a mean absolute decrease in distant recurrence rate of 27.6%. In the SWOG (Southwest Oncology Group) 8814 trial, which randomly assigned node-positive ER-positive patients to tamoxifen versus tamoxifen followed by chemotherapy, a chemotherapy benefit was also observed in patients with high RS (≥ 31); disease-free survival (DFS): HR, 0.59; 95% CI, 0.35 to 1.01), whereas no improvement was seen in patients with low RS (< 18). However, patients with high expression of ER did not benefit from addition of chemotherapy, irrespective of OncotypeDX results. RS was also positively correlated with the likelihood of pathologic complete response (pCR; P = .005), indicating greater sensitivity to neoadjuvant chemotherapy.

In neoadjuvant studies, luminal tumors exhibit lower pCR rates than HER2-enriched and triple-negative breast cancers. When comparing the luminal tumors, results are conflicting: higher pCR rates in luminal B than in luminal A cancers were not homogeneously observed. Nonetheless, neoadjuvant chemotherapy seems effective in reducing tumor volume in luminal B cancers (Table 3). Tumors with high prognostic signature values, according to several GEP classifiers, are associated with a higher probability of pCR.

The latest update from the Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) was unable to ascertain chemosensitivity in luminal tumors or benefit from specific chemotherapies, because of limited data to define luminal tumors. Poor differentiation, defined by local pathologists, was not predictive of chemosensitivity in patients with ER-positive disease. The St Gallen consensus panel considered that both anthracyclines and taxanes should be included in the chemotherapy regimen for luminal B breast cancer.

Three prospective randomized trials—MINDBACT (Microarray in Node-Negative Disease May Avoid Chemotherapy; NCT00433589), TAILORx (Trial Assigning Individualized Options for Treatment; NCT00310180), and RxPONDER (Treatment for Positive Node,
Endocrine Responsive Breast Cancer; NCT01272037)—are testing the usefulness of gene signatures in predicting benefit from adjuvant chemotherapy in patients with ER-positive breast cancer.\(^{61-63}\) Results are expected between 2015 and 2017.

### LUMINAL B AND BENEFIT OF ENDOCRINE THERAPY

The supporting evidence for the relatively lower benefit of endocrine therapy in the luminal B subtype comes from few studies, which, as was the case for chemotherapy, classify luminal tumors through surrogate markers. An EBCTCG meta-analysis evaluating patients enrolled onto endocrine therapy trials showed small benefits with tamoxifen in patients with low ER levels.\(^{64}\) In the BIG (Breast International Group) 1-98 trial, which assigned 8,010 patients to four treatment arms comparing different sequential administrations of letrozole and tamoxifen, patients with lower ER levels had worse DFS than those with high ER levels.\(^{65}\) Patients receiving tamoxifen or no adjuvant systemic treatment with an ER-positive/PgR-negative phenotype had better outcomes than their ER-negative/PgR-negative counterparts but worse outcomes than those with the ER-positive/PgR-positive phenotype.\(^{66}\) Nevertheless, this observation was not confirmed in either in the NSABP B-14 study\(^ {66}\) or in the IES (Inter-group Exemestane Study) comparing exemestane versus tamoxifen.\(^ {67}\)

The ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial evaluated 5 years of anastrozole, tamoxifen, or their combination in postmenopausal women; high OncotypeDX RS (≥31) was associated with disease relapse when compared with low RS (<18).\(^ {68}\) A 78-gene signature\(^ {69}\) showed results similar to RS in evaluating the treatment prognosis in tamoxifen-naïve patients with metastatic breast cancer.\(^ {70}\) However, in the BCIRG (Breast Cancer International Research Group) study, comparing the combination of docetaxel or fluorouracil with doxorubicin and cyclophosphamide, both the luminal A and luminal B subtypes showed significant responses to tamoxifen (luminal B: HR, 0.44; 95% CI, 0.32 to 0.61; P < .001; luminal A: HR, 0.15; 95% CI, 0.07 to 0.33; P < .001).\(^ {71}\)

In contrast to the previously discussed markers that provide only prognostic information, dynamic changes in the expression of Ki67 were able to predict outcome in luminal breast cancer treated with endocrine therapy. In the IMPACT (Immediate Preoperative Anastrozole, Tamoxifen, or Combined With Tamoxifen) trial, patients with higher Ki67 expression after 2 weeks of endocrine therapy had lower recurrence-free survival (P = .004).\(^ {72}\) In the P024 neoadjuvant endocrine therapy trial, baseline Ki67 was associated with higher risk of relapse (HR, 1.3; 95% CI, 1.05 to 1.50; P = .01).\(^ {73}\) Luminal B and A tumors showed similar proportional falls in Ki67 in a neoadjuvant trial with anastrozole; however, patients with luminal B cancers had a poorer outcome, explained by the higher baseline levels of Ki67.\(^ {74}\)

Using clinicopathologic (tumor size, node status, treatment response) and IHC information (ER status, Ki67, histologic grade), a preoperative endocrine prognostic index (PEPI) was developed from a cohort of patients in the P024 trial. This score was independently validated in the IMPACT trial, being able to correctly predict relapse-free survival.\(^ {75}\) The PEPI score was also evaluated in the ACOSOG (American College of Surgeons Oncology Group) Z1031 trial checking for the effect of neoadjuvant anastrozole, letrozole, and exemestane in postmenopausal patients. Luminal B cancers had higher Ki67 at baseline (P < .001), post–aromatase inhibitor treatment (P = .001), and greater change in Ki67 (P < .001). No clinically relevant response differences were found between treatment results among luminal tumors (HR, 1.67; 95% CI, 0.96 to 2.9; P = .07), nor in the rate of breast-conserving surgery, suggesting similar clinical efficacy (HR, 0.75; 95% CI, 0.29 to 1.93; P = .54) despite the better PEPI score among the luminal A group (27.1% vs 10.7%; P = .004).\(^ {76}\) In conclusion, both luminal A and B tumors derive benefit from endocrine treatment, although the magnitude of benefit is larger in luminal A.

In the ATAC trial, ER-positive/PgR-negative patients obtained larger relative benefit from anastrozole than from tamoxifen (HR, 0.42; 95% CI, 0.31 to 0.61), whereas ER-positive/PgR-positive patients showed no differences (HR, 0.84; 95% CI, 0.69 to 1.02).\(^ {77}\) The relative reduction of risk was similar for anastrozole and tamoxifen among patients with different OncotypeDX RS values.\(^ {68}\) In BIG 1-98, high Ki67 predicted greater efficacy of letrozole over tamoxifen (HR, 0.53; 95% CI, 0.39 to 0.72), whereas low Ki67 indicated no difference (HR, 0.81; 95% CI, 0.56 to 1.15; P = .09).\(^ {78}\) Nevertheless, using the surrogate luminal B definition proposed by Cheang et al,\(^ {11}\) no difference was seen between tamoxifen and letrozole.\(^ {78}\)

In a neoadjuvant study randomly assigning postmenopausal ER-positive patients to letrozole or tamoxifen, letrozole was more effective in tumors overexpressing epidermal growth factor receptor or HER2 (clinical response, 88% vs 21%; P < .001).\(^ {79}\) In IMPACT, the response rate for tamoxifen was 22%, whereas for anastrozole, it was 58% in patients presenting HER2 amplification or overexpression (HR, 4.9; 95% CI, 0.53 to 63.22; P = .18).\(^ {80}\) The same trend was observed in the BIG 1-98 trial; patients receiving letrozole had better DFS compared with those receiving sequential administrations of tamoxifen and letrozole or tamoxifen alone (P = .58 and .87, respectively).\(^ {78}\) In a meta-analysis in patients with advanced ER-positive breast cancer, HER2-positive overexpression was identified as increasing the risk of disease recurrence (relative risk [RR], 1.42; 95% CI, 1.32 to 1.52; P < .001), independently from the type of hormone treatment (tamoxifen: RR, 1.33; 95% CI, 1.20 to 1.48; P < .001; other agents: RR, 1.49; 95% CI, 1.36 to 1.64), suggesting no additional benefit from aromatase inhibitors.\(^ {81}\)

Although some studies reported better outcomes with aromatase inhibitors over tamoxifen, these results were not homogeneously observed. These divergences do not allow the drawing of definitive conclusions about the best endocrine agent in patients with luminal B cancer; both choices are acceptable.

### DRUG DEVELOPMENT IN LUMINAL B BREAST CANCER

Many of the mutated genes found in breast cancer occur at a low frequency, rendering their therapeutic exploitation difficult. This requests extensive molecular screening efforts to identify these patients.\(^ {82}\) In addition, functional characterization of these molecular events is needed before they are pursued as therapeutic targets. Findings from high-dimension, clinically and molecularly annotated breast cancer data sets and functional genomic preclinical studies can lead to the development of solid hypotheses on predictive biomarkers, which can be tested prospectively through genotype-driven clinical trials.\(^ {83}\) Figure 2 shows an overview of the signaling pathways under blockade with targeted compounds in luminal breast cancers.
**Phosphatidylinositol-3-Kinase/AKT/Mammalian Target of Rapamycin Signaling Pathway**

In luminal breast cancer, phosphatidylinositol-3-kinase (PI3K) activation is implicated in de novo and acquired endocrine resistance.

Despite lower frequency of PIK3CA mutations, luminal B cancers have higher PI3K activation than luminal A. PIK3CA mutations do not seem to be associated with PI3K pathway activation in early-stage ER-positive breast cancer; a PIK3CA mutation gene signature was identified as a positive prognosticator in this setting.

The most advanced compounds in clinical development are rapamycin analogs called mTOR inhibitors. The combination of everolimus and letrozole has been tested in the metastatic setting. This gene signature indicates a phenotype of low mammalian target of rapamycin (mTOR) C1 signaling, suggesting that PIK3CA mutations might act as weak PI3K pathway activators.

The most advanced compounds in clinical development are rapamycin analogs called mTOR inhibitors. The combination of everolimus and letrozole has been tested in the metastatic setting. This gene signature indicates a phenotype of low mammalian target of rapamycin (mTOR) C1 signaling, suggesting that PIK3CA mutations might act as weak PI3K pathway activators.

The most advanced compounds in clinical development are rapamycin analogs called mTOR inhibitors. The combination of everolimus and letrozole has been tested in the metastatic setting. This gene signature indicates a phenotype of low mammalian target of rapamycin (mTOR) C1 signaling, suggesting that PIK3CA mutations might act as weak PI3K pathway activators.

**FGF Signaling Pathway**

FGFR1 amplification was identified in 16% to 27% of luminal breast cancers as a mechanism of endocrine resistance and as being associated with negative prognosis. The administration of E-3810, a nonselective FGF-blocking agent, to 10 patients with metastatic breast cancer resulted in seven partial responses. A phase II clinical trial assessed dovitinib, another nonselective inhibitor, in heavily pretreated patients with FGFR1-amplified luminal metastatic breast cancer; it produced three partial responses and resulted in stable disease in nine patients. Other studies are assessing the addition of...
Fibroblast Growth Factor (FGF)-blocking agents to endocrine therapy (Appendix Table A1, online only).

**Insulin-Like Growth Factor Signaling Pathway**

The insulin-like growth factor (IGF) signaling pathway causes activation of the PI3K/AKT/mTOR and Ras/Raf/MEK/ERK pathways. In luminal cancers, there is IGF-ER crosstalk; estrogen up-regulates IGF-1 and IGF-1 receptor (IGF-1R), and IGF-1 signaling boosts the proliferative effect of estrogen and is implicated in endocrine resistance. Tamoxifen resistance has been suppressed with an anti-IGF-1R monoclonal antibody, and IGF blocking has synergistic effect with endocrine treatment in luminal breast cancers.

A randomized phase II study testing ganitumab or placebo with exemestane or fulvestrant in postmenopausal women with metastatic hormone receptor–positive disease was unable to demonstrate benefit from this strategy (PFS: HR, 1.17; 95% CI, 0.91 to 1.50; P = .44). However, a promising approach is the combination of mTOR and IGF inhibitors, acting synergistically through the downregulation of IGF-1R–mediated AKT activation induced by mTOR inhibition (Appendix Table A1, online only).

**Cyclin-Dependent Kinases**

Cycle-dependent kinase (CDK) deregulation confers a highly proliferative cellular phenotype and is also linked to endocrine resistance. High expression of cyclin D1 and E1 is associated with poor clinical outcome in tamoxifen-treated women. Cyclin E2 expression is characteristic of luminal B cancer and correlated with shorter distant metastasis–free survival.120 CCND1 (encoding gene for cyclin D1) amplification is found at higher frequencies in luminal B compared with luminal A cancer (58% vs 29%). Furthermore, the METABRIC study identified a bad prognosis molecular subtype of luminal breast cancer, where CCND1 is located.

PD-0332991, a highly specific CDK4 and CDK6 inhibitor, was assessed in a phase II study randomly assigning patients with hormone receptor–positive disease to the combination of PD-0332991 and letrozole versus letrozole alone. The combination caused significant prolongation of PFS (median PFS, 18.2 vs 5.7 months; HR, 0.35; 95% CI, 0.17 to 0.72; P = .006). A phase III trial is ongoing. The patient population in this trial consisted of two groups: a cohort of 66 patients selected according to ER and HER2 status, and a cohort of 99 patients selected according to CCND1 amplification and/or loss of p16. The benefit of the addition of PD-0332991 to letrozole occurred mainly in the biomarker-negative population, in contrast with initial assumptions (HR, 0.37 and 0.19, respectively). This is partially explained by the excessively negative prognosis associated with CCND1 amplification. However, it indicates that patients with both luminal A and B cancers will probably benefit from this strategy.

**ADDITIONAL THERAPEUTIC TARGETS**

TP53 is one of the most commonly mutated breast cancer genes. Luminal B cancers exhibit higher mutation rates than luminal A cancers (32% vs 12%). Higher rates of MDM2 gene amplification, a TP53 antagonist, have been observed in luminal B (32%) than in luminal A cancers (14%). Thus, luminal B cancers exhibit increased rates of TP53 pathway loss of function, which has been identified as a mediator of endocrine resistance. Currently, a new class of targeted agents functioning as MDM2 antagonists is under clinical assessment for patients with advanced solid tumors (NCT01462175, NCT01877382, and NCT01664000), aiming to restore wild-type TP53 function.

The mixed-lineage leukemia gene MLL3 is also a frequently mutated gene in luminal cancers. MLL is a family of histone trimethyltransferases that can regulate gene transcription, including regulation of ESR1 expression. Currently, there is a group of targeted agents under clinical development blocking epigenetic regulation, with histone deacetylase (HDAC) inhibitors being the most advanced class of compounds. At the preclinical level, HDAC inhibitors reactivate gene expression of ESR1 and PGR, whereas when they were combined with DNA methyltransferase inhibitors, enhanced ESR1 gene expression was induced. At the clinical level, the results of a randomized phase II trial assessing exemestane with or without entinostat (HDAC inhibitor) in patients with ER-positive metastatic breast cancer show that the combination significantly prolonged median PFS (4.3 vs 2.3 months; HR, 0.73; P = .055) and OS (28.1 vs 19.8 months; HR, 0.59; P = .036).

**DISCUSSION**

GEP has changed the way breast cancer is perceived. Rather than an aggressive variant of hormone receptor–positive tumors, the luminal B subtype is a distinct entity. However, the application of GEP of luminal B disease in clinical practice has been limited, largely because this classification coincides with traditional characterization by IHC.

So far, the molecular definition of luminal B disease has also been of limited use in tailoring breast cancer treatment. Despite the lower sensitivity of luminal B cancers to endocrine treatment, benefit is still derived from these drugs, and the simple identification of hormone receptor positivity by IHC is enough to justify its use. Therefore, the main clinical challenge relates to the selection of patients who will benefit from adjuvant chemotherapy. Proliferation was identified as a major predictive marker for cytotoxic chemotherapy, because it is also a major discriminant between luminal B and luminal A subtypes. However, with proliferation being a continuous process, defining low and high cutoffs poses an important research question. To date, no chemotherapy predictive biomarker has been validated. However, it is hoped that three ongoing prospective randomized trials testing prognostic gene signatures—MINDACT, TAILORx, and RxPONDER—will shed light on this issue.

Finally, after little more than a decade since the discovery of breast cancer intrinsic subtypes, the characterization of important pathways and molecular alterations is leading to a new portfolio of targeted agents for luminal tumors. Genotype-driven trials are already a reality in breast cancer research and will continue to contribute to the improvement of treatments for patients with luminal B breast cancer.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Although all authors completed the disclosure declaration, the following author(s) and/or an author’s immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a “U” are
those for which no compensation was received; those relationships marked with a “C” were compensated. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None Consultant or Advisory Role: Martine Piccart, sanofi-aventis (C), Amgen (C), Roche (C), GlaxoSmithKline (C), PharmaMar (C) Stock Ownership: None Honoria: Evandro de Azambuja, Roche, GlaxoSmithKline; Martine Piccart, sanofi-aventis, Amgen, Roche, GlaxoSmithKline, PharmaMar Research Funding: None Expert Testimony: None Patents, Royalties, and Licenses: Christos Sotiriou, Gene Expression Grade Index (GGI)

Other Remuneration: Evandro de Azambuja, Roche, GlaxoSmithKline

REFERENCES

28. Grant M, Filipits M, Dubsky P: Predicting risk for late metastasis: The PAM50risk of recurrence (ROR) score after 5 years of endocrine therapy in postmenopausal women with HR+ early breast cancer—A study on 1,478 patients from the ABCSG-8 trial. Presented at the IMPAKT Breast Cancer Conference, Brussels, Belgium, May 2-4, 2013

AUTHOR CONTRIBUTIONS

Conception and design: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors
Luminal B Breast Cancer


63. Gonzalez-Angulo AM, Barlow WE, Gralow J, et al: SWOG S1007: A phase III, randomized clinical trial of standard adjuvant endocrine therapy with or without chemotherapy in patients with one to three positive nodes, hormone receptor (HR)-positive, and HER2-negative breast cancer with recurrence score (RS) of 25 or less. J Clin Oncol 29:88s, 2011 (suppl; abstr TPS104)


marker for antiestrogen resistant human breast cancer cell lines but is not a major growth regulator. Growth Horm IGF Res 16:224-239, 2006


105. Finke R, von der Maase H, et al: Randomized, double-blind, placebo-controlled, phase 2 study of AMG 479 with exemestane (E) or fulvestrant (F) in postmenopausal women with hormone-receptor-positive (HR+) metastatic (M) or locally advanced (LA) breast cancer (BC). Cancer Res 70, 2011 (abstr S1-4)


# Appendix

## Table A1. Ongoing Clinical Trials With Targeted Agents in Luminal Breast Cancers

<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>No. of Patients</th>
<th>Subtype</th>
<th>Setting</th>
<th>Treatment</th>
<th>Primary End Point</th>
<th>Secondary End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pan-PI3K inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKM120</td>
<td>NCT01339442</td>
<td>I</td>
<td>22</td>
<td>ER positive</td>
<td>Metastatic, HT pretreatment</td>
<td>BKM120 plus fulvestrant</td>
<td>MTD, safety</td>
</tr>
<tr>
<td></td>
<td>NCT01610284 (BELLE-2)</td>
<td>III</td>
<td>1,060</td>
<td>HR positive</td>
<td>Metastatic, AI pretreatment</td>
<td>Fulvestrant plus BKM120 or placebo</td>
<td>PFS</td>
</tr>
<tr>
<td></td>
<td>NCT016230060 (BELLE-3)</td>
<td>III</td>
<td>615</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, mTOR inhibitor pretreatment</td>
<td>Fulvestrant plus BKM120 or placebo</td>
<td>PFS</td>
</tr>
<tr>
<td>GDC-0941</td>
<td>NCT01437566</td>
<td>II</td>
<td>270</td>
<td>ER positive, HER2 negative, PIK3CA mutation</td>
<td>Metastatic, AI pretreatment</td>
<td>GDC-0941 or GDC-0980 plus fulvestrant v fulvestrant</td>
<td>PFS, AEs</td>
</tr>
<tr>
<td></td>
<td>NCT01740336</td>
<td>II</td>
<td>180</td>
<td>ER positive, HER2 negative</td>
<td>Metastatic</td>
<td>Paclitaxel plus GDC-0941 or placebo</td>
<td>PFS</td>
</tr>
<tr>
<td><strong>Dual PI3K/mTOR inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEZ235</td>
<td>NCT01248494</td>
<td>I</td>
<td>72</td>
<td>HR positive</td>
<td>Metastatic</td>
<td>BEZ235S or BKM120 plus letrozole</td>
<td>MTD</td>
</tr>
<tr>
<td>XL765</td>
<td>NCT01082068</td>
<td>I/II</td>
<td>72</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, AI pretreatment</td>
<td>XL765 or XL147 plus letrozole</td>
<td>MTD, safety, PFS at 3 months</td>
</tr>
<tr>
<td>GDC-0980</td>
<td>NCT01437566</td>
<td>II</td>
<td>270</td>
<td>ER positive, HER2 negative, PIK3CA mutation</td>
<td>Metastatic, AI pretreatment</td>
<td>GDC-0941 or GDC-0980 plus fulvestrant v fulvestrant</td>
<td>PFS, AEs</td>
</tr>
<tr>
<td><strong>Isoform-selective PI3K inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BYL719</td>
<td>NCT01791478</td>
<td>I</td>
<td>30</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, HT pretreatment</td>
<td>BYL719 plus letrozole</td>
<td>MTD</td>
</tr>
<tr>
<td><strong>AKT inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK2206</td>
<td>NCT01344031</td>
<td>I</td>
<td>54</td>
<td>ER positive</td>
<td>Metastatic, no progression with AIs or fulvestrant</td>
<td>MK2206 plus anastrozole, fulvestrant, or anastrozole plus fulvestrant</td>
<td>MTD, RPTD</td>
</tr>
<tr>
<td></td>
<td>NCT01776008</td>
<td>II</td>
<td>87</td>
<td>ER positive, HER2 negative, PIK3CA mutation</td>
<td>Neoadjuvant</td>
<td>MK2206 plus anastrozole or anastrozole and goserelin</td>
<td>pCR</td>
</tr>
<tr>
<td><strong>mTORC1/2 inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZD2014</td>
<td>NCT01597388</td>
<td>I</td>
<td>30</td>
<td>ER positive</td>
<td>Metastatic</td>
<td>AZD2014 plus fulvestrant</td>
<td>Safety</td>
</tr>
<tr>
<td><strong>FGF inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dovitinib</td>
<td>NCT01484041</td>
<td>I/II</td>
<td>36</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic</td>
<td>Dovitinib plus Al</td>
<td>CBR</td>
</tr>
<tr>
<td></td>
<td>NCT01528345</td>
<td>II</td>
<td>150</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, HT pretreatment</td>
<td>Fulvestrant plus dovitinib or placebo</td>
<td>PFS</td>
</tr>
<tr>
<td>AZD4547</td>
<td>NCT01202591 (GLOW)</td>
<td>I/II</td>
<td>120</td>
<td>ER positive, FGFR1 polysomy or gene amplification</td>
<td>Metastatic, HT pretreatment</td>
<td>Fulvestrant plus AZD4547 or placebo</td>
<td>Safety, PFS</td>
</tr>
<tr>
<td></td>
<td>NCT01791985 (RADICAL)</td>
<td>I/II</td>
<td>99</td>
<td>ER positive</td>
<td>Metastatic, HT pretreatment</td>
<td>AZD4547 plus anastrozole or letrozole v exemestane</td>
<td>Safety, tumor change at 12 weeks</td>
</tr>
</tbody>
</table>

(continued on following page)
<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>No. of Patients</th>
<th>Subtype</th>
<th>Setting</th>
<th>Treatment</th>
<th>Primary End Point</th>
<th>Secondary End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalotuzumab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01605396 (MK-8669-064 AM3)</td>
<td>II 84</td>
<td>ER positive, Ki67 &gt;15%</td>
<td>Metastatic</td>
<td>Dalotuzumab, ridaforolimus, and exemestane v ridaforolimus plus exemestane</td>
<td>PFS</td>
<td>ORR, OS</td>
<td></td>
</tr>
<tr>
<td>NCT00903006</td>
<td>I/II 40</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic</td>
<td>Fulvestrant v fulvestrant, dalotuzumab, and dasatinib</td>
<td>MTD, TTDP</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>BMS-754807</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01225172</td>
<td>II 100</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, AI pretreatment</td>
<td>BMS-754807 plus letrozole v BMS-754807</td>
<td>PFS at 24 weeks</td>
<td>ORR, DOR, safety, TFR, TR</td>
<td></td>
</tr>
<tr>
<td>MEDI-573</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01448159</td>
<td>I 193</td>
<td>HR positive, HER2 negative</td>
<td>Metastatic, HT pretreatment</td>
<td>MEDI-573 plus AI v AI</td>
<td>Safety, PFS</td>
<td>ORR, TTR, DOR, TTP, change in tumor size, OS, PKs, PDs, immunogenicity</td>
<td></td>
</tr>
<tr>
<td>PD0332991</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT01709370</td>
<td>II 45</td>
<td>ER positive, HER2 negative</td>
<td>Neoadjuvant</td>
<td>PD0332991 plus letrozole</td>
<td>ORR</td>
<td>AEs, PRR</td>
<td></td>
</tr>
<tr>
<td>NCT01723774</td>
<td>II 29</td>
<td>ER positive, HER2 negative</td>
<td>Neoadjuvant</td>
<td>PD0332991 plus anastrozole</td>
<td>pCR</td>
<td>Safety, CRR, RRR, Ki67 at 2 weeks, PKs</td>
<td></td>
</tr>
<tr>
<td>NCT01740427</td>
<td>II/III 450</td>
<td>ER positive, HER2 negative</td>
<td>Metastatic, first line</td>
<td>PD0332991 plus letrozole v letrozole</td>
<td>PFS</td>
<td>OS, ORR, DOR, DC, QTc interval, Cmin, QOL, TR</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AE, adverse event; BELLE, Buparlisib Breast Cancer Clinical Evaluation; CBR, clinical benefit rate; Cmin, minimum observed plasma trough concentration; CR, complete response; CRR, clinical response rate; DOR, duration of response; ECOG PS, Eastern Cooperative Oncology Group performance status; ER, estrogen receptor; FGF, fibroblast growth factor; HER2, human epidermal growth factor receptor 2; HT, hormonal therapy; IGF, insulin growth factor; MTD, maximum-tolerated dose; mTOR, mammalian target of rapamycin; NR, not reported; OR, objective response; ORR, objective response rate; OS, overall survival; pCR, pathologic complete response; PD, pharmacodynamic; PI3K, phosphatidylinositol-3-kinase; PK, pharmacokinetic; PR, partial response; PRR, pathologic response rate; QOL, quality of life; RPTD, recommended phase II dose; RRR, radiologic response rate; SD, stable disease; TFR, treatment failure rate; TR, translational research; TTDP, time to disease progression; TTP, time to progression; TTR, time to response.