Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways

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Introduction

Breast cancer is a complex disease including clinical, morphological and molecular very distinct entities. This heterogeneity cannot be explained only by clinical parameters such as tumor size, lymph node involvement, histological grade, age; or by biomarkers like estrogen receptor (ER), progesterone receptor (PGR) and epidermal growth factor receptor 2 (HER2) routinely used in the diagnosis and treatment of patients. During the last decade research has focused in depth on the molecular biology of this disease. Technological breakthroughs and in particular high throughput approaches, have allowed researchers to inquire into the nature of breast cancer revealing that this disease requires the interconnection of several signaling pathways and that both the cellular microenvironment, and the innate characteristics of the patient influence disease pathophysiology, outcome and treatment response. These findings have led us to understand, that this is not just one disease, but many, and that each patient entails a particular case where personalized medicine could play a crucial role.

The therapeutic advances made to date have been achieved by performing large randomized clinical trials. The problem is that these trials were designed to determine the best therapeutic approach for the median population, not for a specific individual. Furthermore, we have learned through trial and error that new targeted therapies have to be developed in targeted populations, selected on the basis of a given biomarker. Consequently, we know...
now that trastuzumab benefits patients with breast and gastric cancer that have the HER2 amplification,1–3 that cetuximab does not benefit K-ras mutated colon cancer patients4 and that erlotinib and gefitinib benefit by large patients diagnosed of lung cancer with EGFR specific mutations.5,6 Clinicians are getting closer to offering personalized medicine, but there are still many unresolved questions and disadvantages. There is a pressing need to identify and validate new molecular markers, especially in those entities lacking clear therapeutic targets. Also, even if a tumor presents a specific druggable oncogenic dependence, tumor cells often display an unexpected resistance that allows them to escape death. The good news is that the molecular studies that have been developed over the past decade have opened a broad field in cancer research that allows basic and translational researchers to look for new potential therapeutic targets.

The following review describes the main advances in the molecular biology over the last decade, focusing on breast cancer. First, there are the new classifications arising from the application of powerful, comprehensive and useful technologies that are still under development. Then, there are the major advances in the knowledge of some of the major signaling pathways in breast cancer, with a special emphasis placed on those that have led to the development of new drugs or the start of clinical trials.

Gene expression profiles

Gene expression microarrays have allowed researchers to carry out simultaneous expression analyses of thousands of genes in a single experiment in order to create the molecular profile of a tumor.7 The information provided by the quantitative assessment of multiple genes is more precise for biological characterization than that offered by the reduced tumor histopathological studies being carried out for diagnosis. In 2000, Perou and colleagues published the first paper classifying breast cancer into intrinsic subtypes based on gene expression profile.7 These data, along with the numerous subsequent contributions of different authors have changed the way researchers understand, classify and study breast cancer. They have led scientists and clinicians to reconsider the way to diagnose and treat patients, and ultimately, how to search for new therapeutic alternatives.

Breast cancer remains the most common cancer diagnosed in women in Europe and the USA. Screening programs, education and improved adjuvant treatment have decreased mortality from this disease. Still, about 20–30% of patients develop metastatic disease that still remains incurable, with a median survival between 2 and 4 years depending on the subtype. As noted above, Perou’s group was the first to provide a molecular classification for breast cancer. Using a cDNA microarray of 38 breast cancer cases, the group defined a list of ‘intrinsic’ genes. The hierarchical cluster analysis revealed four molecular subtypes: luminal, HER2, basal-like and normal breast. The subsequent expansion of this work in a larger cohort of patients showed that the luminal subgroup could be divided into at least two groups (luminal A and B), and that different molecular subtypes were associated with different prognoses (Fig. 1). This new classification validated by independent groups,9 was based on an unsupervised analysis grouping tumors according to their biological characteristics regardless of their clinical or prognostic variables.

Other gene expression based platforms have been developed, but their analysis was supervised, meaning that the prognosis of the cases was known beforehand. The most widely used and best-known analyses are Oncotype DX10 and MammaPrint.16 Both platforms are a clinically useful tool for discerning which patients with breast carcinoma will benefit from hormonal or cytotoxic therapy. Although clinical trials are underway to validate these signatures, MammaPrint16 is already FDA approved. There are other published gene expression signatures that are less known, such as the wound-response or healing model,11 the two-gene expression ratio (HOXB13:IL17BR),12 the Rotterdam 76 gene signature,13,14 and the genomic grade index (also known as MapQuant Dx).15,17 The little or virtually no concordance among the selected genes on each platform led to a comparative study of the five most popular platforms3: MammaPrint, Oncotype DX, wound-response model, the two-genes ratio and the intrinsic subtype model. The study showed that all platforms except two-gene ratio model, predicted progression-free survival (PFS) and overall survival (OS) showing a correlation among the models in terms of risk classification. The main drawback of these platforms is their high cost and the need for fresh-frozen samples in some of them. In addition, the Oncotype DX platform has the added restriction of classifying a percentage of tumors into a subgroup of intermediate

![Image](https://example.com/image1.png)

**Fig. 1.** (A) Features of molecular subtypes of breast cancer. (B) Kaplan–Meier curves of disease-free survival and overall survival based on UNC337 database. Dark blue, luminal A; light blue, luminal B; red, basal-like; pink, HER2-enriched; yellow, Claudin-low.26 (C) Distribution of ER and HER2 in the different subtypes of breast cancer based on mRNA expression (Source: adapted from 91).
risk. This leaves oncologists eagerly awaiting the results of the TAILORx trial in order to decide whether hormone-based treatment is enough in this subgroup of patients.

Of all the platforms mentioned, the intrinsic subtype classification provides the most valuable biological information on breast cancer. This classification groups the tumors into five molecular subtypes that also correlate to prognosis. Recently, new intrinsic subtypes have been added\(^\text{18}\) which make us think that, far from being a closed classification, it is still a model in development that requires improvement and standardization before advancing to clinical practice.

**Luminal A**

The luminal A breast cancer is the most common subtype, representing 50–60% of the total. It is characterized by the expression of genes activated by the ER transcription factor that are typically expressed in the luminal epithelium lining the mammary ducts. It also presents a low expression of genes related to cell proliferation.\(^\text{1,19}\) Based on their molecular profile, all cases of lobular carcinoma in situ are luminal A tumors, as are most of the infiltrating lobular carcinomas. The luminal A immunohistochemistry (IHC) profile is characterized by the expression of ER, PGR, Bcl-2 and cytokeratin CK8/18, an absence of HER2 expression, a low rate of proliferation measured by Ki67 and a low histological grade (Fig. 1). Moreover, the GATA3 marker expresses its highest level in the luminal A subgroup. Patients with this subtype of cancer have a good prognosis; the relapse rate is 27.8% being significantly lower than that for other subtypes.\(^\text{20}\) In addition, survival from the time of relapse is also longer (median 2.2 years). They have a distinct pattern of recurrence with a higher incidence of bone metastases (18.7%) and with respect to other localizations such as central nervous system, liver and lung which represent less than 10%. The treatment of this subgroup of breast cancer is mainly based on third-generation hormonal aromatase inhibitors (AI) in postmenopausal patients, selective estrogen receptor modulators (SERMs) like tamoxifen and pure selective regulators of ER like fulvestrant.\(^\text{21}\)

**Luminal B**

Tumors with the luminal B molecular profile make up between 10% and 20% of all breast cancers. Compared to the luminal A, they have a more aggressive phenotype, higher histological grade and proliferative index and worse prognosis. The pattern of distant relapse also differs, and although the bone is still the most common site of recurrence (30%), this subtype has a higher recurrence rate in sites such as the liver (13.8%). Additionally, the survival from time of relapse is lower (1.6 years).\(^\text{20}\) Luminal A and B both express ER, but, since luminal B’s prognosis is very different, a strong effort to find biomarkers that distinguish between these two subtypes has been made.

The main biological difference between the two subtypes is an increased expression of proliferation genes, such as MKI67 and cyclin B1 in the luminal B subtype which also often expresses EGFR and HER2 (Fig. 1). Oncotype DX\(^\text{\textsuperscript{TM}}\), MammaPrint\(^\text{\textsuperscript{TM}}\) and MapQuantDx platforms classify luminal B tumors in the ER-positive subgroup with poor prognosis.\(^\text{16,17}\) which is expected, since the Recurrence Score is based mainly on proliferation genes. From the immunohistochemical point of view, there have been attempts to differentiate between luminal A and B using the protein expression of Ki67 as a possible marker.\(^\text{22}\) The luminal A subtype has been defined as ER+/HER2− and low Ki67, while the luminal B subtype has tumors with ER+/HER2− and high Ki67 or ER+/HER2+. It is noteworthy that this definition does not include all luminal B subtype tumors (up to 6% of the luminal B tumors are clinically ER−/HER2−). Moreover, the technique used to determine Ki67 (cut-off point to distinguish luminal A and B set at 13.25%)\(^\text{22}\) has not been standardized which adds a variability factor in the assessment of this marker. However, considering that this marker is the most widely used to measure cell proliferation, efforts are being made to reach a consensus on how to evaluate it. In fact, an international consortium has recently published a set of recommendations for Ki67 assessment in breast cancer.\(^\text{23}\)

Luminal B tumors have a worse prognosis than do luminal A tumors despite treatment with tamoxifen\(^\text{\textsuperscript{24}}\) and AI, but they respond better to neoadjuvant chemotherapy achieving pathological complete response (pCR) in 17% of the luminal B tumors (7% in luminal A), however, this is clearly lower than for the HER2+ and basal-like tumors with values of 36% and 43%, respectively.\(^\text{24}\) For these reasons, treatment of this subtype of breast cancer is currently challenging. Many questions about what mechanisms lead to their survival, proliferation and metastatization remain unanswered. Numerous clinical trials are testing inhibitory molecules of the PI3K/AKT/mTOR pathway at different levels, focusing on the treatment of luminal B tumors.\(^\text{25}\)

**HER2 positive**

Fifteen to twenty percent of all breast cancers correspond to this molecular subtype. They are characterized by a high expression of the HER2 gene and other genes associated with the HER2 pathway and/or HER2 amplicon located in the 17q12 chromosome. These cancers exhibit an over-expression of genes related to cellular proliferation. Although this subtype does not express genes of the basal-like cluster, it may show a low expression of characteristic luminal genes. Morphologically, these tumors are highly proliferative, 75% have a high histological grade and more than 40% have p53 mutations. The IHC profile ER−/HER2+ does not correspond perfectly with the intrinsic subtype, since only 70% of HER2+ tumors by microarray have the protein over-expressed by IHC. Conversely, not all tumors with HER2 amplification or over-expression are included in the cluster of HER2 in the analysis of microarrays.\(^\text{26,27}\) In addition, a significant number of tumors clinically ER+/HER2+ are classified molecularly as luminal B (Fig. 1).

HER2 amplified tumors have been further subclassified into three separate subtypes, one with a clearly worse prognosis with a 12% 10 year survival, compared to the 50–55% survival in the other two groups.\(^\text{28}\) This gave rise to the development of a prognostic predictor composed of 158 genes (HER2-derived prognostic predictor (HDPP)) that better stratified the tumors between good and bad prognosis than the initial unsupervised analysis did. In addition, it also had a strong prognostic value in tumors that over-expressed HER2 inside other subgroups of breast cancer. The prognostic capacity of this classification overtook that of the MammaPrint\(^\text{\textsuperscript{TM}}\) and Oncotype DX\(^\text{\textsuperscript{\textsuperscript{TM}}}\) platforms and was additionally useful in basal-like tumors.\(^\text{29}\) Despite the limitations of this study, it is a good starting point for deciphering the subgroups within tumors that over-express HER2. HDPP was not directly related to the expression of proliferation genes or genes from the HER2 pathway, but it was for genes associated with immune response, tumor invasión and metastasis. Furthermore, this classification allows for the identification of an aggressive subgroup within that of the HER2+ tumors with a high invasive capacity and poor immune response.

From the clinical point of view, the HER2 subtype is characterized by a poor prognosis, although in the last decade, anti-HER2 treatment has substantially improved survival in not only the metastatic diseases, but also in the initial stages.\(^\text{7,29–31}\) Both, this subtype as well as the basal-like subgroup have a high chemosensitivity with higher response rates than that for luminal A and B tumors, in the neoadjuvant studies (43% and 36% pCR vs 7% and 17% pCR, respectively).\(^\text{20}\) Today the study of its biology
continues to improve the therapeutic approach. In the 2010 San Antonio Breast Cancer Symposium, the results of the combination of anti-HER2 therapies in neoadjuvance were presented. Clinical trials combining drugs that inhibit the same signaling pathway at different levels showed promising results in terms of response rate and tolerance.32,33

Basal-like

The basal-like subtype represents 10–20% of all breast carcinomas. The term was coined because they express genes usually present in normal breast myoepithelial cells, including high molecular weight cytokeratins CK5 and CK17, P-cadherin, cavelin 1 and 2, nestin, CD44 and EGFR. They also express genes characteristic of luminal epithelium such as CK8/18 and Kit, but at levels significantly lower than those of luminal carcinomas. Clinically they are characterized by their appearance at an early age, predominantly in women of African origin, having a large tumor size at diagnosis, a high histological grade and a high frequency of lymph node affection.34

Basal-like tumors tend to be infiltrating ductal carcinomas with a high mitotic index, tumor necrosis, expanding margins and a clear stromal lymphocytic response.35 The pattern of metastatic relapse is aggressive with predominance for visceral organs, mainly lung, central nervous system and lymph nodes.34,36 One of the most relevant features of this type of tumor is the absence of expression of the three key receptors in breast cancer: ER, PGR and HER2 (Fig. 1). Therefore in clinical practice the terms basal-like and Triple Negative (TN) are often interchanged. They are not, however equivalent terms since a discordance of up to 30% between the two groups has been described.37 Attempts to identify the basal-like group by an IHC profile have led to the selection of five markers (Basal Core Group): ER, PGR, HER2, EGFR and CK5/6. These markers classify this subtype with a specificity of 100% and a sensitivity of 76%.38

Basal-like tumors have a worse prognosis than do luminal ones19,39 with a higher relapse rate in the first 3 years40 despite their presenting a high response to chemotherapy.41 Therefore, it is critical to identify new therapeutic targets and design appropriate treatment strategies. The basal-like subtype tumors have a high rate of p53 mutations, which could explain their enormous aggressiveness and poor prognosis.39 In addition, tumors with germline mutations in the BRCA1 are located in the basal-like subgroup in the classification by intrinsic subtypes.36 The alterations that involve a decrease in the function of the BRCA1 gene, either by mutation or by epigenetic mechanisms, predispose to the development of basal-like tumors, lack of expression of ER and worse prognosis.34 BRCA1 is critical in the DNA repair and its inactivation leads to the accumulation of errors and genetic instability favoring the growth of tumors. These features have been the basis for the development of cytostatic inducers of DNA damage such as platinum salts.

One of the most promising strategies being developed to treat these tumors, and more specifically for TN are the poly-ADP ribose-polymerase-1 (PARP-1) inhibitors. PARP-1 is a key in DNA single strand breaks repair. The inhibition of PARP1 in the context of a defective DNA repair by BRCA1 leads to the accumulation of breaks in the double-stranded DNA and to cell death. Phase I studies evaluating olaparib (AZD2281) as monotherapy in breast cancer with BRCA1 and BRCA2 mutations show a very high response rate and clinical benefit (47% and 63%, respectively).32

Normal breast

These tumors account for about 5–10% of all breast carcinomas. They are poorly characterized and have been grouped into the classification of intrinsic subtypes with fibroadenomas and normal breast samples.7 They express genes characteristic of adipose tissue, presenting an intermediate prognosis between luminal and basal-like and usually do not respond to neo-adjuvant chemotherapy. They lack the expression of ER, HER2 and PGR, so these tumors can also be classified as TN, without being considered basal-like as they are negative for CK5 and EGFR. The clinical significance of these tumors remains to be determined and due to their rarity there are few studies on this subtype. There are doubts about their real existence and some researchers believe they could be a technical artifact from high contamination with normal tissue during the microarrays.43 In fact, in a large series of samples where the neoplastic cells were isolated by micro-dissection no cases of normal breast subtype were found, supporting the technical artifact hypothesis.

Claudin-low

After the initial molecular classification into subtypes of breast cancer, a new intrinsic subtype was identified in 2007.44 It is characterized by a low expression of genes involved in tight junctions and intercellular adhesion, including claudin-3, -4, -7 cingulin, occludin, and E-cadherin hence the name claudin-low. This subtype is located in the hierarchical clustering near the basal-like tumors, suggesting that both subtypes share some characteristic gene expression such as low expression of HER2 and luminal gene cluster. In contrast to the basal-like subtype, this new group over-expresses a set of 40 genes related to immune response indicating a high infiltration of tumors immune system cells.26,45 Claudin-low tumors have a poor prognosis, albeit presenting a low expression of genes related to cell proliferation. Otherwise, they overexpress a subset of genes closely linked to mesenchymal differentiation and epithelial–mesenchymal transition. These features are associated with the acquisition of a cancer stem cell (CSC) phenotype. It is a relatively rare subset of tumors (12–14%) clinically corresponding to high grade infiltrating ductal carcinomas, that can present metastatic or medullary differentiation.45 Immunohistochemically, they are normally TN; but, like with the basal-like tumors, the concordance TN/c Claudin-low is not 100% and about 20% of claudin-low tumors are positive for hormone receptors.27 These tumors show poor long-term prognosis45 and an insufficient response to neoadjuvant chemotherapy with intermediate values between basal and luminal tumors.27

The implications of the molecular classification in the therapeutic approach have been progressively accepted by some International Panels. The St. Gallen International Expert Consensus for Early Breast Cancer 2011 recognized the usefulness of this classification in the therapeutic decision process. Of note, the Panel accepted that the different breast cancer subtypes can be defined not only by genetic array testing but by approximations to this classification using immunohistochemistry. This Expert Consensus established five clinico-pathological definitions, luminal A (ER and/or PGR positive, HER2 negative, Ki67 <14%); luminal B – HER2 negative (ER and/or PGR positive, HER2 negative, Ki67 ≥ 14%); luminal B – HER2 positive (ER and/or PGR positive, HER2 positive, any Ki67); HER2 positive – non luminal (ER and PGR absent, HER2 positive) and Triple Negative (dual) (ER and PGR absent, HER2 negative). The Panel recommended therapeutic strategies for every of the different five clinico-pathological subtypes. In summary, it has been accepted that luminal A disease generally requires only endocrine therapy which also forms part of the therapy of the luminal B subtype. Chemotherapy is considered the recommended treatment for most luminal B, HER2 positive and Triple Negative diseases with the addition of trastuzumab in HER2 positive disease.47 Nevertheless, the NCCN international guidelines 2011 have not yet incorporated the

Given the evidence provided by the multiple studies that evaluate these gene expression based platforms, it can be safely affirmed that they are valuable prognostic tools. Also, the information they provide helps oncologists predict more accurately which patients will benefit from chemotherapy, especially when it comes down to ER positive tumors. It is argued that the predictive capacity of these platforms narrows down to the fact that they measure proliferation and higher proliferation translates into higher chemosensitivity, indiscriminately. But, of course, we aim to predict specific treatment response accurately. Even though efforts are being made to develop signatures that will provide useful information on specific treatment response, none of these are ready for use in the clinical setting.

### Targeting genomic instability

Cancer cells acquire functional capabilities that allow them to survive, proliferate and spread. These skills are acquired by different tumors through various mechanisms and at distinct times during tumor development. One of the most important features that enable the acquisition of these capabilities is genetic instability that generates random mutations and chromosomal rearrangements. Failures in the DNA maintenance system implicate the accumulation of errors that in some cases lead to loss of function of tumor suppressor genes and in other cases gain of function in oncogenes, contributing finally to carcinogenesis. The inactivation by mutation or silencing in BRCA1 is the best known example in breast cancer.

BRCA 1/2 and PARP are some of the essential mechanisms of DNA repair in cells. Thus, treatment with PARP inhibitors in cells lacking BRCA1 is based on the inability of the cell to repair DNA damage induced by the concomitant use of cytotoxic agents such as platinum salts. Cells accumulate chromosomal aberrations that force them to enter into apoptosis.

A phase II clinical trial in patients with metastatic TN breast cancer who received carboplatin–gemcitabine with or without the PARP inhibitor iniparib showed encouraging results in treating these tumors with an improvement in PFS of 2 and 5 months in OS. Recently, however, data from a phase III clinical trial shows iniparib does not reach the objectives of improving PFS and OS, leaving once again a discouraging picture in the treatment of TN breast cancer.

Other drugs that currently are being explored for the treatment of basal-like and TN breast carcinoma in the context of genomic instability are platinum salts. Table 1 summarizes the most relevant clinical trials with published data that explore the role of these cytotoxic agents in TNBC.

### Signaling pathway therapeutic advances in breast cancer

In the last decade, parallel to the effort made to classify breast cancers on the basis of their molecular biology, researchers have investigated the signaling pathways that govern the processes of formation, maintenance and expansion of the tumor. The biologically acquired capabilities that are considered essential for tumor development include the following: sustained proliferative signaling, growth suppression evasion, resistance to cell death, limitless replicative capacity, genomic instability, metabolic re-programming, induction of angiogenesis, invasion and metastasis.

Targeting these hallmarks of tumor development seems the way to go in order to eradicate this disease, and so, a review of the most important achievements with respect to disease control in some of these areas follows.

### Proliferation and survival: the metabolic link

As already mentioned, cancer cells can acquire a proliferative capacity sustained by different mechanisms. These include the

### Table 1

<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase</th>
<th>Study compound</th>
<th>Regimen</th>
<th>N</th>
<th>Efficacy</th>
<th>pCR (n)</th>
<th>RR (n)</th>
<th>SD (n)</th>
<th>CB (n)</th>
<th>PFS (mo)</th>
<th>HR (95CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garber55 (TNBC patients)</td>
<td>II</td>
<td>CDDP</td>
<td>Neoadjuvant CDDP 75 mg/m² q3w + 4 cycles</td>
<td>28</td>
<td>22% (6)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>O'Shaugnessy57</td>
<td>II</td>
<td>Cetuximab</td>
<td>ICb + Cetuximab</td>
<td>103</td>
<td>NA</td>
<td>49% vs. 30%</td>
<td>NA</td>
<td>18% vs. 6%</td>
<td>9% vs. 4%</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>TBCRC 00156</td>
<td>II</td>
<td>Cetuximab; Cb</td>
<td>Cetuximab ± Cb</td>
<td>102</td>
<td>NA</td>
<td>NA</td>
<td>18% vs. 6%</td>
<td>9% vs. 4%</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Gronwald95 (BRCA1) II</td>
<td>CDDP</td>
<td>Neoadjuvant CDDP 75 mg/m² q3w + 4 cycles</td>
<td>25</td>
<td>72% (18)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Ryan96 (TNBC patients)</td>
<td>II</td>
<td>CDDP, Bevacizumab</td>
<td>Neoadjuvant CDDP 75 mg/m² q3w + 4 cycles</td>
<td>51</td>
<td>16% (8)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Baselga BALI-157</td>
<td>II</td>
<td>Cetuximab</td>
<td>CDDP 75 mg/m² C3S + 6 cycles ± Cetuximab weekly</td>
<td>48</td>
<td>NA</td>
<td>20% vs. 10%</td>
<td>NA</td>
<td>3.7 vs. 1.5</td>
<td>0.675 (0.47–0.97)</td>
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<tr>
<td>O'Shaugnessy57</td>
<td>II</td>
<td>BSI-201</td>
<td>Cb-Gem ± BSI-201</td>
<td>120</td>
<td>48% vs. 16%</td>
<td>62% vs. 21%</td>
<td>NA</td>
<td>6.9 vs. 3.3</td>
<td>0.34 (0.2–0.58)</td>
<td>NA</td>
<td>NA</td>
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CDDP: cisplatin; ICb: irinotecan + carboplatin; Cb: carboplatin; Gem: gemcitabine.
pCR: pathologic complete response; RR: response rate (complete response + partial response); SD: stable disease; CB: clinical benefit; NA: not applicable; PFS (mo): progression free survival (months); HR: hazard ratio.

BRCA1: BRCA1 germline mutation carriers.

\(^*\) CB = CR + PR + SD > 6 months.
acquired ability to produce growth factors that stimulate their own proliferation in an autocrine manner or activate the production of paracrine growth factors by surrounding normal stromal cells. Additionally, the signals mediated by membrane receptors can be deregulated by an increase in the amount of the receptor on the cell surface or by structural alterations of the protein that constitutively activates the signal pathway independent of its ligand.

A clear example of pathway de-regulation in breast cancer that leads to sustained proliferative signaling is the HER-2 pathway. This membrane receptor belongs to the human epidermal receptors (HER) family, and after forming homo- or heterodimers its intracellular tyrosine kinase function becomes activated. These receptors can activate three major signaling pathways: Ras/Raf/ MAPK, JAK/Stat and PI3K/AKT/mTOR (Fig. 2). The three are involved in cellular functions such as growth and cell survival, proliferation, division, metabolism, apoptosis and migration capabilities. As specified in the previous section, HER-2+ tumors were historically classified as of poor prognosis. However, the introduction of trastuzumab into the clinic, first in the metastatic disease and subsequently combined with chemotherapy in the adjuvant setting, has reduced the risk of relapses by 50% and improved survival in more than 33% of the cases. This sets a clear example on the importance of identifying and targeting the specific pathways that govern proliferation and survival in each tumor.

Still, resistance to trastuzumab has been described, and all the HER-2+ breast cancer metastatic patients eventually progress under trastuzumab treatment. This set researchers to investigate the possibilities of dual targeting of the HER-2 receptor. The Neo-ALTTO trial proved that a dual inhibition achieves a greater rate of pCR. The results analysis showed that the combination of lapatinib and trastuzumab with paclitaxel improved pathologic complete response rates compared to treatment with paclitaxel and either trastuzumab or lapatinib individually (51.3% vs. 29.5% vs. 24.7%, respectively, \( p = 0.01 \)). These clinical data plus the information that will derive from the paired biopsies collected during the trial will, hopefully, throw more light on the mechanisms of response/resistance to these approved targeted therapies and the nature of the disease. The NeoSPHERE trial has also studied the convenience of a dual blockade of HER-2 with trastuzumab and pertuzumab. The results reported are very encouraging; especially notable is that up to 17.8% of the patients achieved pCR when treated concurrently with both monoclonal antibodies and no docetaxel. These results suggest that in the future it may be possible to design strategies against specific targets for certain patients without adding cytotoxic therapy.

Other recent advances in HER-2+ breast cancer treatment include trastuzumab-DM1 (T-DM1). This is the first drug that combines trastuzumab with maytansine or DM1, an anti-microtubule agent. This cytotoxic agent selectively acts in cells that over-express HER2, limiting systemic toxicity. At the end of 2010 the results of the clinical trial with T-DM1 were published. These revealed response rates around 25% with a progression-free survival of 4.6 months in patients who had previously received several lines of anti-HER2 treatment. The good results and the excellent tolerance have led to the initiation of two phase III trials currently underway (Marianne and Emilia). The first evaluates the activity of T-DM1 compared to that for standard therapy (docetaxel–trastuzumab) in first-line treatment of metastatic disease. The second compares the effectiveness of T-DM1 with capecitabine–lapatinib in second-line treatment. Moreover, there are several phase I

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**Fig. 2.** PI3K/AKT/mTOR and AMPK pathways. The activation of the membrane tyrosine kinase receptors (RTK) as the HER family and IGF1R causes receptor dimerization phenomena, phosphorylation and activation of intracytoplasmic effectors. AKT is activated via PI3K and has multiple targets, including mTOR, which upon activation may exert its function by activating mechanisms of protein synthesis and cell growth. Cellular energy sensor AMPK and LKB1 exert the opposite effect, inhibiting mTOR. The RTK activation also leads to the activation of other routes such as the MAPK and STAT3 (Source: adapted from 66,70).
trials combining T-DM1 with targeted therapies such as PI3K inhibitors, with encouraging data regarding the clinical response.62

The sustained neoplastic cell proliferative capacity implies a deregulation of cell division and growth control. This forces the cell to readjust its metabolism to secure a constant energy source. In 1930, Otto Warburg observed that cancer cells can reprogram their energy metabolism largely limited to the process of glycolysis, a phenomenon known as aerobic glycolysis or the Warburg effect.56

This inefficient mechanism for obtaining energy allows the deviation of glycolytic intermediates to other biosynthetic pathways necessary for the rapid cell division. One of the pathways implicated in cell growth and metabolic regulation is the IGFR1/PI3K/AKT/mTOR pathway, which has been largely looked into the past decade as a target to treat breast cancer (Fig. 2). This pathway is activated under normal conditions when the supply of nutrients is appropriate and it promotes the synthesis of lipids, proteins and glycogen. In case of energy shortages, however, the kinase pathways are activated by adenosine monophosphate (AMPK) leading to the inhibition of earlier biosynthetic processes.63 The IGFR1 activation by IGFR leads to the phosphorylation of tyrosine residues in the intracellular domain of the receptor and phosphorylation of tyrosine and serine residues of the insulin receptor substrate (IRS) and Src which will activate the MAPK and PI3K/AKT/mTOR pathways.63,64 IGF pathway is regulated in many critical points, from ligand availability to negative feedback mechanisms exerted by mTOR.65 As for its role in oncogenesis, preclinical studies show that the overexpression of IGFR1 can induce the formation of tumors and facilitate metastasis formation.60

Given the role of this pathway in cell metabolism regulation, in growth and survival, each of its integral parts is a possible therapeutic target. Furthermore, this pathway frequently presents activating mutations in the membrane receptor, the catalytic subunit of PI3K and AKT. Also, loss of function in the negative regulators, such as PTEN and TSC 1/2, has been described.72 In fact, some retrospective series have defined PI3K mutations as frequently as 20–25% of the breast cancers studied, and this percentage increases up to 35% in ER+ tumors.69 The hyperactivation of this pathway in ER+ tumors has been postulated to confer resistance to conventional anti-estrogen treatment.70 In addition, mutations in this pathway have been associated with resistance to anti-HER2 drugs.71 These data, added to the results obtained in phase I clinical trials with mTOR inhibitors72 have led researchers to change the strategy in the development of this type of targeted therapies. Indeed, mTOR targeting leads to the loss of the negative feed-back loop that it exerts upstream, resulting in an increase of the PI3K activity.72

A phase I trial explored the efficacy of a dual inhibition of this pathway. The combination of ridaforolimus (oral inhibitor of mTOR) and dalotuzumab (monoclonal antibody against IGFR1) showed a promising antitumor activity with acceptable toxicity in ER+ breast cancer with a high proliferative index.73

The AMPK pathway, also involved in the regulation of cellular metabolism in situations of energy shortage and catabolic processes, counteracts PI3K/AKT/mTOR pathway activation through a double inhibitory effect, namely, the phosphorylation of TSC2 and of raptor.74,75 Based on these data, the activation of the AMPK pathway has been proposed as a way of counteracting the mTOR pathway (Fig. 2). Metformin, which mediates activation of AMPK, has been tested pre-clinically. In vitro and in vivo cancer studies show the antiproliferative capacity of this drug, and its ability to induce death in a stem cell model of breast carcinoma.76 Furthermore, a retrospective study linked metformin use with a higher probability of pCR in the neoadjuvant setting.77 Taking all of this together, the use of metformin in breast cancer treatment looks very promising and several clinical trials are exploring the usefulness of this drug in advanced and early breast cancer settings (www.clinicaltrials.gov).

If one reads between the lines of these findings, on a more broad level, it is logical to find diseases closely related to metabolism, such as obesity or diabetes mellitus, epidemiologically linked to breast cancer. Obesity is a risk factor for developing breast cancer in postmenopausal patients and in turn has been associated with an increased risk of relapse and a worse prognosis in overall survival of the patients.78–80

After menopause, the main source of estrogen comes from adipose tissue by conversion of androstenedione to estrone through the action of aromatase. This implies a higher concentration of estrogen in obese women than in women of similar age and normal body mass index (BMI).81,82 A study published in 2010 analyzed the impact of obesity on the prognosis of patients with breast cancer treated with tamoxifen 20 mg/day or anastrozole 1 mg/day within the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial. The results of this study concluded that obese patients had a worse prognosis, with the relative efficacy of AI being higher compared with that for tamoxifen in lean women. The study did not establish a definite biological basis, but suggested the importance of BMI in treatment with AI. To test this hypothesis would require a prospective study to correlate estrogen suppression by AI, estrogen plasma levels, BMI and prognosis.83

The angiogenesis conundrum

The final part of the present review is dedicated to angiogenesis in breast cancer, which has been a matter of long discussion in the past year, especially regarding the relevance of the results obtained in three clinical trials that will be discussed next.

Given the sustained capacity to proliferate of cancer cells, like normal tissues, require an adequate supply of nutrients and oxygen and the removal of metabolic waste and carbon dioxide for their maintenance. The neovascularature generated by the process of angiogenesis meets these needs. Angiogenesis is a process activated in adults, except in very specific physiological situations such as wound healing and the female reproductive cycle. Still, this process is fully functional during tumor development.56 One of the main factors involved in the activation of angiogenesis is the vascular endothelial growth factor (VEGF), which operates mainly through three tyrosine kinase membrane receptors (VEGFR 1–3).84 The tumor neovascularature is characterized by vessel excessive branching, architectural distortion, erratic blood flow, microhemorrhaging, leakiness, apoptosis and abnormal levels of endothelial cell proliferation.85 One of the most important characteristics of the process of angiogenesis is its induction in the early stages of carcinogenesis, even in precancerous phases, and its maintenance throughout the tumor development and metastasis formation. VEGF plays a crucial role in the early stages with predominance over the other growth factors and cytokines involved. Thus, its role in neangiogenesis and its limited performance in adult physiology make this molecule the ideal target to attack this process. The study of the molecules targeting this signaling pathway at different levels has been going on for years, but it was only in 2010 when the scientific community reviewed all the data concerning anti-angiogenesis in breast cancer.

One of the most tested drugs is bevacizumab, a humanized IgG1 monoclonal antibody that recognizes VEGF. Three trials have evaluated its use in advanced HER2 negative disease: Phase III E2100,51 AVADO86 and RIBBON-1.57 In the 2010 ASCO conference a meta-analysis of these three trials (including 2646 patients) was presented.87 All showed an improvement in PFS regardless of the status of hormone receptors, the number of metastatic sites, the prior use of taxanes and the disease-free interval. The conclusion was that the median PFS for the combination of chemotherapy with bevacizumab (9.2 months) was significantly greater than that.
for chemotherapy alone (6.7 months) with a hazard ratio of 0.64 (95% CI 0.57–0.71). In a subset analysis, all subgroups benefited from the combination of bevacizumab and chemotherapy, although, as demonstrated in the individual studies, there was no improvement in OS. A meta-analysis of these three trials\textsuperscript{89} evaluated the impact of the addition of this monoclonal antibody to chemotherapy in patients with TN breast cancer. The study included 621 patients and showed that the combination of bevacizumab to a cytotoxic agent improved the PFS with a median of 8.1 months vs. 5.4 months in the non-antiangiogenic arm (HR 0.68, 95% CI 0.538–0.783) and a higher objective response rate favoring the combination (42% vs. 23%). Moreover, the percentage of patients with primary resistance to treatment was significantly lower in the combination arm (11% vs. 28%). An additional subset analysis in patients previously treated with taxanes\textsuperscript{90} showed the higher benefit in the set receiving taxanes in combination with bevacizumab.

Hence, it seems that targeting angiogenesis, at least in the bevacizumab trials, can be useful, especially in the TNBC subgroup that lacks a specific therapeutic target. Still, there is a pressing need to identify markers that predict response to angiogenic therapy, since it is clear that not all patients will benefit from it.

Conclusions

The implementation of high throughput technologies in breast cancer research over the past decade has led to a classification of this disease from a molecular point of view. The relevance of this new form of classification lies in the additional information provided above and beyond the traditional methods. Nowadays researchers and clinicians know much more about many aspects related to tumors, and can better differentiate between entities that previously were thought to be similar. It can be said that there has been a change in the way we perceive this disease. The downside is that these research tools are far from being applicable to routine clinical practice because of both the substantial economic investment as well as the lack of sufficient validation and standardization. Therefore, in clinical practice, despite the great advances in biological knowledge, we are still using the diagnoses based on immunohistochemical markers. Certainly some markers are gradually being incorporated trying to link the traditional classification to the intrinsic subgroups, such as the routine determination of Ki67 and some cytokeratins (e.g. CK8/18 and CK5), but there is no international consensus yet.

One of the main contributions of this breakthrough in cancer research is the integration of molecular studies into clinical trials. This approach has provided valuable information on the nature of the disease, explaining in part the different responses to treatment and the disparate prognoses. Knowing the pathways regulating the processes involved in neoplastic development should help in the design of clinical trials aimed at patients with specific characteristics that are candidates to benefit from specific treatment. The most important and useful conclusion to be drawn is that if it were possible to identify a tumor’s key mechanisms of regulation, one could attack it more specifically, minimizing toxicities while approaching the ideal of personalized medicine.

Acknowledgments

This work was supported in part by Grants from the Ministerio de Salud Carlos III [Grant numbers P509/01700 and RTICC RD06/0020/0080 to A.L.], and Conselleria de Sanidad [GE-004/09] to P.E.A.B. holds a Rio Hortega fellowship from the Instituto de Salud Carlos III (Ministerio de Ciencia e Innovación) and P.E. is funded from the Instituto de Salud Carlos III under a ‘Miquel Servet’ contract [FI100/0909]. There are no competing financial interests.

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