

Personalized medicine in breast cancer: pharmacogenomics approaches

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Abstract: Breast cancer is the fifth cause of cancer death among women worldwide and represents a global health concern due to the lack of effective therapeutic regimens that could be applied to all disease groups. Nowadays, strategies based on pharmacogenomics constitute novel approaches that minimize toxicity while maximizing drug efficacy; this being of high importance in the oncology setting. Besides, genetic profiling of malignant tumors can lead to the development of targeted therapies to be included in effective drug regimens. Advances in molecular diagnostics have revealed that breast cancer is a multifaceted disease, characterized by inter-tumoral and intra-tumoral heterogeneity and, unlike the past, molecular classifications based on the expression of individual biomarkers have led to devising novel therapeutic strategies that improve patient survival. In this review, we report and discuss the molecular classification of breast cancer subtypes, the heterogeneity resource, and the advantages and disadvantages of current drug regimens with consideration of pharmacogenomics in response and resistance to treatment.

Keywords: breast cancer, personalized medicine, biomarker, pharmacogenomics

Introduction

Providing the most effective treatment is a task of paramount importance in personalized medicine for breast cancer.¹ This approach enables categorizing cancer subtypes and, ultimately, assigning the best treatment regimen based on patient characteristics, medical history and response to therapy.² The combination of pharmacological approaches (eg, pharmacogenetics, and pharmacodynamics) aids personalized medicine therapeutic decisions. Furthermore, pharmacogenetics and pharmacogenomics enable clinicians selecting appropriate drugs and doses to decrease adverse effects and increase efficacy. The systematic study of drug absorption, distribution, and metabolism is crucial for efficacy, and this may depend on genetic and epigenetic variations.³ In addition to the role of genetic polymorphisms, epigenetics, microbiome alterations as well as demographic characteristics are involved in the occurrence of multi-drug resistance.⁴⁻⁶ Historically, pharmacogenetics and pharmacogenomics refer to the effect of genetic variations on drug metabolism, and the influence of the whole genome might have in response to drugs.⁷ These approaches are frequently deployed in the setting of oncology as new strategies to minimize toxicity while maximizing the efficacy of target therapy and chemotherapy. They are also applied to develop new drugs based on genetic profiling and gene expression, this process being facilitated by new innovative techniques and profiling instruments. Here we overview the current molecular

classification of breast cancer, the source of tumor heterogeneity and pharmacogenetics approaches in the targeted therapy and chemotherapy of the main subtypes of breast cancer.

Molecular classification

Based on molecular stratification, breast cancer is categorized into five distinct molecular classes⁸ 1, and 2 hormone receptor positive (luminal A and luminal B), 3 human epidermal growth factor receptor-2 (HER2-positive), 4 basal-like and normal-like based on microarray and gene expression profiling described by Perou and Sorlie,^{9,10} and the 5 claudin-low,¹¹ which is triple-negative breast cancer (TNBC), classified by medullary and metaplastic differentiation.^{12,13} The patterns of each molecular subtype differ in response to treatment^{9,10} The management of the most common class – luminal A (predominantly ER⁺/PR⁺ and HER2-negative) – which accounts for 40%-60% of the disease^{8,14} has low pathological grade and proliferation¹⁵ and shows low response to chemotherapy¹⁶ while being sensitive to endocrine therapy,¹⁷ is focused on anti-endocrine strategies⁸ In luminal B (ER⁺/PR⁺ and HER2-positive), which accounts for 15% of the disease,¹⁴ and compared with luminal A,¹⁵ has higher pathological grade and proliferation, chemotherapy rather than anti-estrogen drugs is beneficial.^{8,18} 10% of breast cancers fall into the HER2 positive category¹⁴ that displays high pathological grade.¹⁵ Although this group of tumors is chemosensitive,¹⁹ the disease prognosis has been dismal up until the introduction of targeted therapeutics.^{20,21} Basal-like tumors that represent 10–25% of the disease,¹³ and are characterized by ER[−]/PR[−] or HER2[−] overlap with triple-negative (TN) tumors, express either HER2 or ER or both, in 15–45% of the cases.²¹ Studies showed that basal-like tumors are chemo responsive,¹⁹ although they are associated with aggressive behavior and poor prognosis^{10,14,22–24}; 3–10% of the cases classified as normal-like type.¹² Those tumors that express gene expression characteristics of adipose tissue show an intermediate prognosis. In some studies, it has been proposed that these cells might represent a technical artifact during the microarrays.²⁵ The type Claudin-low is predominantly present as TNBC that accounts for 7–14% of the tumors and displays low expression of genes claudin and E-cadherin genes²⁶; 15% of these tumors express ER, while HER2 is overexpressed in 15% of the other cases belonging to the claudin-low type.¹² Response to chemotherapy in claudin-low tumors is intermediate.^{12,13}

Table 1 represents subtypes of breast cancer and their corresponding management strategies for cancer therapy. Despite the remarkable improvement achieved upon the use of therapeutic agents, de novo and acquired resistance remains an unsolved issue in breast cancer. The knowledge of the molecular mechanisms involved in the molecular subgroups of breast cancer might guide the development of new therapeutic strategies.^{27,28} Individual or multiple molecules or mutated genes could be deployed as a target of specific therapeutic strategies.

Heterogeneity resources

Heterogeneity of a subtype of breast cancer is the most frequent cause of therapy failure and unexpected outcome. In a distinct subgroup of cancer, alterations in genetic and epigenetic features of a clone of cells lead to changes in the prognosis and drug response.^{5,29} This heterogeneity can be intra-tumoral when involving cells within a tumor in an individual patient, or inter-tumoral when involving cells of the same subgroup of tumors in different patients. Intra-tumoral heterogeneity is named “spatial” when involving a certain part of the tumor; or “temporal” when cells within the same tumor undergo changes during the course of the disease, from the primary tumor to metastasis. The heterogeneity associated with the morphological levels takes from comparing variable cell types in histopathological tests and provides grading systems.³⁰ When considering heterogeneity at a genetic level, alterations are related to the copy number variation (CNV), overexpression and down-regulation of a gene; or from missense, nonsense and frameshift mutations. Nowadays, there is a distinct open source database (COSMIC and TCGA) that provides information related to high throughput techniques. There are different heterogeneity sources for breast cancer patients who categorize in defined groups. These include local and systemic sources.⁵ The COSMIC database contains data from hundreds of breast cancer tumors detected across different platforms and provides information about different analysis, mutation, CNV, methylation, over and underexpression. Table 2 shows the genetic information of different categories of breast cancer categorized based on cellular and molecular signatures. The extensive variation in top 10 genes translates to the high rate of heterogeneity not only across but also within the groups; it would, therefore, be unlikely to observe a certain molecular pattern in all patients even if they all belong to the same group. This variation may be related to spatial or temporal heterogeneity that results in different tumor responses upon exposure to the therapeutic regimens.

Table I Breast cancer classification based on molecular profiling

Molecular class	Chemotherapy response	Target therapy	Resistant factors	Overcome resistant therapy
Luminal A	Low	TAM, fulvestrant, aromatase inhibitor	<i>ER</i> amplification, mutation, methylation, phosphorylation, acetylation, Mutation <i>PI3K/mTOR</i> , <i>FGFR1/2</i> amplification, <i>KRAS</i> and <i>P53</i> mutation, <i>CYP2D6</i> mutation	ARN-810, fulvestrant alone or in combination with CDK4/6 inhibitor (palbociclib), Combination of alpelisib and letrozole, the PI3K inhibitor; epigenetic drugs such as vidaza or decitabine along with histone deacetylase inhibitors such as vorinostat or romidepsin
Luminal B	Intermediate	TAM, Fulvestrant, Aromatase inhibitors	Mutations of <i>PIK3CA</i> , the gain of <i>CCND1</i> and <i>CDK4</i> , moderate PTEN reduction, Up-regulation and autocrine activation of HER2	Alone or in combination mTOR, AKT, or MEK inhibitors with fulvestrant The combination between lapatinib or trastuzumab and aromatase inhibitors (letrozole, anastrozole, exemestane)
HER2-positive	High	HER2 and kinase inhibitor: lapatinib, pertuzumab, trastuzumab and adotrastuzumab emtansine, immune cell activation(Ertumaxomab)	Mutations of <i>PIK3CA</i> , <i>RAS</i> , <i>Src</i> , <i>NF-KB</i> and <i>PTEN</i> , truncated isoforms of HER2, stable HER2 homodimers formation, overexpression of EGFR and HER-3	PI3K/AKT/mTOR inhibitor; Lapatinib, Tyrosine kinase inhibitors
Basal-like Claudin-low	High Intermediate/low	PARP1 inhibitor(olaparib and iniparib), cisplatin	Mutation in <i>TP53</i>	PI3K/AKT/mTOR inhibitor

The pharmacogenomics approach enables identifying all the genetic variations in driver genes that account for the selective growth of cells in tumor bulk. As therapeutic targets, these help the clinician to select the appropriate therapeutic regimens to overcome resistance. The use of anticancer against these driver genes requires several considerations such as the choice of drug combinations, or selected target based on the predominant subclonal population that may undergo adaptive responses or toxic effects.³ The detection of a subclonal population by accuracy methods like the *ex vivo* culture of tumor spheres in the tumor on-chip technique remains a critical issue.^{31,32} Other somatic mutations, introduced as passenger mutations, are those present in genes that help tumor survival. These mutations become acquired when cells are in the normal state or after they have undergone neoplastic transformation.⁵ In some cases, genes implicated in cancer development have not been found to be mutated but are frequently found to be inactivated as a result of epigenetic mechanisms.

Hereditary breast cancer is linked to genetic mutations. *BRCA1*, *BRCA2*, *PALB2*, *TP53*, *CDH1*, and *PTEN* are genes that may undergo mutations associated with cumulative breast cancer risk.³³ Moreover, epigenetic mechanisms,

which are known to play an essential role in the regulation of gene expression, may be involved in a hereditary form of the disease. Previous studies have shown that the tumor suppressor gene – *RASSF1A*– is often silenced following hypermethylation in breast cancer.³⁴ In familial breast cancer, <5% of mutations affect the *BRCA1* and *BRCA2* genes; whereas sporadically arising breast cancer can be associated with 10–15% rate of *BRCA1* methylation. In most subtypes of breast cancer (Figure 1), the molecular pathway³⁵ related to cancer progression include the PI3K/AKT/mTOR and the RAS/RAF/MEK pathways. These are abnormally activated and are associated with resistance. Clinical trials are currently underway to test various PI3K inhibitors, which have been developed to target different components of the pathway. In the PI3K pathway, PTEN inactivation by epigenetic mechanisms is likely to extend the use of drug inhibitors effective in case of PTEN defective cancer cells. Another gene involved in this pathway is *PPP2R2B*, the negative regulator of AKT, and for which promoter methylation occurs in breast cancer.³⁶

In some gene families, overlaps of mutation and methylation are observed, these leading to variable responses. As an example, when considering the RUNX

Table 2 Breast cancer subgroups identified by genome profiling using different analysis platforms in COSMIC database

Cancer types	Cases	Top 10 genes
Acinar cell carcinoma	20	TP53 (40%), PIK3CA (24%), GATA3 (15%), KMT2C (7%), PTEN (6%), ESR1 (6%), ERBB2 (5%), ARID1A (5%), AKT1 (4%), NFI (4%)
Adenoid cystic carcinoma	8	NOTCH1 (67%), KMT2C (67%), PTEN (40%), BRAF (11%), PIK3CA (11%), TP53 (33%), PIMI(33%), TRAF7 (33%), MYOD1 (33%), CREBBP (33%)
Basal (triple-negative) carcinoma	356	TP53 (50%), PIK3CA (12%), RBL (6%), CDKN2A (4%), PTEN (4%), KMT2D (5%), PIK3RI (3%), ATR (4%), ARID1A (3%), BRAF (2%)
Ductal carcinoma	2645	TP53(40%), PIK3CA (24%), GATA3(15%), KMT2C (7%), PTEN (6%), ESR1 (6%), ERBB2 (5%), ARID1A (5%) NFI (4%)
Ductolobular carcinoma	93	PIK3CA (42%), CDH1 (35%), TP53 (13%), NFI (11%), ERBB2 (11%), ESR1 (8%), TBX3 (10%), ARID1A (9%), KMT2C (9%), AKT1 (7%)
ER-HER2-positive	15	TP53 (71%), PIK3Ca (22%), GATA3 (7%), ERBB2 (4%) RBL (14%), JAK2 (7%), SMAD4 (7%), GNAQ (7%), VHL (7%), ATM (7%)
ER-positive carcinoma	345	PIK3CA (32%), TP53 (24%), GATA3 (15%), IL6ST (38%), KMT2C (14%), CDH1 (12%), ESR1 (9%), PTEN (9%), BRCA 2(9%), ERBB2 (9%)
ER-PR-HER2-Positive	28	PIK3CA (29%), TP53 (29%), CDH1(12%), GATA3 (14%), ERBB2 (14%), PCMI (14%), CREBBP (14%), RARA (7%), FOXA1 (7%), ATR (7%)
ER-PR-positive carcinoma	334	PIK3CA (33%), TP53 (22%), GATA3 (17%), ESR1 (10%), CDH1 (9%), KMT2C (10%), AKT1 (7%), SPEN (5%), FAT4 (6%), MAP2K4 (4%)
HER2-positive carcinoma	478	TP53 (46%), PIK3CA (22%), KMT2D (8%), LRP1B (8%), PREX2 (6%), KMT2C (6%), ERBB2(3%), MENI(4%), ZFH3(5%), NCOR2(2%)
Lobular carcinoma	457	CDH1 (55%), PIK3CA (41%), TP53(19%), ERBB2(17%), KMT2C (16%), TBX3(13%), ESR1(7%), FOXA1(7%), ARID1A(6%)
Luminal A carcinoma	23	PIK3CA (48%), RUNX1 (11%), MAP2K4 (7%), TP53 (10%), ASXL1 (4%), EGFR (5%)
Luminal B carcinoma	27	PIK3CA (28%), TP53 (7%), RUNX1 (5%), MAP2K4 (6%), JAK2 (2%), KRAS (2%), BRAF (2%), IDH1 (2%), GNAS (2%), PTEN (2%)
Luminal NS carcinoma	275	TP53 (60%), PIK3CA (56%), GATA3 (44%), CDH1 (42%), MAP2K4 (18%), ATR (16%), RUNX1 (14%), CDKN1B (14%), RBL (12%), KMT2C (11%)
Medullary carcinoma	38	TP53 (76%)
Metaplastic	151	TP53 (64%), PIK3CA (33%), KMT2C (14%), LRP1B (15%), CDKN2A (11%), PTEN (8%), KMT2D (8%), ARID1A (8%), PIK3RI (7%), APC (5%)
Neuroendocrine	25	EP300 (100%), PIK3CA (15%), TBX3 (19%), OXA1(19%), KMT2C (19%), HRAS (8%), FGFR1 (4%), KDR (6%),CTCF (13%), ARID1A (13%)
Normal like carcinoma	4	TP53 (100%), RUNX1 (7%), MAP2K4 (5%)
Carcinoma not specified (NS)	6014	PIK3CA (28%), TP53 (22%), CDH1 (10%), GATA3 (8%), KMT2C (8%), ESR1 (7%), ERBB2 (4%), NCOR1 (4%), SPEN (4%), NFI (3%)
PR-HER2-positive carcinoma	2	PIK3CA (50%), NCOR1 (50%), ERBB4 (50%)
PR-positive	2	SPEN (40%)
Small cell carcinoma	4	PIK3A (40%)

family, *RUNX1* is mutated while *RUNX3* is inactivated by epigenetic mechanisms.^{15,37} DNA methylation can be analyzed in a blood sample using a powerful bisulfite-based PCR technique.¹⁵ The tumor microenvironment, which comprises tumor-associated macrophage, fibroblast, bone marrow-derived cell and lymphatic growth factors,

chemokine, cytokines, and exosome, is also responsible for tumor heterogeneity and contributes to both growth and metastasis.⁵ It is envisaged that in the near future genetic, genomic and immunologic consultants might help the oncologists to implement different strategies and therefore plan for the most successful therapy regimens.

Cancer type

Pathway

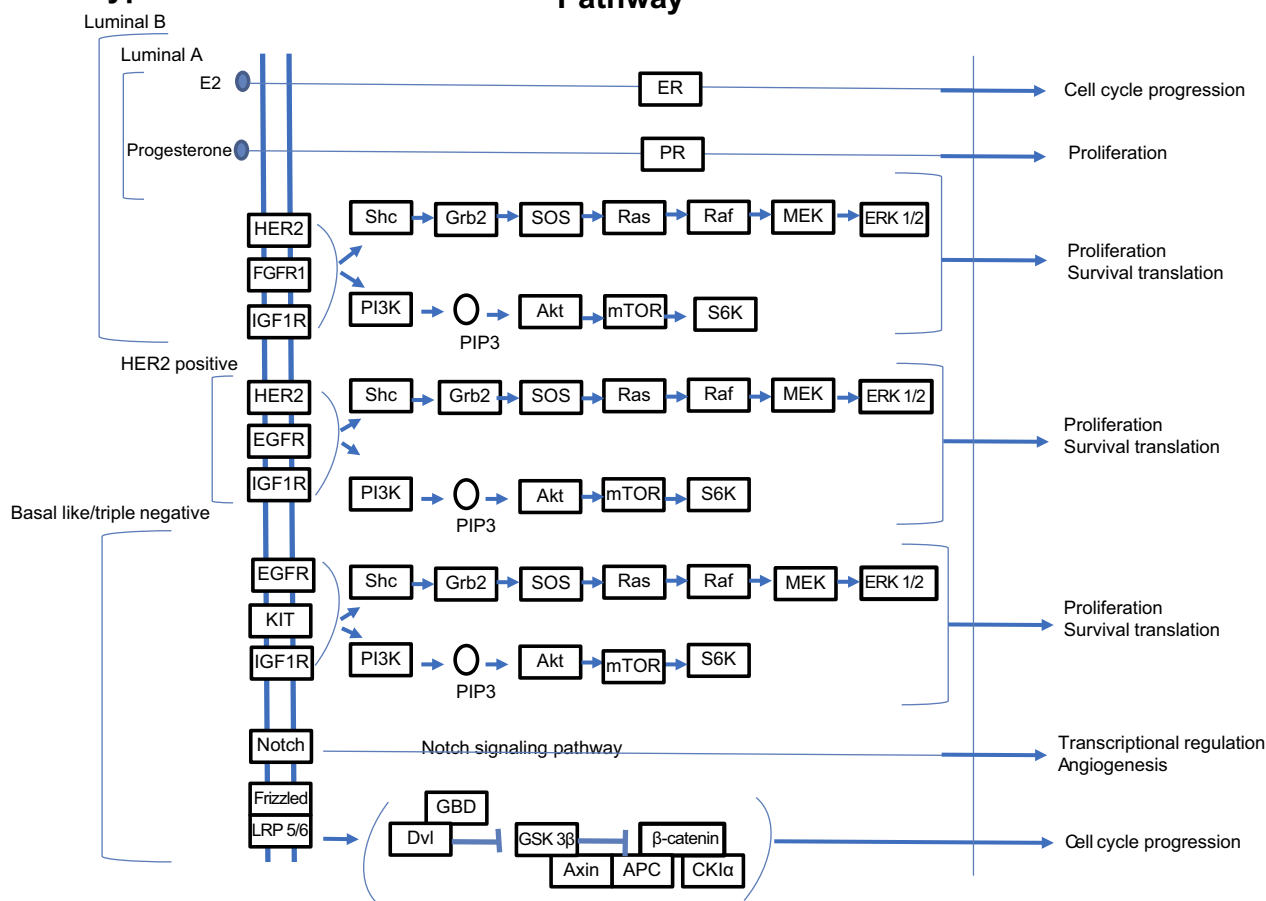


Figure 1 Signaling pathways implicated in different categories of breast cancer.

Notes: Adapted with permission from Kanehisa M, Goto S. KEGG: Breast cancer - Reference pathway; 2018. Available at: https://www.genome.jp/kegg-bin/show_pathway?map05224. Accessed May 15, 2019. Copyright Kanehisa Laboratories.¹⁵⁴

Pharmacogenomics will pave the way for such planning and generation of individualized treatments.

Pharmacogenetics in breast cancer subtypes

Estrogen receptor positive

The predominant type of breast cancer is associated with expression of estrogen receptor, which is used as a predictive marker in the follow up of patients (disease free). These cases benefit from hormonal therapy based on the administration of the artificial estrogen analog tamoxifen (TAM). TAM binds to estrogen receptor and disrupts the activation of the classical pathway that leads to ductal hyperplasia. Subsequent alteration of the tumor microenvironment makes the invasion state.^{38,39} Figure 2 represents the signaling pathways, which modulate tumor cells and tumor microenvironment components, as well as the

effect that certain cancer therapeutic agents exerts on these pathways in patient with ER⁺. The other signaling pathways related to ER overexpression relate to nonclassical functions that facilitate genomics activity in an independent hormone manner. In this context, ER acts through growth factor signaling (FGFR, IGFR, GPCR) that activate intracellular kinase and phosphatase. Several studies determined the effect of kinases in the phosphorylation of ER protein; this phenomenon is associated with sensitivity or resistance to endocrine therapy. Baron et al provided an extensive review of all phosphorylation sites and mRNA splicing regions in ER and associated drug response. Further, the interaction between ER with other transcription factors like C-Fos/C-Jun (AP-1), Sp1 and NF-KB was found to result in tumor cell division, angiogenesis and progression to metastasis⁴⁰ In addition to TAM, the ER down-regulator Fulvestrant has been approved for the treatment of ER⁺ breast cancer. By forming a complex

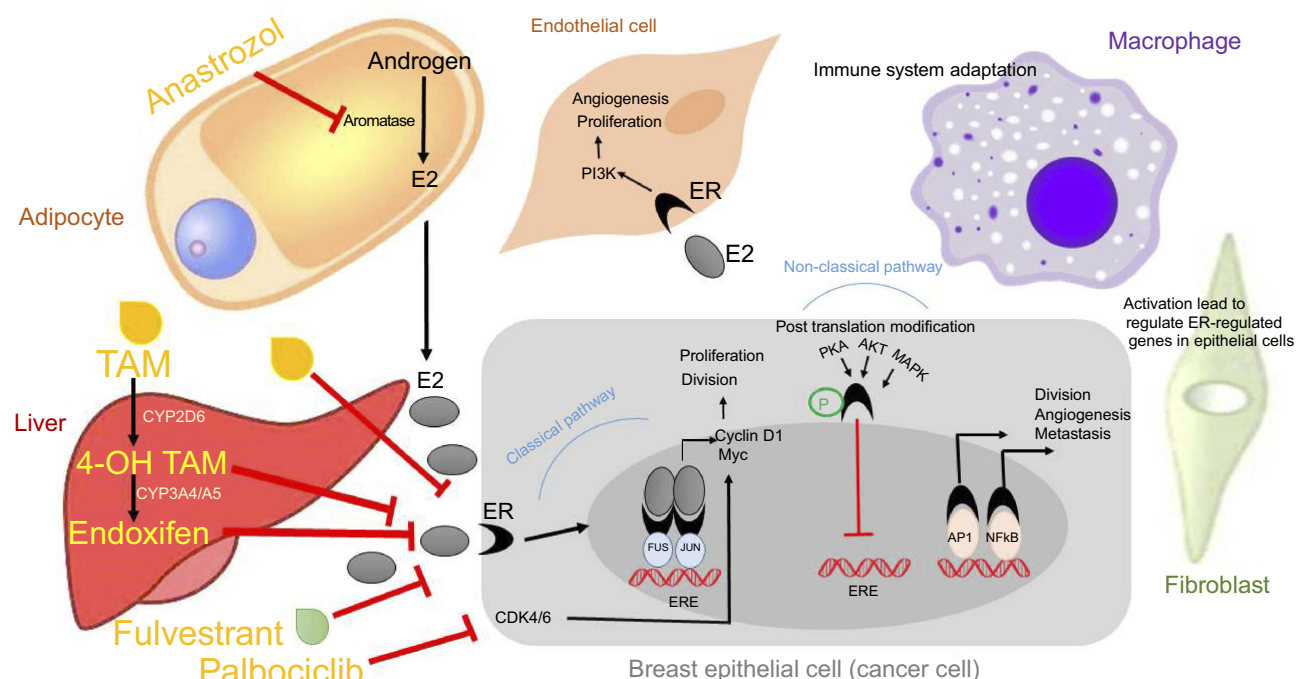


Figure 2 The interaction between tumor microenvironment components, including stromal cells, and tumor cells leads to enhanced cell growth, proliferation, angiogenesis and invasion. Breast cancer epithelial cells increase tumor cell proliferation and invasion while inhibiting apoptosis through either of these following pathways: (1) classical pathway in which estrogen binds to its receptor, ER, or (2) non-classical pathway that involves post-translational modifications of ER by activation kinases, and transcription factors. Anti-cancer agents including tamoxifen (TAM), whose metabolites, 4-OH TAM and endoxifen, have higher affinity for ER when compared with TAM, exert their effects by modulating signaling pathways that regulate tumor cells.

Note: Data adapted from Russell³⁹ and Barone et al.⁴⁰

with ER, Fulvestrant inhibits its dimerization, finally leading to its degradation. Moreover, Anastrozole, an aromatase inhibitor that blocks the conversion of adrenal androgen into estrogen, can be administered to a postmenopausal woman who could also benefit from Fulvestrant treatment. TAM is equally valid regardless of menopausal status.³⁹ As indicated in Table 2, there are variations in hormone receptor subtypes, which are associated with genetic differences. Progesterone receptor gene expression is dependent on ER expression in epithelial cells so that half of the ER⁺ tumors are also PR⁺. Previous work has shown that patients who do not express PR have the worse prognosis since the start of TAM treatment when compared to those who are PR⁺. This group of patients benefits from the administration of Anastrozole.⁴¹ Primary endocrine resistance can occur in half of the cases and finally developed resistance in another half as acquired resistance. Several factors are contributing to resistance, and these include mutation rate, methylation, acetylation and downregulation of ER α , overexpression of ER β as well as crosstalk between ER and growth factor and signaling pathways.^{40,42} ARN-810 is a selective ER α antagonist that induces

proteasomal-mediated degradation of ER α . This drug is active against tumors with the *ESR1* mutation, whereas conventional endocrine therapy is not sufficient.⁴³ Mutations in the ligand binding domain of ER α are associated with ligand-independent transcriptional activity. Hot spots mutations located in the ligand-binding domain include Y537S, Y537N, Y537C, and D538G, these representing >80% of *ESR1* mutations associated with acquired resistance to endocrine therapy.^{44–46} This type of mutations is regarded as the main resistance mechanism that is rare in primary tumors but is reported in >20% of cases of recurrence and metastatic cancer in patients treated with endocrine therapy.^{47–49} Since the frequency mutation of the *ESR1* is higher in metastatic than in primary breast cancer, assessment of this gene mutation in plasma cfDNA could help selecting treatment strategies, eg, administration of Fulvestrant in patients with the *ESR1* mutation was found to improve tumor-free survival.^{48,49} Although the mechanism related to the effect of Fulvestrant alone or in combination with Palbociclib in a patient with ER mutation is not known, patients with metastatic cancer benefit upon exposure to combination therapy.⁵⁰ The COSMIC database reported that the *ESR1* mutation Y537S affects

the phosphorylation of IGF1R.⁵¹ This is associated with shorter overall survival and contributes to resistance to target therapy.⁴² K303R is another significant mutation that was found to reduce sensitivity to TAM through phosphorylation of AKT.⁵² According to the COSMIC database, most of the ER⁺ cases display *PIK3CA* mutations. In luminal B subtype with endocrine resistance generated the moderate PTEN reduction leading enhance PI3K signaling. Alone or in combination mTOR, AKT, or MEK inhibitors with Fulvestrant improved endocrine therapy.⁵³ Luminal B are more aggressive and endocrine therapy resistant than luminal A. Moreover they are known with a different rate of PI3K pathway activation.^{53,54}

Primary and secondary endocrine resistance can be overcome by additional agents like PI3K and CDK4/6 inhibitors. Genetic alterations were noted in sensitive endocrine patients; however, de novo resistance was related to loss of ER, loss of amplification of co-receptor or co-amplificatory molecules as well as to activation of the cyclin D pathway. In the case of acquired resistance, which develops after an initial response to endocrine therapy, the activation of the PI3K pathway represents a common resistance mechanism.⁴³ Previous studies have determined the relationship between *ESR1* and *PIK3CA* mutations with clinical features. Thus, mutations of the PI3K/mTOR pathway observed in one-third of ER⁺ cases associated with good prognosis in early stage and with poor prognosis in the metastatic setting after start of endocrine therapy.⁴³ A recent study that used cfDNA to detect mutations in the *ESR1* and *PIK3CA* genes revealed higher level of heterogeneity in *ESR1* than *PIK3CA*.⁵⁵ *PIK3CA* mutation occurs in the early stage of tumor development;⁵⁶ whereas the *ESR1* mutation occurred later during endocrine treatment and was detected in metastatic lesions.⁵⁷ Therefore, a mutation might result from the pressure endocrine therapy exerts on tumors. Duration of response and development of resistance are related to the rate of *ESR1* mutation.⁵⁷ In ER⁺ patients resistance to endocrine therapy deriving from aberrant activation of PI3K signaling proved to benefit from the combination of a PI3K inhibitor with the antiestrogen. Combination of Alpelisib and Letrozole were more effective in ER⁺/HER2 negative metastatic patients with *PIK3CA* mutation, patients with *FGFR1/2* amplification and *KRAS* and *P53* mutations.⁵⁸ A limited number of clinical trials of luminal B HER2-enriched combination between HER2-targeted therapy (lapatinib or trastuzumab) and aromatase inhibitors provided a clinical benefit.⁵⁹

Further, combinatorial treatment based on cell cycle arrest agents targeting CDK and endocrine therapy has

been proven successful, especially in those cases with primary endocrine resistance. Several studies have investigated the synergistic effect of the CDK4/6 inhibitor Palbociclib and endocrine therapy in both endocrines sensitive and endocrine-resistant cases; the combined action of these drugs leads to improved prognosis. Additional agents could be deployed to target methylation by histone deacetylase; this enabling to reverse resistance.⁴³ Hypermethylation of CPG islands within the promoter of *ESR1* gene is associated with lack of response to TAM. Methylation of *PITX2* has also been related to TAM-resistance. Methylation analysis of candidate genes could help in sparing patients from ineffective treatment by indicating the administration of demethylation agents such as 5-azacytidine (vidaza) or decitabine (5aza 2-deoxycytidine, dacogen) along with histone deacetylase inhibitors like vorinostat or romidepsin.¹⁵ TAM metabolites 4-OH TAM and N-desmethyl TAM (endoxifen) have a higher affinity than TAM for ER. Patients with defective alleles of *CYP2D6*, an enzyme involved in drug metabolism, are less responsive to TAM. In this regard, the mutation G1934A in the *CYP2D6* splicing site resulting in loss of enzyme activity.³ Because the use of TAM, especially in association with chemotherapy, increases the risk of thromboembolic events, breast cancer patients should be screened before prescribing the drug.^{60,61} Although promising, targeted therapy might not be applicable to all patients; therefore the development of new agents based on pharmacogenomics and pharmacogenetics, resistance profile and maintenance of systemic hemostasis is needed. As highlighted above, drug resistance remains one of the most prominent clinical obstacles in breast cancer treatment. Studies have shown that 40–50% of ER⁺ breast cancer patients ultimately develop TAM-resistance. Thus, useful biomarkers are needed for the early diagnosis of these TAM-resistant patients.^{62,63}

HER2 positive

HER2 amplification has been identified in >14% of metastatic breast cancer that associated with increased cell proliferation, angiogenesis, invasion and reduced apoptosis.⁶⁴ It has been found that in HER2⁺ tumors there are compensatory driver genes like *BRF2* and *DSN1*, which undergo amplification or overexpression as oncogenes that bestow a neoplastic advantage.²⁹ HER2⁺ patients are sensitive to HER2 antibodies and kinase inhibitors lapatinib, pertuzumab, trastuzumab, and ado-trastuzumab and emtansine.⁶⁵ Trastuzumab (Herceptin™,

Genentech/Roche, South San Francisco, CA, USA) was the first humanized monoclonal antibody against the domain IV of human epidermal receptor 2 to be developed.⁶⁶ This antibody was the first to receive FDA approval as a targeted drug for breast cancer.⁶⁷ In some clinical studies, a combination of trastuzumab with standard chemotherapy regimens resulted in better response rates than prescription chemotherapy alone.^{68–70} Subsequent studies, however, showed that some patients tend to develop resistance to therapy.⁷¹ The predominant mechanisms of resistance to trastuzumab appear to be related to the HER2 signaling pathway^{71,72} and be associated with activating mutations in *PIK3CA*, *RAS*, *Src*, *NF-KB* and inactivating mutations in *PTEN*, a negative regulator.^{73–75}

Moreover, truncated isoforms of HER2, which lack trastuzumab target epitope,⁷⁶ constitute an active source of HER2 generation due to the formation of stable HER2 homodimers.^{77,78} Overexpression of EGFR and HER-3 – the HER2 co-receptors – and their ligands,⁷³ interaction with adhesion molecules such as MUC1-C or MUC4^{79,80} and incorporation into heterologous receptors (HER-3) with HER2, are additional mechanisms of resistance to trastuzumab. Targeted strategies to overcome resistance to this drug include: 1) use of pan PI3K inhibitor, specific PIK3CA inhibitors, AKT inhibitors, and mTOR inhibitors when resistance results from PIK3CA alterations; 2) Lapatinib chemotherapy to overcome the high level of p95HER2; 3) Tyrosine kinase inhibitors or IGF1R monoclonal antibodies to overcome activation of IGF1R tyrosine kinase receptor; 4) MET inhibitors to overcome MET alterations (mutation and amplification); and 5) immune checkpoints inhibitors to overcome low immune response.⁸¹ Heterogeneity in HER2⁺ breast cancers was associated with failure of target therapy, for example, like in the case of HER2⁺ and luminal molecular subtype that also expresses ER. In this regard, clinical trials highlighted the variability in the efficacy of regimens based on the combination of trastuzumab and endocrine therapy.⁸² As depicted in Table 2, HER2⁺ patients display various molecular alterations and gene expression mutations that may impact prognosis and response to treatment.

Trastuzumab-DM1 (T-DM1: Genentech/Roche, South San Francisco, CA, USA) a novel monoclonal antibody that is conjugated with maytansine – a fungal toxin,⁸⁰ requires a high level of HER2 expression on the targeted cells in order to be effective. Trastuzumab and its metabolites require accumulating in the cytosol of the target

cancer cell to reach an optimum concentration and therefore induce cell death.⁸³ In this context, it appears that low intra-tumor levels of HER2 and poor internalization of the HER2-drug, which is associated with insufficient intracellular expression of DM1, represent the primary mechanisms to drug resistance.⁸⁴ CYD985 is another antibody-drug conjugate administered to and well tolerated in TDM1 pretreated patients.⁸⁵ Lapatinib (Tykerb™, GlaxoSmithKline, Research Triangle Park, NC, USA) is a tyrosine kinase receptor inhibitor that inhibits autophosphorylation of both EGFR and HER2.^{86,87} Administration of lapatinib in combination with trastuzumab represents a strategy to target both intracellular and extracellular domains of HER2.⁸⁸ However, the main issue related to lapatinib is the acquired resistance due to the Presence of HER2 overexpression in tumor cells which are primarily sensitive to lapatinib apoptotic effects.⁸⁹ Activation of AXL, a membrane-bound RTK, constitutes another mechanism of resistance to lapatinib.⁹⁰ Pertuzumab (Omnitarg™, Genentech/Roche, South San Francisco, CA, USA), a humanized monoclonal antibody that binds to an extracellular domain of HER2, different from that bound by trastuzumab, inhibits hetero and homodimerization of HER2⁹¹ and could partially reverse resistance to trastuzumab in metastatic breast cancer patients.⁹² Administration of pertuzumab in combination with lapatinib could be a promising strategy to overcome lapatinib resistance, as demonstrated in an animal model. Pertuzumab inhibited NRG1-induced signaling that is activated 24 hrs following lapatinib administration.⁹³ Ertumaxomab (Rexoman™, Fresenius Biotech, Hamburg, Germany) is an antibody that activates and aggregates T-cells, NK-cells, dendritic cells, and macrophages through the formation of the HER2-drug-CD3 complex.⁹³ Although the drug is still being evaluated in clinical trials, it might represent a promising targeted therapy. The presence of *PIK3CA* mutation might account for resistance to anti-HER2 therapy in HER2⁺ tumors. Neratinib has received increasing interest in the treatment of recurrent and metastatic tumors characterized by non-amplifying HER2 alterations.⁹⁴ Neratinib, a reversible tyrosine kinase inhibitor, can inhibit HER-1, HER2, and HER-4.⁹⁵ In a study including 1,420 women with breast cancer, neratinib significantly improved disease-free survival by 12 months.⁹⁶ In the presence of *HER2* mutations, afatinib, another kinase inhibitor has also been used. Afatinib efficacy has been evaluated both in vivo and in vitro.⁹⁷ The duration of afatinib effects is longer compared with other

reversible EGFR inhibitors.⁹⁸ In a recent study, administration of afatinib with letrozole to metastatic breast cancer patients resulted in stabilization of the disease in 54% of cases, who previously progressed while they were on letrozole therapy.⁹⁹

Triple negative

Triple-negative cancers include three subtypes: normal like, basal-like and non-basal like; 75% of basal-like cancers are TN, and 80% of these TN breast cancer display *P53* mutations (nonsense and frameshift).¹⁰⁰ Basal and non-basal TN also present a different type of mutation, ie, the homologous recombination deficiency (HRD) and homologous recombination repair (HRR). Homologous recombination is the repair mechanism of double-strand DNA breaks (DSB). Several clinical trials on patients with TN cancers highlighted the importance of HRD genomic signature in the prediction of therapeutic response.^{100–102}

The COSMIC database had identified the genes mainly involved in TN variations. These genes have been extensively studied and include *TP53*, *BRCA1*, *PIK3CA*, *RB*, and *PTEN*. In TN tumors, there is no correlation between *TP53* and *BRCA1* mutations,¹⁰³ although the methylation rate of *BRCA1* was found to correlate with the *TP53* mutation.¹⁰⁴

Increased copy number and mutations of the *PIK3CA* gene, loss of *PTEN* and *INPP4B* and overexpression of EGFR were found to activate the PI3K pathway. In the basal-like tumor, 72% of cases are *RB/P16*⁺ and display p53 overexpression, which is correlated with high proliferation. Further, mutations in *BRCA1*, *PTEN*, *ERBB2* genes were correlated with increased risk of metastasis.¹⁰⁰ Genetic and epigenetic alterations in the DNA repair system may act as initial causes of cell transformation. Drugs targeting cells with a deficit in the DSB repair are highly relevant as anti-cancer agents. These are platinum-based compounds that lead to DSB.¹⁰⁵ The PARP inhibitor is effective against cancer cells with a deficit in DNA repair of DSBs. This inhibitor blocks the activity of the PARP enzyme, of which PARP1 is an important target indicating the presence of single-strand breaks. Therefore, the inhibition of PARP1 leads to more “unrepaired” single-strand breaks. These breaks in replication forks lead to the formation of DSB that cannot be repaired in *BRCA1* or *BRCA2* defective cells, thereby leading to the accumulation of DSB and subsequent cellular death.¹⁵ There are similarities between *BRCA1* mutated breast cancer and basal-like breast cancer. The TN cancer due to inherited *BRCA1* mutation is the same as basal-like breast cancer in the presence of acquired

BRCA1 mutations or other mutations in genes within same pathways. This phenotype has been found in the sporadic case of basal-like or TNBC. PARP inhibitors initially gave promising results in TN tumors; however, subsequent trials failed to confirm these findings. Therefore, it remains unclear which subset of TN or inherited *BRCA1* or *BRCA2* can benefit from treatment with the PARP inhibitors olaparib and iniparib.^{106,107} Genetic studies of TN and basal-like cancers have reported defects in *BRCA1* resulting in half of TN tumors having acquired *BRCA1* methylation. Preclinical studies indicate that the use of *BRCA1* methylation might present a promising biomarker of response to PARP inhibitor.¹⁵ In the case of mutation or methylation impacting *BRCA1* and *BRCA2*, defective cells could benefit from platinum-based agents like cisplatin. By crosslinking to DNA, this drug can target the lesions that are ineffectively repaired because of DSB formation. Mutations in the *TP53* gene have been associated with resistance to cisplatin treatment.¹⁰⁸ In TN subtype, mutations in the PI3K/AKT/mTOR pathway could be overcome by target therapy in combination with chemotherapy.⁵

Chemotherapy

One of the cornerstones in the treatment of disease, both when dealing with primary and metastatic breast cancer, is to administer medications systematically.¹⁰⁸ Until now numerous classes of drugs used in the treatment of breast cancer have been given systematically, and these include anthracyclines such as doxorubicin, epirubicin, and mitoxantrone that have pleiotropic effects¹⁰⁹ and induce cell death via a number of proposed mechanisms.^{110–112} Resistance to anthracyclines is related to the increase in expression of P-170 glycoprotein that results in increased drug efflux.¹¹³ A number of approaches have been applied to overcome tumor resistance to anthracyclines, and these are based on the use of fostriecin, merbarone, aclarubicin and bis (2,6-dioxopiperazine) as novel inhibitors of topoisomerase II,^{114,115} altered topoisomerase II,¹¹⁶ use of non-cross-resistant drugs after administration of anthracyclines^{117,118} and changes in the route and time of drug delivery.¹¹⁹ Another class of drugs, the taxanes, includes paclitaxel, docetaxel, and nab-paclitaxel that bind to microtubules with high-affinity; inhibit mitosis and therefore disrupting cell division.¹²⁰ Cancer cells manifest resistance to these drugs by changes in the expression levels of beta-tubulin isotopes and by upregulating caveolin-1.¹²¹ The effects of cyclosporine A, PC833 and verapamil have been evaluated with the view of overcoming resistance to this class of chemotherapeutic agents.¹²² New microtubule inhibitors like

ixabepilone and eribulin were developed to overcome resistance against anthracyclines and taxanes; these were approved by FDA but not by EMA.¹²³ Recently, irinotecan pegol (NKTR-102), a long-acting topoisomerase I inhibitor, has been launched as a new agent to overcome resistance related to anthracyclines and taxanes.^{124,125} A study reported that CPG island hypermethylation in the promoter of the *GSTP1* gene was associated with a therapeutic response to doxorubicin.¹⁵ Antimetabolites such as methotrexate (MTX) and 5-fluorouracil (5-FU) inhibit dihydrofolate reductase (DHFR)¹²⁶ and thymidylate synthase enzymes¹²⁷ respectively. Resistance to MTX may be conferred by a decrease in drug uptake,^{128,129} increase in drug efflux,^{130,131} reduction in polyglutamation,¹³² increase in the level of DHFR¹³³ and expression of Bcl2 during apoptosis.¹³⁴ Resistance to 5-FU results from alterations in enzymes involved in the metabolism of fluoropyrimidine.¹³⁵ Strategies have been evaluated to overcome resistance to antimetabolite drugs. These include manipulation of drug metabolism, using high-dose medications and the development of novel antimetabolites.¹³⁶ Alkylating agents and platinum-based drugs alkylate DNA through the formation of reactive intermediates formation.¹⁰⁹ Tumors can develop resistance to these drugs through decreased alterations linked to transmembrane cellular drug accumulation,¹³⁷ increase in drug inactivation in the cytosol, increase in repair of damaged DNA¹³⁸ and anti-apoptotic mechanisms.¹³⁹ Changes in the regulators of apoptosis have been considered as an approach to overcome resistance observed with the existing drugs.¹⁴⁰ Vinca alkaloids that comprise vincristine, vinblastine, vinorelbine, vindesine, vinflunine, and vinpocetine have clinical uses¹⁴¹ and are also susceptible to multidrug resistance.¹⁴² The mechanism of drug resistance in this class of drugs results from decreased drug accumulation and retention. In this case, methods to overcome resistance have considered administration of DNA polymerase alpha inhibitors like Gemcitabine.¹⁰⁹

New challenges in precision medicine

Despite the progress achieved in targeted therapy, numerous unsolved challenges still exist. The lack of safe and effective drugs is an important issue that might impact the effective control of the disease. Even though the molecular mechanisms underlying the anticancer effects of some common drugs like metformin – an antihyperglycemic drug – remain to be fully understood, growing evidence suggests that

diabetic patients who receive metformin exhibit lower rate of cancers, including breast cancer.¹⁴³ A recent study investigating the effects of metformin on cells overexpressing the tumor suppressor microRNA (miR-200c), showed that the drug inhibits proliferation, migration, and invasion of cancer cells while augmenting the levels of miR-200c.¹⁴⁴ The Authors suggested miR-200c as a potential target for the treatment of breast cancer patients. With the view of revealing the molecular mechanisms involved in the anticancer effects of metformin and to introduce novel therapeutic approaches, a recent proteomics analysis proposed developing further generations of metformin.¹⁴⁵

On the other hand, some factors such as diet and psycho-social status should be considered in personalized medicine. The steroid hormone – vitamin D – which affects almost every cell in the human body plays a significant role in breast cancer prevention.¹⁴⁶

Epidemiological studies have reported the role of vitamin D and vitamin D-binding protein-related genes in the association between vitamin D and the risk of the disease. There is mounting evidence indicating that psycho-social factors such as stress, depression, anxiety, and spiritual status markedly impact the development of breast cancer and response to treatment.^{147–149} It is plausible that the involved molecular mechanisms are used as a step towards precision medicine. Recent studies have shown the potential role for dopamine and serotonin in this context. Experimental research on Iranian women has shown that spiritual intervention causes a decrease in dopamine receptor gene expression in peripheral blood mononuclear cells (PBMCs) of breast cancer patients compared with a control group who did not receive the intervention.¹⁵⁰ Another study on PBMCs of breast cancer patients revealed that serotonin receptor gene expression was increased compared to the healthy group.¹⁵¹ The authors proposed antagonists of serotonin receptors as a new therapeutic agent.

Microbiome profiling is one of the emerging tools in the clarification of the role of environmental factors in the etiology of breast cancer. Recent work indicates there are differences between bacteria present in the normal tumor-adjacent tissue of breast cancer patients and tissue from healthy women.¹⁵²

The advents of modern diagnostics and therapeutics have provided significant advances compared with traditional medicine. Nowadays, novel biomarkers, therapeutic targets and data mining constitute essential parts of

science. Hence, researchers spend a long time analyzing existing databases. Analytic and modeling tools accompanied by experimental modules have been key in providing a comprehensive and meticulous view of the interactions between cellular components in biological processes. This so-called 'systems biology'¹⁵³ proves pivotal in implementing personalized approaches, especially therapeutics.

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