

Review Article

An Intersection Between the Immune System and Cellular Signaling Pathways - The Impact of Anti-HER2 Monoclonal Antibodies on Individualized Approaches to Patients with Breast Cancer

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Abstract

Overexpression or gene amplification of Human Epidermal Growth Factor Receptor 2 (HER2) is present in approximately 20% of breast cancers, and indicates an aggressive tumor behavior. Activated HER homo and heterodimers orchestrate a downstream signaling network that regulates cell growth, proliferation and malignant spread. Trastuzumab, as a monoclonal antibody that inhibits the HER2, has immensely improved the outcome of patients with HER2-positive breast cancer. Since trastuzumab targets abnormal expression of HER2 in tumors, which are 'addicted' to HER2 activation, its therapeutic effects have been attributed to blocking of the intracellular signaling pathways. Moreover, the immune system is involved in the therapeutic impact of trastuzumab or similar anti-HER2 monoclonal antibodies. Various molecular pathways are relevant to HER2 overexpression, as well as the expression of other members of the Epithelial Growth Factor Receptor (EGFR) family. In addition, the estrogen and progesterone activity can interfere with the effects of trastuzumab and/or Chemotherapy (CHT) regimen, especially in patients with metastatic, HER2-positive malignancy.

This article presents some insights to the signaling pathways, relevant to progression of breast cancer. It explains molecular characteristics that play a key role in affecting tumor response and resistance to HER2-targeted treatments. It also highlights the need for patient stratification, to develop the most reasonable de-escalation approaches, using HER2-targeted monoclonal antibodies. In addition, some practical strategies to overcome the resistance to trastuzumab, via targeting the main signaling pathways, in the context of HER2-positive and Hormone Receptor (HR)-positive metastatic or locally advanced breast cancer have been discussed, based on evidence from recent clinical trials.

Keywords: Breast Cancer; Estrogen Receptor (ER); Human Epidermal Growth Factor Receptor 2 (HER2) Monoclonal Antibodies; Trastuzumab; Signaling Pathways

Introduction

Breast cancer has been categorized into certain subtypes, based on the expression of Hormone Receptors (HR) for estrogen and progesterone, and overexpression/amplification of Human Epidermal Growth Factor Receptor 2 (HER2). This expression has been related with specific implications for therapy. Overexpression

and/or gene amplification of HER2, a key member of the HER family of four receptors, is present in approximately 15-20% of breast cancers, and indicates an aggressive tumor behavior [1]. Activated HER homo and heterodimers organize a complex downstream signaling network that regulates cell metabolism, proliferation and metastatic spread. Recently, more and more patients have been diagnosed with HER2-positive breast cancer. As a consequence, some patients can receive targeted therapies, even though they may not be helpful, partially due to a large variability among women classified as having HER2-positive breast tumors. To solve this

problem, a better patient stratification is needed, in order to more accurately predict response to anti-HER2-directed treatments [1].

Monoclonal antibodies have been designed to react against specific antigens on cancer cells, which can help to increase the patient’s immune response and inhibit malignant cell growth. Trastuzumab, as a member of this class, has greatly improved the outcome of patients with HER2-overexpressing breast cancer [2]. Since trastuzumab targets abnormal expression of HER2 in tumors, which are ‘addicted’ to HER2 activation, its therapeutic effects have been attributed to blocking of the intracellular signaling pathways. In addition, the immune system is involved in the therapeutic impact of trastuzumab (or similar monoclonal antibodies). For this reason, trastuzumab should be considered for all patients with early-stage HER2-positive breast cancer in the neoadjuvant (pre-surgical) setting (except from small tumors, which have not spread to lymph nodes). It should be underscored that the breast cancer progression can be related, among other factors, to various mechanisms of resistance to anti-HER2 therapies in HER2-positive tumors [3]. In particular, the Crosstalk of Estrogen (ER) with HER2 and the other members of the EGFR family has often been considered as a contributor to breast cancer progression, and resistance to HER2 targeted or endocrine therapies [4].

This article presents some insights to the signaling pathways, relevant to progression of breast cancer. It explains some molecular characteristics that play a key role in affecting tumor response and resistance to anti-HER2-targeted treatments. It also highlights the need for patient stratification, to develop the most reasonable de-escalation approaches, using HER2-targeted monoclonal antibodies. In addition, some practical strategies to overcome the resistance to trastuzumab, via targeting the main signaling pathways, in the context of HER2-positive and HR-positive metastatic or locally advanced breast cancer (in particular, triple-positive breast cancer) have been discussed, based on evidence from recent clinical trials.

Key Molecular Signaling Pathways Involved in The Response to Trastuzumab In HER2-Positive Breast Cancer

The interactions between the ER and HER2 pathways in patients with breast cancer are very complex. In particular, the growth stimuli binding to receptors such as Epidermal Growth Factor Receptor (EGFR) and HER2 activate intracellular signals, through the PI3K (phosphatidylinositol 3-kinases)/Akt/mTOR (mammalian target of rapamycin) and the MAPK (mitogen activated protein kinases)/ERK (extracellular signal-regulated kinases) pathways. Subsequently, the mTOR phosphorylates downstream kinases, and the S6 kinase activates ER. In consequence, the ER regulates the transcription of many genes (e.g.: PARP (Poly (ADP-Ribose) Polymerase-1), VEGF (Vascular Endothelial Growth Factor), GFR (Guanine nucleotide exchange Factor for Rap1) ligands, and cyclin). The PI3K/Akt/mTOR pathway is a well-known effector of HER2 signaling pathways. It appears that the PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide) mutation or PTEN (phosphatase and tensin homolog protein) loss, despite the HER2 blockage, can allow a treatment escape mechanism (via the PI3K/Akt/mTOR pathway) [4]. In addition, ER that can be activated by Estradiol (E2) and growth factors, can convey its actions through the “non-genomic” patterns (outside of cell nucleus), and influence cellular and mitochondrial membrane proteins [4]. Trastuzumab binds to HER2, and mediates antibody-dependent cellular cytotoxicity against cells, which overproduce HER2, and thus, it is indicated for adjuvant treatment of HER2-overexpressing, node-positive or negative breast cancer (as part of a treatment regimen, including doxorubicin, cyclophosphamide, and either paclitaxel or docetaxel, or as part of a regimen, which also contains docetaxel and carboplatin). In addition, trastuzumab is indicated to be used as a single medication, after anthracycline-based therapy [4].

Pertuzumab, another monoclonal antibody, which binds to the extracellular domain of the HER2 receptor, is indicated for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer (who did not receive previous anti-HER2 therapy or CHT for advanced malignancy), and for the adjuvant treatment of patients with HER2-positive early breast cancer, with high risk of recurrence [4] (Table 1).

Tumor type	HER2 +any ER or PR	HR+ (ER &/or PR) &HER2 -	ER & PR & HER2 - ‘triple negative’	HER2+ ER-
Prevalence	about 15%	about 70% (more common in older patients)	about 15% (more common in younger patients)	more common in younger patients
Molecular subtype	Luminal B &HER2 expressing	Luminal A	Basal-like	HER2+ ER-
Receptor status and tumor behavior	ER+ &/or PR+, HER2+ More aggressive, high grade histology	ER+ &/or PR+, HER2- Less aggressive, low grade histology	Cytokeratin 5/6+ &/or EGFR+ Aggressive, High grade histology	HER2+ ER- Highly aggressive, high grade histology

Type of therapy	anti-HER2 therapy & CHT; Endocrine therapy if HR +	Endocrine therapy; CHT for some patients	CHT	anti-HER2 therapy
Common adjuvant treatment regimens	Docetaxel, Carboplatin Trastuzumab (TCH) Paclitaxel, Trastuzumab (TH) Adriamycin Cyclophosphamide (AC) Dose dense Adriamycin Cyclophosphamide Paclitaxel (AC-T)	Tamoxifen Aromatase inhibitors: Anastrozole Letrozole	Cyclophosphamide Methotrexate 5-fluorouracil (CMF)	
Outcome	improved outcome with anti-HER2 therapies	good outcome, hormone responsive, genetic tests may help select patients who can benefit from CHT	improved outcome with CHT	improved outcome with anti-HER2 therapy
Relapse	<5 years	>5 years	<5 years	
Abbreviations: +, positive; -, negative; &, and; CHT, chemotherapy; HER2, human epidermal growth factor receptor type 2; HR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor				

Table 1: Biologic subtypes of breast cancer - receptor status, pharmacologic therapies and outcomes.

Lapatinib is a Tyrosine Kinase Inhibitor (TKI), which reversibly blocks phosphorylation of EGFR, HER2, Erk (Extracellular Signal-Regulated Kinase)-1 and-2, and AKT kinases [5]. In addition, lapatinib-induced accumulation of HER2 at the cell surface seems to augment immune-mediated trastuzumab-dependent cytotoxicity. According to some studies, inhibition of IGF1R (insulin-like growth factor-1 receptor) activity enhances response to trastuzumab in HER-2-positive breast cancer cells that in turn, may postpone progression of breast malignancy, or decrease resistance to trastuzumab [5]. In particular, a truncated part of the HER2 receptor, also known as p95HER2, and IGF-1R have been known to as biomarkers of resistance to anti-HER2 treatment. Furthermore, a crosstalk between HER2 and IGF-1R has been reported in trastuzumab-resistant cancer cells. Moreover, the blockade of IGF-1R tyrosine kinase activity can contribute to decreased HER2 phosphorylation, and improvement of sensitivity to trastuzumab [5].

Another important component of the signaling pathway are cMET and Src [6,7]. cMET is a receptor tyrosine kinase, which promotes cellular proliferation via MAPK, PI3K, and STAT (signal transducer and activator of transcription) [6]. Src is a proto-oncogene encoding for the non-receptor protein kinase Src, which interacts with Transmembrane Receptor Tyrosine Kinases (RTKs), including HER1 and HER2 [7]. It appears that cMet over-expression, Src activation, in combination with the immune response also represent possible mechanisms, which may allow progression of breast cancer during anti-HER2 treatment [6]. In addition, activation of Src is responsible for tumor progression, after developing resistance to trastuzumab. Also, resistance to lapatinib has been reported in Src activated cell lines [7].

Can we de-escalate therapy of HER2-positive breast cancer? Finding a ‘right’ balance between the response and resistance to treatment

In spite of using anti-HER2-targeted therapies, several HER2-positive breast tumors remain unresponsive, or develop resistance to treatment. Such a therapeutic failure can be related to inaccurate selection of the tumors that are physiologically dependent on HER2 for survival, and thus, would have the biggest advantage from anti-HER2-directed treatment. In such HER2-‘addicted’ tumors (meaning physiologically dependent), resistance can be caused by an incomplete blockage of signaling at the HER receptor. Some clinical studies have reported that the combination anti-HER2 therapy is more effective than a single agent [8].

In addition, HER2 can be activated by mutations or aberrations in HER2, or in other HER family members. Even if a complete HER inhibition is attained, resistance to anti-HER treatment may emerge, due to some other mechanisms, such as alternative signaling pathways (e.g.: the ER, or abnormal downstream signaling of the PI3K pathway), or changes in the tumor microenvironment [8]. It should be pointed out that the majority of the clinical studies, which have explored the efficacy of anti-HER2 therapies were conducted in the context of aggressive CHT regimens. For this reason, the detection of main factors of resistance to the anti-HER2 therapies was difficult [8]. However, current studies have indicated that some HER2-amplified tumors can derive benefits from anti-HER2 therapy, in combination with only a single CHT medication, or even without CHT. At present, such a de-escalation strategy, undergoes clinical investigation.

The combination of endocrine therapy and anti-HER2 therapy - consideration of maintenance treatment for ER positive/HER2 positive metastatic breast cancer

In the past decade, the largest progress among all types of breast cancer, has occurred in the HER2-positive subtype, mostly due to the introduction of targeted anti-HER2 therapies, such as trastuzumab, pertuzumab, or other newer agents from this class [9]. However, this remarkable progress has also caused several adverse effects (e.g., cardiotoxicity), as well as many questions relevant to the most appropriate combinatorial or sequential use of these medications. On the one hand, targeting of the ER by using the selective ER modulators (e.g., tamoxifen - the standard prevention of breast cancer in high-risk women, an adjuvant treatment for breast cancer, and in metastatic breast cancer), aromatase inhibitors (AIs, such as anastrozole or letrozole) or ER downregulating agents (e.g., fulvestrant) represents a reasonable therapeutic option, in a properly selected patient population. On the other hand, however, results of Overall Survival (OS) are mostly derived from trials investigating various combinations of anti-HER2 medications and CHT. In this situation, the role of endocrine therapy combined with anti-HER2 medications for patients with ER positive /HER2 positive breast cancer still needs to be determined. It appears that the TANDEM study, analyzing trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with HER2-positive, HR-positive metastatic breast cancer, shed some light on this common clinical scenario. In particular, in the TANDEM trial, the OS analysis has revealed a small benefit for patients in the combined therapy arm [10].

Combined application of anti-HER2 and endocrine therapy has been especially useful as maintenance treatment for patients with ER positive/HER2 positive breast cancer, after initial cycles of CHT plus anti-HER2 therapy. Although no RCTs are yet available, the existing clinical experience and relatively low toxicity offer this option, which may delay the cancer progression, and the subsequent necessity to use potentially toxic CHT. Since the exact duration of anti-HER2 treatments is still undetermined, in patients with the metastatic breast cancer, it is essential to answer this question, due to possible therapeutic benefits. As an illustration, new data from the German Breast Group 26/breast international group 03-05 study suggest continuing trastuzumab beyond progression in patients with HER2 positive advanced breast cancer. On the other hand, however, the recommended duration of such therapy still remains unknown and there is no evidence to support the use of dual HER2 blockade (e.g., trastuzumab and pertuzumab), beyond neoplastic progression [11]. Another unresolved clinical issue is related to the optimal duration of trastuzumab treatment in patients, who achieved long-term complete remission. This has to be analyzed, keeping in mind toxicity (e.g., cardiac adverse effects), and medical expenses. In absence of concrete data with regard to therapeutic decision making in this scenario, stopping

trastuzumab in clinically stable patients (especially, if treatment re-challenge is possible in case of neoplastic progression), seems to be a reasonable option. Furthermore, it is important to point out that the strict inclusion and exclusion criteria of many Randomized Clinical Trials (RCT) do not involve a patient population, which has been treated in 'real life' hospital practice. To address this gap, the efficacy of anti-HER2 treatment with trastuzumab, pertuzumab and a taxane for HER2-positive metastatic breast cancer, was evaluated in a 'real life' setting, and compared to the results from the CLEOPATRA trial. Findings of this study confirmed that trastuzumab, pertuzumab, and taxane are effective and safe in clinical setting [12].

Also, in HER2 positive early breast cancer, a dual HER2-targeted combination of trastuzumab and pertuzumab, together with neoadjuvant CHT (including taxane-epirubicin-cyclophosphamide) resulted in improved rates of pathologic Complete Response (pCR) [13]. This evidence indicates that the dual blockade with trastuzumab and pertuzumab in combination with CHT, as first line therapy, provides advantages in terms of OS and PFS, and thus, should be considered in the management of patients in a daily practice. There are still some unresolved issues in the care of patients with HER2 positive breast cancer. In particular, there are no data on the role of dual blockade for patients, who relapsed during one year of adjuvant trastuzumab (since majority of such patients were excluded from RCTs). This situation underscores current unmet needs, for which the future studies are necessary. For instance, in the early stage of breast cancer (e.g., in the neoadjuvant setting), the best treatment options for patients, who had relapsed, after treatment with CHT and two anti-HER2 agents (trastuzumab and pertuzumab), would be merited. The above mentioned unresolved issues, as well as the most optimal sequence of therapies for individual patients would be very difficult to assess in RCTs, without using a valuable set of biomarkers. At this point, SystHERs Registry Study, providing documented records of therapies and outcomes, beyond cancer progression in patients with HER2-positive **breast cancer, in the hospital setting** is very useful [14]. In general, in the treatment of HER2 positive **breast cancer**, therapeutic decisions should be based on the tumor molecular characteristics, clinical profile, efficacy and toxicity of therapeutic agents, and patients' choices. Also, it is expected that the results of future RCTs will create more precise recommendations for clinical practice.

Overcoming resistance to trastuzumab - important lessons learned from triple-positive breast cancer

Triple-positive breast cancer has HER2-positive, as well as ER and PR positive status (Table 1). Unfortunately, the estrogen and progesterone activity can unfavorably interfere with the effects of trastuzumab and/or CHT regimen. In particular, in women with metastatic, HER2-positive malignancy, ER expression in over one

third of neoplastic cells may predict a decreased probability of response to treatment with trastuzumab and CHT. In consequence, the management of such patients should include combinations and/or sequences of anti-HER2-targeted medications and CHT, as well as therapies, which block the endogenous production of cancer-stimulating hormones. Endocrine therapies can help prevent the treatment resistance that may occur due to targeting only a single signaling pathway. However, many patients with metastatic triple-positive breast cancer may not have significant advantages, when blocking of the HER2 pathway increases ER signaling. To address this clinical challenge, the PERTAIN trial has been conducted, aiming to explore, whether or not adding endocrine therapy would improve response to dual HER2 blockade, among postmenopausal women with HR-positive and HER2-positive locally advanced metastatic breast cancer [15].

In the PERTAIN trial, patients were randomized to receive pertuzumab, trastuzumab and an Aromatase Inhibitor (AI) (that blocks endogenous production of estrogens), versus trastuzumab and an AI only. In addition, some women have also received CHT (including docetaxel or paclitaxel prior to initiation of the AI/anti-HER2 treatment). The results have revealed that the median PFS was longer in the group that received pertuzumab, trastuzumab, and AI, compared with trastuzumab and an AI only regimen. These findings support adding the AI therapy after CHT in women with triple-positive breast cancer. Therefore, combining an AI with two anti-HER2 medications, after CHT regimen, can be more effective than combining endocrine therapy with CHT [15]. According to current international guidelines [16,17], therapy for patients with advanced HER2-positive breast cancer includes a combination of trastuzumab, pertuzumab, and a taxane (mostly based on evidence from the CLEOPATRA trial [18]).

However, it should be underlined that such a treatment has to be individualized for patients with: ER-positive/HER2-positive malignancy, congestive heart failure, metastases to central nervous system, hypersensitivity to taxanes, as well as in elderly and frail patients. Unquestionably, more precise recommendations with regard to the sequence of CHT, endocrine therapy and anti-HER2-targeted medications, for women with triple-positive breast cancer are needed. Therefore, further research should be focused on the bidirectional crosstalk between the ER and HER2 signaling pathways, and on the detection of subpopulation of patients, who would benefit from estrogen deprivation, in case of triple-positive breast cancer.

ER-positive, HER2-positive or HER2-normal breast cancer - possibilities of using concurrent hormonal therapy and HER2-targeted therapy

In the past, cancers that expressed both ER and HER2 were considered to be resistant to hormone therapy, due to a possible dominant role of the HER2 pathway. However, at present, many

analyses of ER-positive, HER2-positive breast cancers have indicated that there are some cancer subtypes, which can be sensitive to endocrine treatments (Table 1) [19]. Inhibition of HER2 alone in ER-positive, HER2-positive breast cancers may allow ER to embark on an escape pathway. This, in turn, may contribute to resistance to HER2-directed medications [20]. Similarly, it has been suggested that HER2 signaling plays a role in resistance to hormonal treatments [21]. Due to the bidirectional crosstalk between the ER and HER2 pathways, a dual approach, in which both pathways are being blocked concurrently, can represent the best therapeutic strategy to overcome the resistance, related with targeting either pathway alone [21]. An addition of lapatinib (a TKI that targets both EGFR and HER2), to letrozole, among women with ER-positive, HER2-positive metastatic breast cancer has been evaluated. In this study, an improvement in PFS in patients who were randomly assigned to lapatinib arm was reported [22]. Convergently, in the Cancer and Leukemia Group B (CALGB) trial, PFS was also improved in postmenopausal patients with ER-positive, HER2-positive advanced breast cancer, receiving lapatinib and fulvestrant compared to fulvestrant alone [23]. Based on the CALGB trial, it has been demonstrated that there was no benefit for the addition of lapatinib to fulvestrant in patients with ER-positive, HER2-normal cancers [23]. Likewise, no difference in PFS, upon the addition of lapatinib to letrozole, in patients with ER-positive, HER2-normal breast cancer was noted [22].

It appears that inhibition of mTOR, which is a final common protein downstream of these growth factor receptors, seems effective, perhaps be due to a compensatory signaling via other growth factors [24]. As an illustration, a better outcome (improved PFS) was reported in a study exploring inhibition of mTOR pathway with everolimus, added to endocrine therapy, in postmenopausal women with ER-positive, HER2-normal advanced breast cancer [24]. Furthermore, breast cancers which express both HR and HER2 display a great heterogeneity. For instance, about 30% of these cancers represent a luminal A biological subtype (phenotype). It is expected that the stratification, based on these subtypes, to detect which HR-positive, HER2-positive cancers should be treated with a combination of ER and HER2 inhibitors, would be beneficial in clinical practice. In this way, many patients could avoid unnecessary and potentially toxic treatment (e.g., CHT). At present, the double blockade of: HER2 - with lapatinib and ER - with letrozole appears to be a reasonable therapeutic choice for women with ER-positive, HER2-positive advanced breast cancer. The mechanisms underlying resistance to endocrine agents in ER-positive, HER2-normal breast cancers are still unclear. However, it seems that this resistance can be mediated by increased signaling via mediators of the EGFR, or activation of the mTOR.

Conclusion

Monoclonal antibodies that bind to the HER2 receptor (e.g.,

trastuzumab and pertuzumab), have improved survival in both early and late stages of breast malignancy, so that every patient with HER2-positive breast cancer should receive trastuzumab. In addition, co-targeting of ER and HER2, in properly selected patients, can bring therapeutic advantages, without jeopardizing the patient safety. Hormonal therapy should be used when anti-HER2 targeted therapy (e.g.: trastuzumab, pertuzumab or lapatinib) has been used without CHT, or when a break in CHT had been scheduled (e.g., after a good initial therapeutic response to CHT).

It is expected that the therapeutic strategies, including co-targeting of ER and HER2 will be explored in large scale clinical trials, when more information about molecular signaling, relevant to progression of malignant breast tumors become available. Decisions on therapy should be based on evidence from clinical trials, and recent recommendations. However, these decisions need to be individualized, considering not only the efficacy and toxicity profiles, but also the patient comorbidities, prior exposures, preferences, compliance, availability and financial costs. Furthermore, providing patients with the clear information about benefits and risks of each therapy should enhance their active engagement in a long and complicated treatment process.

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