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overview

Role of HER2 signaling pathway in breast cancer: biology, detection and therapeutical implications

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Abbreviatians:

HER	– human epidermal growth factor receptor
ER	– estrogen receptor
p95HER2	– truncated form of HER2 receptor
FDA	– Food and drug administration
Akt	– protein kinase B
HSP90	– heat shock protein 90
IGF1R	– nsulin-like growth factor receptor 1
MAPK	– mitogen-activated protein kinase
mTOR	– mammalian target of rapamycin
PI3K	– phosphatidylinositol 3-kinase
PTEN	– phosphatase and tensin homolog
VEGFR	– vascular endothelial growth factor receptor
OS	– overal survival
DFS	– disease free survival
PFS	– progression-free survival
ORR	– overall response rate
ADCC	– antibody-dependent cell-mediated cytotoxicity

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Abstract

Human epidermal growth factor receptor 2 (HER2), a member of the ErbB family of transmembrane receptor tyrosine kinases, is amplified in 20–30% of invasive breast cancers. HER2 amplification is associated with metastasis and reduced survival. Therapies targeting HER2 receptor are effective in adjuvant treatment and in metastatic disease. Several agents have demonstrated promising activity in HER2 metastatic breast cancer, either as monotherapy or in combination therapy, including the tyrosine--kinase inhibitors lapatinib and neratinib, and the anti-HER2 monoclonal antibodies trastuzumab, pertuzumab and trastuzumab-DM1/T-DM1/. Despite the improvement in overall survival with the addition of HER2--targeted agents to chemotherapy, many patients do not benefit from the agents because of inherent resistance. In addition, many patients who achive an initial response eventually acquire drug resistance. Several mechanism of resistance have been described, including mutations in other signalling pathways, expression of a truncated form of HER2, receptor crosstalk, and autophagy.

INTRODUCTION

Breast cancer is the most common type of cancer and the second most common cause of cancer death in female population. There were reported 1.4 million incident cases worldwide in 2008 *(1)*. According to data of Cancer registry of Croatian National Institute of Public Health there were 2498 new cases of breast cancer in 2008, 2473 in women and 25 in men *(2)*. Almost half of them will relapse and eventually die, as breast cancer in metastatic setting is an incurable disease.

Human epidermal growth factor receptor 1, 2, 3, 4 are family of receptors expressed in many types of cancer and also normal tissues. Human epidermal growth factor receptor-2 (HER2/neu, c-erbB2), one of a family of four membane tyrosine kinases, was found to be amplified in a human breast cancer cell line *(3)*. HER2 amplification resulted in Her2 protein overexpression which has been linked to tumor cell proliferation and cancer progression *(4)*. Targeted therapies are developed to bind specific molecules in signaling pathways important for cancer development and progression, providing most effective therapy in appropriately selected patients.

Biological role of HER2 signaling pathway

HER2 signaling pathway is a complex network comprised of membrane receptor and their ligands, protein kinases and regulating genes

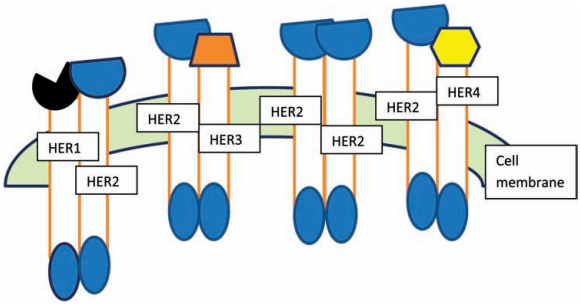

Figure 1. *The HER family of receptors and their homo and heterodimerization pairs.*

that affect various cellular functions. Four transmembrane receptors/tyrosine kinases (TKs) HER1 to HER4 and their multiple ligands form an input layer *(5)*. HER2 is the dominant TK receptor in breast cancer, being amplified in approximately 20% of cases, with unknown ligand. It is activated by homo- or heterodimerization with another family members (Figure 1).

The phosphorylated HER dimers activate downstream cell proliferation (mitogen-activated protein kinase, MAPK pathway), cell survival (phosphoinositide 3-kinase), and signal transducer and activator of transcription pathways *(6)*. HER2 has the strongest catalytic kinase activity among family members, and HER2 containing heterodimers have the strongest signaling activity. HER3 is activated by ligand (heregulin) binding bit it lacks TK activity, and must form heterodimers with another family member to be activated. HER2 can also be activated by complexing with other membrane receptors such as insulin-like growth factor receptor 1 *(7)*.

HER 2 overexpression

Normal tissues express low levels of HER2 membrane protein. Overexpression of HER2 is found in breast and some ovarian and gastric cancers. HER2 gene is amplified on 25–50 copies in breast cancers (8), with 50% in situ carcinomas being HER2 positive, while only 20% of invasive breast cancers are HER2 amplified *(4)*.

HER2 amplified breast cancers have unique biological and clinical characteristics. They are mores sensitive to doxorubicin possibly due to coamplification of the topoisomerase-2 gene, gene also located on chromosome and targeted by the antracyclines, and relatively resistant to hormonal agents *(9)*.

HER2 overexpression detection in breast cancer

HER2 testing is used to select patients for potentially curative and expensive therapy. Currently is testing carried out by several methods: immunohistochemistry (IHC) for detecting protein overexpression, fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and silver enhanced in situ hybridization to evaluate gene amplification. The report of HER2 FISH results must follow the standardized form recommended by ASCO/CAP guidelines *(10)*.

HER2 extracellular domain (ECD) levels can be found elevated in patients with HER2 negative tumours (by IHC or FISH/CISH/SISH) *(11,12)*.

Immunohistochemical detection (IHC) uses monoclonal or polyclonal antibodies that bind to the protein. Currently in the U.S.A. there are two test approved for HER2 assessment: HerceptTest™ (DAKO, Glostrup, Denmark) and HER2/neu (4B5) rabbit monoclonal primary antibody (Ventana, Tucson, Arizona). HER2 testing results by IHC fall into three categories: positive, equivocal and negative, thus triggering different patient management strategies *(13)*.

HER2 molecular analysis has become an integral part of the diagnostic breast cancer patient work-up. The principles of in situ hybridization are simple: use of labeled DNA probes complementary to genomic sequences of interest and hybridization of them to the target tissue.

Fluorescent in situ hybridization (FISH) utilizes fluorescence microscopy and positive result is defined as an average of more than 6 HER2 gene copies per nucleus for test systems without an internal control probe or HER2/CEP17 ratio of more than 2.2 where CEP17 is a centromeric probe for chromosome 17 on which the HER2 gene resides. The negative result is defined as less than four copies of HER2 gene per nucleus for systems without a probe, or HER2/CEP17 ratio of less than 1.8. The equivocal range for HER2 FISH assays is defined as HER2/CEP17 ratios from 1.8 to 2.2 or average gene copy number between 4.0 and 6.0 *(10,14)*.

Chromogenic in situ hybridization (CISH) uses chromogens for signal identification with several advantages over FISH: permanent staining, use of bright field microscopy, easy identification of the target cells *(15)*.

Silver enhanced in situ hybridization (SISH) is a highly sensitive technique with permanent staining, thus allowing specimen archiving *(16)*.

HERmark® test is a new assay that uses the VeraTag system to identify total levels of a cellular protein or two similar or dissimilar proteins in close proximity. Method allows us measuring of total HER2 protein and the amount of HER2 homodimers in breast cancer tissue. According to study results in metastatic HER2 positive breast cancer patients treated with trastuzumab, the assay was superior to FISH and to immunohistochemistry in predicting benefit from trastuzumab therapy *(17)*. In a preliminary report from recently completed adjuvant trial, the HERmark assay declassified 25% of cases originally classified as positive, equivocal, or negative when done by central IHC *(18)*.

Targeting the HER2 receptor signaling pathways

The reason why we need highly accurate determination of HER2 status is current targeted therapy with good

TABLE 1

Therapeutic strategies for HER-2 overexpressing metastatic breast cancer.

Drug name	Class of drug	Mechanisms of action
Trastuzumab	Monoclonal antibody	Binds to extracellular domain, effective against HER-2 homodimers
Lapatinib	Small molecule TKI-reversible	Selective inhibitor of EGFR/HER1 and HER2 intracellular tyrosine kinase, so-called TKI
Neratinib	Small molecule TKI-irreversible	Pan-HER TKI
Pertuzumab	Monoclonal antibody	Binds to different part of extracellular domain than trastuzumab, inhibits hetero-dimerization
T-DM1	Antibody-drug conjugate	Trastuzumab conjugated to an anti-microtubule agent (maytansine)
Afatinib	Small molecule TKI-irreversible	Pan-HER TKI
Ertumaxomab	Trifunctional antibody	Targets HER-2 receptor extracellular domain, CD3 antigen on T cells and activates Fc-gamma receptors

clinical results. Therapeutic strategies for HER2 positive metastatic breast cancer include several anti-HER2 drugs (Table 1), with three drugs being currently FDA approved: trastuzumab, lapatinib and pertuzumab *(19, 20)*.

Trastuzumab is a humanized IgG1 monoclonal antibody against the extracellular domain of the HER2 receptor. Its mechanisms of action is still unclear, but proposed mechanisms include inhibition of HER2 pathway mediated cell proliferation and angiogenesis, immune response against tumor cells via antibody-dependent cell--mediated cytotoxicity (ADCC), acceleration of internalization and degradation of HER2 from the cell membrane leading to receptor downregulation, prevention of homodimerization of truncated HER2 receptors by inhibition of cleavage by metalloproteinases *(21)*. It may also distrupt the HER2/Src interaction. It has sinergistic activity with various chemotherapy drugs, probably by inhibitng important survival pathway. Doxorubicin is more effective when combined with trastuzumab, but high cardiotoxicity rate in concurrent use of trastuzumab and doxorubicine containing regimens demand sequencial use of trastuzumab. Trastuzmab is also beneficial in combination with endocrine therapy in patients with estrogen receptor (ER) positive and HER2 positive tumors. In early-stage breast cancer, adding trastuzumab to neoadjuvant chemotherapy substantially improves overall survival (OS) and reduces the risk of recurrence, both by 33% *(21)*. Similarly, adjuvant trastuzumab has demonstrated a dramatic impact in terms of disease free (DFS) and overal survival (OS). In the adjuvant setting improves DFS by 38% and OS by 34% and reduces the risk of local and distant recurrence by 42% and 40%, respectively *(23, 24)*. Trastuzumab also provides significant benefit for patients with metastatic breast cancer and is moderalely active as single agent in HER2 positive patients with response rates (RR) ranging from 15 to 26% with a median duration of 9 months and a clinical benefits rate, defined as RR plus stable disease >6 months, in 36-48% of the patients. Compared with chemotherapy alone, the combination of trastuzumab and chemotherapy substantially increases the time to progression by 49% and the time to treatment failure by 42% and improves OS by 20% *(25)*.

Lapatinib is a small molecule tyrosine kinase inhibitor that works different then trastuzumab to inhibit HER2 signaling. It blocks both kinases, HER1 and HER2, thus inhibiting downstream signaling for cell proliferation and survival, and induces apoptosis in HER2 and HER1 (EGFR, epidermal growth factor receptor) overexpressing cels. It is expecially important in trastuzumab resistant tumours with shortened form of HER2, so called HER2 p95 *(26)*. These tumors have lost the extracelullar trastuzumab binding domain, but lapatinib should still be effective in blocking tyrosine kinase activity. The most important advantages of lapatinib are oral drug formulation, effective crossing of the blood-brain barrier allowing it to be active in patients with brain metastases. It shows additive effect combined with 5-fluorouracil and its derivates *(27)*.

Novel anti-HER strategies include several agents like pertuzumab, neratinib, afatinib, ertumaxomab and T-DM1. Monoclonal antibody pertuzumab that blocks dimerization of HER2 with other HER receptor family members. This humanized antibody targets a different HER2 extracellular domain than trastuzumab and inhibits HER2 dimerization, shows promising efficacy when added to trastuzumab in several different settings. The recently presented data of CLEOPATRA trial showed progression-free survival (PFS) benefit for dual HER2 blockade *(28)*. In the neoadjuvant setting dual HER2 blockade with pertuzumab and trastuzumab combined with docetaxel showed higher pathologic complete response rate (45.8% vs 29%) than mono HER2 blokade with trastuzumab plus docetaxel *(29)*.

T-DM1, also known as trastuzumab emmtansine, consist of trastuzumab conjugated to an antimicrotubule agent (a derivate of maytansine). The EMILIA study, phase III clinical trial of T-DM1 compared with capecitabin plus lapatinib has shown longer progression-free survival (PFS) and overall response rate (ORR) for patients receiving T-DM1 *(30)*.

Neratinib is orally administered, irreversible pan-HER receptor tyrosine kinase inhibitor. According to results of phase II study of neratinib in patient with prior or no prior trastuzumab *(31),* phase III studies with neratinib are being conducted in women with HER2 postive breast cancer. Afatinib is an oral, small molecule irreversible HER family TKI, investigated in single arm phase II study in patients with trastuzumab refractory metastatic breast cancer *(32)*.

Mechanisms of resistance

Although trastuzumab substantially improves outcomes in both early-stage and metastatic breast cancer, not all patients respond to trastuzumab. This type of trastuzumab resistance is intrinsic or *de novo* HER2 resistance. In early-stage breast cancer, the addition of trastuzumab to neoadjuvant chemotherapy is associated with a complete response (CR) of the breast and lymph nodes in 38%–55% of patients, suggesting intrinsic resistance rate of 45%–62% (21). Some patients respond initially but progress some time after. We explained this resistance as acquired HER2 resistance *(33)*. In patients with metastatic breast cancer treated with trastuzumab and chemotherapy, the median duration of partial or CR is 9.1 months, suggesting that within 1-year patients acquire resistance *(25)*.

There are several proposed mechanisms of HER2 resistance. These include disrupted trastuzumab-HER2 receptor binding, signaling through alternative pathways, upregulation of signaling pathways downstream of HER2, and failure to elicit an appropriate immune response *(34, 35)*. Importantly, activated PI3K/Akt/mTOR signaling appears to play a role in both acquired and intrinsic HER2 resistance *(36)*. Both preclinical and clinical data implicate PTEN loss and PI3CKA mutation in constitutive PI3K/Akt/mTOR signaling and de novo resistance to HER2-targeted therapy. For example, in one study of women with HER2-positive breast cancer treated with trastuzumab, patients who showed the loss of PTEN expression or PI3CKA mutation had a significantly shorter PFS. In another study, the loss of PTEN was associated with a significantly lower overall response rate. In vitro models of HER2-positive breast cancer have shown that the loss of PTEN expression and PI3CKA mutations are also markers of lapatinib resistance *(37)*. Activation of the PI3K/Akt/mTOR pathway is also implicated in acquired resistance to HER2-targeted therapy *(38)*.

Strategies for overcoming Her2 resistance

There are also several therapeutical strategies to overcome HER2 resistance, some are already in clinical use, and some are currently in different phases of clinical trials. We could divide them into two main principles: 1) prolonged HER2 inhibition with maintaining HER2 targeted therapy, 2) strategies based on novel agents that target molecules in alternative signaling pathways *(39)*.

Current strategies based on prolonged HER2 inhibition include: a) continuing trastuzumab and switching chemotherapy regimen, b) switching to a different HER2 targeted therapy c) combining different HER2 inhibitors. Data from preclinical studies indicating additive or synergistic effects of trastuzumab with several cytotoxic agents has led many clinicians to continue trastuzumab therapy beyond progression, in combination with second- or third-line chemotherapeutic agents *(40, 41)*. Switching to a different HER2 inhibitor has been investigated in phase III study of women with HER2-positive metastatic breast cancer that progressed on trastuzumab. Combination therapy with capecitabine and the multityrosine kinase inhibitor lapatinib, which inhibits HER2 and EGFR, substantially extended the time to progression over capecitabine alone (8.4 months versus 4.4 months) *(42)*. The binding of lapatinib to the intracellular domain of HER2 allows it to inhibit both full-length HER2 and truncated p95HER2 therapy *(43)*.

Early clinical data for use of the multityrosine kinase inhibitor neratinib in trastuzumab-refractory disease are also promising *(31)*.

According to second main principle for overcoming HER2 resistance, there are several novel agents designated to target pathways and molecules implicated in HER2 resistance. The strategy with the most available preclinical and clinical data is overcoming HER2 resistance by targeting the PI3K/Akt/mTOR pathway. There are several ongoing studies of PI3K/Akt/mTOR inhibitors in patients with HER2-positive, trastuzumab refractory, metastatic breast cancer. Of these studies, the international, randomized, phase III Breast Cancer Trial of OraL EveROlimus 3 (BOLERO-3), analyzing everolimus in combination with vinorelbine and trastuzumab, is the largest with 572 women expected to enroll *(44)*.

As previously mentioned, there are several agents targeted against other pathways and molecules implicated in HER2 resistance that are in various stages of clinical development. Aside from PI3K/Akt/mTOR inhibitors, other agents currently being investigated in clinical trials of HER2-resistant breast cancer include inhibitors of IGFR1, HSP90, and telomerase. The rationale for assessing IGF1R inhibition in HER2-resistant breast cancer is the hypothesis that cross-talk between IGF1R and HER2 may occur in breast cancer cells, leading to receptor heterodimerization. Preclinical data have shown that HER2 is chaperoned by HSP90, chaperone protein that acts by promoting folding and stabilization of other proteins, and preventing their rapid degradation *(45)*.

CONCLUSIONS

Over the past decade, targeted therapy combined with chemotherapy has made a significant improvement in breast cancer treatment. Increased knowledge in the filed of molecular biology has made us more efficient in treatment of different types of breast cancer. Drugs are designed to target molecules in signaling pathways that are important for cancer cell survival and proliferation. Es-

trogen receptor and HER2 are signaling pathways that are emerged as the most important targets of personalized therapeutical approach to breast cancer patients. Anti-HER2 targeted therapies have substantially improved outcomes for the 15%-23% of patients with HER2 overexpressing breast cancers.

HER2 resistance is a real phenomenon that necessitates the development of novel treatment strategies. Some novel agents like mTOR inhibitor everolimus show promising efficacy in combination with HER2 blocking agent trastuzumab in HER2 positive, trastuzumab resistant metastatic breast cancer. Many agents are investigating in phase II and phase III clinical trial, so we exprect more and more targeted agents in future, leading to prolonged survival and improvements in patients quality of life.

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