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Her2-positive breast cancer: Herceptin and beyond

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ABSTRACT

Breast cancer accounts for approximately 30% of all new cancer cases each year, with an annual incidence of approximately 200,000. Additionally, almost 25% of breast cancers are noted to overexpress Her2, which is an epidermal growth factor receptor. Overexpression of Her2 has been associated with a more aggressive phenotype with decreased survival. Trastuzumab, a recombinant monoclonal antibody against the Her2 receptor, is the only FDA-approved targeted agent for treatment of Her2-overexpressing breast cancer. However, despite the great success achieved with trastuzumab, many women will either not respond or eventually progress despite trastuzumab treatment. As a result, significant efforts have been applied to finding other therapies besides trastuzumab for the treatment of Her2-positive breast cancer. Work has been directed at trying to elucidate the exact mechanism of resistance to trastuzumab and identifying ways to overcome them, at increasing the efficacy of trastuzumab by combining it with other therapeutic agents and at investigating other novel agents.

1. Introduction

Breast cancer accounts for approximately 30% of all new cancer cases each year, with an annual incidence of approximately 200,000.[1] Additionally, almost 25% of breast cancers are noted to overexpress the human epithelial growth factor receptor 2 (Her2), an epithelial growth factor receptor (EGFR)-related tyrosine kinase.[2] Her2 is part of the Her/ErbB2/Neu family of transmembrane receptors which also includes Her1/EGFR, Her3 and Her4. These receptors structurally comprise three specific regions: an extracellular domain (ECD), a membrane spanning region and a cytoplasmic tyrosine kinase domain. The Her2 protein may be cleaved by metalloproteinases, resulting in the production of a truncated membrane-bound fragment known as p95, with release of the ECD into the serum. Several studies have suggested that increased levels of ECD in patients with advanced breast cancer may be predictive of response to hormones, chemotherapy and trastuzumab.[3,4]

Activation of these receptors occurs through the formation of homodimer and heterodimer receptor complexes. These receptor complexes phosphorylate a variety of substrates that result in the activation of distinct intracellular signalling pathways, which are essential to the development and progression of cancers. Although a specific ligand for Her2 has not yet been identified, it is the preferred heterodimerisation partner of this family.[5] Pathways specifically affected by Her2 include the phosphatidylinositol 3-kinase (PI3K)-AKT and the mitogen-activated protein kinase (MAPK) pathways.[6–8]

Activation of the PI3K pathway results in phosphorylation and activation of AKT which in turn can activate multiple targets via phosphorylation. Further downstream effects of the PI3K-AKT pathway include downregulation of cyclin D1 and decreased transcription of p27 (a cyclin dependent kinase 2 inhibitor), processes which eventually result in increased cell

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proliferation and survival.[9] The MAPK cascade is regulated by two main factors, MAP kinase kinase (MEK) and MAPK, which are necessary components of cell growth. Her2 overexpression results in activation of the Ras/MAPK signalling pathway in breast tumour cell lines and carcinomas.[10] Based upon these biological processes, Her2-positive tumours are usually more poorly differentiated, have increased proliferation rates and have more extensive invasion with frequent metastasis.[11] As a result, overexpression of Her2 has been associated with more aggressive tumour behaviour and poorer prognosis.[1]

Additionally, overexpression of Her2 in tumour cells has been associated with increased angiogenesis, which is also essential for tumour survival and metastases. This is thought to be mediated through the ability of Her2 to modulate the equilibrium between proangiogenic factors and antiangiogenic factors.[12,13] Proangiogenic factors that have been identified at higher levels in Her2-overexpressed cancers include VEGF, IL-8 and angiopoietin-2 (Ang-2). The role of VEGF in breast cancer progression is evident from clinical studies showing elevated serum levels of VEGF in invasive breast cancer patients. Additionally, elevated levels of VEGF in breast tumour cytosols have been correlated with increased microvessel density. These findings have supported the hypothesis that the upregulation of VEGF in Her2-overexpressing breast cancers contributes to the aggressive phenotype observed in Her2-positive cases.[14,15]

Because of its prognostic and predictive value, the American Society of Clinical Oncology (ASCO) recommends evaluation of Her2 status on all invasive breast cancers. Currently Her2 expression is by two methods: protein evaluation by immunohistochemistry (IHC) or gene expression via fluorescent in situ hybridisation (FISH). A positive Her2 result is defined as an IHC staining score of 3+, a FISH result of more than six Her2 gene copies/nucleus or a FISH ratio (Her2 gene signal to chromosome 17 signal) greater than 2.2. Because there have been issues regarding the reliable testing and reporting of Her2 expression, to ensure that patients with Her2 overexpression are correctly identified, ASCO has recommended Her2 evaluation by certain accredited laboratories.[16]

In light of the myriad of possible cellular endpoints that are regulated by Her2, it is not surprising that agents directed at Her2 would be investigated in developing agents for treating Her2-overexpressing breast cancers. Trastuzumab (Herceptin, Genentech, South San Francisco, CA) is a recombinant monoclonal antibody to Her2. It was the first humanised monoclonal antibody to be approved for therapeutic use, and the first Her2 inhibitor to obtain FDA approval for use in Her2-overexpressed breast cancer. Great successes have been achieved with trastuzumab, with significant efficacy shown in the treatment of Her2-positive breast cancer, in both the metastatic and adjuvant settings. In fact, its introduction has revolutionised not only how breast cancer, but also how other cancers, can benefit from molecularly targeted agents.

However, despite the successes seen with trastuzumab, many patients will progress. It is estimated that nearly 15% of patients receiving trastuzumab-based adjuvant chemotherapy will go on to develop metastatic disease. Additionally, studies have shown that nearly 60% of patients with Her2-overexpressed MBC will not respond to trastuzumab-based chemotherapy. As a result, significant efforts have been applied to finding other therapies besides trastuzumab for the treatment of Her2-positive breast cancer.

2. Trastuzumab in the treatment of Her2-overexpressed breast cancer

2.1. *Clinical data*

Initial approval of trastuzumab was based on studies in patients with metastatic breast cancer (MBC[17]). Several preclinical trials have shown that trastuzumab inhibits the growth of breast cancer cell lines that overexpress Her2 and inhibits the growth of Her2-positive tumour cells in transgenic mice. Based upon these preclinical trials, trastuzumab has undergone extensive testing in several phase II trials and phase III trials in patients with Her2-overexpressed breast cancer. In the two major phase II clinical trials that eventually lead to its FDA approval, trastuzumab was evaluated in 248 women with Her2-positive MBC (as measured by IHC). In this population of heavily pretreated patients, an overall response rate (ORR) of 12–15% was observed.[18,19]

Use of single agent trastuzumab as a first-line agent in MBC resulted in a median overall response rate of 19–26%, thereby supporting it as an important new option in the treatment of Her2-positive MBC.[20,21] Additionally, when trastuzumab was used in the first-line setting for MBC, in conjunction with other chemotherapeutic agents, such as paclitaxel and an anthracycline, trastuzumab was shown to increase the clinical benefit of first-line chemotherapy as noted by significant increases in time to disease progression, rate of overall response, duration of response and overall survival with a 20% reduction in the risk of death.[22] Subsequent clinical trials have shown that trastuzumab can be combined safely and effectively with a variety of therapeutic agents, including docetaxel,[23] vinorelbine,[24] platinum salts,[25] capecitabine,[26] gemcitabine[27] and aromatase inhibitors.[28,29]

Based upon these encouraging results of trastuzumab in the metastatic setting, it was subsequently evaluated in the adjuvant setting in early breast cancer.[30] Several trials involving almost 10,000 patients with Her2-positive breast cancer have all shown significant increases in time to disease progression and overall survival, with a decrease in risk of recurrence in the first when trastuzumab was used in conjunction with chemotherapy[31,32]; Table 1). Similarly, neoadjuvant clinical trials also showed that the addition of trastuzumab to standard chemotherapy improves the pathologic complete response rate.[33,34]

Although great benefits have been observed with the use of trastuzumab in Her2-positive breast cancer, its benefits are limited due to the potential development of cardiotoxicity, especially when used in conjunction or subsequent to anthracyclines. In the four large trastuzumab adjuvant trials, the incidence of patients who developed severe congestive heart failure (CHF) was up to 4%. Additionally, approximately 25% of patients sustained decreases in their left ventricular ejection fraction (LVEF) to less than 50% six months after their initial diagnosis of CHF.[35] When long-term trastuzumab

Table 1 – Clinical trials of trastuzumab in the adjuvant setting.

Trial	# Pts	Design	Conclusion
HERA[31]	3387	Any chemotherapy ± XRT[d] → Observation versus any chemotherapy ± XRT[d] → trastuzumab q3w[b] × 12 months versus any chemotherapy ± XRT[d] → trastuzumab q3w[b] × 24 months	Trastuzumab resulted in 46% risk reduction of recurrence 2 years after surgery
NSABP B31[32]	3351	AC[a] × 4 → paclitaxel q3w[b] × 4 ± trastuzumab	Result combined due to similarity of studies.
NCCTG N9831[32]		AC[a] × 4 → paclitaxel q1w[c] × 12 versus AC[a] × 4 → paclitaxel q1w[c] × 12 + trastuzumab versus AC[a] × 4 → paclitaxel q1w[c] × 12 → trastuzumab	Trastuzumab resulted in a significant increase in disease-free survival with a 52% reduction in 3-year recurrence when given sequentially and a 33% reduction in risk of death
BCIRG 006[64]	3222	AC[a] × 4 → docetaxel (ACT) versus AC[a] × 4 → docetaxel + trastuzumab × 12 months (ACTH) versus docetaxel + carboplatin × 6 + trastuzumab × 12 months (TCH)	Increased DFS seen when Trastuzumab was added to any regimen (HR 0.49–0.61), with no significant difference between the two trastuzumab-containing arms
FinHER[65]	232	Docetaxel or vinorelbine × 3 ± trastuzumab × 9 wks → FEC[e] × 3	Increased DFS with trend towards better OS benefit similar to other trials in which Trastuzumab used for 1–2 years

a Doxorubicin + cyclophosphamide (AC).
b Every three weeks (q3w).
c Every one week (q1w).
d Radiation therapy (XRT).
e Fluorouracil + epirubicin + cyclophosphamide (FEC).

treatment was evaluated for safety, 28% of patients with MBC treated for at least one year developed a cardiac event (characterised by decreased LVEF). However, cardiotoxicity was reversible in the majority of the patients.[36]

2.2. *Trastuzumab's mechanism of action and modes of resistance*

Although the exact mechanism of how trastuzumab exhibits its effects is not fully elucidated, there are several proposed mechanisms based upon preclinical and clinical studies.[37] These include (a) downregulation of the Her2 receptor resulting in decreased amounts of available receptors, (b) inhibition of Her family dimerisation thus inhibiting activation of the signalling cascades, (c) reduction of the proteolytic cleavage of the ECD, thus preventing the formation of a truncated highly active receptor remnant, (d) inhibition of PI3K/AKT and MAPKs with resultant anti-angiogenesis, (e) the induction of p27 with induction G1 arrest and (f) antibody-dependent cell-mediated cytotoxicity.

To understand the mechanisms of resistance to trastuzumab, it is important to take into account its mechanisms of action. Although Her2 belongs to the HER family of epidermal growth factor receptors, there are actually seven kinase superfamilies. Biological processes are able to proceed through cascades of overlapping signalling processes that are mediated through cross-talk between these receptors. Thus, deregulation of any step along these pathways could result in the development of drug resistance. For example, increased signalling via the PI3K/AKT pathway could contribute to trastuzumab resistance because of activation of multiple downstream receptor pathways.[38] This could include both Her2-related receptors and non-HER receptors such as the insulin-like growth factor 1 receptor (IGF-1), which appears to be involved in a cross-talk with Her2 in resistant cells.[39]

Additionally, loss of function of the tumour suppressor PTEN gene, the negative regulator of AKT, results in heightened AKT signalling that leads to decreased sensitivity to trastuzumab.[40,41] Loss of key cell cycle regulators such as p27kip1 has been associated with the development of trastuzumab resistance *in vitro*.[42] Decreased interaction between trastuzumab and its target receptor Her2, which is due to steric hindrance of Her2 by cell surface proteins such as mucin-4 (MUC4), may block the inhibitory actions of trastuzumab. In addition, the loss of binding site on truncated Her2 receptors (p95) has been associated with trastuzumab resistance *in vitro* and *in vivo*.[43] A better understanding of these aberrant molecular processes has helped in the development of the hypotheses regarding trastuzumab resistance, and in generating ideas of possible further targets.

3. Future directions

Without a doubt, trastuzumab has been shown to have significant efficacy in the treatment of Her2-positive breast cancer, in both the metastatic and adjuvant settings. In fact, its introduction has revolutionised not only how breast cancer, but also how other cancers, can benefit from molecularly targeted agents. However, despite the successes seen with trastuzumab in MBC, all patients will eventually progress, and some will relapse despite adjuvant treatment with trastuzumab. As a result, extensive efforts have been focused on trying to elucidate the exact mechanism of resistance to trastuzumab and trying to find ways to overcome them, on increasing the efficacy of trastuzumab by combining it with other therapeutic agents and on investigating other novel agents.

3.1. Targeting Her2 dimerisation

Since Her2 is activated through Her2 receptor homo- and heterodimerisation, it has been postulated that Her2-positive breast cancer may respond to treatment with agents that prevent Her2 dimerisation. Pertuzumab (2C4; Genentech, South San Francisco, CA) is another Her2-targeted monoclonal antibody. It is the first of a new class of agents known as HER dimerisation inhibitors. In contrast to trastuzumab, pertuzumab sterically blocks HER2 dimerisation with other HER receptors, and blocks ligand-activated signalling between the heterodimers.[44] Preclinical trials with pertuzumab, used in conjunction with trastuzumab, resulted in synergistic decreases in BT474 Her2-overexpressing breast cancer cells.[45,46] While clinical data in breast cancer patients are limited, there have been several studies with pertuzumab in other Her2-positive tumours such as ovarian and prostate cancers. In two phase II studies with ovarian cancer, there was a trend towards improved PFS.[47,48] Several clinical trials are evaluating the safety and efficacy of trastuzumab in combination with pertuzumab in patients with early-stage cancer and MBC.

In an effort to treat microscopic disease, pertuzumab labelled with the low-energy beta emitter ^{177}Lu was investigated as a targeted radiotherapy for the disseminated Her2-positive micrometastases in experimental models. Treatment with ^{177}Lu-pertuzumab delayed tumour progression in BALB/c (nu/nu) mice with Her2-overexpressing xenografts when compared with controls ($p < 0.01$). It is hoped that these results will aid in the planning of clinical trials utilising ^{177}Lu-pertuzumab for therapy.[49]

3.2. Identifying the roles of other HER family members

Newer information is now revealing a more expanded role for other members of the HER family which is not just related to dimerisation. Her3 has been shown to lie upstream of the PI3K/AKT pathway which is critically important in the tumourigenic signalling pathway. Thus it has the potential for cross-talk signalling. In fact, an increasing amount of evidence has shown that Her3 plays a critical role in EGFR- and Her2-driven tumours. Additionally, signalling in *trans* is a key feature of HER family signalling. Studies have shown that this process is mediated predominantly through *trans*phosphorylation of Her3. The importance of this process is seen with angiogenesis which is mediated via transregulation of Her2 by heregulins which binds to Her3 and Her4. Heregulin-beta1 regulates the expression and secretion of VEGF in breast cancer cells, and trastuzumab has been shown to inhibit heregulin-mediated angiogenesis both *in vitro* and *in vivo*.

3.3. Targeting other signalling pathways

Improving trastuzumab responsiveness and preventing the development of resistance are the main goals in the treatment of Her2-positive breast cancer. Several preclinical studies have suggested that activation of downstream signalling pathways is involved in acquired trastuzumab resistance. Thus it is logical that dual blockade of Her2 together with other signalling pathways is a logical approach to improve its responsiveness. Several trials that combine trastuzumab with other signal transduction inhibitors such as small molecular weight tyrosine kinase inhibitors (TKIs) against EGFR and/or Her2 (lapatinib, gefitinib), mTOR inhibitors (sirolemus, temsirolimus), farnesyl transferase inhibitors, histone deacetylase (HDAC) inhibitors and heat shock protein 90 (HSP90) inhibitors have begun (Table 2).

3.3.1. EGFR tyrosine kinase inhibitors

In addition to Her2-directed therapy, therapies targeting other members of the ErbB/HER family, in conjunction with Her2, have been evaluated. It has been shown that Her2 is the most potent dimerisation partner of EGFR/Her1. Additionally, Her2 has been shown to inhibit EGFR degradation and to enhance its membrane recycling, resulting in sustained levels of EGFR. Thus, it has been postulated that agents, which target both Her2 and EGFR, should enhance the efficacy of trastuzumab.

This hypothesis has been supported by preclinical studies and clinical trials using lapatinib, a small molecular weight tyrosine kinase inhibitor that targets both Her2 and EGFR/Her1. Several studies have shown growth inhibition in several tumour lines that overexpress EGFR. Additionally, lapatinib has been shown to retain activity even in cells demonstrating trastuzumab resistance.[50] Phase I and phase II trials showed that lapatinib was effective as a single agent and in combination with capecitabine. A phase III trial randomised 324 patients with Her2-overexpressing MBC who had previously been treated with anthracyclines, taxanes and trastuzumab to capecitabine +/– lapatinib. In this study, the combination of capecitabine and lapatinib improved time to progression and response rates.[51] Based on these results, the US FDA has approved lapatinib in combination with capecitabine in the treatment of MBC that has progressed following treatment with a trastuzumab-containing regimen. Future directions will involve the evaluation of other TKIs in Her2-positive breast cancer.

3.3.2. Mammalian target of rapamycin (mTOR) inhibitors

PI3K/AKT and its upstream regulators are deregulated in Her2-overexpressed breast cancer and are considered key determinants in the biological aggressiveness of these tumours. Thus, agents that target these pathways could have a significant impact in the treatment of this cancer. mTOR is one of the downstream signalling pathways regulated by activated PI3K/AKT.[52] Dysregulation of the mTOR pathway has been found in many human tumours. It is central to the complex intracellular signalling pathways implicated in the promotion of cancer cell growth and survival.[53]

In human breast cancer, mTOR activation has been linked to Her2-positivity as well as decreased disease-free survival.[54] Sirolimus (Rapamycin), the oldest inhibitor of mTOR, was first discovered over 30 years ago. Sirolimus decreases proliferation of a number of mammary epithelial cell lines, particularly those that overexpress Her2/ErbB2, have activated AKT, or have upregulated p70S6K.[55,56] Data from preclinical models of ErbB2/Neu-induced breast cancer have shown that inhibition of the mTOR pathway with sirolimus blocks multiple stages of ErbB2/Neu-induced tumourigenic progression. For example, in c-neu transgenic mice, elevated levels of several mTOR pathway members were noted, either upregulated (p85/P3K and p70S6 kinase) or downregulated (eIF-4E-BP1).

Table 2 – Novel agents under investigation in the treatment of Her2-positive breast cancer.[66]

Agent	Source	Class of compound	Phase of development
Pertuzumab (2C4)	Genentech	Her2 dimerisation inhibitor	Phase I
TAK165	Takeda	Her2 selective TKI[a]	Phase I
CP 724,714	Pfizer	Her2 selective TKI[a]	Phase II
Lapatinib (GW572016; Tykerb)	GlaxoSmithKline	Dual Her1/Her2 TKI[a]	Phase II
BIBW 2992	Boehringer Ingelheim	Dual Her1/Her2 TKI[a]	Phase II
HKI-272	Wyeth	Dual Her1/Her2 TKI[a]	Phase I/II
Sirolimus (Rapamycin)	Genentech	mTOR[b] inhibitor	Phase II
Temsirolimus (CCI-779)	Pfizer	mTOR[b] inhibitor	Phase II
Everolimus (RAD001)	Novartis	mTOR[b] inhibitor	Phase I/II
Tipifarnib (Zarnestra)	Janssen Pharmaceutica	FTI[c]	Phase II
Lonafarnib (SCH66336)	Schering-Plough	FTI[c]	Phase I
Irinotecan (CPT-11; Campto)	Aventis	Camptothecin	Phase II
Bortezomib (PS-341; Velcade)	Millenium Pharmaceuticals	Proteosome inhibitor	Phase I
LBH589	Novartis	HDAC[d]	Phase I/II
Tanespimycin (17-AAG; KOS-953)	Kosan Biosciences	HSP90[e] inhibitor	Phase II
AUY922	Novartis	HSP90[e] inhibitor	Phase I/II
CNF2024	Biogen Idec	HSP90[e] inhibitor	Phase I
SU011248 (Sunitinib)	Pfizer	Angiogenesis inhibitor	Phase I
Epothilone D	Hoffman-LaRoche	Epothilone	Phase II (completed)
Ixabepilone (BMS-247550)	Bristol-Meyers-Squibb	Epothilone	Phase III
Larotaxel (XRP9881)	Sanofi-Aventis	Antimitotic	Phase II
Ertumaxomab	Fresenius Biotech	Trifunctional antibody	Phase II
Trastuzumab-DM1	Genentech	Immunotoxin	Phase II
Her2 vaccine +/– Sargramostim (Leukine)	Various groups/Bayer Pharmaceuticals	Her2 vaccine +/– rhGM-CSF[f]	Phase II

a Tyrosine kinase inhibitor (TKI).
b Mammalian target of rapamycin (mTOR).
c Farnesyltransferase inhibitor (FTI).
d Histone deacetylase (HDAC).
e Heat shock protein 90 (HSP90).
f Recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF).

Additionally when these mice were treated with sirolimus, there was arrest in the growth of the tumours and regression of primary tumours.[57] Thus, the mTOR pathway is considered an important target for anticancer drug development. With the renewed interest in this pathway, several new mTOR inhibitors have recently been developed. These include temsirolimus (CCI-779, Pfizer) and everolimus (RAD001, Novartis). These agents have shown limited efficacy as single agents in patients with MBC. Ongoing clinical trials are evaluating the safety and efficacy of mTOR inhibitors in combination with cytotoxic agents, endocrine therapy and Her2-targeted therapy (e.g. trastuzumab and lapatinib).

3.3.3. *Immunotoxins*

Despite the increased specificity achieved with the use of trastuzumab, the development of resistant clones and the lack of specificity obtained with the use of standard chemotherapeutic agents have spurred studies on the use of even more targeted agents. Efforts have been focused on delivering higher concentrations of cytotoxic agents to specific cell populations. One way this has been achieved is through the development of immunotoxins, compounds in which a tumour-specific antibody or antibody fragment is conjugated to a toxic compound. Previous immunotoxins employed the use of antibodies conjugated to radioisotopes, bacterial toxins such as pseudomonas exotoxin or diphtheria endotoxin or plant products. Several preclinical studies have proven the efficacy of immunotoxin in Her2-positive breast cancer cell lines and human xenograft models.[58,59] In some clinical studies, complete resolution of some Her2-positive tumours was noted.[60,61]

While there have been encouraging results, problems with severe toxicity, including hepatotoxicity and neurotoxicity, have been observed with some of the agents.[62,63] More recent work has been performed combining antibodies and cytotoxic drugs. Based upon preclinical studies, phase I/II studies are currently underway with trastuzumab conjugated to DM1, a maytasinoid derivative which inhibits microtubule formation.

4. Summary

The benefit of trastuzumab in Her2-positive breast cancers has been well documented as noted by prolonged survival. However, many patients develop progressive disease despite treatment with trastuzumab. Thus, there is a need for the development of other treatment strategies. As our understanding of trastuzumab resistance has emerged, newer agents targeting these processes are being studied. Additionally, use of trastuzumab in combination with other well-known chemotherapeutic agents is also evaluated and appears very promising. Nevertheless, there is still a need for the development of novel agents that can be used in this patient population. Luckily there are many new products in the clinical pipeline being evaluated that show promise.

Conflict of interest statement

Dr. Windy Dean-Colomb: none declared. Dr. Francisco J. Esteva has served as a paid consultant for Genentech, Inc., GlaxoSmithKline and Novartis Pharmaceuticals.

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