review

Targeting the HER2 Receptor in Metastatic Breast Cancer

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The advent of targeted therapies has revolutionized the treatment of certain types of cancer. Identification of molecular targets on cancer cells has led to the design of novel drugs, which either used as single agents or in combination with chemotherapy, has prolonged survival in metastatic disease, or contributed to curative treatment in the adjuvant setting. A literature review was conducted to identify and present current knowledge on the molecular function of the HER2 receptor, its role in the pathogenesis of breast cancer and anti-HER2 targeted drugs in use or under development. Many molecular targets have been identified in breast cancer, with the HER family of receptors being the ones most extensively studied. Trastuzumab and lapatinib target the HER2 receptor and are approved drugs for the treatment of metastatic breast cancer. Several other targeted agents, including T-DM1, pertuzumab, neratinib, afatinib and ertumaxomab, are currently being tested in vivo as well as in clinical studies. The use of targeted therapies in metastatic breast cancer has improved prognosis, increased survival and dramatically changed the way we treat breast cancer patients today.

Breast cancer is the most common type of cancer and the second most common cause of cancer death in the female population.[1] Despite the fact that over 90% of women with breast cancer will be diagnosed at a loco-regional stage, almost half of them will relapse and eventually die, as metastatic breast cancer is currently an incurable disease.[1] Chemotherapy has traditionally been the mainstay of treatment of metastatic disease.

The advent of targeted therapies has revolutionized the treatment of many types of cancer, including breast cancer. Targeted therapies are specifically designed to target certain molecules that are expressed mainly on cancer cells and to a lesser degree on normal tissues. These targets may be located on the cell membrane,[2,3] inside or outside the cancer cell and when mutated or overexpressed, their activation may lead to increased proliferation, angiogenesis and inhibition of apoptosis thus giving the malignant phenotype.[4]

The concept of targeted therapy is not new in oncology. The first targeted therapy used in breast cancer was tamoxifen. Tamoxifen targets the intracellular steroidal estrogen receptor (ER). Women with breast cancer whose tumors express ERs benefit by tamoxifen use, when given as adjuvant treatment or in the metastatic disease setting.[5] Other selective estrogen receptor modulators, as well as selective estrogen receptor down-regulators (eg, fulvestrant), aromatase inhibitors (AIs; eg, anastrazole, letrozole, exemestane) and gonadotrophin-releasing hormone analogs (eg, goserelin) can all be considered targeted therapies. Currently, when we discuss targeted therapies, we are referring to drugs such as tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (moAbs) which interfere with and block the function of their specific targets, thus inhibiting proliferation, the metastatic potential of cancer cells and angiogenesis while promoting cell death, or apoptosis.

Molecular subtypes of breast cancer and potential targets

Traditionally, prognostic predictions and recommendations for adjuvant therapy for patients with early stage breast cancer were based on pathologic determinations of tumor size, lymph node status, ER and human epidermal growth factor receptor 2 (HER2) status.[6] Today however, these are considered only crude indicators. A new molecular classification, based on gene expression microarrays, identified five different molecular subtypes

of breast cancer and proved the remarkable heterogeneity of breast cancer.[7] These subtypes are luminal A, luminal B, basal-like, HER2 positive and normal-like. The luminal A subtype has the best prognosis, basal like and HER2 positive the worst and luminal B has an intermediate prognosis. Luminal A is characterized by high expression of ERs and/or progesterone receptors (PRs), no expression for HER2 and low expression of other proliferation markers. Luminal B is ER and/or PR positive, but also HER2 positive and with higher expression of proliferation markers. The HER2-positive subtype is negative for ER and PR expression. Basal-like is usually negative for ER, PR and HER2, but positive for CK5/6 and/or epidermal growth factor receptor (EGFR).[8] However, it has now become clear that not all basal-like tumours demonstrate a triple (ER, PR, HER2) negative phenotype and conversely, not all triple negative tumors express a basal-like gene expression profiling.[9] Normal-like are unclassified breast carcinomas which are negative for all markers (**Table 1**).[10]

Hereditary breast cancer arising in carriers of mutations in the *BRCA1* and *BRCA2* genes[11] differs from sporadic breast cancer and from non-BRCA1/2 familial breast carcinomas. The majority of cancers that carry a *BRCA1* germline mutation have a triple negative phenotype and are shown to express basal cytokeratins and EGFR by immunohistochemistry (IHC). These similarities, along with some other common cytogenetic abnormalities (p53 mutations, loss of 5q and loss of X chromosome inactivation) suggest that even in the absence of a germline BRCA1 mutation, the BRCA1 pathway function is compromised in basal-like tumors.[12] The basal-like phenotype is only occasionally found in BRCA2 carcinomas, which tend to be ER and PR positive. Sporadic basal-like tumors usually, but not always, harbor BRCA1 mutations.[13]

The surface of cancer cells express hundreds of different types of receptors. Each receptor has its own specific ligands which function mostly as growth factors. One ligand can activate more than one receptor while a receptor can be activated by more than one ligand.[4] Usually these receptors form dimers (eg, HER1 and HER2) before their activation. There are also intracellular receptors (eg, ER). The binding of a ligand to its receptor, which acts as tyrosine kinase (phosphorylation of a tyrosine residue) activates an intracellular signal transduction cascade to the nucleus. Each receptor has its own signal transduction pathway but often some steps are common for many pathways.[14] Overexpression of growth factors, mutation or overexpression of receptors or their corresponding signal transducers are common findings in cancer and they lead to increased proliferation, metastasis, angiogenesis and inhibition of apoptosis.[4] Interruption of any of the steps of a given pathway could potentially lead to cell death. Therefore, drugs are currently being designed to target these molecules; strategies to improve their use, as combination with other drugs, potentiation of their action and overcoming resistance, are subjects of intense study. We present current knowledge on HER2 receptor and the main anti-HER2 targeted drugs in use or under development (**Figure 1, Table 2**).

Targeting the HER2 receptor—trastuzumab

Human epidermal growth factor receptors (HER) 1, 2, 3 and 4 (EGFR/ErbB1, ErbB2, ErbB3, ErbB4), are a family of receptors expressed in normal tissues and in many types of cancer.[15] All members of the HER family consist of an extracellular ligand binding site, a transmembrane domain, and an intracellular tyrosine kinase domain. Dimerization is required to activate tyrosine kinase phosphorylation.[15] Dimerization can be either homodimerization, between two molecules of the same type, or heterodimerization between two molecules of different types. The phosphorylated HER dimers activate downstream cell proliferation (mitogen-activated protein kinase), cell survival (phosphoinositide 3-kinase), and signal transducer and activator of transcription pathways.[16] HER1 (EGFR) and HER2 are the most significant and better studied so far. HER2 is overexpressed and amplified in 20% to 30% of human breast cancers[17] which follow a more aggressive course of disease and correlate with poor prognosis, including high risk of recurrence, metastasis and reduced overall survival.[18] HER2 overexpression is also a predictive factor of response

Table 1. Classification of breast cancer based on hormone and surface receptor expression.

Luminal A	Luminal B	HER2 (+)	Basal-like/BRCA+	Normal-like
ER(+) PR(+) HER2(-)	ER(+) PR(+) HER2(+)	ER(-) PR(-) HER2(+)	ER(-) PR(-) HER2(-) CK5/6 (+) EGFR (HER1) (+)	All markers negative

to chemotherapy and hormonal treatment. Therefore, HER2 seems to play a significant role in the pathogenesis of breast cancer. Several mechanisms have been proposed to associate HER2 overexpression/amplification with carcinogenesis including:

- Formation of HER2 containing heterodimers which lead to enhanced downstream signaling[19]
- Increased recycling of EGFR to the cell surface that also leads to enhanced downstream signaling[20]
- Formation of HER2 homodimers, some truncated and constitutively active
- Activation or suppression of signal transduction pathways, important in tumor development and growth.[21]

Assessment of HER2 status of patients is currently being performed by using either immunohistochemistry to evaluate protein overexpression, or fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and silver-enhanced in situ hybridization (SISH) to evaluate gene amplification.[22,23] HER2 extracellular domain (ECD) levels can be found elevated in patients with HER2 negative (by IHC or FISH/CISH/SISH) breast cancer and recent reports suggest that these patients do benefit from HER2 targeting therapy.[24,25]

Trastuzumab is a humanized IgG1 monoclonal antibody against the extracellular domain of the HER2 receptor.[26] Proposed potential mechanisms to explain its antitumor effects include:

- Inhibition of proliferation and angiogenesis mediated through HER2 signaling.[27]
- Immune response against tumor cells via antibody-dependent cell-mediated cytotoxicity (ADCC).[28]
- Prevention of homodimerization of truncated HER2 receptors through inhibition of cleavage of the HER2 ECD by metalloproteinases.[29]
- Acceleration of the internalization and degradation of HER2 from the cell membrane.[30]

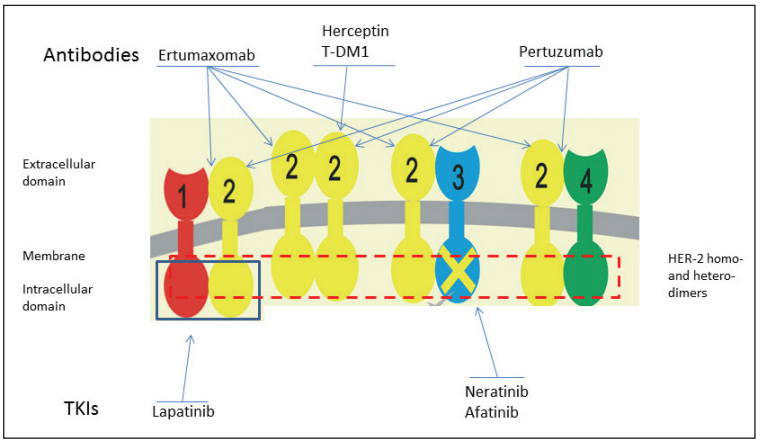

Figure 1. The HER family of receptors and their corresponding targeted drugs.

Table 2. Overview of therapeutic strategies for HER2 positive metastatic breast cancer.

Agent	Type	Mechanism of action
Trastuzumab	Monoclonal antibody	Extracellular domain, effective against HER2 homodimers
Lapatinib	Reversible TKI	Selective inhibitor of EGFR/HER1 and HER2 intracellular tyrosine kinases
T-DM1	Antibody-drug conjugate	Trastuzumab conjugated to an anti-microtubule agent (maytansine). Maytansine is a potent anti-microtubule agent
Pertuzumab	Monoclonal antibody	Extracellular domain different than trastuzumab, inhibits hetero-dimerization
Neratinib	Irreversible TKI	Pan-HER TKI
Afatinib	Irreversible TKI	Pan-HER TKI
Ertumaxomab	Trifunctional, bispecific antibody	Targets both HER2 Receptor (extracellular domain different epitope), CD3 antigen on T cells and activates Fc-gamma receptors

TKI: Tyrosine kinase inhibitor.

Trastuzumab as first line treatment

Initial studies evaluated trastuzumab as single agent and found an overall response rate (ORR) of 11% to 15% and 26% in pretreated[31] and untreated[32] patients respectively. The first randomized phase III trial to study the combination of trastuzumab with chemotherapy in the first line setting enrolled 469 women with HER2 positive (IHC: 2+,3+) metastatic breast cancer.[33] Patients received either an anthracycline (doxorubicin or epirubicin) with cyclophosphamide (if they had not received anthracyclines as adjuvant treatment) or paclitaxel with or without trastuzumab. Patients in the chemotherapy only arm could cross over to receive trastuzumab at disease progression. The study met its primary endpoint—time-to-progression (TTP) was prolonged from 4.6 to 7.4 months ($P<.001$) for patients who received trastuzumab. ORR (50% vs. 32%, $P<.001$), median duration of response (9.1 vs. 6.1, $P<.001$) and median overall survival (OS) (25.1 vs. 20.3 months, $P<.046$) were all significantly improved in favor of the trastuzumab arm. This study led to the approval of trastuzumab in combination with chemotherapy for the treatment of metastatic breast cancer in the first line.

Several other chemotherapy agents were tested in phase II studies with trastuzumab and the combination was found to be active with response rates ranging from

Table 3. Main phase III trials of trastuzumab and lapatinib in HER2-positive metastatic breast cancer.

Characteristics	Number of patients	Regimen	Endpoints/outcome		References
Trastuzumab					
First-line setting	469	A + C or Pac +/- Trast (Arm A: +Trast, Arm B: -Trast)	TTP: 7.4 vs 4.6 mo (*P*<.001)	OS: 25.1 vs 20.3 mo (*P*<.046)	33
Trast-pretreated	482	Cap +/- Trast (Arm A: +Trast, Arm B: -Trast)	PFS: 8.2 vs 5.6 mo (*P*=.03)		43
ER+, postm (TAnDEM trial)	207	Anastr +/- Trast (Arm A: +Trast, Arm B: -Trast)	PFS: 4.8 vs 2.4 mo (*P*=.0016)	ORR: 20.3 vs 6.8% (*P*=.018)	75
Lapatinib					
A, T, Trast pretreated	324	Cap +/- Lap (Arm A: +Lap, Arm B: -Lap)	TTP: 8.4 vs 4.4 mo (*P*<.001)		62
First-line setting (initially unknown HER2 status)	579	Pac+/- Lap [Arm A (49/291=HER2+): +Lap], [Arm B (37/288=HER2+): -Lap)], Analysis of HER2+ cases	TTPn: 46.4 vs 25.1 w (*P*=.005)	ORR: 63.3 vs 37.8% (*P*=.023)	63
Second-line setting (pretreated on A-T regimen, progressed on Trast)	296	Lap +/- Trast (Arm A: +Trast, Arm B: -Trast)	PFS: 12 vs 8.4 w (*P*=.029)	CBR: 24.7 vs 12.4% (*P*=.02)	66
ER+	1,286 (HER2+= 219)	Letr +/- Lap [Arm A (111=HER2+): +Lap], [Arm B (108=HER2+): -Lap)]	PFS: 8.3 vs 3 mo (*P*=.019)	ORR: 28 vs 15% (*P*=.02)	76

Anastr: anastrazole, A: anthracycline, Cap: capecitabine, C: cyclophosphamide, Lap: lapatinib, Letr: letrozole, Pac: paclitaxel, T: taxane, Trast: Trastuzumab, ER: estrogen receptor, Postm: postmenopausal, OS: overall survival, ORR: overall response rate, TTP: time to progression, Mo: months, W: weeks, CBR: clinical benefit ratio

20% to 68%. Vinorelbine,[34] capecitabine,[35] docetaxel,[36] paclitaxel,[37] platinum agents[38] and gemcitabine[39] are all now considered good partners of trastuzumab. Increased rates of cardiac toxicity when trastuzumab is combined with anthracyclines inhibits their concurrent use,[40] although the combination seems very active; recent reports from patients who received both drugs as neoadjuvant treatment suggest that cardiotoxicity is not a major issue.[41] The trend at present, however, is not to use these two drugs concurrently.

Trastuzumab at disease progression

How should patients be treated when they progress on trastuzumab? The experience derived from the use of chemotherapy denotes that when disease progresses while on treatment with a certain chemotherapeutic agent, that agent should be discontinued and another one should be used, but with trastuzumab, disease progression does not necessarily reflect inactivity of the drug. Possibly, the HER2 signaling pathway is triggered by another pathway because of intracellular crosstalk, but trastuzumab acts by other mechanisms (eg, ADCC cytotoxicity) which are probably still active and could act synergistically with another chemotherapy drug.[42] A similar paradigm is the case of prostate cancer where luteinizing hormone-releasing hormone (LHRH) analogs are used even when the disease becomes hormone resistant.

A phase III study confirmed the above assumption. Patients with HER2-positive metastatic breast cancer who had progressed on trastuzumab-containing therapy were treated either with capecitabine with trastuzumab or capecitabine alone. Of the planned 482 patients, only 156 were enrolled as the study was halted by an independent data monitoring committee. At a median follow up of 15.6 months, progression-free survival (PFS) was improved in favor of the combination arm (8.2 vs. 5.6 months) (*P*=.03).[43]

Are there any predictors for activity of second line trastuzumab-based therapy? The only study to address this question is a retrospective study by Bartsch and colleagues who reviewed the data of 97 patients treated with more than one line of a trastuzumab-containing regimen.[44] In a multivariate analysis, no clinical or histopathological features could reliably predict the activity of second-line therapy. Interestingly though, TTP

Table 4. Main trials on novel HER2 therapeutic strategies in HER2-positive metastatic breast cancer.

Trial/Characteristics	Number of patients	Arms/Regimen	Endpoints/outcomes				References
T-DM1							
EMILIA (Phase III trial, Trast-T pretreated pts)	978	T-DM1 vs Cap - Lap	PFS: 9.6 vs 6.4 mo (HR: 0.65, 95% CI: 0.55-0.77, *P*<.0001)	OS: improved for T-DM1 arm, (median has not yet been reached)		Obj. RR: 43.6 vs 30.8% (95% CI: 6-19.4, *P*=.0002)	91
Pertuzumab							
CLEOPATRA (Phase III trial)	808	Doc - Trast +/- Per	PFS: 18.5 vs 12.4 mo (*P*<.001)				96
NEOSPHERE (Phase III trial, neo-adjuvant)	417	Doc-Per vs Doc-Trast vs Doc-Trast-Per vs Trast-Per	pCR: 45.8 vs 29% (3-drug vs Doc-Trast, *P*=.014)				95
Neratinib							
Phase II (LABC, MBC)	136	Ner 240 mg, 66 pts (prior treated with Trast) 70 pts (not treated with Trast)	PFS: 40 w 22 w		CBR: 56% 24%		97

Doc: docetaxel, Lap: Lapatinib, Ner: neratinib, Per: pertuzumab, LABC: locally advanced breast cancer, MBC: metastatic breast cancer, pCR: pathologic complete response, PFS: progression free survival, OS: overall survival, Ob. RR: objective response rate, Mo: months, W: weeks, CBR: clinical benefit rate

was seven months, being almost the same (8 months) for first line, indicating a continuum of activity despite progression. OS was 43 months suggesting improved survival for those patients. Of course, this was a highly selected cohort as patients who progressed rapidly after first-line therapy were excluded. Nevertheless, these data indicate that eligible patients benefit from trastuzumab-based treatment in multiple lines. An important observation was that 40.2% of the patients in the study developed brain metastases and this can be easily explained by the inability of trastuzumab to pass the blood-brain barrier and the longer survival achieved with multiple systemic treatments. Conflicting results regarding the utility of continuing trastuzumab beyond progression were reported by Montemurro and colleagues.[45] They retrospectively compared two groups of patients, who either received or did not receive trastuzumab after progression and found no statistically significant difference in their response rate (RR) (28% vs. 30%), TTP (8.4 vs. 7 months) and median post-progression survival (20.6 vs. 15.4 months).

Mechanisms of resistance to trastuzumab

Despite the fact that many patients with HER2-positive metastatic breast cancer will benefit from successive lines of trastuzumab-containing therapy, they will all eventually become resistant. Proposed mechanisms of resistance include:

- Heterodimerization between HER2 and IGF-1R and thus signaling through other growth factors.[46]
- Shedding of the extracellular domain of the receptor leaving the truncated form of the receptor (p95) which does not bind to trastuzumab but remains active.[47]
- Increased expression of the MUC4 membrane glycoprotein, which inhibits the interaction of trastuzumab with the HER2 receptor.[48]
- Activating mutations of AKT and decreased levels of phosphatase and tensin homolog (PTEN).[49]
- Transcriptional upregulation of HER2 gene expression.[50]
- Down-regulation of p27kip1, a downstream effector of multiple growth factor receptor pathways.[51]
- Expression of low-affinity Fc receptor polymorphisms on immune effector cells.[52]
- Decreased expression of the cyclin-dependent kinase inhibitor p27.[53]

Targeting the HER2 receptor—lapatinib

Lapatinib is an orally administered, reversible, selective

inhibitor of the intracellular tyrosine kinase domains of EGFR/ErbB1 and HER2/ErbB2.[54] Lapatinib exerts its anti-tumor activity through:

- Inhibition of tumor growth by means of inhibition of activation of downstream signalling for cell proliferation and survival.[55]
- Induction of apoptosis in HER2- and EGFR-overexpressing cells.[56]
- Inhibition of phosphorylation of the truncated form of the HER2 receptor (HER2p95).[57]

There is no cross-resistance between lapatinib and trastuzumab, while lapatinib induces apoptosis in trastuzumab-resistant cell lines.[49] It effectively crosses the blood-brain barrier and is active in central nervous system (CNS) metastatic disease.[58] An additive in vitro effect was also seen when combined with 5-fluorouracil.[59]

Lapatinib as first-line treatment

Initially, phase I and II studies evaluated lapatinib as a single agent, achieving RR between 1.4% to 7.7%[60] in heavily pretreated patients and 24% when given as first line.[61] Two phase III trials evaluated lapatinib in combination with chemotherapy as second- and first-line treatment, respectively, and led to the approval of lapatinib by the FDA. The first trial[62] enrolled patients with advanced HER2-positive breast cancer previously treated with a taxane, an anthracycline and trastuzumab. Patients were treated with either single agent capecitabine, 2500mg/m^2 daily for 14 days on a 21-day cycle or capecitabine 2000 mg/m^2 on the same schedule plus lapatinib 1250 mg daily. After the accrual of 324 patients, an interim analysis showed a significant improvement in TTP from 4.4 months to 8.4 months in favor of the combination arm [HR: 0.49 (CI 0.34-0.71) P<.001)] that was the primary study endpoint. Accrual was terminated after recommendation from an independent data safety monitoring committee.

The second phase III study,[63] tested lapatinib in combination with paclitaxel versus paclitaxel and placebo in the first line setting in patients with HER2 negative or uncharacterized metastatic breast cancer. In a total of 579 evaluable patients, no significant differences were observed in TTP, event-free survival and OS between the two arms. However, in the subset of patients with HER2-positive tumors (49 of 291 in the combination arm and 37 of 288 in the monotherapy arm) ORR (63.3% vs. 37.8%; P=.023), clinical benefit ratio (CBR) (69.4% vs. 40.5%; P=.011) and TTP (46.4 vs. 25.1 weeks; P=.005) were significantly improved.

Lapatinib as second-line treatment

Phase II studies that evaluated lapatinib as single agent after trastuzumab-based therapy showed minimal activity with RR ranging from 1.4% to 5.1%. However, in another study in which lapatinib monotherapy[64] was given in patients with HER2-overexpressing relapsed or refractory inflammatory breast cancer, 39% had a partial response. Median PFS was 14.6 weeks (95% CI 12.1-16.0) with a median duration of response of 20.9 weeks (12.7-32.1). The likelihood of response to lapatinib was not affected by previous treatment with trastuzumab.

Pre-clinical data suggested a synergistic effect between trastuzumab and lapatinib in HER2-overexpressing tumors.[65] It seems that lapatinib can partially restore sensitivity to trastuzumab in patients previously exposed to the drug. This synergistic effect can be explained by a lapatinib stabilization and accumulation of inactive HER2 receptors at the cell surface, which leads to enhanced trastuzumab-dependent, immune-mediated cytotoxicity.

A randomized phase III study presented initially at ASCO 2008[66] evaluated women with HER2-positive MBC who had received prior anthracycline and taxane therapy and had progressed on trastuzumab. Two hundred ninety-six patients were randomized to receive either lapatinib (1500 mg daily) or lapatinib (1000 mg daily) plus trastuzumab (2 mg/kg weekly after 4 mg/kg loading dose). If patients progressed on the lapatinib arm, they could cross over to the combination arm. PFS was 8.4 weeks vs. 12 weeks (P=.029) and CBR 12.4% vs. 24.7% (P=.02) for the lapatinib-only arm vs. the combination arm, respectively. RR and median OS were also improved but their differences were not statistically significant. Both trastuzumab and lapatinib have shown significant activity when combined with chemotherapy in the first and second line treatment of metastatic HER2-positive breast cancer. Several questions though still remain unanswered:

1. Which agent should be used first, trastuzumab or lapatinib?
2. Should lapatinib be used in combination with a different chemotherapy drug after progression on lapatinib-based chemotherapy in the first line?
3. Is the combination of trastuzumab and lapatinib plus chemotherapy better than either agent plus chemotherapy in the first line?

Currently, a randomized, open-label, phase III study of taxane-based chemotherapy with lapatinib or trastuzumab first line therapy for women with HER2 positive metastatic breast cancer is designed to determine which is the best anti-HER2-targeted agent to be used in combination with chemotherapy in the first-line setting.[67] Another phase III trial will test whether the

addition of lapatinib to trastuzumab in patients who either responded or achieved stable disease with trastuzumab-based chemotherapy is better than continuing trastuzumab alone.[68]

Lapatinib for the treatment of CNS disease

CNS metastases are more common in patients with HER2-positive tumors.[69] Increased frequency of brain metastases has been observed in patients treated with trastuzumab, which can be as high as 40% due to the inability of trastuzumab to cross the blood-brain barrier (BBB).[70] Lapatinib on the other hand is a small molecule that crosses the BBB and can be effective in CNS disease. In the largest clinical trial which examined the effect of lapatinib monotherapy[71] in patients with CNS disease, 15 (6%) out of 242 patients had an objective response with a mean absolute CNS lesion volumetric reduction of 3.2 cm^2 (range, 0.7-29.7 cm^2). Response was defined as a 50% volumetric reduction of CNS lesions without development of a new lesion or increase in the dose of steroids, progressing neurologic symptoms and signs or progressing non-target lesions. Patients who progressed on treatment were treated with the combination of capecitabine and lapatinib. Ten (20%) of 51 patients had more than 50% and another 18 (35%) patients had more than 20% volumetric reduction of CNS metastases making the combination of capecitabine and lapatinib the treatment of choice for patients with brain metastases even after progression on lapatinib monotherapy. Currently, the combination of lapatinib with temozolomide[72] is being tested in clinical trials. The main trials on trastuzumab and lapatinib are summarized in **Table 3**.

Combining anti-HER2 targeted drugs with hormonal treatments

There is a crosstalk between downstream pathways of HER2 and/or EGFR and ER.[73] Almost half of HER2 positive breast cancers co-express ERs. There are studies that provide evidence that the concurrent use of trastuzumab and tamoxifen can restore sensitivity to tamoxifen in resistant lines. The same has been observed when lapatinib was combined with tamoxifen. Both anti-HER2 agents have been combined with aromatase inhibitors and it seems that the two drugs act synergistically.

A meta-analysis that included 2379 patients from 12 studies[74] showed a high correlation between HER2 overexpression and hormonal treatment failure (tamoxifen, AIs, megestrol acetate and others) that was even higher when the few ER-negative patients were excluded.

The results from the TAnDEM (Trastuzumab and Anastrazole Directed Against Estrogen Receptor-Positive, HER2-Positive Mammary Carcinoma)[75] trial have been recently reported. Two hundred and seven postmenopausal breast cancer patients with ER and HER2 positive tumors were randomly assigned to anastrazole plus trastuzumab (103 patients) or anastrazole monotherapy (104 patients). The primary endpoint was PFS. Median PFS was 4.8 months for the combination vs. 2.4 months for the anastrazole monotherapy group (HR: 0.63, CI, 0.47 to 0.84, *P*=.0016). Furthermore, median PFS was longer in both arms (5.6 vs. 3.8 months, *P*=.006) in those patients who were found to be ER positive by central assessment. ORR was also higher for the combination vs. the monotherapy group (20.3 vs. 6.8%, *P*=.018) as was the CBR (42.7 vs. 27.9%, *P*=.026). OS was also prolonged (28.5 vs. 23.9 months, *P*=.325). Although not statistically significant, this improvement in survival was observed despite the crossover of 73 patients from the monotherepy to the combination arm at disease progression.

Another randomized phase III trial examined the combination of lapatinib and letrozole vs. letrozole and placebo. Of 1286 postmenopausal women with ER+ metastatic breast cancer who received either lapatinib 1500 mg with letrozole 2.5mg or letrozole 2.5mg or placebo, 219 (17%) patients were HER2-positive of which 111 received the combination and 108 received letrozole with placebo.[76] The addition of lapatinib to letrozole in this patient population significantly decreased the risk of disease progression with PFS being 8.3 months for the combination vs. 3 months for the letrozole-placebo arm (HR=0.71; 95% CI, 0.53 to 0.96; *P*=.019). ORR was 28% vs. 15% (odds ratio OR=0.4; 95% CI, 0.2 to 0.9; *P*=.021). CBR was also improved (48% vs. 29%, respectively, OR=0.4; 95% CI, 0.2 to 0.8; *P*=.003). A statistically significant increase in the overall survival has not been observed with OS being 33.3 months for the combination and 32.5 months for the letrozole-placebo arm (HR=0.74; 95% CI, 0.5 to 1.1; P=.114). An interesting observation in this study was that in the 952 patients with HER2 negative tumors, the researchers identified a subgroup (200 patients) who showed a non-significant trend toward prolonged PFS (8.3 vs. 3.1 months (HR=0.78; 95% CI, 0.57 to 1.07; *P*=.117). These patients had experienced relapse less than six months since prior tamoxifen discontinuation. A possible explanation could be the fact that an enhancement of growth factor activity can be seen in association with endocrine resistance, and indeed, 20% of patients with HER2 negative tumors can be found HER2 positive if re-biopsied at the the time of disease progression/hormone resistance.

Evolving novel anti-HER2 strategies

Novel approaches targeting the HER2 pathway are now available in the armamentarium. These approaches showed efficacy when tested in preclinical models.[77-81] Initially, phase I trials tested their safety and due to their tolerable profile further investigation has been performed.[82-85] We present the most important agents such as T-DM1, pertuzumab, neratinib, afatinib and ertumaxomab and related phase II and III trials that have been conducted. The main trials are summarized in **Table 4**.

Trastuzumab conjugated to an anti-microtubule agent

T-DM1 consist of trastuzumab conjugated to an anti-microtubule agent (a derivative of maytansine). Also known as trastuzumab emmtansine, it is a novel approach for overcoming resistance to trastuzumab. DM1 is a potent anti-microtubule agent with in vitro cytotoxicity more than 1000 times higher than any other chemotherapeutic agent.[86] In a phase II study, 107 patients with metastatic trastuzumab refractory disease were assessed after T-DM1 administration. Complete response was observed in one patient and partial response in 41. The objective RR was 25% and the CBR 34.8%.[87]

Another phase II trial enrolled 110 patients who had previously received a median of seven prior agents. An objective RR of 32.7% and a CBR of 44.5% were evaluated.[88] In addition, another randomized phase II trial evaluated T-DM1 vs trastuzumab/docetaxel in first-line, HER2-positive MBC. A significant increase of PFS was reported with the T-DM1 arm (14.2 vs 9.2 months). In 137 patients enrolled, the objective RR in T-DM1 arm was of 47.8% as compared with 41.4% for the other arm.[89] Interestingly, a single arm phase II study evaluated the combination of T-DM1 and pertuzumab in relapsed (n=46) and previously untreated patients (n=21). A RR of 57.1% and 34.8% was observed in untreated and relapsed group of patients respectively.[90] Patient tolerability was good with fatigue, nausea and thrombocytopenia the most common adverse events. Left ventricular ejection fraction (LVEF) decline has been reported but seems to be rare.

Recently, the results of the EMILIA study, a phase III trial of T-DM1 compared with capecitabine plus lapatinib provided convincing evidence. OS was improved for patients receiving T-DM1. Furthermore, median OS has not been reached vs 23.3 mo (HR=0.621, 95% CI: 0.475–0.813, *P*=.0005). Median PFS was improved also; 9.6 months in the T-DM1 arm compared with 6.4 months (HR=0.650, 95% CI: 0.55–0.77, *P*<.0001). The ORR was significantly higher in the T-DM1 group at 43.6% vs 30.8% in the (95% CI: 6.0–19.4, *P*=.0002). Also greater safety was demonstrated; the incidence of adverse events, toxicity was in favor for the T-DM1 arm; cardiac toxicity was not increased.[91]

Pertuzumab

Pertuzumab is a monoclonal antibody that targets HER2 extracellular domain at a different epitope than trastuzumab. Trastuzumab, although effective against HER2 homodimers, is not active against ligand-induced HER2 heterodimers. EGFR/HER2 and HER2/HER3 heterodimers are important in breast cancer proliferation through the HER2 pathway and also in bypassing trastuzumab inhibition. Pertuzumab inhibits dimerization with other HER receptors and downregulates MAPK and PI3K/Akt signaling pathways.[92] In a single arm, phase II study, the pertuzumab-trastuzumab combination was evaluated in 11 patients with HER2 positive MBC. The median TTP was 6 weeks and the objective RR 18% (no CR was observed).[93] Six cases with left ventricular dysfunction were reported (3 grade I, 2 grade II, 1 grade III), but using a lower limit of LVEF cut-off of 55%.

In addition, Baselga and co-investigators evaluated the same combination in 66 patients trastuzumab refractory HER2 positive MBC. The median PFS was 5.5 months with an objective RR of 24.2% (CR: 7.6%, PR: 16.7%).[94] Contrary to the previous study, cardiotoxicity was not a major issue with only three patients with an asymptomatic LVEF decline >10%. In two patients LVEF recovery was seen without treatment interruption. Diarrhea, fatigue, nausea and rash were the most common toxicities.

In the NEOSPHERE trial, patients with operable locally advanced or inflammatory breast cancer received the subsequent regimens in the neoadjuvant setting. Of 417 patients were randomized to receive docetaxel-pertuzumab, docetaxel-trastuzumab, docetaxel-trastuzumab-pertuzumab or trastuzumab-pertuzumab, the three-drug combination presented the higher pathologic complete response rate (45.8% vs 29% of DOCTRAST combination, *P*=.014). Even in the arm that received only targeted therapies without chemotherapy a pCR rate of 16.8% was observed.[95] The recently presented data of CLEOPATRA trial showed that dual HER2 blockade improves PFS. The 808 patients enrolled in this phase III trial received docetaxel plus trastuzumab combined with pertuzumab or placebo. The pertuzumab arm presented significantly prolonged PFS (18.5 vs 12.4 months; *P*<.001). No decrease of LVEF was evaluated. In the pertuzumab group the rates of febrile neutropenia and diarrhea of grade 3 or above were higher than in the control group.[96]

Neratinib

Neratinib is an orally administered, low molecular weight, irreversible pan-HER receptor tyrosine kinase inhibitor. A phase II trial tested the activity of neratinib in patients with HER2 positive by FISH in locally advanced or metastatic breast cancer who either received or did not receive trastuzumab therapy in the past.[97] Patients with prior (n=66) on no prior trastuzumab treatment (n=70) were recruited and received 240 mg of neratinib once daily. Median PFS was 40 and 22 weeks, and the CBR was 56% and 24%, respectively. Based on these results, phase III studies with neratinib are being conducted in women with HER2-positive breast cancer.

Afatinib

Afatinib is an oral, small molecule irreversible HER family TKI. In a single arm, phase II study, 41 patients with HER2 positive, trastuzumab refractory MBC were enrolled. PR was evaluated in 4 patients and stable disease in 8. Diarrhea (22%) and rash (9.8%) were the most common adverse events.[98]

Ertumaxomab

Ertumaxomab is a trifunctional, bispecific monoclonal antibody that targets both HER2 receptor, CD3 antigen on T cells and activates fcgamma receptors. The complex induces the activation of T cells, dendritic cells, natural killers and macrophages that through phagocytosis provoke tumor death.[99] In a phase I trial a response was evaluated in 15 patients with MBC. The most common adverse events were nausea, vomiting, fever, lymphocytopenia and elevated liver enzymes.[100] The results of further phase II studies are eagerly awaited.

Conclusions

Over the past decade, we have witnessed a shift in the treatment of breast cancer, from chemotherapy to combinations of chemotherapy and targeted therapies. Increased knowledge of the molecular biology of breast cancer has enabled us to be more specific in the treatment of the different types of breast cancer. Drugs are now designed to target molecules that are important for the survival of cancer cells. The ER and HER2 receptors have emerged as the most important targets and determinants of individualized treatment. In the following years we expect to see an increasing number of targeted agents, promising prolonged survival and better quality of life for cancer patients.

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