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Resistance to therapy in estrogen receptor positive and human epidermal growth factor 2 positive breast cancers: progress with latest therapeutic strategies

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Abstract: In this article, we focus on the subtype of estrogen receptor (ER)-positive, human epidermal growth factor 2 (HER2)-positive breast cancer (BC). Preclinical and clinical data indicate a complex molecular bidirectional crosstalk between the ER and HER2 pathways. This crosstalk probably constitutes one of the key mechanisms of drug resistance in this subclass of BC. Delaying or even reversing drug resistance seems possible by targeting pathways implicated in this crosstalk. High-risk patients currently receive anti-HER2 therapy, chemotherapy and endocrine therapy in the adjuvant setting. In metastatic cases, most patients receive a combination of anti-HER2 therapy and chemotherapy. Only selected patients presenting more indolent disease are candidates for combinations of anti-HER2 therapy and endocrine therapy. However, relative improvements in progression-free survival by chemotherapy-based regimens are usually lower in ER-positive patients than the ER-negative and HER2-positive subgroup. Consequently, new approaches aiming to overcome endocrine therapy resistance by adding targeted therapies to endocrine therapy based regimens are currently explored. In addition, dual blockade of HER2 or the combination of trastuzumab and phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOP) inhibitors targeting the downstream pathway are strategies to overcome resistance to trastuzumab. This may lead in the near future to the less frequent use of chemotherapy-based treatment options in ER-positive, HER2-positive BC.

Keywords: breast cancer, endocrine therapy, human epidermal growth factor 2, resistance, therapy, trastuzumab

Introduction

Breast cancer (BC) is a very heterogeneous disease. Gene-expression profiling has identified four molecular classes of BC: basal-like, luminal-A, luminal-B and human epidermal growth factor 2 (HER2)-positive BC [Perou *et al*. 2000]. These four classes are very close to the clinical classifications based on proliferation markers, histological grade, expression of estrogen and progesterone receptors (ER and PgR) and overexpression of HER2.

In this article, we focus on ER- and HER2-positive BC subclasses. HER2-positive BC represents approximately 20–25% of BC [Slamon *et al*. 1989]. Half of HER2-positive BC expresses ER or PgR. Consequently, the subgroup we discuss here represents only about 10% of all BC cases. However, this subgroup needs specific systemic treatment approaches. Although tumor cells express ER, these tumors respond poorly to endocrine therapy alone, particularly tamoxifen. These have a short disease-free survival (DFS) [Azim and Piccart, 2010]. There is growing preclinical and clinical evidence indicating a complex molecular bidirectional crosstalk between ER and HER2 pathways [Arpino *et al*. 2008; Massarweh and Schiff, 2007]. This crosstalk probably constitutes one of the key mechanisms of drug resistance in this subclass of BC. A better understanding of this crosstalk is of considerable clinical interest. Delaying or even reversing drug resistance seems

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possible by targeting pathways implicated in this crosstalk.

Estrogen receptor and HER2 pathways: bidirectional molecular crosstalk

Genomic and nongenomic action of estrogen receptor

ER is mainly a nuclear protein with a genomic nuclear activity also known as 'nuclear initiated steroid signaling'. Through this activity, ER functions as a ligand-dependent (estrogen) transcription factor of genes implicated in BC cell proliferation and survival [Osborne and Schiff, 2005]. ER also has an inhibitory function on a subclass of genes, which are mainly transcriptional repressors or genes with antiproliferative or proapoptotic functions [Frasor *et al.* 2003]. The transcriptional activity of ER is regulated by the binding to coactivator or corepressor proteins. Their nuclear levels can considerably influence ER signaling [Arpino *et al.* 2008].

For basic comprehension of bidirectional crosstalk between the ER and HER2 pathways, it is essential to insist on the fact that signaling from different growth factor receptor dependent kinases phosphorylates various factors in the ER pathway, including ER itself. This potentiates ER genomic signaling activity on gene transcription [Arpino *et al.* 2008]. Thus, in the presence of hyperactive growth factor receptor signaling, as often occurs in BC (e.g. HER2 overexpression), an excessive phosphorylation of ER and its coregulators may severely weaken the inhibitory effects of various endocrine therapies [Arpino *et al.* 2008]. It may increase ER transcriptional activity in a ligand-independent mode or even in the presence of selective ER modulators (SERMs) like tamoxifen [Schiff *et al.* 2003].

ER can also induce rapid stimulatory effects on different signal transduction pathways independent of gene transcription. This is called 'membrane initiated steroid signaling', and it can be activated by both estrogen and SERMs like tamoxifen [Massarweh and Schiff, 2007]. This mode of action can directly or indirectly activate epidermal growth factor receptor (EGFR), HER2 and insulin-like growth factor receptor 1 (IGFR1) [Lee *et al.* 2000]. This, in turn, activates the EGFR downstream kinase cascades (i.e. RAS/MEK/mitogen-activated protein kinases (MAPK) and PI3K/AKT). These downstream kinases phosphorylate and activate ER and its coregulators, increasing genomic activities of ER [Schiff *et al.* 2004]. The genomic and nongenomic actions of ER are complementary and not mutually exclusive.

Mechanisms of resistance to endocrine therapy

Several mechanisms of resistance to endocrine therapy have been described. They include the loss or modification of ER expression, epigenetic mechanisms regulating ER expression and crosstalk between ER and different signaling pathways [Garcia-Becerra *et al.* 2013]. The loss of ER expression can be explained by epigenetic changes, such as aberrant methylation of the ER promoter, limiting its transcription. Epigenetic changes are frequent and reversible events. Thus, the inhibition of these mechanisms could be a potential therapeutic strategy for the treatment of endocrine-resistant BC. Hypoxia, overexpression of EGFR or HER2 and MAPK hyperactivation have also been proposed to explain the loss of ER expression. Different models suggest that overexpression of EGFR and HER2 contribute to transcriptional repression of ER gene. In recent years, interest was focused on modification in ER expression and, particularly, in gain-of-function mutations in ESR1, the gene encoding ER. These mutations are clustered in a hotspot within the ligand-binding domain of ER and lead to ligand-independent ER activity [Jeselsohn *et al.* 2015]. Finally, we insist on the crosstalk described between ER and different signaling pathways as the main mechanism of endocrine therapy resistance.

ER can be phosphorylated and activated by intracellular kinases following activation of EGFR or IGFR by their ligands. This can result in ligand-independent activation of the receptor [Schiff *et al.* 2003]. Another mechanism of resistance is activation by the agonist effect of SERMs like tamoxifen. Phosphorylation of ER coactivators is as important as phosphorylation of ER itself. The coactivator amplified in breast cancer 1 (AIB1) can be activated by multiple cellular kinases.

Two retrospective studies demonstrated that tumors with high levels of both AIB1 and HER receptors (HER2 or HER3) are less responsive to treatment by tamoxifen. This supports the hypothesis that increased signaling from the HER family activates downstream kinases, which in turn activates ER and AIB1 to increase transcriptional activity even in the presence of tamoxifen

[Osborne *et al.* 2003; Kirkegaard *et al.* 2007]. This is called *de novo* resistance to tamoxifen. Furthermore, the nongenomic action of ER can be activated by high levels of growth factor receptors and their ligands [Shou *et al.* 2004]. Various studies have shown that enhanced expression of EGFR and HER2 with activation of downstream signalization by p42/44 MAPK and PI3K/AKT/mTOR is also clearly implicated in acquired resistance to hormonal therapy (tamoxifen, estrogen depletion and aromatase inhibitor therapy) [Arpino *et al.* 2008; Knowlden *et al.* 2003; Brodie *et al.* 2007; Jeng *et al.* 2000].

PgR-negative tumors are less responsive to endocrine therapy than PgR-positive tumors. Some authors think that an increased activity in growth factor receptor pathways is also responsible for the loss of PgR [Cui *et al.* 2003; Petz *et al.* 2004; Arpino *et al.* 2005]. They hypothesize that suppressed or reduced PgR levels may derive from and indicate hyperactivity in the signaling cascade generated by EGFR, HER2 and other kinase activation. This could explain the endocrine resistance [Arpino *et al.* 2008].

The current understanding is that growth factor receptors play central roles in resistance to various endocrine therapies. Different clinical observations support these models of endocrine resistance.

A meta-analysis examining the interaction between HER2 expression and response to endocrine treatment in metastatic disease clearly shows that HER2-positive BC is less responsive to any type of endocrine treatment [De Laurentiis *et al.* 2005]. It is interesting to note that EGFR overexpression is also associated with a poorer response to tamoxifen in patients with metastatic BC [Arpino *et al.* 2004]. Furthermore, results from neoadjuvant settings also support the role of HER2 and EGFR in resistance to treatment by tamoxifen [Ellis *et al.* 2001; Zhu *et al.* 2004; Dowsett *et al.* 2007]. These neoadjuvant studies tend to show a higher efficacy of aromatase inhibitors compared with tamoxifen in HER2-positive tumors, but no robust conclusion can be made. In addition, both agents are associated only with short-lived responses [Azim and Piccart, 2010].

Signaling *via* HER2/MAPK appears to be a main mechanism of resistance to different endocrine therapies. It is also attractive to target growth factor receptor signaling in addition to ER itself to optimize the treatment benefits [Massarweh and Schiff, 2007]. Indeed, xenograft studies have confirmed that HER2 targeting in combination with endocrine therapy in HER2-overexpressing xenografts restores tamoxifen sensitivity and significantly delays resistance to estrogen deprivation or fulvestrant [Shou *et al.* 2004; Massarweh *et al.* 2006]. There is also a growing interest in adding mTOR inhibitors to endocrine therapy to delay endocrine resistance because this treatment improved progression-free survival (PFS) compared with endocrine therapy alone in patients with advanced BC who had relapsing disease on treatment with aromatase inhibitors in the BOLERO-2 study [Baselga *et al.* 2012c; Yardley *et al.* 2013].

Mechanisms of resistance to anti-HER2 therapies

As for resistance to endocrine therapy, there are different mechanisms for resistance to anti-HER2 therapies [Tortora 2011; Rexer and Arteaga, 2012]. It can be a mechanism intrinsic to the target itself, with masking of the antibody binding epitope, truncated forms with kinase activity and an extracellular domain which neutralizes the HER2 antibody, or even with mutations in the tyrosine kinase domain.

Resistance can arise from defects in the apoptosis pathway and cell cycle control in tumor cells or in host factors that participate in drug action. As an example, defects in ADCC immunomodulatory function of trastuzumab can contribute to the resistance.

Resistance can also mainly involve parallel bypass signaling pathways to overcome HER2 inhibition. It includes upregulation of ligands and heterodimerization with EGFR or HER3. There are also interactions with other membrane receptors such as IGF1R or MET. Loss of phosphatase and tensin homolog (PTEN) or activating mutations of the PIK3CA gene promote persistent PI3K activation, even in the presence of anti-HER2 therapy.

An inverse relationship has been observed between expression of growth factor receptors and ER [Massarweh and Schiff, 2007]. ER content is inversely correlated with EGFR/HER2 levels in ER-positive, HER2-positive tumors [Konecny *et al.* 2003]. Preclinical data support the hypothesis that increased growth factor signaling downregulates ER expression [Stoica *et al.* 2000a, 2000b; Tang *et al.* 1996; Oh *et al.* 2001]. This can

lead to a complete loss of ER expression and consequently represents a potential mechanism of resistance to endocrine therapy [Massarweh *et al.* 2006]. Recent observations support the hypothesis that some HER2-overexpressing tumors that are apparently ER negative may actually revert to ER positivity after treatment with anti-HER2 therapy [Munzone *et al.* 2006; Xia *et al.* 2006]. A preclinical model published by Giuliano and colleagues has reported that ER and Bcl2 expression are simultaneously increased in BC xenografts treated with anti-HER2 therapies [Giuliano *et al.* 2015]. A combination of endocrine and anti-HER2 therapies given simultaneously might benefit ER+/HER2+ cell lines, including those with low ER levels [Wang *et al.* 2011]. This bidirectional crosstalk between ER and HER2 pathways may also contribute to resistance to anti-HER2 therapy. Moreover, as mentioned above, resistance to trastuzumab is often thought to be mediated by the loss of PTEN, resulting in the activation of the PI3K/AKT/mTOR pathway [Nahta and O'Regan, 2010; Jensen *et al.* 2012; Razis *et al.* 2011]. Thus, activation of mTOR plays a central role in the crosstalk. Due to the fact that many of the known resistance mechanisms can bypass the HER2-targeted agents, there is intense interest in defining new therapeutic targets downstream from HER2.

Resistance to endocrine therapies and anti-HER2 therapies is a crucial downstream element of intracellular signaling. From this point of view, in addition to the interest in the PI3K/AKT/mTOR pathway, the CDK4/6 complexes represent a new promising target downstream of HER2 at the interface between proliferation signaling pathways and the cell cycle machinery [Witkiewicz *et al.* 2014]. There are also downstream of the majority of processes driving resistance to HER2-targeted therapies and endocrine therapies.

Preclinical models: proof of concept of dual targeting estrogen receptor and HER2

In a xenograft model, Sabnis and colleagues showed that ERα expression is reduced while HER2 expression is increased in letrozole-resistant cancer cells [Sabnis *et al.* 2009]. In these cells, the addition of trastuzumab to letrozole increases levels of ERα and restores sensitivity to letrozole. The combination of trastuzumab and letrozole provided higher anticancer effects compared with trastuzumab or letrozole alone. In another model, lapatinib in combination with tamoxifen effectively inhibited the growth of tamoxifen-resistant HER2 overexpressing Michigan Cancer Foundation-7 (MCF-7) mammary tumor xenografts [Chu *et al.* 2005]. Xia and colleagues confirmed that acquired resistance to lapatinib is mediated by a switch in cell survival dependence [Xia *et al.* 2006]. This regulation from HER2 results in codependence on ER and HER2. Increased ER signaling in response to treatment by lapatinib is enhanced by the activation of factors facilitating the transcriptional activity of ER. These findings provided the rationale for preventing the development of acquired resistance to lapatinib by simultaneously inhibiting both ER and HER2 signaling pathways. In their model, Giuliano and colleagues reported that neoadjuvant treatment with lapatinib leads to a rapid increase in ER expression in HER2 BC and demonstrated that cotargeting ER along with HER2-targeted therapies circumvents this type of resistance. They also found that endocrine therapy delays tumor progression in the presence of restored ER expression in xenograft tumors treated with anti-HER2 therapy [Giuliano *et al.* 2015]. These preclinical models are a proof of concept that dual targeting of ER and HER2 is of considerable clinical interest.

Clinical evidence: results of phase II/III trials

We would like to emphasize the importance of this 'new entity' of 'triple-positive breast cancer' by analyzing, if mentioned, results of this subgroup in phase III and some important phase II trials in the field of HER2-positive BC. Different outcomes based on ER status is the basis for the design of new clinical trials evaluating specific treatment approaches for ER-positive, HER2-positive BC.

Neoadjuvant phase II/III trials

The pathological complete remission (pCR) rate is lower in ER-positive, HER2-positive BC *versus* ER-negative HER2-positive BC in neoadjuvant settings (Table 1). The difference in pCR rates between ER-positive and ER-negative cancers varies between trials according to the type of chemotherapy and the HER2-directed agent under study. The pCR rate has a clear prognostic value in HER2-positive ER-negative BC [Nahta and O'Regan, 2012], but this relationship between pCR and outcome in HER2-positive ER-positive BC has not been demonstrated.

Table 1. pCR according to ER status in HER2-positive breast cancer after neoadjuvant therapy.

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	pCR	*p*	pCR rateHR+	pCR rate HR−	*p*	Other results regarding hormonal status
GeparQuattro [ClinicalTrials.gov identifier: NCT00288002]	Untch *et al.* [2010]	Phase III Multicenter Open label Randomized HER2+ *versus* − Three arms	HER2+/− Stages II–III HR−/+ if cN or pN (SLN)	pCR	1495 HER2+: 445 HER2−: 1050	HER2+: 1. EC×4 → docetaxel (D)×4 + trastuzumab 2. EC×4 → D + capecitabine (C)×4 + trastuzumab 3. EC×4 → D×4 → C×4 + trastuzumab HER2−: 1. EC×4 → D×4 2. EC×4 → D+C×4 3. EC×4 → D×4 → C×4	31.7% 32.7% 31.3% 34.6% 15.7% 17.6% 14.4% 17.5%	NR	23.40%	43.50%	<**0.001**	
GeparQuinto [ClinicalTrials.gov identifier: NCT00567554]	Untch *et al.* [2012]	Phase III Multicenter Open label Randomized Two arms	HER2+ Stages II–III HR−/+ if cN or pN (SLN)	pCR	615 Trastuzumab: 307 Lapatinib: 308	4×EC → 4×docetaxel + trastuzumab 4×EC → 4×D + lapatinib	30.3% 22.7%	**0.04**	NR	NR	NR	Odds ratio for pCR according to hormonal status: − *versus* + = 0.49 (0.34–0.70)
NeoALLTO [ClinicalTrials.gov identifier: NCT00553358]	Baselga *et al.* [2012a]	Phase III Multicenter Open label Randomized Three arms	HER2+ Stages II–III	pCR	455 A = trastuzumab: 149 B = lapatinib: 154 C = trastuzumab + lapatinib: 152	Trastuzumab (T)×6 → T + weekly paclitaxel (P)×12 Lapatinib (L)×6 weeks → L + weekly P×12 T + L×6 → T + L + weekly P×12	29.5% 24.7% 51.3%	A *versus* B = 0.34 A *versus* C = **0.0001**	22.67% 16.25% 41.56%	36.49% 33.78% 61.33%	NR NR NR	
NOAH [ClinicalTrials.gov identifier: ISRCTN86043495]	Gianni *et al.* [2010, 2014]	Phase III Multicenter Open label Randomized Two arms HER2−: control	HER2+ Stage III Control arm HER2−	Event-free survival Secondary endpoint: pCR	235 Trastuzumab: 118 Without trastuzumab: 117 Control arm: 99	Doxorubicine (D)+ paclitaxel (P)×4 → P×4 → T + cyclophosphamide + methotrexate + fluorouracil (CMF)×3 D + P×4 → P×4 → CMF×3 D + P×4 → P×4 → CMF×3	38% 19% 16%	**0.0001**	NR NR	NR NR		Odds ratio for EFS according to hormonal status in HER2+ (Trastuzumab *versus* control): HR−: 0.46 (0.27–0.8) HR+: 0.87 (0.43–1.74)
NSABP B41 [ClinicalTrials.gov identifier: NCT00486668]	Robidoux *et al.* [2013]	Phase III Multicenter Open label Randomized Three arms	HER2+ Stages II–III	pCR	519 A = trastuzumab: 177 B = lapatinib: 171 C = trastuzumab + lapatinib: 171	Doxorubicine–cyclophosphamide (AC) ×4 → paclitaxel weekly 3 weeks/4×4 + trastuzumab AC×4 → paclitaxel 3 weeks/4×4 + lapatinib AC×4 → paclitaxel 3 weeks/4×4 + trastuzumab + lapatinib	49.4% 47.4% 60.2%	A *versus* B = 0.78 A *versus* C = 0.056	45.5% 42% 54.6%	58.2% 54.9% 69.8%	NR NR NR	
ACOZOG Z1041 [ClinicalTrials.gov identifier: NCT00513292]	Buzdar *et al.* [2013]	Phase III Multicenter Open label Randomized Two arms	HER2+ Stages II–III	pCR	280 A = sequential: 138 B = concurrent: 142	FEC75×4 → paclitaxel weekly + trastuzumab×12 Paclitaxel weekly + trastuzumab×12 → FEC75×4 + trastuzumab weekly	56.5% 54.2%	0.9	47.6% 38.1%	70.4% 77.6%	NR NR	

(Continued)

Table 1. (Continued)

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	pCR	*p*	pCR rateHR+	pCR rate HR−	*p*	Other results regarding hormonal status
CALGB 40601 [ClinicalTrials.gov identifier: NCT00770809]	Carey *et al.* [2016]	Phase III Multicenter Open label Randomized Three arms	HER2+ Stages II–III	pCR	305 A = trastuzumab: 120 B = trastuzumab + lapatinib: 118 C = lapatinib: 67	Paclitaxel weekly + trastuzumab×16 Paclitaxel weekly + trastuzumab + lapatinib×16 Paclitaxel weekly + lapatinib×16 → early closing	46% 56%	0.13	41% 41%	54% 79%	NR NR	
NeoSPHERE [ClinicalTrials.gov identifier: NCT00545688]	Gianni *et al.* [2012]	Phase II Multicenter Open label Randomized Four arms	HER2+ Stages II–III	pCR	417 A = trastuzumab + docetaxel: 107 B = trastuzumab + pertuzumab + docetaxel: 107 C = trastuzumab + pertuzumab: 107 D = pertuzumab + docetaxel: 96	Trastuzumab (T) + docetaxel (D)×4 T + pertuzumab (P) + D×4 T + P×4 P + D×4	29% 45.8% 16.8% 24%	A *versus* B = **0.0141** A *versus* C = **0.0198** B *versus* D = **0.003**	20% 26% 5.9% 17.4%	36.8% 63.2% 27.3% 30%	NR NR NR NR	
CHER-LOB [ClinicalTrials.gov identifier: NCT00429299]	Guarneri *et al.* [2012]	Phase II Multicenter Open label Randomized Three arms	HER2+ Stages II–III	pCR	121 A = trastuzumab: 36 B = lapatinib: 39 C = trastuzumab + lapatinib: 46	Paclitaxel weekly×12 + trastuzumab → FEC75×4 + trastuzumab Paclitaxel weekly×12 + lapatinib → FEC75×4 + lapatinib Paclitaxel weekly×12 + trastuzumab + lapatinib → FEC75×4 + trastuzumab + lapatinib	25% 26.3% 46.7%	C *versus* A–B = **0.019**	28.8%	41.3%	NR	
TRYPHAENA [ClinicalTrials.gov identifier: NCT00976989]	Schneeweiss *et al.* [2013]	Phase II Multicenter Open label Randomized Three arms	HER2+ Stages II–III	Incidence of symptomatic LVSD Decline of LVEF > 10% Secondary endpoint: pCR	225 A = concurrent anthracycline based: 73 B = sequential anthracycline based: 75 C = concurrent non-anthracycline based: 77	FEC100 + trastuzumab + pertuzumab×3 → docetaxel + trastuzumab + pertuzumab×3 FEC100×3 → docetaxel + trastuzumab + pertuzumab×3 Docetaxel + carboplatine + trastuzumab + pertuzumab×6	61.6% 57.3% 66.2%	NR	46% 49% 50%	79% 65% 84%	NR NR NR	
TBCRC006 [ClinicalTrials.gov identifier: NCT00548184]	Rimawi *et al.* [2013]	Phase II Multicenter Single arm	HER2+ Stages II–III	pCR	64 ER+: 39 ER−: 25	Trastuzumab + lapatinib + letrozole (+LHRH if premenopausal) 12 weeks Trastuzumab + lapatinib 12 weeks	21% 36%	NR	21%	36%	NR	

ER, estrogen receptor; HER2, human epidermal growth factor 2; LHRH, luteinizing hormone releasing hormone; LVEF, ;eft ventricular ejection fraction; LVSD, left ventricular systolic dysfunction; NR, not reported; pCR, pathological complete remission. Bold *p* values : differences are statistically significant.

Indeed, in a recent meta-analysis including more than 6000 patients, von Minckwitz and colleagues showed that pCR is not prognostic in HER2-positive ER-positive BC [von Minckwitz *et al.* 2012]. Furthermore, a pooled analysis of 12 international trials including a total of 11,955 patients does not allow validation of pCR as a surrogate endpoint for improved event-free survival and overall survival in BC [Cortazar *et al.* 2014]. Although long-term follow up of these neoadjuvant trials is not yet available, it seems that pCR has no prognostic value. Bhargava and colleagues divided HER2-positive BC into three subgroups based on the level of expression of ER and PgR [Bhargava *et al.* 2011]. They reported an inverse correlation between the pCR rate and the level of ER expression. These data suggest that treatment options other than chemotherapy should be evaluated for these HER2-positive BC cases and high levels of ER expression. This option could associate anti-HER2-targeted therapies and endocrine therapy. This strategy was evaluated in the phase II neoadjuvant trial TBCRC006 [Rimawi *et al.* 2013]. The aim of the study was to show that an optimal blockade of the HER pathway and its potential escape mechanisms (activation of the ER pathway) without chemotherapy may induce pCR in many patients. Although inhibition of ER using letrozole with or without goserelin in combination with trastuzumab and lapatinib resulted in tumor responses, the pCR rate was unfortunately low [Nahta and O'Regan, 2012].

It is interesting to note that in NeoALLTO [Baselga *et al.* 2012a] and NeoSPHERE [Gianni *et al.* 2012], dual anti-HER2 therapy compared with single-agent anti-HER2 treatments was associated with higher pCR rates independent of the receptor status. These findings support the hypothesis that complete blockade of the HER receptor family in HER2-positive BC, along with targeting of ER simultaneously when coexpressed, may be necessary for optimal therapy.

All current data in the neoadjuvant setting suggest the existence of a subset of ER-positive, HER2-positive BC that behaves more like an ER-positive, HER2-negative BC. Low pCR rates after neoadjuvant chemotherapy with or without HER2-directed agents are observed in these patients; pCR is not predictive of outcome in this subtype of BC. High levels of ER expression are observed in these patients. These patients might need systemic treatments associating anti-HER2-targeted therapies and hormonal therapy; thus, chemotherapy can be avoided [Nahta and O'Regan, 2012]. Continued follow up of the reported neoadjuvant trials is important to monitor patients for late recurrences similar to what is seen in ER-positive, HER2-negative BC. The role of extended adjuvant endocrine therapy in ER-positive, HER2-positive cancers must be evaluated further.

Phase III trials in the adjuvant setting

As summarized in Table 2, a similar benefit in terms of relative improvement in DFS is obtained by adding trastuzumab to standard systemic therapy in ER-positive and ER-negative subgroups. However, patients presenting an ER-positive BC have a longer DFS in these studies. This agrees with previous observations indicating that a subset of ER-positive and HER2-positive BC cases behave similar to ER-positive and HER2-negative BC in terms of late relapse risk.

It is very interesting to note that only one trial, the TEACH trial that evaluated the addition of lapatinib to standard therapy after the end of chemotherapy, showed a significant difference in relative improvements in DFS in ER-positive and ER-negative patients. Lapatinib was sometimes administered very late during long-term follow up.

One of the possible explanations for poorer performance in ER-positive tumors is that 19% of these patients did not receive endocrine therapy concomitantly with lapatinib in the TEACH trial [Goss *et al.* 2013]. In other trials, endocrine therapy was administered in every patient with ER-positive tumors. This fact might have diminished the efficacy of lapatinib in this group by considering the bidirectional molecular crosstalk. These data, if confirmed, suggest that endocrine therapy should be evaluated earlier in the course of adjuvant therapy for patients with ER-positive, HER2-positive early-stage BC relative to current recommendations [Nahta and O'Regan, 2012].

Phase III trials in the advanced/metastatic setting

Important studies describing the outcome according to ER status are summarized in Table 3. The EGF104900 trial in patients with heavily pretreated HER2-positive metastatic BC demonstrates the benefit of dual targeting by trastuzumab and lapatinib only in patients who are ER negative. These data support the hypothesis that ER

Table 2. Adjuvant trials in HER2-positive breast cancer: results according to ER status.

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	DFS	*p*	Results according Hormonal status	*p*
HERA [ClinicalTrials.gov identifier: NCT00045032]	Piccart-Gebhart *et al.* [2005] Goldhirsch *et al.* [2013]	Phase III Multicenter Open label Randomised 3 arms	HER2+ N+/N− > 1 cm Completely resected At least four cycles of chemotherapy (neoadjuvant/ adjuvant)	DFS	5081 A = observation arm: 1693 B = trastuzumab 1 year: 1694 C = trastuzumab 2 years: 1694	Chemotherapy completed before randomization Selection from a list of approved regimens A: no anti-HER2 treatment B: trastuzumab 1 year C: trastuzumab 2 years Hormonotherapy if HR+	First interim analysis, 2005: Median follow up 1 year (0–36 months) B *versus* A HR: 0.54 (0.43–0.67) Final analysis, 2013: Median follow up 8 years (1–10 years) C *versus* B HR: 0.99 (0.85–1.14) B *versus* A HR: 0.76 (0.67–0.86)	**p < 0.0001** p = 0.86 **p < 0.0001**	First interim analysis, 2005: Median follow up 1 year (0–36 months) B *versus* A HR−: HR = 0.52 (0.39–0.69) HR+: HR ER−/PgR+ = 0.67 (0.24–1.84) HR ER+/PgR− = 0.63 (0.34–1.17) HR ER+/PgR+ = 0.61 (0.38–1) Final analysis, 2013: Median follow up 8 years (1–10 years) C *versus* B HR−: HR = 0.93 (0.76–1.14) HR+: HR = 1.05 (0.85–1.29)	NR NR NR NR *p* = 0.51 *p* = 0.67
NSABP B-31 NCCTG N9831 Joint analysis [ClinicalTrials.gov identifier: NCT00004067 NCT00005970]	Romond *et al.* [2005] Perez *et al.* [2014]	Joint analysis Phase III Multicenter Open label Randomized NSABP B-31: two arms NCCTG N9831: three arms	HER2+ N+/N− high risk only in N9831 Completely resected	DFS	NSABP B-31: 2102 1 = control arm: 1047 2 = trastuzumab concurrent: 1055 NCCTG N9831: 3160 A = control arm: 971 B = trastuzumab sequential: 1216 C = trastuzumab concurrent: 973 Joint analysis: 4046 1+A = control arm: 2018 2+C = trastuzumab concurrent: 2028	NSABP B-31 1: doxorubicine + cyclophosphamide (AC)×4 → paclitaxel (P) weekly×12 *or* P 1×/3 weeks×4 2: AC×4 → P weekly×12+ trastuzumab *or* P 1×/3 weeks×4 + trastuzumab → trastuzumab (1 year in total) NCCTG N9831: A: AC×4 → P weekly×12 B: AC×4 → P weekly×12 → trastuzumab (1 year in total) C: AC×4 → P weekly×12 + trastuzumab → trastuzumab (1 year in total) Hormonotherapy if HR+	First interim analysis, 2005: Median follow up 2 years 2+C *versus* 1+A HR: 0.48 (0.39–0.59) Final analysis, 2014: Median follow up 8.4 years 2+C *versus* 1+A HR: 0.6 (0.53–0.68)	**p < 0.0001** **p < 0.001**	First interim analysis, 2005: Median follow up 2 years 2+C *versus* 1+A HR−: HR = 0.51 (0.39–0.67) HR+: HR = 0.44 (0.32–0.61) Final analysis, 2014: Median follow up 8.4 years 2+C *versus* 1+A HR−: HR = 0.62 (0.52–0.73) HR+: HR = 0.61 (0.51–0.72)	NR NR NR NR
BCIRG 006 [ClinicalTrials.gov identifier: NCT00021255]	Slamon *et al.* [2011]	Phase III Multicenter Open label Randomized	HER2+ N+/N− high risk Completely resected	DFS	3222 A = control arm: 1073 B = anthracyclines and trastuzumab: 1074 C = no anthracyclines and trastuzumab: 1075	A: cyclophosphamide (AC)×4 → docetaxel×4 B: AC×4 → ×4 + trastuzumab → trastuzumab (1 year in total) C: docetaxel + carboplatine + trastuzumab×6 → trastuzumab (1 year in total) Hormonotherapy if HR+	Median follow up 65 months B *versus* A: HR = 0.64 C *versus* A: HR = 0.75	**p < 0.001** **p = 0.04**	B *versus* A HR−: HR = 0.64 (0.49–0.83) HR+: HR = 0.65 (0.49–0.85) C *versus* A HR−: HR = 0.65 (0.5–0.84) HR+: HR = 0.88 (0.68–1.13)	NR NR NR NR

Table 2. (Continued)

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	DFS	*p*	Results according Hormonal status	*p*
PHARE [ClinicalTrials.gov identifier: NCT00381901]	Pivot *et al.* [2013]	Phase III noninferiority Multicenter Open label Randomized Two arms	HER2+ Completely resected At least four cycles of chemotherapy and 6 months of Trastuzumab	DFS	3380 A = 6 months: 1690 B = 12 months: 1690	A: trastuzumab 12 months B: trastuzumab 6 months Hormonotherapy if HR+	Median follow up 42.5 months B *versus* A: HR = 1.28 (1.05–1.56)	p = 0.29	B *versus* A ER–: HR = 1.34 (1.02–1.76) PgR–: HR = 1.28 (1.01–1.64) ER+: HR = 1.23 (0.92–1.65) PgR+: HR = 1.24 (0.87–1.75)	 NR NR NR NR
TEACH [ClinicalTrials.gov identifier: NCT00374322]	Goss *et al.* [2013]	Phase III Multicenter Double blind Randomized Two arms	HER2+ Completely resected Adjuvant chemotherapy ended Never trastuzumab At any time from diagnostic	DFS	3147 A = placebo: 1576 B = lapatinib: 1571	A: placebo once a day, 1 year B: lapatinib once a day, 1 year Hormonotherapy if HR+ (19% of HR+: no hormonotherapy)	Median follow up 48 months B *versus* A: HR = 0.83 (0.7–1)	p = 0.053	B *versus* A HR–: HR= 0.68 (0.52–0.89) HR+: HR= 0.98 (0.77–1.25)	**p = 0.006** p = 0.89
ALLTO [ClinicalTrials.gov identifier: NCT00490139]	Piccart-Gebhart *et al.* [2016]	Phase III Multicenter Open label Randomized Four arms	HER2+ Completely resected N+/N– >1 cm	DFS	8381 A = trastuzumab: 2097 B = lapatinib: 2100 → early closing C = sequential trastuzumab and lapatinib: 2091 D = concurrent trastuzumab and lapatinib: 2093	DESIGN 1 = neoadjuvant or adjuvant chemotherapy completed before randomization; anti-HER2 agents were given alone DESIGN 2 = anthracycline component of adjuvant chemotherapy before randomization; taxanes were given concomitantly with anti-HER2 agents DESIGN 2B = non- anthracycline chemotherapy was given concomitantly with anti-HER2 agents A: chemotherapy + trastuzumab (1 year in total) B: chemotherapy + lapatinib (1 year in total) C: chemotherapy + trastuzumab → lapatinib (1 year in total of anti-HER2 drugs) D: chemotherapy + trastuzumab + lapatinib (1 year in total)	Median follow-up: 4.5 years (1 day–6.4 years) B *versus* A at first interim analysis (2011): HR = 1.52 (1.23–1.88) → early closing C *versus* A: HR = 0.96 (0.8–1.15) D *versus* A: HR = 0.84 (0.7–1.02)	p = 0.61 p = 0.048	D *versus* A HR–: HR = 0.82 (0.62–1.08) HR+: HR = 0.87 (0.66–1.13)	p = 0.7 NR

DFS, disease-free survival; ER, estrogen receptor; HER2, human epidermal growth factor 2; HR, hazard rate; NR, not reported; PgR, progesterone receptor.

Table 3. Trials in advanced HER2-positive breast cancer.

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	PFS	*p*	Results according to hormonal status	*p*
CLEOPATRA [ClinicalTrials.gov identifier: NCT00567190]	Baselga *et al.* [2012b] Swain *et al.* [2015]	Phase III Multicenter Randomized Double blind Two arms	HER2+ Locally recurrent unresectable or metastatic Maximum one hormonal treatment for metastatic BC before randomization No central nervous system metastases	PFS Secondary endpoint: OS	808 A = placebo: 406 B = pertuzumab: 402	A: placebo + trastuzumab + docetaxel 1×/3 weeks until disease progression or unmanageable toxic effects B: pertuzumab + trastuzumab+ docetaxel 1×/3 weeks until disease progression or unmanageable toxic effects	First interim analysis, 2012: Median follow up: 19.3 months Median PFS: 18.5 months for B *versus* 12.4 months for A HR B *versus* A = 0.62 (0.51–0.75) OS: HR B *versus* A = 0.64 (0.47–0.88) Final analysis, 2015: Median follow up: 50 months Median PFS: 18.7 months for B *versus* 12.4 months for A HR B *versus* A = 0.68 (0.58–0.80) Median OS: 56.5 months for B *versus* 40.8 months for A HR B *versus* A = 0.68 (0.56–0.84)	<0.001 0.005 ($p < 0.0012$) <0.001 <0.001	First interim analysis, 2012: Median follow-up: 19.3 months PFS B *versus* A HR+: HR = 0.72 (0.55–0.95) HR–: HR = 0.55 (0.42–0.72) Final analysis, 2015: Median follow up: 50 months PFS B *versus* A HR+: HR = 0.73 (0.58–0.91) HR–: HR = 0.64 (0.51–0.81) OS B *versus* A HR+: HR = 0.71 (0.53–0.96) HR–: HR = 0.61 (0.47–0.81)	NR NR 0.38 0.47
EMILIA [ClinicalTrials.gov identifier: NCT00829166]	Verma *et al.* [2012]	Phase III Multicenter Randomized Open label Two arms	HER2+ Progression of unresectable, locally advanced, or metastatic BC Previously treated with a taxane and trastuzumab	PFS OS Safety	991 A = lapatinib:496 B = T-DM1: 495	A: lapatinib once a day + capecitabine 1×/12 h 14 days/21 until disease progression or unmanageable toxic effects B: T-DM1 1×/3 weeks until disease progression or unmanageable toxic effects	First interim analysis, January 2012: Median follow up: 13 months Median PFS: 9.6 months for B *versus* 6.4 months for A HR B *versus* A = 0.65 (0.55–0.77) OS: HR B *versus* A = 0.62 (0.48–0.81) Second interim analysis, July 2012: Median follow up: 19 months Median OS: 30.9 months for B *versus* 25.1 months for A HR B *versus* A = 0.68 (0.55–0.85)	**<0.001** 0.0005 ($p = 0.0003$) **<0.001**	First interim analysis, January 2012: Median follow-up: 13 months PFS B *versus* A HR+: HR = 0.72 (0.58–0.91) HR–: HR = 0.56 (0.44–0.72)	NR NR
TH3RESA [ClinicalTrials.gov identifier: NCT01419197]	Krop *et al.* [2014]	Phase III Multicenter Randomized (2:1) Open label Two arms	HER2+ Progressive advanced (unresectable locally advanced or recurrent or metastatic) BC Two or more HER2– directed regimens including trastuzumab and lapatinib in the advanced setting and previous therapy in any setting	PFS OS	602 A = control: 198 B = T-DM1: 404	A: treatment of physician's choice (chemotherapy/ endocrine therapy/ HER2-directed therapy) until disease progression or unmanageable toxic effects B: T-DM1 1×/3 weeks until disease progression or unmanageable toxic effects	Median follow up: 7 months Median PFS: 6.2 months for B *versus* 3.3 months for A HR B *versus* A = 0.528 (0.422–0.661) First interim analysis for OS: HR B *versus* A = 0.552 (0.369–0.826)	**<0.0001** 0.0034 ($p < 0.0000016$)	Median follow-up: 7 months PFS B *versus* A HR+: HR = 0.56 (0.41–0.76) HR–: HR = 0.51 (0.37–0.71)	NR NR

Table 3. (Continued)

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	PFS	*p*	Results according to hormonal status	*p*
EGF104900 [ClinicalTrials.gov identifier: NCT00320385]	Blackwell *et al.* [2010, 2012]	Phase III Multicenter Randomized Open label Two arms	HER2+ Progressive metastatic BC on the most recent treatment regimen which must have contained trastuzumab	PFS Secondary endpoint: OS	291 A = lapatinib: 145 B = lapatinib + trastuzumab: 146	A: lapatinib once a day until disease progression or unmanageable toxic effects B: lapatinib once a day + trastuzumab weekly until disease progression or unmanageable toxic effects	First interim analysis, 2010: Median PFS 12 weeks for B *versus* 8.1 weeks for A HR B *versus* A = 0.73 (0.57–0.93) OS HR B *versus* A = 0.75 (0.53–1.07) Final analysis, 2012: Median follow up: 12.8 months for B and 8.7 months for A Median PFS 11.1 weeks for B *versus* 8.1 weeks for A HR B *versus* A = 0.74 (0.58–0.93) Median OS 14 months for B *versus* 9.5 months for A HR B *versus* A = 0.74 (0.57–0.96)	 **0.008** 0.106 **0.010** **0.021**	Final analysis, 2012: Median follow up: 12.8 months for B and 8.7 months for A Median OS ER+ = 12 months for B *versus* 11.2 months for A HR ER+ B *versus* A= 0.85 (0.57–1.26) ER– = 16.5 months for B *versus* 8.9 months for A HR ER– B *versus* A= 0.68 (0.47–0.98)	 0.404 **0.012**
BOLERO-1 [ClinicalTrials.gov identifier: NCT00876395]	Hurvitz *et al.* [2015]	Phase III Multicenter Randomized (2:1) Double blind Two arms	HER2 + advanced BC First line for advanced disease except endocrine therapy	PFS In the full study population In the HR– population	719 A = placebo: 239 B = everolimus: 480	A: placebo once a day + trastuzumab weekly + paclitaxel weekly 3 weeks/4 until disease progression or unmanageable toxic effects B: everolimus once a day + trastuzumab weekly + paclitaxel weekly 3 weeks/4 until disease progression or unmanageable toxic effects	Median follow up: 41.3 months Median PFS: 14.95 months for B *versus* 14.49 months for A HR B *versus* A = 0.89 (0.73–1.08)	 0.1166 ($p < 0.0174$)	Median follow-up: 41.3 months Median PFS in HR–: 20.27 months for B *versus* 13.08 months for A HR B *versus* A = 0.66 (0.48–0.91)	 0.0049 ($p < 0.0044$)
BOLERO-3 [ClinicalTrials.gov identifier: NCT01007942]	André *et al.* [2014]	Phase III Multicenter Randomized Double blind Two arms	HER2+ advanced BC Trastuzumab resistant Previous chemotherapy including a taxane No more than three previous lines of chemotherapy for advanced disease	PFS	569 A = placebo: 285 B = everolimus: 284	A: placebo once a day + trastuzumab weekly + vinorelbine weekly in 3-week cycle until disease progression or unmanageable toxic effects B: everolimus once a day + trastuzumab weekly + vinorelbine weekly in 3-week cycle until disease progression or unmanageable toxic effects	Median follow up: 20.2 months Median PFS: 7 months for B *versus* 5.78 months for A HR B *versus* A = 0.78 (0.65–0.95)	**0.0067**	Median follow up: 20.2 months Median PFS: HR–: HR B *versus* A = 0.65 (0.48–0.87) HR+: HR B *versus* A = 0.93 (0.72–1.20)	 NR NR

(Continued)

Table 3. (Continued)

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	PFS	*p*	Results according to hormonal status	*p*
AVEREL [ClinicalTrials.gov identifier: NCT00391092]	Gianni *et al.* [2013]	Phase III Multicenter Randomized Open label Two arms	HER2+ advanced BC (locally recurrent inoperable or metastatic) No prior trastuzumab or chemotherapy for advanced disease No central nervous system metastases	PFS	424 A = no bevacizumab: 208 B = bevacizumab: 216	A: docetaxel (six cycles) + trastuzumab every 3 weeks until disease progression or unmanageable toxic effects B: docetaxel (six cycles) + trastuzumab + bevacizumab every 3 weeks until disease progression or unmanageable toxic effects	Median follow-up: 26 months Median PFS: 16.5 months for B *versus* 13.7 months for A HR B *versus* A = 0.82 (0.65–1.02)	0.0775	Median follow up: 26 months Median PFS: HR−: HR B *versus* A = 0.81 (0.59–1.12) HR+: HR B *versus* A = 0.81 (0.59–1.11)	 NR NR
COMPLETE NCIG CTG MA.31 [ClinicalTrials.gov identifier: NCT00667251]	Gelmon *et al.* [2015]	Phase III Multicenter Randomized Open label Two arms Noninferiority trial	HER2+ metastatic BC No prior therapy with cytotoxics or biologics for recurrent or advanced disease No central nervous system metastases	PFS	652 A = trastuzumab: 326 B = lapatinib: 326	A: taxane + trastuzumab weekly or every 3 weeks during 24 weeks → trastuzumab every 3 weeks until disease progression B: taxane + lapatinib once a day during 24 weeks → lapatinib once a day until disease progression	Median follow up: 21.5 months Median PFS: 9 months for B *versus* 11.3 months for A HR B *versus* A = 1.37 (1.13–1.65)	**0.001**	Median follow up: 21.5 months PFS HR B *versus* A adjusted for ER/PgR = 1.41 (1.16–1.71)	 **0.001**
EGF104535 [ClinicalTrials.gov identifier: NCT00281658]	Guan *et al.* [2013]	Phase III Multicenter Randomized Double blind Two arms	HER2+ metastatic BC (stage IV) No prior treatment except hormonal treatment No central nervous system metastases	OS Secondary endpoint = PFS	444 A = placebo: 222 B = lapatinib: 222	A: paclitaxel weekly 3 weeks/4 (six or more cycles) + placebo until disease progression or unmanageable toxic effects B: paclitaxel weekly 3 weeks/4 (six or more cycles) + lapatinib once a day until disease progression or unmanageable toxic effects	Median OS 27.8 months for B *versus* 20.5 months for A HR B *versus* A = 0.74 (0.58–0.94) Median PFS 9.7 months for B *versus* 6.5 months for A HR B *versus* A = 0.52 (0.42–0.64)	**0.0124** <**0.001**	OS HR− *versus* HR +: HR = 1.16 (0.9–1.49)	 0.2546

Table 3. (Continued)

Study	Source	Design	Inclusion	Primary endpoint	Number of patients	Chemotherapy regimen	PFS	*p*	Results according to hormonal status	*p*
TAnDEM [ClinicalTrials.gov identifier: NCT00022672]	Kaufman *et al.* [2009]	Phase III Multicenter Randomized Open label Two arms	HER2+ and hormone receptor positive Postmenopausal Metastatic BC No prior chemotherapy for metastatic disease No central nervous system metastases	PFS	207 A = anastrozole: 104 B = anastrozole + trastuzumab: 103	A: anastrozole once a day until disease progression B: anastrozole once a day + trastuzumab weekly until disease progression	Median PFS 4.8 months for B *versus* 2.4 months for A HR B *versus* A = 0.63 (0.47–0.84)	**0.0016**		
EGF30008 [ClinicalTrials.gov identifier: NCT00073528]	Johnston *et al.* [2009]	Phase III Multicenter Randomized Double blind Two arms	Hormone receptor positive locally advanced or metastatic BC No prior therapy for advanced or metastatic disease HER2+/–	PFS	1286 A = letrozole: 644 B = letrozole + lapatinib: 642 HER2+: 219/1286 A: 108/644 B: 111/642	A: letrozole once a day + placebo until disease progression or unmanageable toxic effects B: letrozole once a day + lapatinib once a day until disease progression or unmanageable toxic effects	Median follow up: 1.8 years Median PFS Full study population 11.9 months for B *versus* 10.8 months for A HR B *versus* A = 0.86 (0.76–0.98) HER2+ 8.2 months for B *versus* 3 months for A HR B *versus* A = 0.71 (0.53–0.96) HER2– HR B *versus* A = 0.9 (0.77–1.05)	0.026 **0.019** 0.188		
eLEcTRA [ClinicalTrials.gov identified: NCT00171847]	Huober *et al.* [2012]	Phase III Multicenter Randomized Open label Three arms	Postmenopausal newly diagnosed metastatic or locally advanced BC Hormone receptor positive HER2+ (A–B)/ HER2– (C) No prior treatment	TTP	93 HER2+ A = letrozole: 31 B = letrozole + trastuzumab: 26 HER2– C = letrozole: 35	HER2+ A: letrozole once a day until disease progression B: letrozole once a day + trastuzumab weekly until disease progression HER2– C: letrozole once a day until disease progression	Median TTP B *versus* A 14.1 months *versus* 3.3 months HR = 0.67 (0.35–1.29) C *versus* A 15.2 months *versus* 3.3 months HR = 0.71 (0.52–0.96)	 0.23 0.03		

BC, breast cancer; HER2, human epidermal growth factor 2; HR, hazard rate; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

signaling constitutes one of the mechanisms of resistance in anti-HER2 targeted therapy. In other words, the absence of ER expression could enhance HER signaling dependence. This explains the difference in the efficacy of dual targeting between these two subgroups [Blackwell *et al* 2010, 2012]. In the BOLERO-1 trial, despite the fact that this trial did not meet its primary endpoint, a relevant prolongation of PFS is seen in ER-negative BC if everolimus is added to taxane- and trastuzumab-based therapy for advanced disease. The effect of everolimus seems to differ depending on the expression of ER in HER2-positive advanced BC if the patients do not receive endocrine therapy. Again, the bidirectional crosstalk could explain that ER signaling is an escape mechanism for HER2-targeted therapy independent of the fact that this therapy produces a more complete blockade of HER2 signaling. Inhibition of HER2 alone increases signaling through ER. Thus, we suppose that the efficacy of the combination therapy with everolimus and trastuzumab might be enhanced with the inhibition of ER signaling in HER2- and ER-positive BC.

Different ongoing trials assess the benefits of adding a PI3K/mTOR inhibitor to endocrine therapy and HER2-targeted therapy in patients with ER-positive, HER2-positive advanced BC [Hurvitz *et al*. 2015]. A similar observation was already made in the BOLERO-3 trial [André *et al*. 2014]. In more heavily pretreated patients, the addition of everolimus to vinorelbine and trastuzumab resulted only in improved outcome in the ER-negative subpopulation during a hypothesis-generating analysis. The TAnDEM trial was the first phase III study to evaluate the combination of an endocrine therapy and trastuzumab without chemotherapy as a treatment for HER2-positive and ER-positive metastatic BC. It met its primary endpoint, although outcomes are poor in both treatment arms for most patients.

It is interesting to note that approximately 15% of patients who received trastuzumab plus anastrozole did not experience disease progression for at least 2 years, suggesting that the use of HER2-targeted therapy with an aromatase inhibitor can substantially delay chemotherapy in some patients [Kaufman *et al*. 2009]. The EGF30008 trial [Johnston *et al*. 2009] included patients not selected for HER2 status and compared the combination of lapatinib and letrozole with letrozole alone. As previously mentioned for TAnDEM, EGF30008 also supports the hypothesis that combined inhibition of both pathways (ER and HER2) delays the development of resistance and prolongs PFS *versus* treatments targeting only ER. As a result, the onset of palliative chemotherapy is also delayed.

Although there are some differences in the design of these two studies, a relevant difference in the median PFS between the combination arms of the two studies can also be explained by alternative hypotheses. It might be partially explained by the dual inhibition of HER-1 and HER-2 by lapatinib to provide a more complete blockade of the HER pathway [Azim and Piccart, 2010]. The eLEcTRA trial confirmed the results of the two previous studies. Although the results did not reach statistical significance, the trends are globally similar to the results seen in TAnDEM and EGF30008.

This lack of statistical significance may be attributable to the small number of patients [Huober *et al*. 2012]. The median time to progression observed in these three studies in ER-positive, HER2-positive BC treated by endocrine therapy alone is very short. These results highlight the aggressive nature of these cancers and their low sensitivity to endocrine therapy alone. Although these studies confirm the superiority of the combined approach over the endocrine treatment alone, nearly 50% of patients derive no benefit from this combination. This is probably due to common mechanisms of resistance involving downstream signaling pathways [Azim and Piccart, 2010]. In light of the above explanation, we can speculate on the implication of the PI3K/AKT/mTOR pathway. However, these studies have a small subset of patients with ER-positive and HER2-positive BC that benefits from endocrine therapy alone. These cancers behave more like ER-positive, HER2-negative cancers and are mainly driven by ER signaling [Nahta and O'Regan, 2012].

In summary, metastatic studies indicate that ER-positive, HER2-positive BC is a distinct entity *versus* ER-negative, HER2-positive BC. Within this entity, a heterogeneous response to therapies can be observed. Some tumors behave more like ER-positive, HER2-negative BC. The ER signaling pathway mainly drives these. Others can benefit from the combination of endocrine therapy and anti-HER2 targeted therapy, which provides an opportunity to delay the onset of palliative chemotherapy. The last ones are resistant

to this treatment combination and are actually best treated by chemotherapy and anti-HER2-targeted therapy. Today, the best results remain those obtained with chemotherapy and trastuzumab [Prat and Baselga, 2008]. One of the main future challenges is to identify these subsets in ER-positive, HER2-positive BC to tailor the treatment. This would also better explain the mechanisms of treatment resistance.

Therapeutic implications

In the adjuvant setting, systemic therapy includes anti-HER2 therapy, chemotherapy and endocrine therapy if systemic therapy is indicated. The current ongoing discussion concerns optimal treatment in advanced disease settings.

The American Society of Clinical Oncology Clinical Practice Guidelines about systemic therapy for patients with advanced HER2-positive BC offers specific treatment recommendations for ER-positive, HER2-negative BC [Giordano *et al.* 2014]. As the most appropriate first-line treatment, the experts strongly recommend an association of HER2-targeted therapy with chemotherapy. The association of endocrine therapy plus trastuzumab or lapatinib may be an option in selected cases. This is a moderately strong recommendation. An endocrine therapy alone may also be considered in selected cases with a weak strength of recommendation. Unfortunately, there are no direct comparisons between endocrine therapy combined with HER2-trageted therapy and chemotherapy combined with HER2-targeted therapy. The studies evaluating chemotherapy and HER2-targeted therapy have the best results and are the only ones with an overall survival benefit. Because some patients can benefit from the association of endocrine therapy and HER2-targeted therapy and because this treatment is much less toxic than chemotherapy, this combination therapy may be considered as an option. For endocrine therapy alone, there are insufficient data, but experts think that patients who have low-volume disease, long disease-free interval, indolent disease, significant comorbidities (heart failure), a preference to avoid intravenous chemotherapy or additional toxicity are the most appropriate candidates for this treatment.

The National Comprehensive Cancer Network guidelines contain less specific recommendations for this subgroup [Gradishar *et al.* 2016]. The experts recommend pertuzumab plus trastuzumab in combination with a taxane as a preferred option for the first-line treatment of patients with HER2-positive metastatic BC. The combination of trastuzumab and endocrine therapy is another option to be considered for patients with HER2-positive metastatic BC who are ER positive, but this is not the preferred regimen according to these guidelines.

The European School of Oncology–European Society for Medical Oncology second international consensus guidelines for advanced breast cancer (ABC2) state that for patients with ER-positive, HER2-positive metastatic breast cancer for whom endocrine therapy was chosen, anti-HER2 therapy plus endocrine therapy should be considered with the initiation of endocrine therapy since anti-HER2 therapy in combination with endocrine therapy has shown substantial PFS benefit compared with endocrine therapy alone [Cardoso *et al.* 2014]. The authors clarified that the addition of anti-HER2 therapy in this setting has not led to a survival benefit. Ninety percent of the experts support this recommendation.

New perspectives

Preclinical and clinical data indicate that ER-positive, HER2-positive BC is a subset of BC that needs specific treatment approaches considering the bidirectional crosstalk between the ER and the HER2 pathways. Standard endocrine monotherapy improves outcomes if added to anti-HER2 agents, but it does not allow long-term disease control. Dual HER2 blockade compared with single blockade by trastuzumab improves outcome when added to docetaxel in ER-positive and ER-negative HER2-positive BC [Swain *et al.* 2015]. Although BOLERO 1 and BOLERO 3 [Hurvitz *et al.* 2015; André *et al.* 2014] failed to define a new standard therapy by adding everolimus, evaluating other drugs targeting the PI3K/AKT/mTOR pathway remains of considerable interest in HER2-positive BC as hyperactivation of this pathway is one of the mechanisms of resistance to trastuzumab-based therapy. Data available in the neoadjuvant setting indicate that the pCR rate after standard anti-HER2-based therapy is lower in tumors presenting PI3K mutations [Loibl *et al.* 2014; Majewski *et al* 2015].

Optimization of the ER pathway blockade seems mandatory to further improve disease control in

the ER-positive, HER2-positive subgroup. The addition of a targeted therapy to endocrine therapy *versus* endocrine therapy alone allows more than a doubling in the median PFS in ER-positive, HER2-negative BC [Baselga *et al.* 2012c; Turner *et al.* 2015]. The next step is to evaluate this new strategy in the clinic. We expect that many new clinical trials will be designed in the near future to test this hypothesis. Some are already recruiting.

For example, Mayer and colleagues are investigating the combination of the α-specific PI3K inhibitor BYL719 with letrozole and trastuzumab [ClinicalTrials.gov identifier: NCT01791478] and Wheler and colleagues are assessing everolimus plus letrozole and trastuzumab [ClinicalTrials.gov identifier: NCT02152943] in patients with HER2-positive, ER-positive advanced BC. Of course, the ultimate goal is not only to obtain better disease control in the metastatic setting but also to identify a subgroup of ER-positive, HER2-positive BC in which chemotherapy can be deleted in the adjuvant setting. The NA-PHER2 trial [ClinicalTrials.gov identifier: NCT02530424] is ongoing and investigates the combination of trastuzumab, pertuzumab, palbociclib and fulvestrant in the neoadjuvant setting. Inhibition of the CDK4/6 complexes represents a great hope as these complexes act downstream of the majority of anti-HER2 and endocrine resistance mechanisms. Total ER and HER2 pathway blockades may be obtained through this ambitious experiment. However, there are still many steps before this can become a reality. In particular, we do not want to take the risk of undertreating patients who can be cured today by more aggressive approaches. Strong data are needed in the metastatic setting before trials can ethically be designed in this field.

Conclusion

ER-positive, HER2-positive BC requires specific treatment approaches that consider bidirectional crosstalk between the ER and HER2 pathways. In the adjuvant setting, high-risk patients have to receive anti-HER2 therapy and chemotherapy. Deletion of chemotherapy is an important objective, but in this curative setting we cannot risk undertreatment. Consequently, we have to wait for additional convincing data in the metastatic setting before starting well designed adjuvant trials aiming to develop new standard chemotherapy-free regimens for this subgroup of BC. In the metastatic setting, dual HER2 blockade has already demonstrated improved outcomes *versus* standard blockade by trastuzumab alone. The addition of drugs acting downstream of the HER2 receptor, and in particular inhibitors of the PI3K/AKT/mTOR pathway and inhibitors of the CDK4/6 complexes, need further evaluation to optimize anti-HER2 therapy while considering the mechanism of resistance. Chemotherapy remains the standard systemic therapy associated with anti-HER2 therapy for most patients. Only these therapies have been proven to impact overall survival. Endocrine therapy combined with anti-HER2 therapy is considered for patients with more indolent disease. New directions include the association of an agent targeted to endocrine therapy and anti-HER2 therapy to overcome endocrine therapy resistance. If these approaches are more effective than the results observed with the same regimens without the targeted agent, then chemotherapy may become much less frequently used in advanced settings. The bidirectional crosstalk between the ER and HER2 pathway is the basis for increasing interest in these strategies. We can expect many important additional data in this field in the near future because many drugs targeting endocrine resistance are currently under development.

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