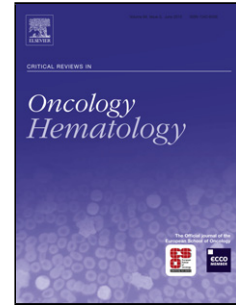


Accepted Manuscript

Title: Current challenges in HER2-positive breast cancer

Author: Fabio Puglisi Caterina Fontanella Vito Amoroso
Giulia Valeria Bianchi Giancarlo Bisagni Cristina Falci
Andrea Fontana Daniele Generali Lorenzo Gianni Antonio
Grassadonia Luca Moscetti Ilaria Portarena Emanuela Rossi
Paolo Marchetti



PII: S1040-8428(15)30065-2
DOI: <http://dx.doi.org/doi:10.1016/j.critrevonc.2015.10.016>
Reference: ONCH 2067

To appear in: *Critical Reviews in Oncology/Hematology*

Received date: 4-4-2015
Revised date: 6-10-2015
Accepted date: 28-10-2015

Please cite this article as: Puglisi Fabio, Fontanella Caterina, Amoroso Vito, Bianchi Giulia Valeria, Bisagni Giancarlo, Falci Cristina, Fontana Andrea, Generali Daniele, Gianni Lorenzo, Grassadonia Antonio, Moscetti Luca, Portarena Ilaria, Rossi Emanuela, Marchetti Paolo. Current challenges in HER2-positive breast cancer. *Critical Reviews in Oncology and Hematology* <http://dx.doi.org/10.1016/j.critrevonc.2015.10.016>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TITLE: Current challenges in HER2-positive breast cancer

AUTHORS: Fabio Puglisi^{1,2}, Caterina Fontanella^{1,2}, Vito Amoroso³, Giulia Valeria Bianchi⁴, Giancarlo Bisagni⁵, Cristina Falci⁶, Andrea Fontana⁷, Daniele Generali⁸, Lorenzo Gianni⁹, Antonio Grassadonia¹⁰, Luca Moschetti¹¹, Ilaria Portarena¹², Emanuela Rossi¹³, Paolo Marchetti¹⁴.

AFFILIATIONS: ¹Department of Medical and Biological Science, University of Udine, Udine, Italy; ²Department of Oncology, University Hospital of Udine, Udine, Italy; ³Department of Oncology, Spedali Civili Hospital, Brescia, Italy; ⁴Department of Oncology, National Cancer Institute, Milano, Italy; ⁵Department of Oncology, Arcispedale Santa Maria Nuova Hospital, Reggio Emilia, Italy; ⁶Department of Oncology, Istituto Oncologico Veneto, Padova, Italy; ⁷Department of Oncology, Hospital and University of Pisa, Italy; ⁸Department of Oncology, Istituti Ospitalieri Hospital, Cremona, Italy; ⁹Department of Oncology, Infermi Hospital, Rimini, Italy; ¹⁰Department of Oncology, G. D'Annunzio University - SS Annunziata Hospital, Chieti, Italy; ¹¹Department of Oncology, Belcolle Hospital, Viterbo, Italy; ¹²Department of Oncology, Tor Vergata Hospital, Roma, Italy; ¹³Department of Oncology, SG Moscati Hospital, Avellino, Italy; ¹⁴Department of Oncology, S. Andrea Hospital – Sapienza University, Roma, Italy.

CORRESPONDING AUTHOR: *Fabio Puglisi, MD, PhD

Address: Department of Oncology, University Hospital of Udine, Piazzale S.M. Misericordia, 33100 Udine, Italy

Phone: + 39 (0) 432 559309 Fax: +39 (0) 432 552762 e-mail: fabio.puglisi@uniud.it

TABLE OF CONTENTS

1. Introduction
2. Primary systemic HER2-targeted therapy
 - 2.1 Patient selection and correlation between pCR and long-term outcome
 - 2.2 Prediction and predictors of response
 - 2.3 Primary systemic chemotherapy in combination with HER2-targeted agents
3. Adjuvant HER2-directed therapy
 - 3.1 Update on cardiac safety and optimal duration of adjuvant therapy
 - 3.2 Adjuvant trastuzumab in elderly patients
 - 3.3 Adjuvant trastuzumab in T1a/b N0 tumors.
 - 3.4 Trastuzumab and pregnancy
4. HER2-directed therapy for metastatic BC
5. Future perspectives

ABSTRACT:

The introduction of trastuzumab into routine clinical practice has had a dramatic effect on the outlook for patients with HER2-positive breast cancer. Nevertheless, answers to some long unresolved questions about the optimal use of trastuzumab (such as its role in small tumors or low-risk disease, cardiac safety in the elderly, and treatment duration) have emerged only relatively recently. Moreover, with the availability of new highly effective HER2-directed therapies, including pertuzumab and trastuzumab-emtansine (T-DM1), the treatment algorithm for HER2-positive breast cancer continues to evolve.

This review provides a summary of the latest evidence providing insight into the management of early and advanced HER2-positive breast cancer and delineates future perspectives of study and treatment for these patients.

KEYWORDS: breast cancer, dual targeting, HER2, lapatinib, neoadjuvant, pertuzumab, trastuzumab, trastuzumab-emtansine

SHORT BIOGRAPHY OF THE CORRESPONDING AUTHOR:

Fabio Puglisi (MD, PhD) is an associate professor of medical oncology and head of the school of medical oncology at the University of Udine, Italy. He is a senior staff member of the Department

of Medical Oncology, University Hospital of Udine, Italy. In 1993, he received his degree in Medicine (with honors) from the University of Palermo, Italy. He undertook his specialist training in cancer medicine at the University of Udine, receiving its certification (with honors) in 1997. In January 2002, professor Puglisi pursued the title of PhD in Diagnostic Quantitative Pathology from the University of Siena, Italy. He is author of several publications in scientific peer-reviewed journals, especially in his main fields of interest (i.e. clinical and translational studies on breast cancer).

1. Introduction

Breast cancer (BC) is the most common cancer and the second leading cause of cancer death in women [1]. It is estimated that in 2015, close to 232,000 new cases will be diagnosed and over 40,000 women will die from the disease in the USA [1]. Data suggest that in most Western European countries, life expectancy for patients with BC is increasing [2,3], mainly as a result of improved mammographic screening and adjuvant treatment [4], but also through advances in the treatment of metastatic disease [5].

Overexpression of the membrane tyrosine kinase receptor HER2 in BC cells has long been established as a major negative prognostic factor [6,7]. Overexpression occurs in approximately 20–25% of cases and is detected either as gene amplification (by fluorescence in situ hybridization) or as protein expression (by immunohistochemistry) [8,9]. HER2 is a member of the epidermal growth factor receptor (EGFR) family, along with HER1 (also known as EGFR), HER3 and HER4. These receptors, functioning as homo- or heterodimers, activate multiple cellular pathways such as the p44/42 mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3-kinase (PI3K) pathways, and stimulate cell growth, survival and differentiation [10,11]. Unlike other members of the family, HER2 is not activated by a specific ligand and is always in an active conformational state, ready to interact with other ligand-activated EGFR receptors [12], particularly HER3 [13].

The introduction into the clinic of the anti-HER2 humanized monoclonal antibody trastuzumab was associated with a considerable improvement in the outcome of patients with HER2-overexpressing BC. Clinical studies have shown that in the subgroup of patients with HER2-positive disease, the addition of trastuzumab to chemotherapy significantly improves both recurrence-free survival (RFS) and overall survival (OS) in the adjuvant setting [14-20], increases the rate of pathologic complete response (pCR) in the primary systemic therapy (PST) setting [21,22], and also improves OS in the metastatic setting [23,24]. Nevertheless, unanswered questions remain and our understanding of the most appropriate use of anti-HER2 therapy across treatment settings and biological subtypes is continuing to evolve. In this article, we review the latest scientific evidence informing the management of HER2-positive BC and delineate future perspectives of study and treatment for this subset of patients. Specifically, in the adjuvant setting we assess available evidence on the treatment of elderly patients, women in populations with small/low-risk tumors, and subjects at high risk of cardiac toxicity; moreover, we consider the optimal treatment duration. We also evaluate data on dual HER2 targeting, combining trastuzumab with other HER2-targeted agents in both the early and the metastatic settings.

2. Primary systemic HER2-targeted therapy

2.1 Patient selection and correlation between pCR and long-term outcome

PST should be considered a standard approach in the routine management of operable BC based on the identical disease-free survival (DFS) and OS outcomes compared with adjuvant therapy demonstrated in several randomized clinical trials and a meta-analysis [25]. Generally, any patient who is a candidate for adjuvant systemic chemotherapy can be considered for PST with the additional expectation of needing less extensive surgery. The main candidates for PST in the HER2-positive treatment setting are patients with a high likelihood of achieving a pCR, for example, those with highly proliferative/high-grade tumors. Furthermore, in patients with inoperable or inflammatory BC, NST should always be recommended [26,28].

pCR has been adopted as the primary endpoint for clinical trials of neoadjuvant therapy but to date is not uniformly defined. At least four different definitions of pCR have been adopted, taking into consideration the absence of residual disease in both breast and axilla or only in breast, and permitting or not permitting residual in situ disease in the breast. Both the NSABP B-18 and the NSABP B-27 trials reported that patients achieving a pCR after neoadjuvant therapy had better DFS and OS than patients with residual disease remaining at surgery [29,30]. However, other trials failed to show a predictive role of pCR on outcome [26]. Recently, two meta-analyses have highlighted emerging questions in the primary treatment of BC. von Minckwitz and colleagues analyzed pCR and its association with long-term outcome in 6,377 patients with primary BC receiving neoadjuvant anthracycline- and taxane-based chemotherapy in seven randomized trials [31]. DFS was found to be significantly superior in patients with no invasive and no in situ residual disease in breast or nodes (ypT0 ypN0) compared with patients with gross invasive residual disease or less stringent definitions of pCR (residual ductal carcinoma in situ [DCIS] only, no invasive residual disease in the breast but involved nodes, only focal-invasive disease in the breast; ypT0/Tis ypN0). The hazard ratio (HR) for DFS in patients with versus without pCR was lowest when the most stringent definition of pCR was used (ypT0 ypN0), and increased with broadening of the pCR definition. A second meta-analysis, from the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC), included 11,955 patients treated in 12 randomized neoadjuvant trials [32]. Results indicated that patients who obtain a pCR, defined as either the absence of invasive and in situ carcinoma in breast and axilla or only invasive carcinoma in both breast and axilla, have a more favorable long-term outcome than those without a pCR (HR for OS = 0.36 with both definitions; HR for event-free survival [EFS] = 0.44 with ypT0 ypN0, and HR = 0.48 with ypT0/Tis ypN0).

[32]. Thus, in the CTNeoBC analysis the presence or absence of DCIS showed no correlation with prognosis. Taken together, both meta-analyses confirmed the correlation between absence of invasive carcinoma in both breast and axilla and a favorable outcome. Moreover, they also reinforced the importance of a standard stringent definition of pCR across clinical trials and the need for different neoadjuvant approaches in different tumor subtypes.

2.2 Prediction and predictors of response

Evaluation of tumor response during PST is important for several reasons. On-treatment tumor response may be used as a predictor of pCR or a predictor of long-term outcome. Alternatively, it may be used to aid decision-making for surgery or to select patients who may or may not benefit from a change of systemic therapy. Assessment of early or mid-course response to PST is relevant as it helps in understanding the chemosensitivity of individual tumors and the intrinsic probability of a pCR at surgery. Early assessment of tumor response to chemotherapy is crucial in avoiding unnecessary toxicity in patients who are unlikely to benefit from further treatment [33].

Neoadjuvant chemotherapy response is usually assessed by monitoring changes in tumor size using clinical examination, mammography, and/or ultrasound [34]. In the clinical trial setting, magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT) scan have been recently implemented. Literature data show that dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) was consistently performed after 1-2 cycles of PST and compared to a pre-PST baseline scan, in the vast majority of the studies. Based on descriptive presentation of the results, sensitivity/specificity pairs for prediction of pathologic response were lower in studies measuring reductions in uni- or bidimensional tumor size, but it can be increased by three-dimensional volume measurement, quantitative dynamic contrast measurements (volume transfer constant [K_{trans}], exchange rate constant [k_(ep)], and early contrast uptake [ECU]) [35]. The use of PET/CT is based on the idea that, during PST, changing in metabolic activity can predict the final response after the first cycles of chemotherapy [36,37]. A recent study enrolling 57 women with HER2-positive BC, treated with trastuzumab plus taxane-based PST, showed that a Maximum Standardized Uptake Value (SUV_{max}) ≤ 2.0 after the first cycle of chemotherapy was the strongest predictor of pCR; in multivariate analysis, tumor SUV_{max} ≤ 2.0 predicted pCR with an Odds Ratio (OR) of 14.3 ($p=0.004$), with both negative and positive predictive values of 76% [38]. Accordingly, the AVATAXHER trial confirmed that early PET/CT assessment may help to identify non-responders to PST with docetaxel plus trastuzumab therapy [39]. In this study, PET/CT was performed after two cycles of docetaxel plus trastuzumab and the change in SUV_{max} was used to predict pCR: patients who were predicted to be responders on PET continued to receive standard therapy; non-responders were randomly assigned to receive four cycles of docetaxel and

trastuzumab plus bevacizumab (group A) or continue on docetaxel plus trastuzumab alone (group B). pCR were documented in 53.6% of the PET responders, 43.8% of those in group A, and 24.0% of those in group B.

There have also been efforts to identify clinical features, such as body mass index and age role [40,41], and biological markers that predict response or likelihood of pCR at surgery in patients receiving PST [40]. Currently, a lower response rate to PST is typical of invasive lobular carcinomas (6.2% of pCR in invasive lobular carcinoma versus 17.4% in invasive ductal carcinoma, $p < 0.001$) [42]. By contrary, the most important predictive markers for response to taxane- and anthracycline-based PST are a high proliferation index and negative hormone receptor (HoR)-status [43,44], and HER2 signaling is associated with high rates of response and pCR with neoadjuvant trastuzumab-containing regimens [21,45]. Thus current evidence suggests that multiple biological markers, rather than a single one, seem to be important in differentiating between a high or low probability of clinical response and pCR. In addition, recent gene expression profiling studies suggest that the processes and pathways involved in pCR with PST vary between different BC subtypes [46].

2.3 Primary systemic chemotherapy in combination with HER2-targeted agents

In HER2-positive BC, trastuzumab in combination with primary systemic chemotherapy resulted in favorable long-term outcome with minimal late toxicity. In an early MD Anderson Cancer Center trial comparing trastuzumab-containing therapy versus chemotherapy alone, the pCR rate was doubled (67% versus 25%) with trastuzumab, leading to early termination of the trial (table 1) [21]. Additional single-arm phase II studies and the GeparQuattro phase III trial supported the high activity of the combination regimen [47-50]. The randomized phase III NOAH trial conducted by Gianni and colleagues confirmed the significant pCR and EFS benefit of combining trastuzumab with neoadjuvant chemotherapy and continuing adjuvant trastuzumab after surgery in HER2-positive disease [22]. Furthermore, it showed that combining trastuzumab with anthracycline-based chemotherapy is tolerable and is not associated with an increase in cardiac toxicity. At the 2013 American Society of Clinical Oncology (ASCO) Annual Meeting the updated results have been presented. After a median follow-up of 5.4 years, the EFS benefit with trastuzumab was confirmed and a strong trend towards improved OS was observed. Cardiac tolerability was good despite concurrent administration of trastuzumab with doxorubicin [51]. Accordingly, a more recent study showed a 92.9% 4-year RFS in patients who achieved pCR after trastuzumab-based PST versus 72.4% without pCR. All cases of symptomatic cardiotoxicity have been resolved during follow-up [52]. On the basis of the NOAH results, the European Medicines Agency (EMA) extended the

approved indication for trastuzumab to include its use in combination with neoadjuvant chemotherapy followed by adjuvant trastuzumab monotherapy for locally advanced and inflammatory HER2-positive disease or for tumors >2 cm in diameter [53].

The impressive results observed with trastuzumab in the neoadjuvant setting led to the initiation of several trials evaluating new HER2-targeted agents with or without trastuzumab. In the GeparQuinto trial, the direct comparison of the monoclonal antibody trastuzumab versus small tyrosine-kinase inhibitor lapatinib showed a pCR rate significantly lower with the lapatinib-based chemotherapy compared with the trastuzumab-based chemotherapy (table 1) [54]. Consequently, the authors concluded that outside clinical trials, lapatinib should not be used as single anti-HER2 treatment in combination with neoadjuvant chemotherapy. Subsequently, the NSABP B-41 and the NeoALTTO trials compared trastuzumab single agent, lapatinib single agent, and the vertical dual HER2 blockade by combination of trastuzumab plus lapatinib. The same approach with dual vertical HER2 inhibition has been explored in the randomized phase II CHER-LOB trial [55] (table 1). In these studies the substitution of lapatinib for trastuzumab in combination with chemotherapy resulted in similarly high pCR rates (table 1) [56,57]. Moreover, although the combination of trastuzumab and lapatinib plus chemotherapy led to a higher pCR rate compared to antiHER2 single agent plus chemotherapy treatment in the NeoALTTO trial (51.3% with combination versus 29.5% with single agent; $p = 0.0001$), it did not reach statistical significance in the NSABP B-41 (pCR 52.5% with trastuzumab versus 62.0% with combination, $p = 0.095$). Furthermore, in the long term follow-up of the NeoALTTO trial, neither EFS nor OS did differ between the lapatinib and trastuzumab groups (EFS: HR 1.06, $p = 0.81$; OS: HR 0.86, $p = 0.65$) [58].

The vertical dual HER2 blockade with trastuzumab plus lapatinib has been also investigated in adjuvant setting in the ALTTO trial [59]. It was the largest-ever adjuvant clinical trial in HER2-positive BC, involving more than 8,000 patients. Disappointingly, despite a statistically significant benefit in pCR in the NeoALTTO trial, there were no statistically significant differences in DFS (4-year DFS rates were 86% with trastuzumab, 88% with concurrent trastuzumab plus lapatinib, and 87% with trastuzumab followed by lapatinib) and OS (4-years OS rates were 94%, 95%, and 95%, respectively) in the ALTTO trial. To note, in the CTNeoBC meta-analysis the EFS HR of achieving a pCR versus non-pCR was 0.39, meaning a 61% reduction in the risk of relapse; however, in the NeoALTTO study the combination strategy improved the pCR by 20%, this means that 1 out of 5 patients moved from the non-pCR to the pCR group. This improvement in pCR is probably too marginal to be translated into a significant improvement in survival outcomes [60].

Trastuzumab has also been combined in a horizontal blockade strategy with the monoclonal antibody pertuzumab. In the NeoSphere trial [61], the combination of trastuzumab, pertuzumab, and

docetaxel demonstrated a significantly higher pCR rate than was seen with trastuzumab and docetaxel alone (pCR 29.0% with docetaxel plus trastuzumab, 45.8% with docetaxel plus trastuzumab and pertuzumab, 16.8% with trastuzumab and pertuzumab alone, and 24.0% with docetaxel and pertuzumab), without substantial differences in tolerability (table 1). Moreover, the TRYPHAENA randomized trial suggested that combining pertuzumab and trastuzumab with standard neoadjuvant chemotherapy posed no cardiac safety concerns and it demonstrated a pCR rate higher than 66% with an anthracycline-free regimen (table 1) [62]. These findings justify the EMA approval of pertuzumab as primary systemic therapy for HER2-positive early breast cancer [63].

Taking into account the results of all these clinical trials, neoadjuvant treatment should be offered to patients with HER2-positive locally advanced or inflammatory BC.

3. Adjuvant HER2-directed therapy

3.1 Update on cardiac safety and optimal duration of adjuvant therapy

Notwithstanding the impressive efficacy demonstrated with adjuvant trastuzumab, cardiac toxicity remains a significant consideration. Cardiac toxicity may manifest as any of a broad spectrum of effects, ranging from an asymptomatic decrease in ejection fraction to symptomatic congestive heart failure (CHF) resulting in cardiac death. The lack of a precise and widely accepted definition of cardiotoxicity prevents meaningful or appropriate cross-trial comparison of incidences of cardiac adverse events in the large randomized trials of adjuvant trastuzumab [15,19,20]. For example, at 8-year median follow-up, in the HERA trial the incidence of severe CHF was 0.8% in both trastuzumab-containing arms and 0.0% in trastuzumab-free arm, and the incidence of left ventricular dysfunction was 7.2% with 2 years of adjuvant trastuzumab, 4.1% with 1 year, and 0.9% without trastuzumab; more than 80% of patients with confirmed LEVF decrease reached acute recovery [64]. Similarly, in the BCIRG 006 trial, the 4-year follow-up results showed a stable incidence of CHF compared with the incidences reported at the first analysis (2% in the sequential trastuzumab arm and 0.4% in the concomitant trastuzumab arm) [20,65]. To note, a joint analysis of data from NCCTG N9831 and NSABP B-31 with 4 years' follow-up enabled exclusion of a significant increase in cardiac events [17].

In contrast with the knowledge that trastuzumab-induced cardiac toxicity is generally promptly reversible, the BCIRG 006 trial revealed that in 33% of cases, a >10% reduction in LVEF persisted for at least 4 years. These data highlight the importance of balancing the real efficacy of

anthracycline- and trastuzumab- combinations as adjuvant therapy in light of longer-term cardiac tolerability.

Moreover, a recent meta-analysis demonstrated that concomitant administration of anthracyclines and trastuzumab was moderately associated with increased risk of cardiac-related adverse events in both early BC setting (relative risk [RR] 1.51, 95 % CI 1.10–2.07, $p = 0.01$) as well as in palliative setting (RR 3.92, 95 % CI 2.11–7.27, $p < 0.001$) [66].

Standing these concerns, anthracyclines-free strategies have been investigated especially in patients treated with curative intent. In a phase III trial, adjuvant cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regimen sequentially combined with epirubicin has been compared with CMF alone in 705 patients with early BC. In HER2-positive disease, no benefits were documented by the addition of epirubicin to CMF [67]. Consistently, a neoadjuvant study showed that 24 weeks of neoadjuvant paclitaxel plus trastuzumab could be a reasonable treatment option in HER2-positive early BC patients. This anthracyclines-free strategy led to a pCR rate of 50%, without documented severe cardiotoxicity. Moreover, in the early 2014, a negative phase III trial showed no differences in progression-free survival (PFS) ($p = 0.174$) and OS ($p = 0.083$) by the addition of nonpegylated liposomal doxorubicin to paclitaxel plus trastuzumab in first-line setting for advanced HER2-positive BC [68]. Over the last decade, in order to limit the cardiac toxicity, the strategy of shortening trastuzumab exposure in the adjuvant setting has been explored. In the FinHer trial women received 9 weeks of trastuzumab plus docetaxel or vinorelbine; at the 60-month follow up, median LVEF remained stable in the trastuzumab arm compared with a decrease from 66% to 62% in the control arm [69]. However, the first results of the PHARE trial suggested a greater benefit from the standard 1-year adjuvant trastuzumab when compared with shorter duration. The trial demonstrated a higher 2-year DFS rate in patients receiving trastuzumab for 12 months compared with those treated for only 6 months (94% versus 91%, respectively; HR 1.28, 95% CI 1.05–1.56) and the authors suggested that the DFS results in the subset of patients with estrogen receptor-positive tumors or receiving concomitant chemotherapy and trastuzumab were inconclusive [70]. Further evaluation of shorter trastuzumab durations is ongoing in two European academic trials: the Italian Short-HER study is comparing 12 versus 3 months of trastuzumab following traditional anthracycline- and taxane-based chemotherapy [71] and the Finnish Synergism Or Long Duration (SOLD) trial is comparing concomitant trastuzumab plus docetaxel followed by FEC versus the same regimen followed by single-agent trastuzumab for a total trastuzumab duration of 1 year [72]. Both trials are still recruiting and the final results are expected in 2015.

In conclusion, as outlined in a recent Cochrane review [14], the survival benefit gained from adjuvant trastuzumab may be partially impaired by cardiac risk. Therefore the decision to

administer 12 months of trastuzumab should be considered carefully in women with low-risk BC or higher cardiovascular risk.

3.2 Adjuvant trastuzumab in elderly patients

At present, there are limited efficacy and safety data supporting the role of adjuvant trastuzumab in elderly patients. In the largest trials evaluating adjuvant trastuzumab [15,17,19,20], the study populations were significantly younger than those observed in daily clinical practice. For example, in the HERA, NSABP B-31, and NCCTG N9831 trials, only 16% of the intent-to-treat population was aged ≥ 60 years [15-17]. In the BCIRG 006 trial, the distribution of the study population by age is not specified beyond the fact that 48% of patients were aged ≥ 50 years, and no subgroup analysis according to age was reported [20]. Finally, the FinHer [69] and FNCLCC PACS 04 [70] trials excluded patients aged > 65 years. The under-representation of elderly patients in such trials prevents extrapolation of the results to nearly half of the patients currently encountered in daily practice.

Recently, Brollo and colleagues [73] attempted to analyze DFS and cardiac safety in pooled datasets of patients aged ≥ 60 years treated in the HERA, NSABP B-31, and NCCTG N9831 trials. DFS results showed a HR of 0.53 (95% CI, 0.36–0.77) in elderly patients receiving trastuzumab compared with chemotherapy alone. Cardiac safety analysis showed a 5% incidence of cardiac events (95% CI, 4–7%) in elderly patients receiving trastuzumab. However, rates were not available for age-matched control patients.

A small number of retrospective studies have attempted to assess the activity and safety of trastuzumab in elderly patients, but their small sample size significantly limits the applicability of the results. Barthelemy and colleagues [74] reviewed medical records of 102 patients aged > 70 years with early-stage HER2-positive BC. Although most patients presented with large tumors ($pT \geq 2$) and/or lymph node involvement, the retrospective analysis showed that only 63 patients (62%) received trastuzumab-based adjuvant therapy. Among these 63 trastuzumab-treated patients, a $\geq 10\%$ decrease in LVEF was observed in 29% of patients, and 37% required a delay in administration of trastuzumab, most commonly because of cardiac toxicity.

In a recent Spanish retrospective analysis of 45 trastuzumab-treated patients aged ≥ 70 years, three (12.5%) of the 24 patients with early BC developed asymptomatic cardiotoxicity but none developed symptomatic heart failure [75]. The presence of cardiovascular risk factors, such as diabetes or a history of cardiac disease, was significantly associated with a higher incidence of trastuzumab-related cardiac events.

Very recently, data from 2,208 women aged ≥ 66 years (including almost 600 aged ≥ 75 years) and treated with adjuvant trastuzumab were published [76]. Factors associated with a lower likelihood of completing adjuvant trastuzumab therapy were age ≥ 80 years and a comorbidity score of 2. Furthermore, the incidence of significant cardiac events was higher in those who did not complete trastuzumab.

In conclusion, elderly patients with HER2-positive BC appear to derive the same benefit as younger patients from the addition of trastuzumab to traditional adjuvant chemotherapy. However, considering the lack of robust cardiac safety data generated within prospective trials, elderly patients should be carefully assessed for cardiovascular risk and closely monitored during adjuvant trastuzumab treatment.

3.3 Adjuvant trastuzumab in T1a/b N0 tumors.

Amplification and/or overexpression of HER2 in breast tumors has been reported to be the main prognostic factor for OS and BC-related mortality [6,21]. HER2 positivity usually correlates with poorly differentiated tumors, high proliferative rate (high ki-67), and lack of estrogen and/or progesterone receptor expression. With improvements in screening programs, the incidence of breast tumors < 1 cm has significantly increased [77]. Within this subset of small node-negative cancers, HER2 positivity seems to play a key role in survival and BC-related mortality. Data from a series of near 1,000 patients with T1a/b tumors indicated that 5-year RFS was significantly worse in HER2-positive tumors than in HER2-negative cancers (77.1% versus 93.7%, respectively; $p < 0.001$), patients with HER2-positive tumors had 5.09 times the rate of recurrences compared with patients who had HoR-positive tumors ($p < 0.0001$) [78]. Notably, the poorer prognosis was observed irrespective of HoR-status, with worse 5-year RFS in patients with HER2-positive disease than in those with either HoR-positive or TNBC [54]. In an old retrospective study by Black and colleagues, patients with HER2-positive T1a/b tumors showed a moderate risk of tumor recurrence, similar to that in T1c patients [79]. However, in patients with pT1a/b cancer, there were lower recurrences in patients receiving chemotherapy (4%) as compared with patients who did not receive it (17%) [79]. More recently, data reported by Curigliano and colleagues confirmed that patients with node-negative HER2-positive T1/b tumors had a worse prognosis than those with HER2-negative tumors, particularly in HoR-positive BC [80]. Another study suggested that patients with small HER2-positive tumors have a prognosis similar to that of patients with TNBC [81]. In the last five years several retrospective studies have been reported focused on this area. In a retrospective series on 16,975 consecutive patients with T1a/b HER2-positive disease, 5-year invasive distant recurrence-free interval was 99.0% for T1a patients and 97.0% for T1b patients

[82]. Another retrospective analysis on 128 patients with node-negative, HER2-positive BC ≤ 2 cm showed a lower DFS and OS in women who did not received adjuvant trastuzumab (3-year follow-up, DFS 100% with trastuzumab versus 79.2% without trastuzumab; OS 100% versus 92.6%, respectively) [83]. Similarly, data from the Memorial Sloan-Kettering Cancer Center suggested a significant benefit from trastuzumab in terms of locoregional invasive RFS (98% versus 92% with no trastuzumab; $p = 0.0137$) and invasive DFS (97% versus 82%, respectively; $p < 0.0001$) in patients with HER2-positive node-negative BC with tumors ≤ 2 cm treated at the [84]. Rodrigues and colleagues also reported a significantly reduced risk of recurrence with adjuvant trastuzumab-based chemotherapy in a retrospective analysis of 276 patients treated with or without trastuzumab [85]. An Italian real-world study demonstrated a 3-year RFS and OS significantly lower in patients who received adjuvant chemotherapy alone compared with those who received trastuzumab ($p < 0.0001$). In multivariate analysis, factors related to relapse were younger age, advanced stage at diagnosis, absence of hormonal and of trastuzumab therapy. The benefit derived from the addition of trastuzumab was independent of nodal status and hormonal receptors expression [86].

Unfortunately, the major adjuvant trials on BC excluded patients with T1a/b tumors because of their good prognosis and few data are available on treatment options. The nonrandomized Adjuvant Paclitaxel and Trastuzumab (APT) prospectively evaluated the beneficial effect of adjuvant trastuzumab in small tumors [87]. Women diagnosed with HER2-positive tumors < 3 cm received paclitaxel 80 mg/m^2 plus trastuzumab 2 mg/kg for 12 weeks, followed by 9 months of trastuzumab alone at a dose of 6 mg/kg every 3 weeks. After a median follow-up of 3.6 years, 2.5% patients experienced a recurrence or death. Overall 3-year DFS was 98.7%; this included 98% of patients with tumors >1 cm and 99.5% of those with tumors ≤ 1 cm. Moreover, DFS rates were 98.5% in HoR-positive disease and 99.2% in HoR-negative disease. Two patients developed symptomatic congestive heart failure. The investigators concluded that paclitaxel plus trastuzumab could be considered a reasonable approach for the majority of patients with stage I HER2-positive BC. Similarly, a subgroup analysis of the PHARE trial showed that in very-low risk patients (node negative and tumor ≤ 2 cm) the absolute benefit of 1 year adjuvant trastuzumab was small and its clinical relevance questionable [88]. These patients experienced an overall 3-year cumulative incidence for metastases of only 1.7%; no differences were observed between patients receiving 6 versus 12 months of trastuzumab.

On the basis of this combined evidence, National Cancer Comprehensive Network (NCCN) and German Arbeitsgemeinschaft Gynakologische Onkologie (AGO) guidelines have recently updated treatment recommendations to suggest consideration of trastuzumab-based adjuvant therapy for T1b tumors, particularly in patients with HoR-negative disease [89,90]. For T1a node-negative HER2-

positive tumors, no recommendations are available and clinicians must make treatment decisions on a case-by-case basis.

3.4 Trastuzumab and pregnancy

The possibility of pregnancy after a diagnosis of malignant disease has become a reality [91,92]. The effects of chemotherapy on fetal development are well known but only a few case reports are available describing trastuzumab use during pregnancy. Administration of trastuzumab has been associated with cases of oligohydramnios and oligohydramnios sequence [92,93], probably due to the presence of HER2 protein in the embryonic kidney, which could influence amniotic fluid dynamics and fetal renal function. Pulmonary hypoplasia, skeletal abnormalities and neonatal death have also been reported in trastuzumab-treated women [93-97]. In 2012, Azim and colleagues published data on the effect of trastuzumab on pregnancy outcome in women treated in the HERA trial [95]. Patients were divided into three groups on the basis of their exposure to trastuzumab: pregnancy during or up to 3 months after trastuzumab exposure; pregnancy >3 months after stopping trastuzumab; and pregnancy in patients who did not receive trastuzumab. If patients became pregnant during treatment and continued their pregnancy, trastuzumab treatment was stopped. No congenital anomalies were reported in fetuses exposed to trastuzumab in utero and trastuzumab did not affect fetal outcome. The only difference between the groups was a higher rate of spontaneous abortion in women having pregnancy during after stopping trastuzumab compared with the general population.

A recent systematic review including 17 studies synthesized available data on the safety of trastuzumab during pregnancy [98]. Notably, in 55.6% of cases, trastuzumab was administered in the metastatic setting. Occurrence of oligohydramnios was the most common adverse event; it accounts for 73.3% of pregnancies exposed to trastuzumab during the last two trimester. Trastuzumab also increased the risk of early delivery, the mean gestational age at delivery was 33.8 weeks, and the mean weight of babies at delivery was 2,261 gr. In 52.6 % of cases, a healthy neonate was born, but all children exposed to trastuzumab in utero exclusively in the first trimester were completely healthy at birth.

A new US registry (MoTHER) has been established as a prospective, observational study of women with HER2-positive BC who were treated with trastuzumab with or without pertuzumab during pregnancy or within six months before conception [99]. The primary objective of this study is to describe adverse pregnancy complications, fetal outcomes, and birth defects. Nevertheless, in the absence of robust evidence on pregnancy in trastuzumab-exposed women, women of childbearing

potential should be counseled to use effective contraception during treatment and for at least seven months after treatment has completed.

4. HER2-directed therapy for metastatic BC

In the setting of BC relapse, treatment decisions are typically based on assessment of HER2 and HoR status. However, several studies have demonstrated a discordance in terms of HER2 and HoR expression between primary BC and metastatic sites [100]. A recent analysis demonstrated a discordance rate of 6.8% in HER2 status between primary BC and paired metastatic lesions, mainly from positive to negative status (16.0% from positive to negative versus 4.6% from negative to positive) [101]. A change in HER2 status was associated with a worse prognosis; the HER2-loss patients showed both worse OS ($p < 0.001$) and post-recurrence survival ($p < 0.001$) when compared with the concordant-positive cases. Conversely, no significant association of HER2 gain with prognosis was observed.

Considering that HER2-positivity has shown mixed prognostic and predictive values, concern regarding HER2-status discordance may lead to the need of re-biopsying metastatic disease; especially because the landscape of HER2-positive metastatic BC treatment is evolving rapidly.

In the past decade, first-line trastuzumab combined with a taxane (paclitaxel or docetaxel) or vinorelbine has been considered the most active and effective treatment choice for HER2-positive metastatic BC, with response rates of 50–60% and median time to progression (TTP) ranging from 7 to 15 months [23–25]. After disease progression on first-line trastuzumab-based therapy, continued HER2 blockade with lapatinib plus capecitabine [102,103] or trastuzumab plus capecitabine [104] has been considered a standard in clinical practice.

In post-menopausal, chemotherapy-free therapeutic options have been successfully explored in the management of first-line HER2-positive and HoR-positive metastatic BC with highly endocrine-sensitive non-life-threatening and/or slowly progressive disease. A chemotherapy-free first-line regimen of trastuzumab plus anastrozole demonstrated a significant improvement in PFS (median 5 versus 2 months, respectively) and response rate (20% versus 7%, respectively) compared with anastrozole alone [105]. Similar results have been obtained with lapatinib plus letrozole (median PFS 8 versus 3 months, respectively; $p = 0.019$; clinical benefit rate 48% versus 29%, respectively; $p = 0.003$) [106]. In both randomized trials, patients had not previously received trastuzumab.

Starting from 2012, a paradigm shift was observed in the management of HER2-positive metastatic BC following release of results from the CLEOPATRA [107], the EMILIA [108], the TH3RESA [109], and the MARIANNE [110] trials (figure 1).

The double-blind placebo-controlled phase III CLEOPATRA trial compared the combination of trastuzumab and docetaxel plus pertuzumab or placebo as first-line treatment in 808 patients with HER2-positive metastatic BC [107]. At the first analysis, the addition of pertuzumab significantly improved PFS (18.5 months with pertuzumab-containing therapy versus 12.4 months in the control arm, HR 0.62, 95% CI 0.51,0.75; $p < 0.001$). The response rate was 80% versus 69%, respectively ($p=0.001$), and a strong trend towards an OS improvement was shown with the combination of pertuzumab, trastuzumab and docetaxel. Lately, after a median follow-up of 50 months, the final analysis showed an unprecedented 16 months improvement in OS (56.5 months in the pertuzumab arm versus 40.8 months in the placebo arm, HR= 0.68; $p = 0.0002$) [111]. The main criticism of the trial was that only a minority of patients had previously received trastuzumab (11% of patients) or a taxane (23% of patients) in the (neo)adjuvant setting, which was perceived as unrepresentative of everyday clinical practice. Moreover, patients had to have a disease-free interval of at least 12 months after completion of (neo)adjuvant therapy. However, subgroup analyses suggested a similar benefit from pertuzumab-containing therapy irrespective of prior (neo)adjuvant chemotherapy (HR 0.61 in patients who had received (neo)adjuvant chemotherapy versus 0.63 in those who had not). With respect to safety, pertuzumab did not add to trastuzumab cardiotoxicity.

The phase III EMILIA trial compared the antibody drug conjugate trastuzumab-emtansine (T-DM1) with lapatinib plus capecitabine in 991 patients with HER2-positive metastatic BC who had previously been treated with a taxane and trastuzumab [108]. T-DM1 was associated with significantly improved PFS (9.6 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine; HR 0.65, 95% CI 0.55-0.77, $p < 0.001$), OS (30.9 versus 25.1 months, respectively; HR 0.68, 95% CI 0.55-0.85, $p < 0.001$), and response rate (44% versus 31%, respectively; $p < 0.001$). Of note, the benefit of T-DM1 was observed irrespective of treatment line and even in patients whose disease progressed less than 6 months after completing (neo)adjuvant trastuzumab. Furthermore, the incidence of grade 3/4 adverse events was lower with T-DM1 than with lapatinib plus capecitabine (41% versus 57%, respectively).

The phase III TH3RESA trial randomized 602 patients with progressive disease after two or more HER2-targeted regimens metastatic HER2-positive BC to receive T-DM1 or physician's choice therapy [109]. As like as for the EMILIA trail, median PFS was significantly improved with T-DM1 compared with physician's choice (6.2 months versus 3.3 months, HR 0.528, $p < 0.0001$). Interim OS analysis showed a trend favoring T-DM1 (HR 0.552, 95% CI 0.369-0.826, $p = 0.0034$), but the stopping boundary was not crossed. Moreover, a lower incidence of grade 3-4 adverse events was reported with T-DM1 than with physician's choice (32% versus 43%, respectively). The

Authors concluded that T-DM1 should be considered as a standard for patients with HER2-positive metastatic BC who have previously failed trastuzumab and lapatinib.

The phase III MARIANNE randomly assigned 1,095 patients with metastatic HER2-positive BC to receive T-DM1 plus pertuzumab, T-DM1 plus placebo, or taxanes plus trastuzumab [110]. Quite unexpectedly, both T-DM1-containing regimens did not show superiority over control arm. The median PFS was 15.2 months with T-DM1 plus pertuzumab arm (HR 0.87, 95% CI 0.69-1.08, $p = 0.14$), 14.1 months with T-DM1 alone (HR 0.91, 95% CI 0.73-1.13, $p = 0.31$) compared with 13.7 months with taxanes plus trastuzumab. However, the median duration of response was 21.2 months with T-DM1 plus pertuzumab, 20.7 months with T-DM1 alone, and 12.5 months with standard treatment.

As the efficacy of these first- and second-line therapies in HER2-positive metastatic BC is substantial, oncologists now encounter new clinical challenges in managing patients whose disease progresses after two lines of HER2-targeted therapy. Currently, prospective data are sparse in this setting: in the EGF104900 trial [112], the combination of lapatinib plus trastuzumab demonstrated improved OS compared with lapatinib alone in patients with heavily pretreated disease (median three prior lines of trastuzumab). The HR for OS was 0.74 (95% CI 0.57-0.97; $p=0.026$) and the 1-year OS rate was 56% with the combination versus 41% with lapatinib alone). An exploratory analysis showed that combining the two anti-HER2 agents was particularly effective in subgroups of patients with HoR-negative tumors or treated with fewer than four prior lines of trastuzumab. However, in Europe, the combination of lapatinib plus trastuzumab is not approved and the current practice in patients whose disease progresses on trastuzumab plus a taxane or on lapatinib plus capecitabine is to rechallenge with trastuzumab combined with an alternative chemotherapy, even though the evidence supporting this approach is derived only from retrospective studies [113].

The perhaps surprisingly high proportion of patients (53%) with an initial diagnosis of stage IV disease in the CLEOPATRA trial [107] raises another important question in the management of metastatic BC. Anthracyclines, to which HER2-positive BC is well-known to be highly sensitive, are usually administered in the adjuvant setting. However, as patients with de-novo metastatic BC have received no previous (neo)adjuvant chemotherapy, first-line anthracycline-containing treatment may be an option. However, the role of these agents in newly diagnosed stage IV BC is not precisely known. In a retrospective series of 450 patients treated with trastuzumab-based therapies between 1999 and 2008 [114], only 40 (21%) of 190 eligible patients received an anthracycline after trastuzumab in the metastatic setting.

The estimated improvement in 5-year OS with the addition of trastuzumab to first-line chemotherapy is 10.2% (from 13.2% to 23.4%) [115]. The current practice of continuing

trastuzumab until progression in responding patients is based on protocols of pivotal trials, and long-term trastuzumab treatment does not appear to be associated with an excess in cardiac toxicity [115]. A retrospective analysis of data from two institutions with differing approaches to trastuzumab duration in patients achieving a complete response showed a higher rate of durable complete response in patients continuing trastuzumab for 5 years compared with only 2 years (11% versus 6%, respectively), particularly in patients with HoR-negative tumors and liver metastases [116]. No late cardiac toxicity was reported, although the small number of patients precludes any definitive conclusion about the cardiac safety of trastuzumab administered for such a long duration in an unselected population.

Recently, the role of mTOR inhibition in HER2-positive BC has been evaluated in two large phase III trials. The first study, named BOLERO-3, randomized 569 women with HER2-positive, trastuzumab-resistant, metastatic BC who had previously treated with taxane therapy to receive vinorelbine plus trastuzumab with or without everolimus [117]. After a follow-up of 20.2 months, median PFS was 7.00 months with the addition of everolimus and 5.78 months with placebo (HR 0.78, 95% CI 0.65-0.95, $p = 0.0067$). The most common grade 3-4 adverse events were hematological toxicity followed by stomatitis and fatigue. Two on-treatment deaths due to adverse events occurred in each group. Later, the BOLERO-1 trial randomly assigned 719 patients who had not received previous trastuzumab or chemotherapy for advanced BC to receive trastuzumab plus paclitaxel with or without everolimus [118]. In contrast with the BOLERO-3, no differences in PFS were observed with the addition of everolimus to first-line paclitaxel plus trastuzumab therapy ($p = 0.1166$). Moreover, on-treatment adverse event-related deaths were reported in 4% of patients in the experimental arm and none in the standard arm.

To summarize, following the remarkable results of recent first- and second-line trials in HER2-positive metastatic BC, the optimal treatment algorithm has been revisited (Figure 1). At the third disease progression in a patient still fit enough for active treatment, enrollment in a clinical trial is warranted; alternatively, rechallenge with trastuzumab combined with a non-cross-resistant chemotherapy or with single-agent anthracycline therapy may be considered.

5. Future perspectives

Continued attempts to optimize our use of HER2-directed therapies have led to improved understanding of trastuzumab and newer anti-HER2 agents. For patients with T1b tumors, NCCN and AGO guidelines now recommend trastuzumab-based adjuvant chemotherapy, although the optimal adjuvant strategy for T1a tumors remains less clear-cut and patients should be made aware of the risk of recurrence, the availability of HER2-directed therapy, and the possible side effects

during treatment decision making. The OS benefit with adjuvant trastuzumab should be balanced with the cardiac risk, particularly in patients with low-risk BC [14]. Similarly, the balance of safety and efficacy should be kept in mind when considering treatment options in elderly patients, in whom the presence of other cardiovascular risk factors correlates with a higher incidence of trastuzumab-related cardiac events [75]. Serum cardiac biomarkers, including troponins and natriuretic peptides, represent possible tools to detect cardiotoxicity at a preclinical level, and may represent an important means of selecting treatment to avoid cardiac side effects [119]. Ongoing studies continue to explore the possibility of shorter adjuvant trastuzumab exposure that may be associated with an improved cardiac safety profile.

The present challenge is to improve these results while maintaining low toxicity and financial burdens. Refining the ASCO/CAP criteria for HER2 positivity and improving the accuracy of HER2 testing to avoid exclusion from trastuzumab treatment [120], identifying the most appropriate duration of adjuvant trastuzumab, and introducing more selective tumor targeting with antibody–drug conjugates are very important achievements in recent research. The goal of overcoming treatment resistance remains a high priority. Several mechanisms of resistance to trastuzumab have been identified, including increased MUC4 expression [121], presence of a truncated form of HER2 receptor [122], activation of the PI3K-AKT pathway through loss of PTEN or PIK3CA mutation [123], increased signalling through HER2/HER3 heterodimerization [13], and activation of hormonal receptor signalling [124]. Targeting the pathways involved in trastuzumab resistance is a novel avenue of clinical research and new biological agents are expected to improve the outcome of patients with HER2-positive tumors.

The benefit of dual HER2 targeting in primary therapy has been demonstrated in the NeoALTTO and CHER-LOB trials evaluating the combination of trastuzumab and lapatinib [55,58] and the NeoSphere trial evaluating trastuzumab and pertuzumab combined with docetaxel [61]. Similarly, in the metastatic setting, dual HER2 blockade with pertuzumab and trastuzumab plus docetaxel significantly improved PFS in the CLEOPATRA phase III trial with no increase in cardiac toxic effects [107]. In the phase III EMILIA trial, T-DM1 was significantly more effective and better tolerated than capecitabine plus lapatinib in taxane- and trastuzumab-pretreated metastatic BC [108]. Moreover, other antibody-conjugated drugs have been recently developed. The on-going phase II HERMIONE trial randomizes anthracycline naïve, trastuzumab-, pertuzumab- and T-DM1-pretreated HER2-positive locally advanced BC to receive MM-302, a HER2-targeted liposomal doxorubicin, plus trastuzumab or chemotherapy of physician's choice (vinorelbine, capecitabine, or gemcitabine) plus trastuzumab (Clinical trial information: NCT02213744). Recruitment is expected to be complete in late 2016.

Another interesting on-going phase II trial is exploring the activity of margetuximab, a chimeric anti-HER2 monoclonal antibody with an Fc domain engineered which exhibited enhanced antibody dependent cell cytotoxicity (ADCC) against HER2-positive BC cells, including trastuzumab-resistant and HER2-low expressing cells (Clinical trial information: NCT01828021).

The promising strategy of combining pertuzumab, trastuzumab and chemotherapy is now being tested in newly diagnosed HER2-positive early BC in the Adjuvant Pertuzumab and Herceptin in Initial Therapy in Breast Cancer (APHINITY) trial (NCT01358877), which has already completed accrual.

In conclusion, the efficacy and safety of novel anti-HER2 agents demonstrated in the metastatic setting represents a major advance. Results of trials evaluating these agents in the neoadjuvant and adjuvant settings may enable clinicians to improve clinical outcomes and increase rates of cure in patients with BC.

Acknowledgements

We would like to thank for their precious cooperation Dr. Francesca Adami (Mantova, Italy), Dr. Maria Rosa Coccaro (Rionero, Italy), Dr. Saverio Danese (Torino, Italy), Dr. Roberto Dottori (Grosseto, Italy), Dr. Fernando Gaion (Camposampiero, Italy), Dr. Marianna Giampaolo (Frosinone, Italy), Dr. Giuseppe Grimaldi (Salerno, Italy), Dr. Maria Malagoli (Trieste, Italy), Dr. Lucia Moraca (Foggia, Italy), Dr. Nina Antonina Olmeo Sassari, Italy), Dr. Silvana Saracchini (Pordenone, Italy), Dr. Clementina Savastano (Salerno, Italy), Dr. Emiliana Tarenzi (Milano, Italy), Dr. Anna Turletti (Torino, Italy), Dr. Milvia Zambetti (Milano, Italy), Dr. Valentina Zanfagnin (Torino, Italy).

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Siegel RL, Miller K, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5–29.
2. La Vecchia C, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P, et al. Cancer mortality in European, 2000–2004, and an overview of trends since 1975. *Ann Oncol* 2010;21:1323–60.
3. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Peto R, Davies C, Godwin J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet* 2012;379:432–44.
4. Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al; Cancer Intervention and Surveillance Modeling Network (CISNET) Collaborators. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med* 2005;353:1784–92.
5. Giordano SH, Buzdar AU, Smith TL, Kau SW, Yang Y, Hortobagyi GN. Is breast cancer survival improving? *Cancer* 2004;100:44–52.
6. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177–182.
7. Prat A, Baselga J. The role of hormonal therapy in the management of hormonal-receptor-positive breast cancer with co-expression of HER2. *Nat Clin Pract Oncol* 2008;5:531–42.
8. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al; American Society of Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*. 2013;31:3997–4013.
9. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. *Oncologist* 2009;14:320–68.
10. Rubin I, Yarden Y. The basic biology of HER2. *Ann Oncol* 2001;12 Suppl 1:S3–8.
11. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006;7:505–16.
12. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J* 1997;16:1647–55.
13. Lee-Hoeflich ST, Crocker L, Yao E, Pham T, Munroe X, Hoeflich KP, et al. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. *Cancer Res* 2008;68:5878–87.

14. Moja L, Tagliabue L, Balduzzi S, Parmelli E, Pistotti V, Guarneri V, et al. Trastuzumab containing regimens for early breast cancer. *Cochrane Database Syst Rev* 2012 Apr 18;4:CD006243.
15. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, et al; Herceptin Adjuvant (HERA) Trial Study Team. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659–72.
16. Gianni L, Dafni U, Gelber RD, Azambuja E, Muehlbauer S, Goldhirsch A, et al; Herceptin Adjuvant (HERA) Trial Study Team. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol* 2011;12:236–44.
17. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clin Oncol* 2011;29:3366–73.
18. Perez EA, Suman VJ, Davidson NE, Gralow JR, Kaufman PA, Visscher DW, et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. *J Clin Oncol* 2011;29:4491–7.
19. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673–84.
20. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med* 2011;365:1273–83.
21. Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J Clin Oncol* 2005;23(16):3676–85
22. Gianni L, Eiermann W, Semiglazov V, Booser DJ, Thomas ES, Theriault RL, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet* 2010;375:377–84.

23. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783–92.
24. Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first line treatment: the M77001 study group. *J Clin Oncol* 2005;23:4265–24.
25. Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. *J Natl Cancer Inst* 2005;97:188–94.
26. Kaufmann M, von Minckwitz G, Mamounas EP, Cameron D, Carey LA, Cristofanilli M, et al. Recommendations from an international consensus conference on the current status and future use of neoadjuvant systemic therapy in primary breast cancer: an update. *Ann Surg Oncol* 2012;19:1508–16.
27. Berruti A, Generali D, Kaufmann M, Puztai L, Curigliano G, Aglietta M, et al. International expert consensus on primary systemic therapy in the management of early breast cancer: highlights of the fourth symposium on primary systemic therapy in the management of operable breast cancer, Cremona, Italy (2010). *J Natl Cancer Inst Monogr* 2011;2011:147–51.
28. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus of Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 2013;24:2206-23.
29. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. *J Clin Oncol* 2008;26:778–85.
30. Bear HD, Anderson S, Smith RE, Geyer CE Jr, Mamounas EP, Fisher B, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project protocol B-27. *J Clin Oncol* 2006;24:2019–27.
31. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol* 2012;30:1796–1804.
32. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014;384:164-72.

33. von Minckwitz G, Fontanella C. Selecting the neoadjuvant treatment by molecular subtype: how to maximize the benefit? *Breast* 2013;22 Suppl2:S149-51.
34. Marinovich ML, Houssami N, Macaskill P, von Minckwitz G, Blohmer JU, Irwig L. Accuracy of ultrasound for predicting pathologic response during neoadjuvant therapy for breast cancer. *Int J Cancer*. 2015 Jun 1;136:2730-7.
35. Marinovich ML, Sardanelli F, Ciatto S, Mamounas E, Brennan M, Macaskill P, et al. Early prediction of pathologic response to neoadjuvant therapy in breast cancer: systemic review of the accuracy of MRI. *Breast* 2012;21:669-77
36. Humbert O, Cochet A, Coudert B, Berriolo-Riedinger A, Kanoun S, Brunotte F, et al. Role of positron emission tomography for the monitoring of response to therapy for breast cancer. *Oncologist* 2015;20:94-104.
37. Pahk K, Rhee S, Cho J, Seo M, Lee S, Park T, et al. The role of interim 18F-FDG PET/CT in predicting early response to neoadjuvant chemotherapy in breast cancer. *Anicancer Res* 2014;34:4447-55.
38. Humbert O, Cochet A, Riedinger JM, Berriolo-Riedinger A, Arnould L, Coudert B, et al. HER2-positive breast cancer: ¹⁸F-FDG PET for early prediction of response to trastuzumab plus taxane-based neoadjuvant chemotherapy. *Eur J Nucl Med Mol Imaging*. 2014;41:1525-33
39. Coudert B, Pierga JY, Mouret-Reynier MA, Kerrou K, Ferrero JM, Petit T, et al. Use of [(18)F]-FDG PET to predict response to neoadjuvant trastuzumab and docetaxel in patients with HER2-positive breast cancer, and addition of bevacizumab to neoadjuvant trastuzumab and docetaxel in [(18)F]-FDG PET-predicted non-responders (AVATAXHER): an open-label, randomised phase 2 trial. *Lancet Oncol*. 2014;15:1493-502
40. Loibl S, von Minckwitz G, Untch M, Denkert C. Predictive factors for response to neoadjuvant therapy in breast cancer. *OncolRes Treat* 2014;37:563-8.
41. Fontanella C, Lederer B, Gade S, Vanoppen M, Blohmer JU, Costa SD, et al. Impact of body mass index on neoadjuvant treatment outcome: a pooled analysis of eight prospective neoadjuvant breast cancer trials. *Breast Cancer Res Treat*. 2015;150:127-39.
42. Ignatiadis M, Singhal SK, Desmedt C, Haibe-Kains B, Criscitiello C, Andre F, et al. Gene modules and response to neoadjuvant chemotherapy in breast cancer subtypes: a pooled analysis. *J Clin Oncol* 2012;30:1996–2004.
43. Loibl S, Volz C, Mau C, Blohmer JU, Costa SD, Eidtmann H, et al. Response and prognosis after neoadjuvant chemotherapy in 1,051 patients with infiltrating lobular breast carcinoma. *Breast Cancer Res Treat*. 2014;144:153-62

44. Ingolf JB, Russalina M, Simona M, Julia R, Gilda S, Bohle RM, et al. Can ki-67 play a role in prediction of breast cancer patients' response to neoadjuvant chemotherapy? *Biomed Res Int* 2014;2014:628217.
45. von Minckwitz G, Fontanella C. Pathway-oriented concepts in adjuvant and neoadjuvant breast cancer therapy. *Breast Care (Basel)*. 2013;8:246-7.
46. Denkert C, Lobl S, Muller BM, Eidtmann H, Schmitt WD, Eiermann W, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. *Ann Oncol* 2013;24:2786-93
47. Coudert BP, Arnould L, Moreau L, Chollet P, Weber B, Vanlemmens L, et al. Pre-operative systemic (neo-adjuvant) therapy with trastuzumab and docetaxel for HER2-overexpressing stage II or III breast cancer: results of a multicenter phase II trial. *Ann Oncol* 2006;17:409–14.
48. Hurley J, Doliny P, Reis I, Silva O, Gomez-Fernandez C, Velez P, et al. Docetaxel, cisplatin, and trastuzumab as primary systemic therapy for human epidermal growth factor receptor 2-positive locally advanced breast cancer. *J Clin Oncol* 2006;24:1831–8.
49. Untch M, Fasching PA, Konecny GE, Hasmüller S, Lebeau A, Kreienberg R, et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: Results from the TECHNO trial of the AGO and GBG Study Groups. *J Clin Oncol* 2011;29:3351–7.
50. Untch M, Rezai M, Loibl S, Fasching PA, Huober J, Tesch H, et al. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. *J Clin Oncol* 2010;28:2024–31.
51. Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, et al. Follow-up results of NOAH, a randomized phase III trial evaluating neoadjuvant chemotherapy with trastuzumab (CT+H) followed by adjuvant H versus CT alone, in patients with HER2-positive locally advanced breast cancer. *J Clin Oncol* 31, 2013 (suppl; abstr 503)
52. Mayer EL, Gropper AB, Harris L, Gold JM, Parker L, Kuter I, et al. Long-Term Follow-Up After Preoperative Trastuzumab and Chemotherapy for HER2-Overexpressing Breast Cancer. *Clin Breast Cancer*. 2015;15:24-30
53. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000278/WC500126896.pdf Accessed on March 22nd, 2015.
54. Untch M, Loibl S, Bischoff J, Eidtmann H, Kaufmann M, Blohmer JU, et al; German Breast Group (GBG); Arbeitsgemeinschaft Gynäkologische Onkologie-Breast (AGO-B) Study Group. Lapatinib versus trastuzumab in combination with neoadjuvant anthracycline-taxane-based

- chemotherapy (GeparQuinto, GBG 44): a randomised phase 3 trial. *Lancet Oncol* 2012;13:135-44.
55. Guarneri V, Frassoldati A, Bottini A, Cagossi K, Bisagni G, Sarti S, et al. Preoperative chemotherapy plus trastuzumab, lapatinib, or both in human epidermal growth factor receptor 2-positive operable breast cancer: results of the randomized phase II CHER-LOB study. *J Clin Oncol* 2012;30:1989–95.
56. Robidoux A, Tang G, Rastogi P, Geyer CE Jr, Azar CA, Atkins JN, et al. Lapatinib as a component of neoadjuvant therapy for HER2-positive operable breast cancer (NSABP protocol B-41): an open-label, randomised phase 3 trial. *Lancet Oncol* 2013;14:1183–92.
57. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, et al; NeoALTTO Study Team. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2012;379:633–40.
58. de Azambuja E, Holmes AP, Piccart-Gebhart M, Holmes E, Di Cosimo S, Swaby RF, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): survival outcomes of a randomised, open-label, multicentre, phase 3 trial and their association with pathological complete response. *Lancet Oncol*. 2014;15:1137-46
59. Piccart-Gebhart MJ, Holmes AP, Baselga J, et al: First results from the phase III ALTTO trial (BIG 2-06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone, trastuzumab alone, their sequence, or their combination in the adjuvant treatment of HER2-positive early breast cancer. ASCO Annual Meeting. Abstract LBA4. Presented June 1, 2014.
60. Fontanella C, Loibl S, von Minckwitz G. Clinical usefulness and relevance of intermediate endpoints for cytotoxic neoadjuvant therapy. *Breast*. 2015. pii: S0960-9776(15)00158-7.
61. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol* 2012;13:25–32.
62. Schneeweiss A, Chia S, Hickish T, Harvey V, Eniu A, Hegg R, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: A randomized phase II cardiac safety study (TRYPHAENA). *Ann Oncol* 2013;24:2278-84.
63. <http://www.roche.com/media/store/releases/med-cor-2013-03-05.htm> Accessed March 22nd, 2015.

64. de Azambuja E, Procter MJ, van Veldhuiden DJ, A gbor-Tarh D, Metzger-Filho O, Steinseifer J, et al. Trastuzumab-associated cardiac events at 8 years of median follow-up in the Herceptin Adjuvant trial (BIG 1-01). *J Clin Oncol* 2014;32:2159-65
65. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Pawlicki M, et al. Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC/T) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (AC/TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER-2 positive early breast cancer patients: BCIRG 006 study. *Breast Cancer Res Treat* 2005;94(Suppl 1):(Abstr 1).
66. Du F, Yuan P, Zhu W, et al. Is it safe to give anthracyclines concurrently with trastuzumab in neo-adjuvant or metastatic settings for HER2-positive breast cancer? A meta-analysis of randomized controlled trials. *Med Oncol.* 2014;31:340.
67. Rocca A, Bravaccini S, Scarpi E, et al. Benefit from anthracyclines in relation to biological profiles in early breast cancer. *Breast Cancer Res Treat.* 2014 Apr;144(2):307-18. doi: 10.1007/s10549-013-2819-0. Epub 2014 Jan 1. Erratum in: *Breast Cancer Res Treat.* 2014;144:319.
68. Baselga J, Manikhas A, Cortés J, et al. Phase III trial of nonpegylated liposomal doxorubicin in combination with trastuzumab and paclitaxel in HER2-positive metastatic breast cancer. *Ann Oncol.* 2014;25:592-8.
69. Joensuu H, Bono P, Kataja V, Alanko T, Kokko R, Asola R, et al. Fluorouracil, epirubicin, and cyclophosphamide with either docetaxel or vinorelbine, with or without trastuzumab, as adjuvant treatments of breast cancer: final results of the FinHer trial. *J Clin Oncol* 2009;27:5685–92.
70. Pivot X, Romieu G, Debled M, Pierga JY, Kerbrat P, Bachelot T, et al. 6 months versus 12 months of adjuvant trastuzumab for patients with HER2-positive early breast cancer (PHARE): a randomised phase 3 trial. *Lancet Oncol* 2013;14:741-8.
71. Guarneri V, Frassoldati A, Bruzzi P, D'Amico R, Belfiglio M, Molino A, et al. Multicentric, randomized phase III trial of two different adjuvant chemotherapy regimens plus three versus twelve months of trastuzumab in patients with HER2- positive breast cancer (Short-HER Trial; NCT00629278). *Clin Breast Cancer* 2008;8:453–6.
72. <https://clinicaltrials.gov/ct2/show/NCT00593697> Accessed on March 22nd, 2015.
73. Brollo J, Curigliano G, Disalvatore D, Marrone BF, Criscitiello C, Bagnardi V, et al. Adjuvant trastuzumab in elderly with HER-2 positive breast cancer: a systematic review of randomized controlled trials. *Cancer Treat Rev* 2013;39(1):44–50.

74. Barthelemy P, Bassot K, Joly F, Coquard IR, Freyer G, Dufour PR, et al. Adjuvant treatment of early-stage HER2+ elderly breast cancer patients: A retrospective, multicenter French study. *J Clin Oncol* 2012;30(suppl): Abstract 636.
75. Serrano C, Cortés J, De Mattos-Arruda L, Bellet M, Gómez P, Saura C, et al. Trastuzumab-related cardiotoxicity in the elderly: a role for cardiovascular risk factors. *Ann Oncol* 2012;23:897–902.
76. Vaz-Luis I, Keating NL, Lin NU, Lii H, Winer EP, Freedman RA. Duration and toxicity of adjuvant trastuzumab in older patients with early-stage breast cancer: A population-based study. *J Clin Oncol* 2014; 32:2142-50.
77. Lehtimäki T, Lundin M, Linder N, Sihto H, Holli K, Turpeenniemi-Hujanen T, et al. Long-term prognosis of breast cancer detected by mammography screening or other methods. *Breast Cancer Res.* 2011;13:R134.
78. Gonzales-Angulo AM, Litton JK, Broglio KR, Meric-Bernstam F, Rakhit R, Cardoso F, et al. High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. *J Clin Oncol* 2009;27:5700–6.
79. Black D, Younger J, Martei Y. Recurrence risk in T1a/b, node-negative, HER-2 positive breast cancer. *Breast Cancer Res Treat* 2006;100:S92(Abst 2037).
80. Curigliano G, Viale G, Bagnardi V, Fumagalli L, Locatelli M, Rotmensz N, et al. Clinical relevance of HER2 overexpression/amplification in patients with small tumor size and node-negative breast cancer. *J Clin Oncol* 2009;27:5693–9.
81. Amar S, McCullough AE, Tan W, Geiger XJ, Boughey JC, McNeil RB, et al. Prognosis and outcome of small (≤ 1 cm), node-negative breast cancer on the basis of hormonal and HER-2 status. *Oncologist* 2010;15:1043–9.
82. Fehrenbacher L, Capra AM, Quesenberry CP Jr, Fulton R, Shiraz P, Habel LA. Distant invasive breast cancer recurrence risk in human epidermal growth factor receptor 2-positive T1a and T1b node-negative localized breast cancer diagnosed from 2000 to 2006: a cohort from an integrated health care delivery system. *J Clin Oncol.* 2014 Jul 10;32:2151-8.
83. Tognela A, Beith J, Kiely B, Bastick P, Lynch J, Descallar J, et al. Small HER2-Positive Breast Cancer: Should Size Affect Adjuvant Treatment? *Clin Breast Cancer.* 2015 Jan 9. pii: S1526-8209(15)00014-2.
84. McArthur HL, Mahoney KM, Morris PG, Patil S, Jacks LM, Howard J, et al. Adjuvant trastuzumab with chemotherapy is effective in women with small, node-negative, HER2-positive breast cancer. *Cancer* 2011;117:5461–8.

85. Rodrigues MJ, Peron J, Frénel JS, Vano YA, Wassermann J, Debled M, et al. Benefit of adjuvant trastuzumab-based chemotherapy in T1ab node-negative HER2-overexpressing breast carcinomas: a multicenter retrospective series. *Ann Oncol* 2013;24:916-24.
86. Vici P, Pizzuti L, Natoli C, Moscetti L, Mentuccia L, Vaccaro A, et al. Outcomes of HER2-positive early breast cancer patients in the pre-trastuzumab and trastuzumab eras: a real-world multicenter observational analysis. The RETROHER study. *Breast Cancer Res Treat.* 2014;147:599-607.
87. Tolaney SM, Barry WT, Dang CT, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. *N Engl J Med.* 2015;372:134-41.
88. Kramar A, Bachelot T, Madrange N, et al. Trastuzumab duration effects within patient prognostic subgroups in the PHARE trial. *Ann Oncol.* 2014;25:1563-70.
89. https://www.nccn.org/store/login/login.aspx?ReturnURL=http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf Accessed on October 05nd, 2015.
90. http://www.ago-online.de/fileadmin/downloads/leitlinien/mamma/maerz2014/en/2014_englisch_all.pdf Accessed on October 05nd, 2015.
91. Pant S, Landon MB, Blumenfeld M, Farrar W, Shapiro CL. Treatment of breast cancer with trastuzumab during pregnancy. *J Clin Oncol* 2008;26:1567–9.
92. Beale JM, Tuohy J, McDowell SJ. Herceptin (trastuzumab) therapy in a twin pregnancy with associated oligohydramnios. *Am J Obstet Gynecol* 2009;201:e13–4
93. Bader AA, Schlembach D, Tamussino KF, Pristauz G, Petru E. Anhydramnios associated with administration of trastuzumab and paclitaxel for metastatic breast cancer during pregnancy. *Lancet Oncol* 2007;8:79–81.
94. Witzel ID, Müller V, Harps E, Janicke F, Dewit M. Trastuzumab in pregnancy associated with poor fetal outcome. *Ann Oncol* 2008;19:191–2.
95. Azim HA Jr, Metzger-Filho O, de Azambuja E, Loibl S, Focant F, Gresko E, et al. Pregnancy occurring during or following adjuvant trastuzumab in patients enrolled in the HERA trial (BIG 01-01). *Breast Cancer Res Treat* 2012;133:387-91.
96. Ring AE, Smith IE, Jones A, Shannon C, Galani E, Ellis. Chemotherapy for breast cancer during pregnancy: an 18-year experience from five London teaching hospitals. *PA J Clin Oncol* 2005;23:4192–7.
97. McGrath SE, Ring A. Chemotherapy for breast cancer in pregnancy: evidence and guidance for oncologists. *Ther Adv Med Oncol* 2011;3:73–83.

98. Zagouri F, Sergentanis TN, Chrysikos D, Papadimitriou CA, Dimopoulos MA, Bartsch R. Trastuzumab administration during pregnancy: a systematic review and meta-analysis. *Breast Cancer Res Treat.* 2013;137:349-57.
99. Brown VD, EB Andrews, LI Wang, LK Chu, C Trudeau, and KM Keppler-Noreuil. MotHER: A registry developed for women with breast cancer who have received trastuzumab within 6 months prior to conception or during pregnancy. *Cancer Research* 2012;71:OT1-02-11.
100. Rossi S, Basso M, Strippoli A, et al. Hormone Receptor Status and HER2 Expression in Primary Breast Cancer Compared With Synchronous Axillary Metastases or Recurrent Metastatic Disease. *Clin Breast Cancer.* 2015;15:307-12.
101. Yang YF, Liao YY, Yang M, et al. Discordances in ER, PR and HER2 receptors between primary and recurrent/metastatic lesions and their impact on survival in breast cancer patients. *Med Oncol.* 2014;31:214.
102. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 2006;355:2733–43.
103. Cameron D, Casey M, Oliva C, Barinoff J, Mundhenke C, Cufer T, et al. Lapatinib plus capecitabine in women with HER2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. *Oncologist* 2010;15:924:34.
104. von Minckwitz G, du Bois A, Schmidt M, Barinoff J, Mundhenke C, Cufer T, et al. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a German Breast Group 26/Breast International Group 03-05 study. *J Clin Oncol* 2009;27:1999–2006.
105. Kaufmann B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol* 2009;27:5529–37.
106. Johnston S, Pippin Jr J, Pivot X, Lichinitser M, Sadeghi S, Dieras V, et al. Lapatinib combined with letrozole and placebo as first line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J Clin Oncol* 2009;27:5538–46.
107. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med* 2012;366:109–19.
108. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012;367:1783–91.

109. Krop IE, Kim SB, González-Martín A, LoRusso PM, Ferrero JM, Smitt M, et al; TH3RESA study collaborators. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2014;15:689-99.
110. Ellis PA, Barrios CH, Eiermann W. Phase III, randomized study of trastuzumab emtansine (T-DM1) ± pertuzumab (P) vs trastuzumab + taxane (HT) for first-line treatment of HER2-positive MBC: Primary results from the MARIANNE study. *J Clin Oncol* 33, 2015 (suppl; abstr 507).
111. Blackwell KL, Burstein HJ, Storniolo AM, Rugo HS, Sledge G, Aktan G, et al. Overall survival benefit with lapatinib in combination with trastuzumab for patients with human epidermal growth factor receptor 2–positive metastatic breast cancer: Final results from the EGF104900 study. *J Clin Oncol* 2012;30:2585–92.
112. Swain S. Et al, 350O_PR Final Overall Survival (Os) Analysis From The Cleopatra Study Of First-Line (11) Pertuzumab (Ptz), Trastuzumab (T), And Docetaxel (D) In Patients (Pts) With Her2-Positive Metastatic Breast Cancer (Mbc). *Ann Oncol* (2014) 25 (suppl 4): doi: 10.1093/annonc/mdu438.7
113. Gori S, Montemurro F, Spazzapan S, Metro G, Foglietta J, Bisagni G, et al. Retreatment with trastuzumab-based therapy after disease progression following lapatinib in HER2-positive metastatic breast cancer. *Ann Oncol* 2012;23:1436–41.
114. Montemurro F, Rossi V, Nolè F, Redana S, Donadio M, Martinello R, et al. Underuse of anthracyclines in women with HER-2+ advanced breast cancer. *The Oncologist* 2010;15:665–72.
115. Tripathy D, Slamon DJ, Cobleigh M, Arnold A, Saleh M, Mortimer JE, et al. Safety of treatment of metastatic breast cancer with trastuzumab beyond disease progression. *J Clin Oncol* 2004;22:1063–70.
116. Gullo G, Zuradelli M, Sclafani F, Santoro A, Crown J. Durable complete response following chemotherapy and trastuzumab for metastatic HER2-positive breast cancer. *Ann Oncol* 2012;23:2204–5.
117. André F, O'Regan R, Ozguroglu M, et al. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol.* 2014;15:580-91.
118. Hurvitz SA, Andre F, Jiang Z, et al. Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer

- (BOLERO-1): a phase 3, randomised, double-blind, multicentre trial. *Lancet Oncol.* 2015;16:816-29.
119. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, et al. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. *Circ Cardiovasc Imaging* 2012;5:596–603.
120. Perez EA, Dueck AC, McCullough AE, Reinholz MM, Tenner KS, Davidson NE, et al. Predictability of adjuvant trastuzumab benefit in N9831 patients using the ASCO/CAP HER2-positivity criteria. *J Natl Cancer Inst* 2012;104:159–62.
121. Nagy P, Friedländer E, Tanner M, Kapanen AI, Carraway KL, Isola J, et al. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res* 2005;65:473–82.
122. Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* 2007;99:628–38.
123. Chandarlapaty S, Sakr RA, Giri D, Patil S, Heguy A, Morrow M, et al. Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. *Clin Cancer Res* 2012;18:6784–91.
124. Rexer BN, Arteaga CL. Intrinsic and acquired resistance to HER2-targeted therapies in HER2 gene-amplified breast cancer: mechanisms and clinical implications. *Crit Rev Oncol* 2012;17:1–16.
125. Gianni L, Eiermann W, Semiglazov V, Lluch A, Tjulandin S, Zambetti M, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. *Lancet Oncol.* 2014;15:640-7.

Figure Legend

Figure 1. Proposed treatment algorithm from the first to the fourth line of systemic therapy

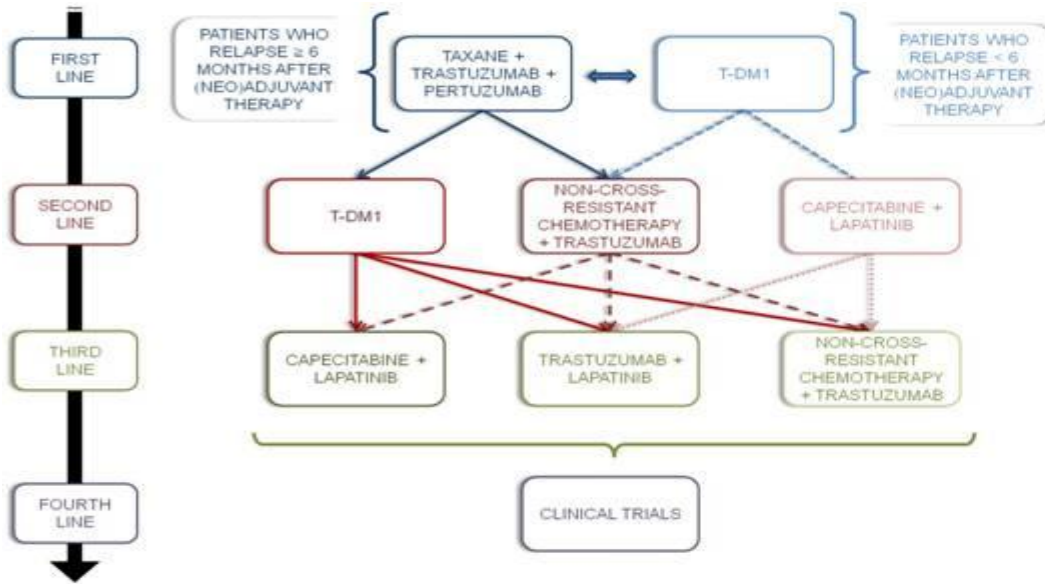


Table 1. Summary of efficacy data from main neoadjuvant clinical trials

TRIAL	N	ARMS	pCR			ADDITIONAL OUTCOMES
			N	%	p-value	
NOAH ^[22,115]	235	CT alone	23	19.5	0.001	EFS HR 0.64, p = 0.0016; OS HR 0.66, p = 0.055
		CT + H	45	38.5		
GeparQuinto ^[54]	620	ECH-TH	93	30.3	0.04	
		ECL-TL	70	22.7		
NSABP B-41 ^[55]	529	AC-PwH	93	52.5	0.095	
		AC-PwL	91	53.2		
		AC-PwHL	106	62.0		
NeoALTTO ^[56,57]	455	PwH*	44	29.5	0.34	3-year DFS HR 1.06, p = 0.81; 3-year OS HR 0.86, p = 0.65
		PwL	38	24.7		
		PwHL	78	51.3		
CHER-LOB ^[58]	121	PwH-FECH	9	25.0	0.019	
		PwL-FECL	10	26.3		
		PwHL-FECHL	21	46.7		
NeoSphere ^[59]		HD*	31	29.0	0.0141	
		PHD	49	45.8		
		PH	18	16.8		

				PD		23	24.0			
TRYPHAENA ^[60]		225		FECHP-DHP		45	61.6	NR		
				FEC-DHP		43	57.3			
				DCarbHP		51	66.2			

