The Breast 19 (2010) 128–132

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Preoperative therapy with trastuzumab and oral vinorelbine (± endocrine therapy) in patients with HER2-positive breast cancer

Monica Iorfida [a,i,*], Vincenzo Bagnardi [e,f,g], Alessandra Balduzzi [a,i], Silvia Dellapasqua [a,i], Anna Cardillo [a,i], Alberto Luini [d], Mattia Intra [d], Ida Minchella [h], Paolo Veronesi [d], Giuseppe Viale [b,c], Aron Goldhirsch [i], Marco Colleoni [a,i]

[a] Unit of Research in Medical Senology, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy
[b] Division of Pathology, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy
[c] University of Milan School of Medicine, Milan, Italy
[d] Division of Senology, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy
[e] Division of Epidemiology and Biostatistics, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy
[f] Department of Statistics, University of Milan-Bicocca, Milan, Italy
[g] Frontier Science and Technology Research Foundation, Southern Europe, Milan, Italy
[h] Medical Care Unit, European Institute of Oncology Milan, via Ripamonti 435, Milan 20141, Italy
[i] Department of Medicine, European Institute of Oncology Milan, via Ripamonti 435, Milan 20141, Italy

ARTICLE INFO

Article history:
Received 28 August 2009
Received in revised form
20 November 2009
Accepted 27 December 2009

Keywords:
Preoperative therapy
Trastuzumab
Oral vinorelbine
Breast cancer

ABSTRACT

Background: Combined trastuzumab and intravenous vinorelbine yielded high clinical activity as preoperative treatment in patients (pts) with HER 2/*neu* positive breast cancer.
Patients and methods: We tested a preoperative combination of trastuzumab with oral vinorelbine (oV) in pts with locally advanced (T2-T4 N0-3 M0) HER2-positive breast cancer. Trastuzumab was administered i.v q 3 wks and oV was administered at the dose of 55 mg/sqm on days 1 and 3 q 3 wks, for 8 courses. Pts with ER $\geq$ 10% tumors received endocrine therapy with letrozole 2.5 mg/day, plus monthly triptorelin if premenopausal.
Results: Forty-five pts entered the study. The overall response rate (CR + PR) was 76% (95% CI: 60%–87%). pCR was observed in 4 pts (10%). Among ER-positive tumors 21/25 pts obtained a clinical response (84%) and two pts obtained a pCR (8%).
Conclusions: The combination of trastuzumab and oral vinorelbine demonstrated encouraging activity in patients with HER 2 positive ER-positive tumors. Alternative strategies should be investigated in patients with endocrine non responsive disease.

Introduction

Substantial activity has been shown for the combination of trastuzumab and several cytotoxic agents in the preoperative setting with objective response rate ranging from 70 to 90% and pathological complete remissions (pCR) reported in 7–78%.[1]

Vinorebine is characterized by a favourable safety profile compared with other drugs showing in preclinical models synergism with trastuzumab. High clinical activity was also shown in the advanced disease [2] using the combination of weekly trastuzumab and intravenous vinorelbine. A small randomized study showed equivalent activity for vinorelbine and taxanes in combination with trastuzumab for HER2-positive advanced breast cancer.[3] When used as preoperative treatment the combination yelded a 20% of pCR rate.[4]

The oral and the i.v. formulations of vinorelbine had equivalent activity, with a better tolerance profile for the former. Chemical phlebitis is avoided, sparing the patient the implant of a central venous access. Oral vinorelbine has been routinely introduced in polychemotherapy regimens for advanced breast cancer with substantial clinical activity and high patient compliance.

In particular, the combination of 3-weekly trastuzumab and oral vinorelbine has provided a response rate ranging from 43% to 68% in HER2-positive advanced breast cancer.[5,6]

We reported the results of a prospective phase II study investigating the activity of the combination of 3-weekly trastuzumab and oral vinorelbine in patients with HER2-positive locally advanced breast cancer. Patients with estrogen receptor positive

* Corresponding author. Tel.: +39 02 57489502; fax: +39 02 94379205.
E-mail address: monica.iorfida@ieo.it (M. Iorfida).

0960-9776/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.breast.2009.12.011

disease (ER ≥ 10% of the cells) also received endocrine therapy with letrozole, in combination with ovarian suppression if premenopausal.[7]

Patients and methods

Patients with stage II-III (cT2-4a-d, N0-2, M0), HER2-positive, any ER and PgR, breast cancer consecutively admitted at the Department of Medicine of the European Institute of Oncology (EIO) from July 2004 to October 2006 were enrolled into this study. A core biopsy was performed for diagnosis and for the assessment of the biological variables of the tumor. Investigations (chest X-ray, abdomen ultrasound and bone scan) were performed to exclude distant metastasis and blood tests were performed to assess bone marrow, renal and hepatic function. Cardiac function was assessed at baseline by ECG and echocardiography. An LVEF >50% and no impairment of ventricular kinesis were required for enrolment in the study.

ER and PgR status, assessment of the proliferative fraction (% of Ki-67 stained cells) and over-expression of HER2 were determined both on core biopsies at the time of diagnosis, as previously described,[8] and on residual tumor after surgery. Steroid hormone receptors status was classified as negative (ER and PgR < 10% of the cells), or positive (ER and/or PgR ≥ 10% of the cells). The results were recorded as the percentage of immunoreactive cells over at least 2000 neoplastic cells. The value of Ki-67 labelling index was used as a cut-off in distinguishing tumors with low (<20%) and high (≥20%) proliferative fraction. The value of 20% was selected based on previous data from our group indicating that this threshold significantly correlated with higher response rate to preoperative chemotherapy.[9]

Only nuclear reactivity was taken into account for ER, PgR, and Ki-67 antigen.

HER2-positive status was defined either as the occurrence of intense and complete immunostaining in >10% of the neoplastic cells, or as gene amplification by dual color fluorescence in situ hybridization (FISH).

Immunostaining experiments for the localization of ER and PgR, HER2 protein and Ki-67 antigen were performed on consecutive tissue sections of the core biopsies obtained before primary treatment, as previously reported.[8] The following primary antibodies were used: the monoclonal antibody (MAb) to ER (clone 1D5 at 1/100 diluition, Dako, Glostrup, Denmark), the Mab to PgR (clone 1A6, 1/800, Dako), the MIB-1 Mab to the Ki-67 antigen (Dako, 1/1200), the polyclonal antiserum (Dako, 1/3200) to the HER2 protein.

Patients received a loading dose of trastuzumab 8 mg/kg as a 90′ iv infusion followed by a dose of 6 mg/kg every 3 weeks for 8 courses. Vinorelbine was administered orally at the dosage of 55 mg/sqm on day 2 and 4 of the 1st cycle and thereafter on day 1 and 3 for 8 courses, as previously reported.[6] Patients were instructed to swallow the tablets without chewing. Standard anti-hemetic premedication with oral granisetron was administered 30′ before vinorelbine.

Letrozole 2.5 mg/day was started concomitantly with chemo-immunotherapy in postmenopausal women with ER ≥ 10% tumors and after achievement of postmenopausal estradiol levels in premenopausal women with ER ≥ 10% tumors who received monthly triptorelin 3.75 mg as intramuscular injection. Estradiol levels were monitored periodically during treatment.

Patients were assessed at each course for clinical response, by physical examination with a caliper, and at baseline, at the 4th cycle and after the completion of the 8th cycle of trastuzumab and vinorelbine for instrumental response with breast ultrasound and mammography.

Clinical responses were evaluated according to both radiological (breast ultrasound or mammography) and clinical evaluation, by measuring the largest diameters of the tumor and were graded according to standard RECIST criteria.[10]

Pathological complete remissions (pCR) were evaluated according to Kuerer et al.[11] A pCR was defined as a total disappearance of invasive tumor both in the breast and in the axilla. The presence of intraductal carcinoma qualified for pCR.

Toxicity was recorded and classified according to the NCITC-CTG Criteria. The treatment was postponed by one week if the blood count on day 21 showed a neutrophil count <1000/mm3 and/or platelet count <100,000 mm^3. In case of febrile neutropenia, or anemia, mucositis, hand & foot syndrome, gastrointestinal, biochemical and neurological toxicity ≥ grade 2, dose reduction by 25% of the related drug was performed.

Surgery was performed approximately 28 days after the last cycle of chemoimmunotherapy to allow recovery from toxicity.

Written informed consent was obtained from all patients. The protocol was notified to the Ethical Committee.

The primary end point of this study was pCR rate. The secondary endpoints were the objective response rate, toxicity and disease free survival (DFS).

Confidence intervals for proportions were calculated using the normal approximation to the binomial distribution. The Fisher exact test was used to evaluate differences in the distribution of categorical variables.

DFS was defined as the length of time from the date of the start of treatment to any event such as relapse (including ipsilateral breast recurrence), appearance of a second primary cancer (including contralateral breast cancer) or death, whichever occurred first. For survivors, DFS was censored at the last follow-up visit. The DFS distribution was estimated using the Kaplan–Meier method.

All *p*-values were two sided. The statistical analyses were run using SAS version 8.2 (SAS Institute Inc, Cary, NC).

Results

From July 2004 to October 2006 forty-five patients were enrolled in a prospective study (mean age, 46 years; range 31–67 years). Patients' and tumors' characteristics are summarized in

Table 1
Characteristic at presentation.

	N	% col
Age, years		
Mean [Range]	46 [31–67]	
Menopausal status		
Premeopausal	35	78
Postmenopausal	10	22
Clinical T		
T2	24	53
T3	16	36
T4	5	11
Clinical Nodal status		
N0	2	4
N1	42	94
NX	1	2
Hormone receptor status		
ER-negative, PgR negative	20	44
ER-positive,[a] PgR negative	7	16
ER-positive,[a] PgR positive[a]	18	40
HER 2/neu status		
3+	45	100
Ki-67		
>=20%	45	100
Nuclear grade		
2	25	56
3	20	44

[a] ER ≥ 10% of the cells.

Table 1. All patients underwent surgery. Four patients with T4d and one patient with T4b tumor were included. Forty-three patients had clinically positive nodes assessed at ultrasound. At baseline all patients were HER2 3+ at IHC and all patients had highly proliferating (≥20%) tumors (median value 40). At surgery 2 patients with ER-positive tumors switched to a negative phenotype while PgR was downregulated in all but 2 patients. At the end of treatment HER2 turned out to be negative (at IHC and at FISH) in 2 cases.

Twelve patients discontinued treatment before completing the planned 8 cycles. Only one of these patients stopped treatment because of axillary progression after the 3rd cycle and was submitted to surgery immediately. Three patients, with stable disease after the 1st clinical evaluation who were considered inoperable by the surgeon, were switched to the combination of trastuzumab and docetaxel up to the completion of the 8 courses, one obtained a pCR and the other two obtained a partial response. These 3 patients were considered evaluable for clinical response but not for pathological response.

A complete clinical response was observed in 4 patients and a partial clinical response was observed in 30 patients with a cumulative response rate of 76% (95%CI: 60%–87%). Ten patients had stable disease and 1 patient had progressive disease.

Among 42 evaluable patients a pCR was observed in 4 patients (10%, 95% CI: 3%–23%) and 18 patients had pathological negative nodes (*p*N0) (43% 95% CI 28%–59%). One patient who obtained a pCR had stable disease at clinical evaluation. One patient had pCR in the breast and only 1 micrometastatic node, 2 patients were *p*N0 and had residual tumor < 2 mm in size in the breast and 1 patient had pathological negative nodes and multiple foci of microinvasive tumor in the breast (Table 2).

Overall conservative surgery was performed in 25 patients (56%, 95% CI 32%–62%). When patients with T4 tumor, who were candidated to radical mastectomy irrespective of clinical response and patients who switched to docetaxel were excluded, quadrantectomy was feasible in 63% of patients (95% CI 46%–78%).

Results of clinical and pathological response according to hormone receptor status are reported in Table 3. Clinical response, pCR, *p*N0 and conservative surgery rate were not different among the 2 groups, while the reduction of the proliferative fraction was significantly greater in the ER-positive group.

After surgery 16 patients (36%) received anthracycline- or taxane-based chemotherapy and 33 patients (73%) received further trastuzumab, up to the completion of 1-year treatment. 27% of the patients did not receive futher trastuzumab due to unavailability of the drug as adjuvant treatment at the time of the study.

Median follow up is 35 months (range 15–54 months). Thirteen patients had an event: 2 patients had a second primary (colorectal cancer and cervical cancer) and have not experienced disease recurrence; 3 patients had loco-regional recurrence and 8 patients had distant relapse. Four patients died. The 3-yr DFS was 69% (95%CI: 55%–83%) (Fig. 1).

Table 2
Response.

	N	% col
Pathological Complete Response		
No	38	90
Yes	4	10
Nodal Status at surgery		
N0	18	43
N1	9	21
N1 mic	4	10
N2	6	14
N3	5	12

Table 3
Response rates by hormonal status.

	pCR % [95%IC]	Partial/Complete clinical % [95%IC]	No % [95%IC]
ER-negative	2/17 12 [1–36]	13/20 65 [41–85]	7/17 41 [18–67]
ER-positive	2/25 8 [1–26]	21/25 84 [64–95]	11/25 44 [24–65]
p-value	1.00	0.18	1.00

Toxicity was mild. Toxicities grade ≥ 2 are shown in Table 4. There were no treatment-related death. Grade 3 hematologic toxicities occurred only in 2 patients. Grade 3 non-hematologic toxicities included liver dysfunction (2 cases). No hematologic toxicities were of G4 severity. Only 1 patient experienced a Grade 4 transaminitis, which completely resolved after dose reduction.

No patient showed clinical evidence of cardiac toxicity.

Discussion

The administration of preoperative systemic therapy has proven to increase the rate of breast conserving surgery and to provide the opportunity to assess the tumor biology and response.

In fact, the achievement of a pCR can be considered a surrogate marker of treatment efficacy and a predictor of outcome.[11,12]

The best available treatment strategy in early breast cancer should focus mainly on targeted therapies wherever possible, though acknowledging that supplementation with less target-specific chemotherapy is often required. However, the selection of preoperative therapy did not commonly take into account biological characteristics of the tumor in the past. Preoperative chemotherapy has been given almost universally to patients with large tumors with few exceptions of small series of elderly women for whom an endocrine preoperative therapy seemed to be the only treatment which could be proposed.[13]

Only recently a change in the algorithm was proposed with the expression of steroid hormone receptors and HER2 considered to be pivotal factors in selecting a program of neoadjuvant therapy.[8,14,15]

Despite the low pCR rate for preoperative chemotherapy given to patients with endocrine responsive tumors, only few studies attempted the effects of combined chemotherapy and endocrine therapies.

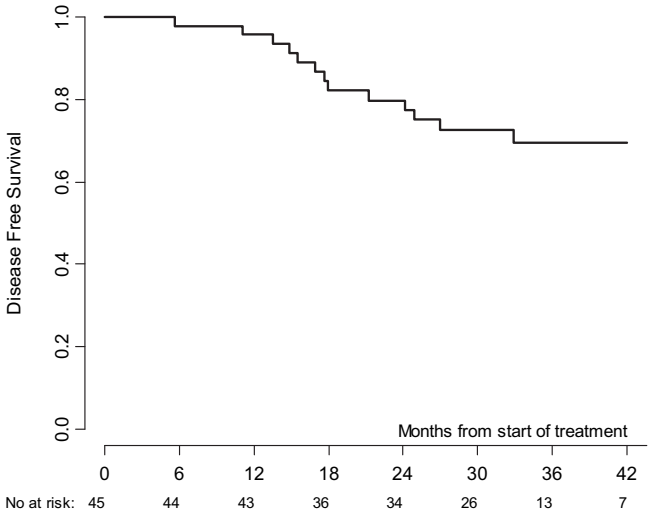

Fig. 1. Disease Free Survival.

Table 4
Toxicity.

	Grade		
	2	3	4
Leucopenia	1	1	–
Neutropenia		2	–
Anemia	1		–
Nausea	6	–	–
Vomiting	1	–	–
Diarrhea	6	–	–
Constipation	8	–	–
Mucositis	3	–	–
Peripheral neuropathy	–	1	
Liver disfunction	5	2	1
Fever	3	–	–
Gastric pain	2	–	–
Fatigue	6	–	–
Hot flushes	7	–	–

A phase II trial evaluated the role of concurrent chemotherapy and tamoxifen as compared with chemotherapy alone in the preoperative setting. pCR rate observed in the two arms was similar although treatment duration was short (8–10 weeks).[16]

In the adjuvant setting, three randomized phase III trials addressing the chemotherapy-tamoxifen question favored the sequential approach over concurrent treatment.[17]

The interaction of aromatase inhibitors with chemotherapy might be different from the known negative interactions of concomitant chemotherapy with tamoxifen.

Preclinical data support the combination of chemotherapy and aromatase inhibitors. In fact, the expression of angiogenic factors was found to be significantly reduced in MCF-7 and T47D cells after exposure to high drug concentrations of chemotherapeutic agents together with aromatase inhibitors.[18]

Our experience on chemo-endocrine therapy in the preoperative setting with aromatase inhibitors is based on three prospective studies including aromatase inhibitors alone or combined with GnRH analogue, administered concurrently with chemotherapy.[19–21]

In the present study we developed a tailored preoperative therapy based upon the expression of specific targets, namely the expression of steroid hormone receptors and the over-expression of HER2.

Despite the targeted treatment delivered in our series we obtained overall a 10% pCR rate (*p*T0pN0) which is lower as compared with other series. In particular in the population with ER-negative disease a pCR rate of only 12% was observed. Many studies investigated different combinations of preoperative trastuzumab and cytotoxic therapy, showing different pCR results ranging from 7% to 78%.[1] In particular a study with weekly trastuzumab plus intravenous vinorelbine published by Harris et al. obtained a 20% of pCR.[4] This could be due to the choice of a different chemotherapeutic agent and schedule. As conventional vinorelbine at the dose of 25 mg/m^2 is equivalent to 60 mg/m^2 of oral vinorelbine, we used an oral vinorelbine dose slightly lower than the conventional dose although for a longer period. Also the schedule used (day 1 and 3) significantly differs from the weekly continuous administration. Moreover, almost all previous trials used trastuzumab administered weekly.

Conversely, the activity (8% of pCR) observed in the population with ER-positive is encouraging if compared with the results observed of our previous experience. In fact a pCR rate of 0–3% was previously observed with chemo-endocrine therapy.[19]

The increased pCR rate observed might be related to the already observed higher responsiveness of HER2-positive tumors if compared with HER2 negative tumors.[12,22]

Also the introduction of trastuzumab has been shown to increase the response rate.[23,24] A recently published study showed that ER- and HER2-positive breast cancers benefit more from the addition of trastuzumab to chemotherapy compared to HR-negative HER2-positive tumors.[25]

In the present study, concurrent endocrine therapy was administered with trastuzumab in the ER-positive population. Previous preclinical studies demonstrated a bidirectional cross talk between the HER2 and ER signal transduction pathways and that estrogen can modulate HER2 proto-oncogene expression.[26–28] Clinical studies support the hypothesis that trastuzumab may enhance the efficacy of hormonal therapies by interfering with such pathway, thus supporting this combination.[29,30]

Regarding the relation between tumor response and Ki-67, we found a statistically significant reduction in Ki-67 labelling index $< 20\%$ at surgery in ER-positive as compared to ER-negative tumors (48% vs 6%, $p = 0.005$). It has been shown that a low Ki-67 at final surgery in ER-positive disease might correlate with an improved outcome.[31]

Ki-67 is a marker of cell proliferation and its high expression is a sign of poor prognosis.[32,33] Many cell culture model studies in ER-positive tumors have found that over-expression of HER2 and the activation of HER 2/mitogen-activated protein kinase (MAPK) pathway leads to the resistance to tamoxifen as well as aromatase inhibitors. The concomitant use of trastuzumab, blocking such pathway, may enhance the antitumor activity of letrozole and could explain the greater Ki-67 rate reduction in patients with ER-positive tumors.

As regard toxicity, the present study showed that trastuzumab and oral vinorelbine has a favourable safety profile and tolerability. In fact no grade 4 hematologic toxicity was observed.

In conclusion, the limited activity in terms of pCR and Ki-67 decrease observed in the subgroup of patients with ER-positive tumors might support the combination only in selected patients not suitable for more intensive regimen (e.g. taxanes containing regimen).

Other trastuzumab-based combinations should be explored in the population with HER2-positive endocrine non responsive disease.

Conflict of interest statement

The authors indicated no potential conflicts of interest.

Acknowledgement

We thank the patients, nurses and physicians at the European Institute of Oncology

References

1. Lazaridis G, Pentheroudakis G, Pavlidis N. Integrating trastuzumab in the neoadjuvant treatment of primary breast cancer: accumulating evidence of efficacy, synergy and safety. *Crit Rev Oncol Hematol* 2008;**66**:31–41.
2. Chan A. A review of the use of trastuzumab (Herceptin®) plus vinorelbine in metastatic breast cancer. *Ann Oncol* 2007;**18**:1152–8.
3. Burstein HJ, Keshaviah A, Baron AD, Hart RD, Lambert-Falls R, Marcom PK, et al. Trastuzumab plus vinorelbine or taxane chemotherapy for HER2-overexpressing metastatic breast cancer: the trastuzumab and vinorelbine or taxane Study. *Cancer* 2007;**110**(5):965–72.
4. Harris LN, You F, Schnitt SJ, Witkiewicz A, Lu X, Sgroi D, et al. Predictors of resistance to preoperative trastuzumab and vinorelbine for HER2-positive early breast cancer. *Clin Cancer Res* 2007;**13**:1198–207.
5. Bartsch R, Wenzel C, Altorjai G, Pluschnig U, Bachleitner-Hoffmann T, Locker GJ, et al. Results from an observational trial with oral vinorelbine and trastuzumab in advanced breast cancer. *Breast Cancer Res Treat* 2007;**102**:375–81.
6. Catania C, Medici M, Magni E, Munzone E, Cardinale D, Adamoli L, et al. Optimizing clinical care of patients with metastatic breast cancer: a new oral vinorelbine plus trastuzumab combination. *Ann Oncol* 2007;**18**:1969–75.
7. Torrisi R, Bagnardi V, Pruneri G, Ghisini R, Bottiglieri L, Magni E, et al. Antitumour and biological effects of letrozole and GnRH analogue as primary

therapy in premenopausal women with ER and PgR positive locally advanced operable breast cancer. *Br J Cancer* 2007;**97**(6):802–8.
8. Colleoni M, Viale G, Zahrieh D, Pruneri G, Gentilini O, Veronesi P, et al. Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. *Clin Cancer Res* 2004;**10**:6622–8.
9. Colleoni M, Minchella I, Mazzarol G, Nole F, Peruzzotti G, Rocca A, et al. Response to primary chemotherapy in breast cancer patients with tumors not expressing estrogen and progesteron receptors. *Ann Oncol* 2000;**11**:1057–9.
10. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;**92**:205–16.
11. Kuerer HM, Newman LA, Smith TM, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 1999;**17**:460–9.
12. Guarneri V, Broglio K, Kau SW, Cristofanilli M, Buzdar AU, Valero V, et al. Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. *J Clin Oncol* 2006;**24**:1037–44.
13. Kaufmann M, von Minckwitz G, Smith R, Valero V, Gianni L, Eiermann W, et al. International expert panel on the use of primary (preoperative) systemic treatment of operable breast cancer: review and recommendations. *J Clin Oncol* 2003;**21**:2600–8.
14. Colleoni M, Viale G, Zahrieh D, Bottiglieri L, Gelber RD, Veronesi P, et al. Expression of ER, PgR, HER1, HER2, and response: a study of preoperative chemotherapy. *Ann Oncol* 2008;**19**:465–72.
15. Kaufmann M, von Minckwitz G, Bear HD, Buzdar A, McGale P, Bonnefoi H, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. *Ann Oncol* 2007;**18**:1927–34.
16. von Minckwitz G, Costa SD, Raab G, Blohmer JU, Eidtmann H, Hilfrich J, et al. Dose-dense doxorubicin, docetaxel, and granulocyte colony-stimulating factor support with or without tamoxifen as preoperative therapy in patients with operable carcinoma of the breast: a randomized, controlled, open phase IIb study. *J Clin Oncol* 2001;**19**:3506–15.
17. Albain KS. Adjiuvant chemo-endocrine therapy for breast cancer: combined or sequential? *The Breast* 2003;**12**:12.
18. Fersis N, Smyczek-Gargya B, Armeanu S, Gagulic E, Pantic L, Relakis K, et al. Changes in vascular endothelial growth factor (VEGF) after chemoendocrine therapy in breast cancer. *Eur J Gynecol Oncol* 2004;**25**:45–50.
19. Torrisi R, Dellapasqua S, Ghisini R, Viale G, Veronesi P, Luini A, et al. Preoperative concurrent chemo- and endocrine therapies for women with large operable breast cancer expressing steroid hormone receptors. *The Breast* 2008;**17**:654–60.
20. Torrisi R, Bagnardi V, Cardillo A, Bertolini F, Scarano E, Orlando L, et al. Preoperative bevacizumab combined with letrozole and chemotherapy in locally advanced ER- and/or PgR-positive breast cancer: clinical and biological activity. *Br J Cancer* 2008;**99**:1564–71.
21. D'Alessandro C, Dellapasqua S, Orlando L, Santoro L, Maisonneuve P, Torrisi R, et al. Role of endocrine responsiveness and HER2/neu overexpression in inflammatory breast cancer treated with multimodality preoperative therapy. *Breast J* 2008;**14**:435–41.
22. Andre F, Chafika M, Cornelia L, Kau SW, Frye D, Green M, et al. HER2 expression and efficacy of preoperative paclitaxel/FAC chemotherapy in breast cancer. *Breast Cancer Res Treat* 2008;**108**:183–90.
23. 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.
24. Gianni L, Eiermann W, Semiglazov V, Manikhas G, Lluch A, Tjulandin S, et al. Neoadjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer: primary efficacy analysis of the NOAH trial. *Cancer Res* 2009;**69**(Suppl. 2). Abstr 31.
25. Peitinger F, Buzdar AU, Kuerer HM, Mejia JA, Hatzis C, Gonzalez-Angulo AM, et al. Hormone receptor and pathologic response of HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab. *Ann Oncol* 2008;**19**:2020–5.
26. Shou J, Massarwesh S, Osbourne CK, Wakeling AE, Ali S, Weiss H, et al. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2 cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst* 2004;**96**:926–35.
27. Lipton A, Ali SM, Leitzel K, Demers L, Chinchilli V, Engle L, et al. Elevated serum HER-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. *J Clin Oncol* 2002;**20**:1467–72.
28. Read LD, Keith D, Slamon DJ, Katzenellenbogen BS. Hormonal modulation of HER-2/neu protooncogene messenger ribonucleic acid and p1875 protein expression in human breast cancer cell lines. *Cancer Res* 1990;**50**:3947–51.
29. Marcom PK, Isaacs C, Harris L, Wong ZW, Kommarreddy A, Novielli N, et al. The combination of letrozole and trastuzumab as first or second-line biological therapy produces durable responses in a subset of HER2 positive and ER positive advanced breast cancers. *Breast Cancer Res Treat* 2007;**102**:43–9.
30. Mackey JR, Kaufman B, Clemens M, Bapsy PP, Vaid A, Wardley A, et al. Trastuzumab prolongs progression free survival in hormone-dependent and HER-2 positive metastatic breast cancer. *Breast Cancer Res Treat* 2006;**100**(Suppl. 1):S5 (Abstr 3).
31. Ellis MJ, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, et al. Outcome prediction for estrogen receptor-positive breast cancer based on post-neoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008;**100**(19):1380–8.
32. Locker AP, Birrelli K, Bell JA, Nicholson RI, Elston CW, Blamey RW, et al. Ki67 immunoreactivity in breast carcinoma: relationships to prognostic variables and short term survival. *Eur J Surg Oncol* 1992;**18**:224–9.
33. Urriticoechea A, Smith IE, Dowsett M. Proliferative marker Ki-67 in early breast cancer. *J Clin Oncol* 2005;**23**:7212–20.