Quantitative Expression of Estrogen Receptor on Relapse Biopsy for ER-positive Breast Cancer: Prognostic Impact

MARIA VITTORIA DIECI[1,2], FEDERICO PIACENTINI[3], MASSIMO DOMINICI[3], CLAUDIA OMARINI[3], AICHA GOUBAR[4], GUIDO FICARRA[5], PIERFRANCO CONTE[1,2] and VALENTINA GUARNERI[1,2]

[1]*Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy;*
[2]*Division of Medical Oncology 2, Venetian Oncology Institution IRCCS, Padova, Italy;*
[3]*Department of Medical and Surgical Sciences of Mother, Child and Adult, Modena, Italy;*
[4]*INSERM U981, Gustave Roussy Institute, Villejuif, France;*
[5]*Department of Pathology, University Hospital, Modena, Italy*

Abstract. *Background: The aim of this study was to evaluate the prognostic impact of quantitative estrogen receptor (ER) expression at relapse for ER-positive breast cancer with ER-positive recurrence. Patients and Methods: A total of 81 patients with ER-positive primary breast cancer and ER-positive paired recurrence were included. ER expression was evaluated as the percentage of tumor cells staining for ER under immunohistochemistry. Samples were defined as ER-high (ER >50%) or ER-low (ER ≥10% and ≤50%). Results: Quantitative ER expression on relapse biopsy was an independent prognostic factor for overall survival in multivariate analysis, both as a continuous (hazard ratio=0.8; 95% confidence interval=0.7-0.92, p=0.001) and as a categorical (ER-high vs. ER-low; hazard ratio=0.26; 95% confidence interval=0.11-0.59, p=0.001) variable. Patients whose status changed from ER-high (primary BC) to ER-low (relapse) had the poorest outcome, with a 10-year overall survival rate of 14%. Conclusion: Even in the case of maintenance of ER-positivity on primary and relapse of breast cancer, recurrence biopsy provides prognostic information.*

Breast cancer (BC) is a heterogeneous disease. Routinely performed pathological assessments allow the identification of at least three different BC subtypes: the estrogen receptor (ER)-positive, the human epidermal growth factor receptor-2 (HER2)-positive and the triple-negative group. Molecular classification based on gene expression profiling has confirmed the devision of BC into at least four disease subtypes (luminal A, luminal B, HER2-enriched and basal-like) somewhat, although not perfectly, overlapping the pathology classification (1). Up to 10 different BC molecular subtypes with different prognoses can be identified by next-generation sequencing techniques (2). Intra-tumor heterogeneity is also frequently observed and studies evaluating differences in molecular features among cells from the same tumor are increasing (3). Moreover, molecular characteristics of tumor cells may evolve during cancer progression. A recent meta-analysis of published data concluded that changes in ER and HER2 expression from primary to relapse occur at rates of 20% and 8%, respectively (4). Whether this phenomenon is due to true biological changes, to a Darwinian selection of more aggressive clones, or is merely a consequence of technical errors is not fully understood. However, these discrepancies may induce a change in treatment decision in up to 14% of cases (5). Our group and others have previously described the prognostic impact of phenotype changes from primary tumor to recurrence (6, 7). Nowadays, tissue confirmation of recurrent BC is suggested by international guidelines whenever possible (8, 9).

We have previously reported that patients with ER-positive BC who become ER-negative at relapse have a poorer overall survival (OS) compared to those still ER-positive at relapse (7). However, ER-positive tumors are heterogeneous and they do not all behave in the same way. Our aim was to evaluate whether, among the group of good-prognosis patients with an ER-positive status on both primary and recurrence, the level of ER expression at relapse and the change in ER level from primary tumor to relapse may be of prognostic value.

Patients and Methods

Case selection. As described elsewhere (7), we constructed a mono-institutional database including all the consecutive cases of patients who underwent biopsy or surgical resection of suspected recurrent BC between January 1994 and December 2011 (n=139) and who

Correspondence to: Maria Vittoria Dieci, Division of Medical Oncology 2, Istituto Oncologico Veneto IRCCS, via Gattamelata 64, 35128, Padova, Italy. Tel: +39 0498215295, Fax: +39 0498215932, e-mail: mariavittoria.dieci@unipd.it

Key Words: Metastatic breast cancer, estrogen receptor, receptor discordance.

were treated at the Medical Oncology Division of the Modena University Hospital, Italy. Patient characteristics, including stage at diagnosis, adjuvant therapies, site of relapse, site of biopsy and treatments for advanced disease, were recorded. Information about those patients who underwent recurrence biopsy between 1994 and December 2007 were retrospectively retrieved. Since 2007, a dedicated database has been prospectively maintained. For the purpose of the present analysis, only patients with ER-positive (cut-off: ≥10% of tumor cells staining for ER) primary BC and ER-positive paired recurrence were considered (n=81).

Pathology. All the pathological assessments were performed at the Pathology Division of our Institution by a dedicated breast pathologist (GF) (7). The same assays and standardized methodology were applied to each sample (7) .The methods applied to evaluate ER and HER2 status have been previously described (7). The cut-off for ER-positivity was IHC staining in ≥10% of tumor cells. For each sample, the percentage of tumor cells staining for ER was recorded.

Statistics. All data are presented descriptively as medians, means or proportions. Continuous variables were compared by using Wilcoxon signed-rank test. For survival analyses, ER expression levels were considered both as continuous variables (per 10% increase) and as categorical variables, by adopting a cut-off of 50% of tumors cells staining for ER to distinguish between ER-high (IHC staining in >50% of the cells) or ER-low samples (IHC staining in ≥10% and ≤50% of the cells). This cut-off was predefined previously to any statistical analysis. OS was calculated from the time of primary BC diagnosis to the date of death or last follow-up. The Kaplan–Meier method was used to estimate survival curves and the log-rank test was used to test for differences between groups. Hazard ratios (HRs) and their confidence intervals (CIs) were calculated by using the Cox regression model. Results were considered statistically significant if the *p*-value was less than 0.05. Data were analysed using R software (R 3.0.2 version, ref. http://www.r-project.org/).

Results

Patients' characteristics. Eighty-one consecutive patients who had both ER-positive primary and ER-positive relapsed BC were identified and included in the analysis. Patients' characteristics are reported in Table I. Eighty percent of patients received endocrine treatment as part of their adjuvant therapy. Seventy-four (91%) patients received hormone therapy for relapsed disease at some time point. Overall, 86% of the patients received hormone treatment before relapse biopsy either as adjuvant therapy or as treatment for advanced disease or both.

ER expression of primary and relapsed tumors. The mean ER expression level was 77% and 81% for primary and relapsed tumors, respectively (range 20% to 100%, both groups). No significant difference in ER expression between primaries and recurrences was observed (p=0.19). The quantitative absolute changes in ER expression for each patient are represented in Figure 1.

Table I. *Patients' characteristics*.

	N (%)
Patients with ER-positive primary and ER-positive recurrent BC	81 (100)
Median age at diagnosis: years (range)	52 (26-87)
Stage at diagnosis	
I	16 (20)
II	30 (37)
III	29 (36)
IV	6 (7)
Histologic type	
Ductal	61 (75)
Lobular	13 (16)
Other	7 (9)
Histologic grade	
1/2	32 (39)
3	37 (46)
NA	12 (15)
HER2-status at diagnosis	
HER2-positive	12 (15)
HER2-negative	69 (85)
HER2-status at relapse	
HER2-positive	17 (21)
HER2-negative	64 (79)
Site of relapse biopsy	
Distant	61 (75)
Locoregional	20 (25)
Adjuvant hormone therapy	
Yes	65 (80)
No	11 (14)
NA	5 (6)
Hormone therapy for advanced disease	
Yes	74 (91)
No	7 (9)
Hormone therapy before relapse biopsy (either adjuvant or for advanced disease)	
Yes	70 (86)
No	11 (14)
Neo/Adjuvant chemotherapy	
Yes	55 (68)
No	22 (27)
NA	4 (5)
Chemotherapy for advanced disease	
Yes	68 (84)
No	11 (14)
NA	2 (2)

n, Number; ER, estrogen receptors; BC, breast cancer; NA, not available.

As shown in Table II, each sample was classified as ER-high or ER-low according to the percentage of tumor cells staining for ER (cut-off: >50%). Table II also summarizes the rates of concordance/discordance in ER levels from samples of primary and recurrent BC.

Prognostic value of ER expression on relapse biopsy. Quantitative ER expression on the relapse biopsy as continuous variable significantly correlated with OS. For each 10%

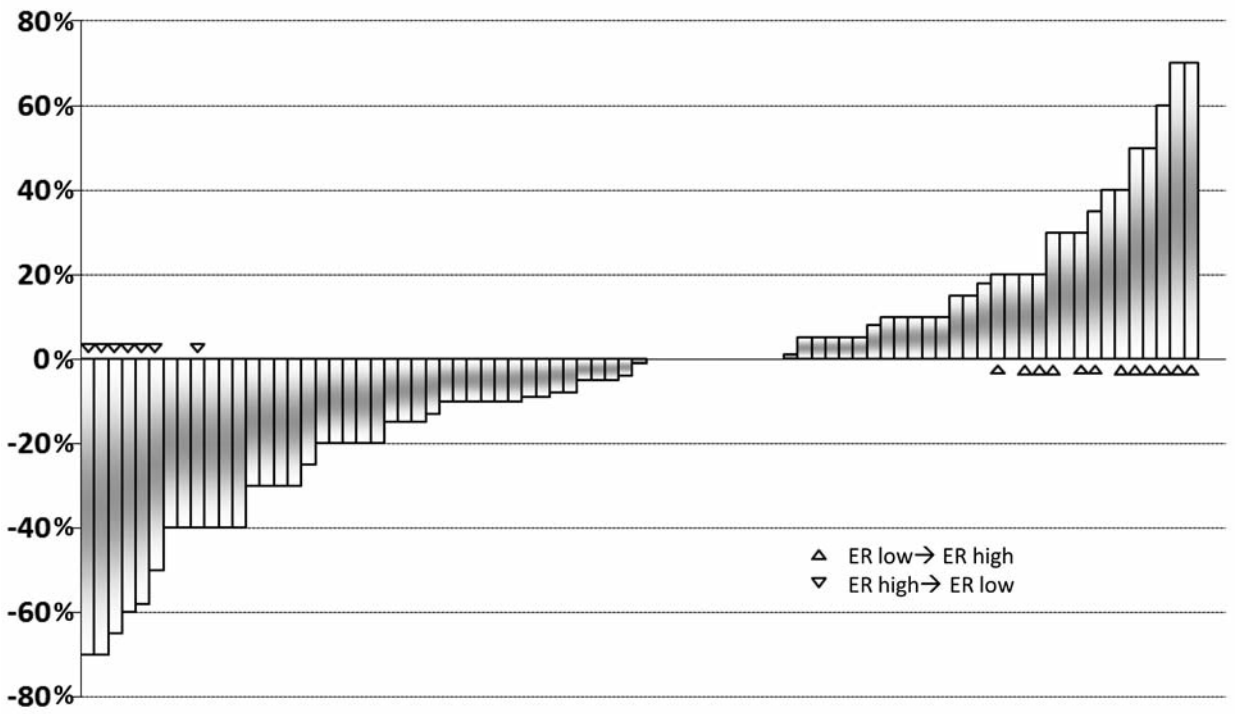


Figure 1. *Waterfall plot showing the absolute percentage change in ER (estrogen receptor) expression from primary tumor to relapse biopsy. Triangles indicate those cases that changed from ER-low (≤50% of stained tumor cells) to ER-high (>50% of stained tumor cells) or vice versa.*

increment in the ER expression on the relapse sample, a 21% reduction in the risk of death was observed (unadjusted HR=0.79, 95% CI=0.69-0.91, $p<0.001$). When applying the predefined cut-off of 50% to discriminate between ER-high and ER-low samples, patients with an ER-high relapse had a significantly better OS than patients with an ER-low relapse (unadjusted HR=0.29, 95% CI=0.13-0.62, $p<0.001$). ER expression of the primary tumor did not significantly correlate with prognosis, neither as a continuous nor categorical variable (Table III). ER expression of the relapse sample maintained its prognostic value in multivariate analysis (HR=0.80; 95% CI=0.7-0.92, $p=0.001$ for continuous variable; HR=0.26, 95% CI=0.11-0.59, $p=0.001$ for ER-high *vs*. ER-low), as shown in Table III. The other strong independent prognostic factor was tumor stage at diagnosis ($p\leq0.01$).

Prognostic impact of ER expression level changes. No difference in OS was observed between those patients who maintained the same ER level (ER-concordant group) during disease progression compared to those patients whose ER levels changed (ER-discordant), as shown in Figure 2A. Ten-year OS rates were 51% for the ER-concordant group compared to 35% for the ER-discordant group (HR=1.39, 95%CI=0.74-2.59, $p=0.30$). However, those who changed from ER-high to ER-low status had a particularly poor prognosis (Figure 2B). Ten-year OS rates were 51% for the ER-concordant group, 50% for patients changing from ER-low to ER-high and only 14% for patients changing from ER-high to ER-low (ER-concordant *vs*. ER-high to ER-low: HR=2.94, 95% CI=1.29-6.66, $p=0.01$).

Table II. *Changes in estrogen receptor (ER) level from primary to relapse according to the dichotomization into ER-high and ER-low samples based on the cutoff of 50% of ER-positive tumor cells by immunohistochemistry.*

	N/total (%)
Primary ER-high	66/81 (81)
Primary ER-low	15/81 (19)
Relapse ER-high	71/81 (88)
Relapse ER-low	10/81 (12)
ER-level concordant:	62/81 (77)
Primary ER-high→ Relapse ER-high	59/62 (95)
Primary ER-low→ Relapse ER-low	3/62 (5)
ER level discordant:	19/81 (23)
Primary ER-high→ Relapse ER-low	7/19 (37)
Primary ER-low→ Relapse ER-high	12/19 (63)

N, Number.

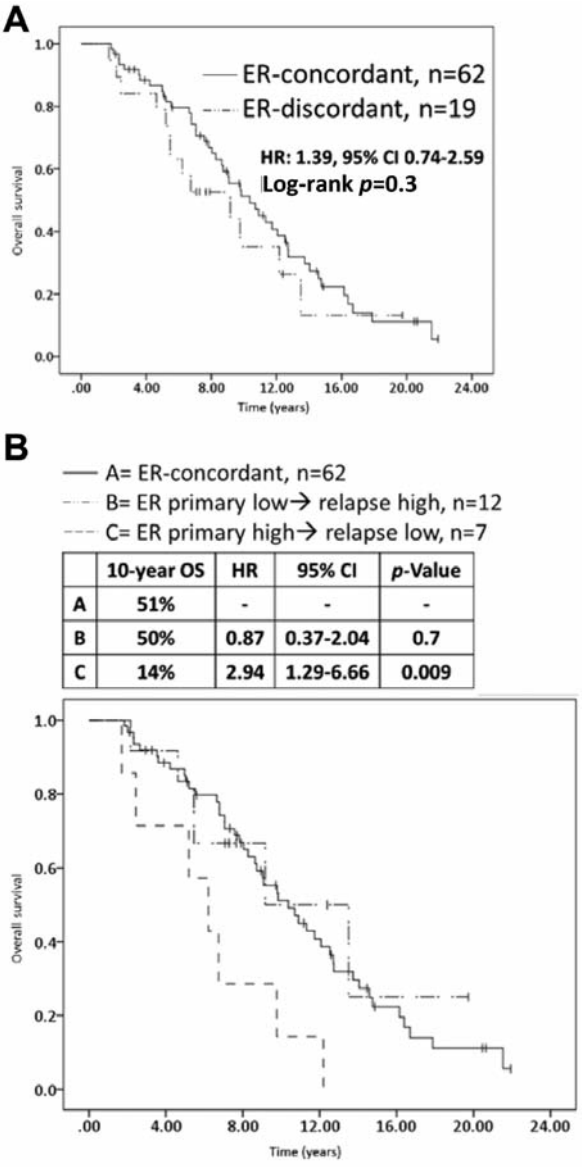
Discussion

In our work, ER expression levels of the relapse biopsy (continuous and categorical variable) and not of the primary tumor was a strong independent prognostic factor for patients with ER-positive primary BC and ER-positive paired recurrence. The fact that for patients with ER-positive BC, ER expression levels may have a prognostic/predictive impact in the early and advanced setting has been established (10-13).

In a recently published work, 698 patients with ER-positive primary BC were evaluated. The percentage of tumor cell nuclei positively stained for ER was significantly and independently correlated to BC-specific mortality risk (p_{trend}=0.006) as a continuous variable (12). Dowsett *et al*. evaluated quantitative expression of ER as prognostic/predictive value in 950 patients with ER-positive BC enrolled into the monotherapy arms (adjuvant anastrozole or tamoxifen) of the ATAC trial. ER expression was calculated as the H-score, taking into considerations both the percentage of stained cells and the staining intensity. Low ER H-scores were associated with a shorter time to relapse (p=0.78 and p=0.0009 for tamoxifen and anastrozole-treated patients, respectively) (11).

Evidence has also been produced as regards the metastatic setting. As an example, the percentage of ER-positive tumor cell nuclei was associated with the cumulative probability of survival in a cohort of 205 patients with ER-positive metastatic BC treated with tamoxifen within the SWOG protocol 8228 (10). More recently, in the context of the phase III EGF30008 trial comparing lapatinib alone *vs*. lapatinib plus letrozole for advanced BC, Finn *et al*. investigated whether ER expression levels predicted response to the combination treatment in a total of 821 patients with ER-positive/HER2-negative BC. Only those patients presenting a low ER expression level (first H-score quartile) derived benefit from the addition of lapatinib to letrozole (13).

However, all the studies conducted in the advanced setting evaluated ER expression mainly of the primary tumor. Nowadays, in the era of the rapidly increasing knowledge in cancer biology, this approach may be considered as inadequate. Indeed, many contributions support the concept of BC heterogeneity and the fact that molecular features of BC may change during tumor progression (5-7). How these changes affect patient outcome and management is starting to be evaluated and represents a new field for cancer research. This is the main reason why we decided to evaluate to what extent ER expression level of the relapse biopsy may influence the prognosis of patients with ER-positive primary BC and ER-positive relapse. To the best of our knowledge, this is the first time that the degree of ER expression on the metastasis sample is described as a strong independent prognostic factor.



	10-year OS	HR	95% CI	*p*-Value
A	51%	-	-	-
B	50%	0.87	0.37-2.04	0.7
C	14%	2.94	1.29-6.66	0.009

Figure 2. *A: Kaplan–Meier overall survival curves for patients with the same estrogen receptor (ER) expression level for both primary and recurrent tumor samples (ER-concordant, i.e. primary and relapse both ER-high or both ER-low) vs. patients with a change in ER expression level from primary to relapse (ER-discordant, i.e. primary ER-high and relapse ER-low or vice versa). B: Kaplan–Meier overall survival curves for ER-concordant patients, patients whose status changed from ER-low to ER-high, and those whose changed from ER-high to ER-low. HR: Hazard ratio; CI: Confidence interval.*

Moreover, patients with the worst prognosis were those whose ER-level changed from high (>50% of positive tumor cells) for the primary tumor to low (≤50% of positive tumor cells) at relapse compared to patients who changed in the opposite direction and patients who maintained the same ER level for both samples.

Table III. *Univariate and multivariate Cox regression analysis of overall survival.*

	Univariate			Multivariate 1*			Multivariate 2‡		
Variable	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
ER of relapse: (per 10% increase)	0.79	0.69-0.9	<0.001	0.8	0.7-0.92	0.001			
ER of primary: (per 10% increase)	1	0.89-1.12	1						
ER of relapse									
ER-low	1						1		
ER-high	0.3	0.15-0.61	<0.001				0.26	0.11-0.59	0.001
ER of primary									
ER-low	1								
ER-high	0.92	0.45-1.89	0.8						
Grade of primary									
1/2	1			1			1		
3	2.07	1.13-3.76	0.02	1.73	0.94-3.19	0.08	1.59	0.85-2.98	0.14
Age at diagnosis									
≤50 years	1								
>50 years	1.33	0.76-2.31	0.3						
Stage at diagnosis									
I/II	1			1			1		
III/IV	2.07	1.22-3.52	0.007	2.15	1.19-3.88	0.01	2.42	1.32-4.44	0.004
HER2 of primary									
Negative	1								
Positive	0.95	0.37-2.42	0.9						
HER2 of relapse									
Negative	1								
Positive	0.82	0.38-1.75	0.6						
PgR of relapse									
Negative (<10%)	1								
Positive (≥10%)	0.84	0.5-1.42	0.5						
PgR of primary									
Negative (<10%)	1								
Positive (≥10%)	0.65	0.35-1.21	0.2						
Distant relapse									
No	1								
Yes	2.88	0.89-9.35	0.08						

*Including ER levels at relapse as continuous variable (per 10% increase), grade of primary tumor and stage at breast cancer diagnosis. ‡Including ER expression at relapse as categorical variable (ER-high vs. ER-low based on the 50% of ER-positive tumor cell cut-off), grade of primary tumor and stage at breast cancer diagnosis. HR, Hazard ratio; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.

One possible criticism of our study may be that technical errors might have accounted for discrepancies in ER level between the primary and relapsed tumors. However, this risk is reduced by the rigid methodology that was applied in our work. Indeed, all specimens from both primaries and recurrences were evaluated at the same laboratory, by the same pathologist and adopting the same procedures and techniques. Nevertheless, some potential bias deserves to be mentioned. This is a retrospective study with the potential bias of enriching for cases with unusual clinical course. Moreover, relapse biopsy was not taken at the same time point for all the patients during their disease history. However, the study population was mostly represented by patients with ER-positive advanced BC whose disease progressed after a previous antiestrogen treatment (either as adjuvant treatment or as therapy for advanced disease) before relapse biopsy was performed.

In conclusion, besides BC relapse confirmation and receptor status evaluation, we provide evidence to support another rationale for performing histological evaluation of recurrent BC. We demonstrate that even with the same positive ER status on matched primary and relapsed tumor samples, the biopsy of recurrence provides relevant additional prognostic information. Although it is unclear whether a change from ER-high to ER-low at relapse reflects a real switch to a more aggressive phenotype or is the consequence of prior treatments, the particularly poor outcome of this group of patients suggests that they might deserve more aggressive treatments.

Our results strengthen the concept of BC heterogeneity, widening the spectrum of prognostic phenotype changes that may be observed during clinical progression. In the future, the application of next-generation sequencing techniques to paired BC primaries and recurrences might provide further evidence to this concept (14) and, hopefully, will allow prognostic and predictive parameters to be defined at the genomic level that might help in treatment decision.

Conflicts of Interest

Authors declare they have no conflicts of interest in regard to this study.

Acknowledgements

Supported by the Monica Boscolo Research Grant 2012 and a Ministry of Health Research Grant (RF-2009-1472600). No funding source was involved in the study design, collection, analysis and interpretation of data, in the writing of the manuscript, nor in the decision to submit this article for publication.

References

1 Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE and Børresen-Dale AL: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA *98*: 10869-10874, 2001.

2 Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Gräf S, Ha G, Haffari G, Bashashati A, Russell R, McKinney S; METABRIC Group, Langerød A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowetz F, Murphy L, Ellis I, Purushotham A, Børresen-Dale AL, Brenton JD, Tavaré S, Caldas C and Aparicio S: The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature *486*: 346-352, 2012.

3 Ng CK, Pemberton HN and Reis-Filho JS: Breast cancer intratumor genetic heterogeneity: causes and implications. Expert Rev Anticancer Ther 12: 1021-1032, 2012.

4 Aurilio G, Disalvatore D, Pruneri G, Bagnardi V, Viale G, Curigliano G, Goldhirsch A, Munzone E, De Vita F and Nolè F: A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. Eur J Cancer 50: 277-289, 2014.

5 Amir E, Miller N, Geddie W, Freedman O, Kassam F, Simmons C, Oldfield M, Dranitsaris G, Tomlinson G, Laupacis A, Tannock IF and Clemons M: Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer. J Clin Oncol *30*: 587-592, 2012.

6 Niikura N, Liu J, Hayashi N, Mittendorf EA, Gong Y, Palla SL, Tokuda Y, Gonzalez-Angulo AM, Hortobagyi GN and Ueno NT: Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. J Clin Oncol *30*: 593-599, 2012.

7 Dieci MV, Barbieri E, Piacentini F, Ficarra G, Bettelli S, Dominici M, Conte PF and Guarneri V: Discordance in receptor status between primary and recurrent breast cancer has a prognostic impact: a single-institution analysis. Ann Oncol *24*: 101-108, 2013.

8 Cardoso F, Harbeck N, Fallowfield L, Kyriakides S and Senkus E; ESMO Guidelines Working Group: Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol *23(Suppl 7)*: vii11-9, 2012.

9 NCCN Clinical Practice Guidelines in Oncology. Breast Cancer. Version 3.2014. http://www.nccn.org/professionals/physician_gls/pdf/breast. Last accessed april, 2014.

10 Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, Ravdin P, Martino S and Osborne CK: Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: A Southwest Oncology Group Study. Int J Cancer *89*: 111-117, 2000.

11 Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, Cuzick J, Houghton J, Williams N, Mallon E, Bishop H, Ellis I, Larsimont D, Sasano H, Carder P, Cussac AL, Knox F, Speirs V, Forbes J and Buzdar A: Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination Trial. J Clin Oncol *26*: 1059-1065, 2008.

12 Ma H, Lu Y, Marchbanks PA, Folger SG, Strom BL, McDonald JA, Simon MS, Weiss LK, Malone KE, Burkman RT, Sullivan-Halley J, Deapen DM, Press MF and Bernstein L: Quantitative measures of estrogen receptor expression in relation to breast cancer-specific mortality risk among white women and black women. Breast Cancer Res *15*: R90, 2013.

13 Finn RS, Press MF, Dering J, O'Rourke L, Florance A, Ellis C, Martin AM and Johnston S: Quantitative ER and PgR Assessment as Predictors of Benefit from Lapatinib in Postmenopausal Women with Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer. Clin Cancer Res *20*: 736-743, 2014.

14 Bidard F, Ng CK, Piscuoglio S, Pierga JY, Cottu P, Norton L, Weigelt B, Sigal B and Reis-Filho JS: High-depth massively parallel sequencing reveals heterogeneity between primary tumor and metastatic deposits in *de novo* metastatic breast cancer patients prior to exposure to systemic therapy. Presented at San Antonio Breast Cancer Symposium, December 10-14 2013, San Antonio, Texas. Abstract: S6-06.

Received April 1, 2014
Revised May 27, 2014
Accepted May 28, 2014