

Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases?

C. Simmons^{1,2}, N. Miller³, W. Geddie³, D. Gianfelice⁴, M. Oldfield^{1,2}, G. Dranitsaris¹ & M. J. Clemons^{1,2*}

¹Division of Hematology and Medical Oncology, Princess Margaret Hospital; ²Department of Medicine; ³Division of Pathology, University Health Network and ⁴Department of Interventional Radiology, University Health Network, University of Toronto, Canada

Received 9 January 2009; accepted 15 January 2009

Background: Decisions about systemic treatment of women with metastatic breast cancer are often based on estrogen receptor (ER), progesterone receptor (PgR), and Her2 status of the primary tumor. This study prospectively investigated concordance in receptor status between primary tumor and distant metastases and assessed the impact of any discordance on patient management.

Materials and methods: Biopsies of suspected metastatic lesions were obtained from patients and analyzed for ER/PgR and Her2. Receptor status was compared for metastases and primary tumors. Questionnaires were completed by the oncologist before and after biopsy to determine whether the biopsy results changed the treatment plan.

Results: Forty women were enrolled; 35 of them underwent biopsy, yielding 29 samples sufficient for analysis; 3/29 biopsies (10%) showed benign disease. Changes in hormone receptor status were observed in 40% ($P = 0.003$) and in Her2 status in 8% of women. Biopsy results led to a change of management in 20% of patients ($P = 0.002$).

Conclusions: This prospective study demonstrates the presence of substantial discordance in receptor status between primary tumor and metastases, which led to altered management in 20% of cases. Tissue confirmation should be considered in patients with clinical or radiological suspicion of metastatic recurrence.

Key words: breast cancer, metastatic, receptor discordance

introduction

The diagnosis of metastatic breast cancer is usually made by a combination of clinical signs and symptoms and by radiological evaluation. Confirmatory biopsy of suspected metastatic lesions is rarely carried out at most centers, even if many years have passed since diagnosis of primary breast cancer. Systemic management of metastatic breast cancer with endocrine therapy, chemotherapy, or biologic agents (such as trastuzumab or lapatinib) is therefore based usually on tumor characteristics from the patient's original breast surgery. However, certain characteristics such as estrogen receptor (ER), progesterone receptor (PgR), and Her2 status may change with recurrent disease. Retrospective reviews have shown discordance between ER and PgR status between the primary tumor and metastases in 15%–40% of women [1–18] and 7%–26% for Her2 status [19–21]. Therefore, it is possible that many patients with metastatic disease might be offered suboptimal therapy.

Previous studies of discordance in hormone receptor and Her2 status between primary tumor and metastases have limitations. Most were retrospective [8, 17], used older pathological techniques [1–6], utilized different staining procedures between primary tumor and metastases [22], or included heterogeneous groups of patients including those with local recurrences [14, 17, 23, 24]. In addition, retrospective reviews have inherent selection biases such that patients may have been selected for biopsy based on suspicion of other pathological abnormality, atypical presentation of metastases, prolonged time from primary disease, or the perceived ease and acceptability of performing a biopsy [25]. Also, the impact of the biopsy results on clinical management cannot be captured from retrospective reviews. Finally, prior studies have not been able to provide information about the feasibility of a biopsy procedure or its acceptability to patients.

The primary objective of the present study was to prospectively evaluate possible changes that occur in ER, PgR, and Her2 status between primary tumor and distant metastases before initiation of therapy. Secondary objectives were to determine whether any discordance between primary disease and metastases altered patient management and to demonstrate

*Correspondence to: Dr M. J. Clemons, Division of Hematology and Medical Oncology, Princess Margaret Hospital, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada. Tel: +1-416-946-4534; Fax: +1-416-946-2983; E-mail: mark.clemons@uhn.on.ca

feasibility and acceptability of performing biopsies in this patient population.

materials and methods

study design

This prospective cohort study took place in a single institution with a large breast medical oncology practice and accrued patients over a 1-year period from September 2006 to October 2007. Patients were identified at the time of suspected clinical or radiological recurrence by their primary oncologist. Patients were excluded if they had operable breast or axillary recurrence with no evidence of metastatic disease or if they had already started on therapy for metastatic disease. Patients were also excluded if the location of the lesion was not amenable to biopsy by the following criteria: rib lesion, brain metastases, lesion <1 cm in size, or lesion in a location that could not be reached by core biopsy techniques available with interventional radiology. Further exclusion criteria included an international normalized ratio or partial thromboplastin time above the upper limit of normal for our institution.

Patients who met all eligibility criteria were provided with written information about the study. The proportion of patients approached who provided written informed consent for this study was collected as a surrogate marker of acceptability of a biopsy procedure. Reasons for declining to participate were documented. The proportion of patients who consented to biopsy but for whom a biopsy was subsequently not feasible was recorded as a surrogate marker of feasibility of obtaining biopsies. The study was approved by the research ethics board at the University Health Network, Toronto, Canada.

Consenting patients underwent core biopsy by an interventional radiologist, fine needle aspirate by a diagnostic pathologist, or drainage of pleural fluid by ultrasound guidance in a dedicated procedure clinic. An interventional radiologist reviewed X-rays or scans of suspicious lesions and appropriateness for biopsy was determined by anatomic location of the lesion. In patients with multiple lesions, the lesion in the safest and most practical location was chosen for biopsy. Biopsy samples were fixed in formalin and paraffin embedded before analysis. Core biopsy specimens from bone metastases were not decalcified, in order to ensure that interpretation of ER and PgR was not compromised. Samples were analyzed pathologically to confirm metastatic disease and secondly to evaluate ER and PgR by immunohistochemistry (IHC; Ventana 6F11 and Ventana clone 16, respectively) and Her2 status by FISH.

The assessment of ER, PgR, and Her2 for the metastatic tissue was then compared with that for the primary tumor. The pathologist analyzing the samples was blinded as to the patients' original hormone receptor and Her2 status. Further, primary samples were reassessed to confirm the original hormone receptor status and Her2 status. There were no significant changes on reassessment of the primary tumor. For samples that previously had not reported percentage of cells staining for ER and PgR, this was recorded at the time of reassessment. The threshold values for reporting positivity were 10% for ER and PgR as per local institutional guidelines. As mentioned, the percentage of cells expressing ER and PgR was reported for both the primary tumor and the metastasis. For Her2 evaluation, FISH was employed for all metastatic samples due to the high rate of false positivity when IHC is completed on core biopsy samples due to edge artifact [26, 27].

The primary oncologist caring for the patient completed a questionnaire both before and after the biopsy to assess if a change in management had occurred and if the change was related to the biopsy results. In order to assess patient acceptance and feasibility of carrying out a biopsy, patients completed a questionnaire at the end of the study and were also asked if they perceived a delay in initiation of their treatment due to the time required to obtain a biopsy.

Information about adjuvant therapy (if any) received by each patient and time between initial diagnosis and metastatic presentation were also documented.

statistical methods

To fulfill the primary objective of determining the probability of discordance in receptor status, 27 paired samples were required to detect a discordance rate of $\geq 20\%$ with 80% power using a one-sided alpha of 5%. For the secondary objective of determining effect on treatment, if we assume that 90% of the time oncologists treat women with metastatic breast cancer based on the characteristics of the primary tumor and that a change in treatment of 20% of patients is clinically significant, a sample size of 27 was also adequate (under the same null hypothesis as above). The sample size was subsequently increased to 35 to allow for treatment driven by patient wishes and to allow for 10% of cases where a biopsy may not be feasible and 5% rate of patient withdrawal from the study. Data were analyzed using Stata statistical software version 10.0; McNemar's test or Fisher's exact test was used where appropriate.

role of funding source

The sponsor of this study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The primary and corresponding authors had access to all data in the study and were responsible for submission of this paper for publication.

results

participants

Forty-nine patients were approached for this study. Of these, nine patients declined participation for the following reasons (in decreasing frequency): advised not to participate by another physician, language barrier to understanding and obtaining informed consent, fear of biopsy causing tumor spread, and participation in another clinical trial that did not allow enrollment. Forty patients (82%) provided written informed consent to participate. Of these, three patients were found after initial screening to have metastatic lesions not amenable to biopsy due to location. A further two patients were found on subsequent radiological investigation in preparation for biopsy to have complete resolution of their initial suspected metastatic lesion. Thirty-five patients had biopsy of their metastatic lesions, from which 29 analyzable samples were obtained (Figure 1).

characteristics of patients

Of the women recruited to the study, 61% had received adjuvant chemotherapy, 53% had received endocrine therapy, and 2% had received trastuzumab; 26% were still receiving endocrine therapy when metastases were diagnosed. Median duration between diagnosis of the primary tumor and identification of a metastatic lesion was 2.4 years (interquartile range 1.2–6.5 years). All patients underwent biopsy before receiving any treatment of metastatic disease. The sites of metastases biopsied are described in Table 1; most were from bone, the most common site of metastasis from breast cancer.

comparison of receptor status between primary and metastatic lesion

Of the 29 analyzable samples, three diagnosed as benign disease (two bone biopsies and one cerebrospinal fluid) and one sample

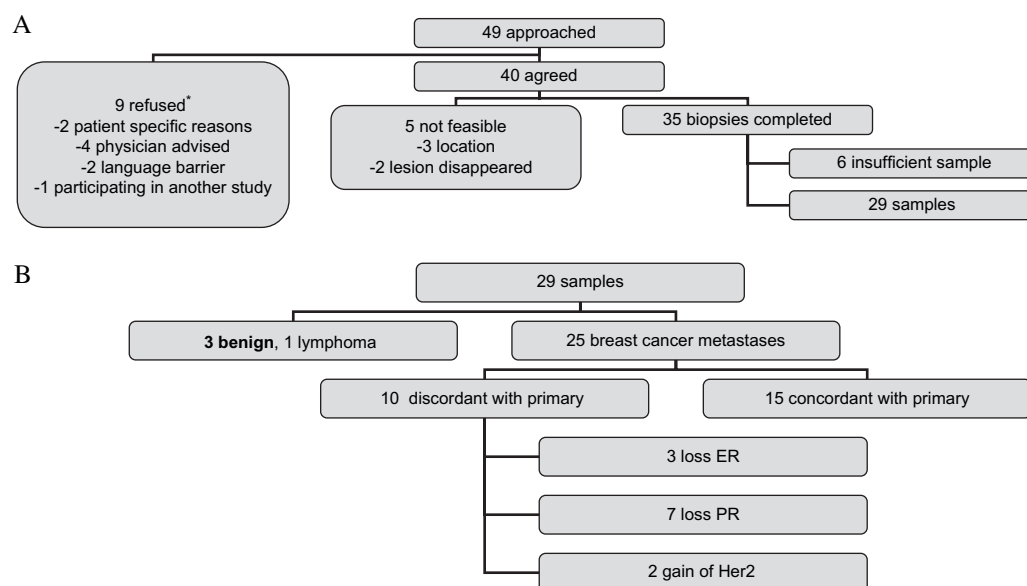


Figure 1. (A) Organization chart of participants. (B) Organization chart of results.

Table 1. Baseline information of all patients who underwent biopsy attempt

	n (%)
Histology primary disease (n = 35)	
Infiltrating ductal	28 (80)
Infiltrating lobular	5 (14)
Mixed lobular and ductal	1 (3)
DCIS with microinvasion	1 (3)
Stage at diagnosis (N = 35)	
1	6 (17)
2a	5 (14)
2b	8 (23)
3a	9 (26)
3b	3 (10)
3c	4 (11)
Hormone status primary (n = 35)	
ER+/PgR+	13 (37)
ER+/PgR-	10 (29)
ER-/PgR-	12 (34)
ER-/PgR+	0
Her2 status primary (n = 35)	
Her2+	13 (37)
Her2-	22 (63)
Sites of biopsy (n = 29)	
Bone	11 (38)
Soft tissue (not surgically curable)	10 (34)
Pleural effusion	3 (10)
Liver	3 (10)
Lung	1 (3)
CSF	1 (3)

DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; PgR, progesterone receptor; CSF, cerebrospinal fluid.

diagnosed as low-grade follicular lymphoma. On further follow-up, these women all remain free of breast cancer recurrence at 1 year. The remaining 25 samples provided histological

confirmation of metastatic breast cancer. Based on the primary tumor, 16 of 25 patients were ER+, 9 of 25 patients were PgR+, and 4 of 25 patients were Her2+ based on original report. The primary tumour slides for these 25 patients were reassessed to confirm and quantify ER and PgR status. Of the 25 paired samples from primary and metastatic lesions, 10 were discordant for ER/PgR and two were discordant for Her2 (Figure 2), with a complete change in receptor status. This resulted in a 40% overall discordance rate for ER and PgR ($P = 0.026$) and an 8% discordance rate for Her2. All changes in hormone receptor status involved a complete loss of ER, PgR, or both. Both changes in Her2 status involved a complete gain in Her2 overexpression compared with the primary tumor.

The percentage of cells expressing ERs and/or PgRs for the paired samples is presented in Figure 2. A change in ER or PgR status was reported if quantitative staining changed from negative (<5%) to positive (>10%) or vice versa. Three patients had an increase in percentage positivity of ER from primary to metastatic lesion of $\geq 10\%$, but none of these resulted in a complete gain in ER status. Five patients had a quantitative decrease in expression of ER positivity between primary tumor and metastasis, of whom three became ER negative by the above definition. Almost all the tumors that were positive for PgR in the primary had some loss of PgR in the metastatic lesion, ranging from 15% to 90% absolute decrease in number of cells with PgR expression (Figure 2B). Only one patient had an increase in percent positivity of PgR from 10% to 100% in a metastasis. All the tumors that were triple negative in the primary tumor ($n = 6$) did not change on analysis of the metastatic lesion.

changes in clinical management

Six independent medical oncologists accrued patients to this study. Of the 29 patients who underwent biopsy, 20% (6 of 29) had a change in their treatment plan based on the results of the

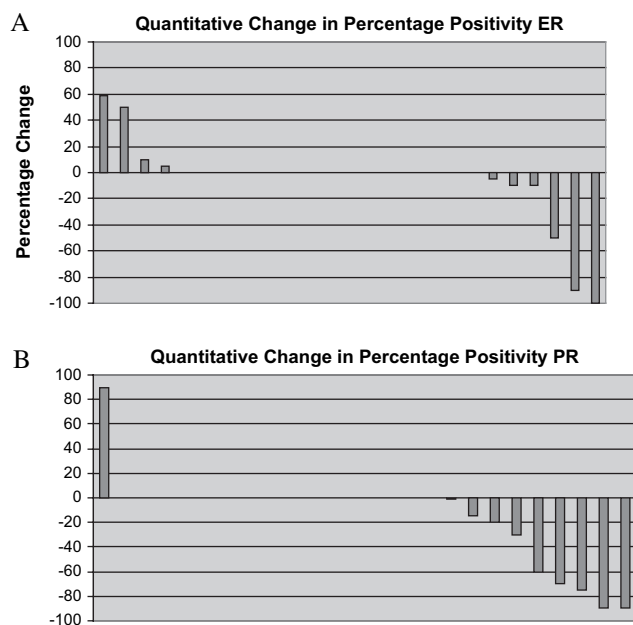


Figure 2. (A) Percentage change in positivity of estrogen receptor (ER). (B) Percentage change in positivity of progesterone receptor (PgR).

biopsy ($P = 0.002$). For the four patients who had no evidence of metastatic breast cancer on biopsy, not only did their treatment plan change but also the biopsy results drastically changed their prognosis. A gain in Her2 overexpression resulted in change of management for two patients, both of whom qualified for trastuzumab or for a clinical trial, based on the result of the biopsy.

patient acceptance

Forty patients consented over a period of just >1 year. For the patients who underwent biopsy, 38% reported some anxiety before the procedure. Pain associated with the biopsy was reported by 48% of women, with 28% reporting pain as mild and 20% as moderate. No patient reported pain as severe. Of those who experienced pain, 25% reported that the pain lasted for >1 day after the biopsy. Overall, patients were satisfied with the biopsy procedure. The main concern expressed by patients was the time required to obtain the biopsy. Most women underwent a biopsy within 2 weeks of consenting to the study, but treatment was delayed in two patients by 30 days due to scheduling of the biopsy procedure. For patients who underwent a biopsy that did not result in a change in management, they reported reassurance at having tissue confirmation of metastatic disease.

discussion

Changes in molecular markers in women with breast cancer between primary lesion and metastatic disease are increasingly important because of increasing use of targeted therapies [28, 29]. While previous retrospective studies have indicated that change in receptor status may occur, we are unaware of prior prospective studies to evaluate discordance between primary and metastatic lesions.

This current study demonstrated 40% discordance in ER/PgR status and 8% discordance in Her2 status in a cohort of patients who presented with new lesions suspicious for metastatic breast cancer. The rate of ER and PgR discordance is similar if not higher than that reported in previous retrospective reviews, and all changes involved a loss in receptor status. While interlaboratory variability may occur with IHC assessment [30], in the present study, all samples (primary tumor and metastases) were reviewed centrally with the same staining technique to reduce interlaboratory variability. Additionally, the observed changes were verified by a single (blinded) pathologist, further decreasing interobserver variability. This is in contrast to prior retrospective studies which in large part relied on information gained from pathology reports alone, prepared by a number of different pathologists [22, 24, 30]. In addition, all Her2 analysis was carried out with FISH, unlike prior retrospective studies which for the most part relied on IHC quantification of Her2 overexpression [22–24]. The problem with relying on IHC for core biopsy specimens is that a high rate of false-positive Her2 overexpression may be reported due to edge artifact [26, 27].

Potential mechanisms for the observed discordance between primary tumor and metastases may include either treatment-induced selection or progression of tumor cells to a more malignant phenotype. Selective killing of ER+ and PgR+ tumor cells by adjuvant hormonal treatment might result in selection of untreated or undertreated ER– and PgR– cells, with which time may lead to metastatic recurrence [31]. Also, molecular changes may result in decreased expression or complete loss of hormone receptors or an increase in expression of Her2, leading to a more aggressive phenotype.

In the present study, we have linked changes in ER/PgR and Her2 status with impact on management. Based on the biopsy results, 20% of patients had a significant change in management. For four of these patients in whom the biopsy was either benign or another cancer, the biopsy results changed their diagnosis, prognosis, and life expectancy. While one might expect that the 10 patients who had a change in receptor status would have had a change in management, only six of them had a change in management plan. Reasons for this were a combination of patient wishes, physician practice/acceptance of results, and other tumor characteristics.

Clinicians are often reluctant to request a biopsy from a patient perhaps due to their belief that she may experience discomfort or inconvenience at a time when she has just been informed that she has metastatic disease. In this study, we demonstrated that patients were motivated to undergo biopsy to confirm their metastases, and even where the biopsy did not affect management, they reported reassurance in having tissue confirmation of their disease. Accrual to this study was faster than expected, and anxiety and discomfort associated with obtaining a tissue biopsy was mild to moderate.

The results of this study are generalizable to other women with an initial diagnosis of metastatic breast cancer as most biopsies were obtained from bone lesions and (unlike prior retrospective studies) local recurrences were excluded. We have studied the population for whom a change in receptor status would have the most clinical significance. All patients in this prospective study were assessed before receiving any therapy for

metastatic disease which allowed not only for assessment of molecular markers in the absence of new therapy but also demonstration of the clinical significance of obtaining a confirmatory biopsy. A limitation of the study is its relatively small size such that any subgroup analysis would be unreliable: it does not allow for changes to be related to type of adjuvant treatment, location of metastases, or disease-free interval.

Our study has implications for the design of clinical trials that evaluate targeted therapies. Mandating a biopsy of metastases at study entry would ensure that the target is expressed and would allow for a more efficient study to be carried out.

In summary, this prospective study has demonstrated a substantial rate of discordance in pathology and molecular markers between primary and suspected metastatic lesions in women with breast cancer that is sufficient to alter management in 20% of them. Tissue confirmation should be considered standard of care in patients with clinical and/or radiological suspicion of metastatic recurrence and lesions amenable to biopsy.

funding

Canadian Breast Cancer Foundation, Ontario Chapter.

acknowledgements

The authors would like to thank the patients for participating in this research study. Further, we would like to acknowledge Rebecca Dent, Candi Flynn, Nadia Salvo, Julie Napolskikh, Mike Fralick, Ritu Kumar, Jaqueline Galica, Nirosha Nathan, Kathy Pritchard, May-Lynn Quan, Frances Wright, Robyn Pugash, Sandra Gardener, Harriette Kahn, and Ian Tannock for reviewing the manuscript.

Prior presentations—Parts of the data from this study have been presented at the following meetings: San Antonio Breast Cancer Symposium 2007, American Society of Clinical Oncology General Meeting 2008, and American Society of Clinical Oncology Breast Cancer Symposium 2008.

references

- Brennan MJ, Donegan WL, Appleby DE. The variability of estrogen receptors in metastatic breast cancer. *Am J Surg* 1979; 137: 260–262.
- Allegra JC, Barlock A, Huff KK, Lippman ME. Changes in multiple or sequential estrogen receptor determination in breast cancer. *Cancer* 1980; 45: 792–794.
- Holdaway IM, Bowditch JV. Variation in receptor status between primary and metastatic breast cancer. *Cancer* 1983; 52: 479–485.
- Hull DF, Clark GM, Osborne CK et al. Multiple estrogen receptor assays in human breast cancer. *Cancer Res* 1983; 43: 413–416.
- Raemaekers JM, Beex LV, Koenders AJ et al. Concordance and discordance of estrogen and progesterone receptor content in sequential biopsies of patients with advanced breast cancer: relation to survival. *Eur J Cancer Clin Oncol* 1984; 20: 1011–1018.
- Gross GE, Clark GM, Chamness GC et al. Multiple progesterone receptor assays in human breast cancer. *Cancer Res* 1984; 44: 836–849.
- Mobbs BG, Fish EB, Pritchard KI et al. Estrogen and progesterone receptor content of primary and secondary breast cancer: influence of time and treatment. *Eur J of Cancer Clin Oncol* 1987; 23(6): 819–826.
- Rasmussen BB, Thorpe SM, Nørgaard T et al. Immunohistochemical detection of estrogen receptors in paraffin sections from primary and metastatic breast cancer. *Pathol Res Pract* 1989; 185: 856–859.
- Hawkins RA, Tesdale AL, Anderson ED et al. Does the oestrogen receptor concentration of a breast cancer change during systemic therapy? *Br J Cancer* 1990; 61: 877–880.
- Johnston SR, Saccani-Jotti G, Smith IE et al. Changes in estrogen receptor, progesterone receptor, and pS2 expression in tamoxifen-resistant human breast cancer. *Cancer Res* 1995; 55: 3331–3338.
- Li BD, Byskosh A, Molteni A, Duda RB. Estrogen and progesterone receptor concordance between primary and recurrent breast cancer. *J Surg Oncol* 1994; 57(2): 71–77.
- Kuukasjarvi T, Kononen J, Helin H et al. Loss of ER in recurrent breast cancer is associated with poor response to endocrine therapy. *J Clin Oncol* 1996; 14: 2584–2589.
- Umekita Y, Sagara Y, Yoshida H. Estrogen receptor mutations and changes in estrogen receptor and progesterone receptor protein expression in metastatic or recurrent breast cancer. *Jpn J Cancer Res* 1998; 89: 27–32.
- Bachleitner-Hofmann T, Pichler-Gebhard B, Rudas M et al. Pattern of hormone receptor status of secondary contralateral breast cancers in patients receiving adjuvant tamoxifen. *Clin Cancer Res* 2002; 8: 3427–3432.
- Gutierrez MC, Detre S, Johnston S et al. Molecular changes in tamoxifen-relapsed breast cancer: relationship between ER, HER2 and P38-MAP-kinase. *J Clin Oncol* 2005; 23(11): 2469–2476.
- Robertson J, Gutteridge E, Cheung KL et al. Oestrogen receptor expression in human breast cancer during long-term fulvestrant treatment. *Proceedings of ASCO* 2004; 22 (Suppl): 14S (Abstr 536).
- Franco A, Col N, Chlebowski RT et al. Discordance in estrogen (ER) and progesterone receptor (PR) status between primary metastatic breast cancer: a meta-analysis. *ASCO Annual Meeting Proceedings (Post-Meeting Edition)*. *J Clin Oncol* 2004; 22: 14S (Abstr 539).
- Lower EE, Glass EL, Bradley DA et al. Impact of metastatic ER and PR status on survival. *Breast Cancer Res Treat* 2005; 90: 65–70.
- Lipton A, Leitzel K, Ali SM et al. Serum HER-2/neu conversion to positive at the time of cancer progression in metastatic breast patients treated with letrozole vs. tamoxifen. *Cancer* 2005; 104(2): 257–263.
- Regitnig P, Schippinger W, Lindbauer M et al. Change of HER-2/neu status in a subset of distant metastases from breast carcinomas. *J Pathol* 2004; 203(4): 918–926.
- Fehm T, Jäger W, Kraemer S et al. Changes of serum Her2 status during clinical course of metastatic breast cancer patients. *Anticancer Res* 2004; 24(6): 4205–4210.
- Broom R, Tang P, Simmons C et al. Changes in estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu status with time: discordance between primary and metastatic breast pathology samples. *Proceedings of ASCO* 2007; 25 (Suppl): Part 1, 18S (Abstr 1024).
- McFarlane R, Speers C, Masoudi H, Chia S. Molecular changes in the primary breast cancer versus the relapsed/metastatic lesion from a large population-based database and tissue microarray series. *Proceedings of ASCO* 2008; 26 (Suppl): (Abstr 1000).
- Broglio K, Moulder SL, Hsu L et al. Prognostic impact of discordance/concordance of triple-negative expression between primary tumor and metastasis in patients with metastatic breast cancer. *Proceedings of ASCO* 2008; 26 (Suppl): (Abstr 1001).
- Agulnik M, Oza AM, Pond GR, Siu LL. The impact and perception of mandatory tumour biopsies for correlative studies in clinical trials of novel anticancer agents. *J Clin Oncol* 2006; 24(30): 4801–4807.
- Hanna W, O'Malley FP, Barnes P et al. Updated recommendations from the Canadian National Consensus Meeting on HER2/neu testing in breast cancer. *Curr Oncol* 2007; 14(4): 149–153.
- Wolff AC, Hammond ME, Schwartz JN et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007; 25(1): 118–145.

28. Goss PE. Factors mediating endocrine therapy response and resistance. Proceedings of ASCO 2008; 26 (Suppl): 15S. Session discussant (Abstr 1000 & 1001).
29. Piccart-Gebhart MJ. Adjuvant ovarian suppression combined with tamoxifen or anastrozole, alone or in combination with zoledronic acid, in premenopausal women with hormone-responsive, stage I and II breast cancer: first efficacy results from ABCSG-12. Proceedings of the ASCO 2008; 26 (Suppl): 15S. Plenary session discussant (Abstr 248).
30. Rhodes A, Jasani B, Barnes DM et al. Reliability of immunohistochemical demonstration of oestrogen receptors in routine practice: interlaboratory variance in the sensitivity of detection and evaluation of scoring systems. J Clin Pathol 2000; 53: 125–130.
31. Kurbel S. Selective reduction of estrogen receptor (ER) positive breast cancer occurrence by estrogen receptor modulators supports etiological distinction between ER positive and ER negative breast cancers. Med Hypotheses 2005; 64: 1182–1187.