Brain Metastases in Patients with Advanced Breast Cancer

G. SANNA[1], L. FRANCESCHELLI[1], N. ROTMENSZ[2], E. BOTTERI[2], L. ADAMOLI[1],
C. MARENGHI[1], E. MUNZONE[1], M. COSSU ROCCA[1], E. VERRI[1], I. MINCHELLA[1],
M. MEDICI[1], C. CATANIA[1], E. MAGNI[1], A. GOLDHIRSCH[1] and F. NOLÈ[1]

Departments of [1]Oncology and [2]Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy

Abstract. *Background: The incidence of brain metastases (BM) is apparently rising in patients with advanced breast cancer (ABC). We performed a case control study to define current features of breast cancer related to central nervous system (CNS) metastases. Patients and Methods: From March 1999 to May 2006, we identified 72 patients with symptomatic BM of breast cancer. A comparison group was randomly selected assigning to each case two patients with primary breast cancer and no BM, matched for year of diagnosis, age and tumour stage (pT status and nodal status). Results: Cases had a significantly higher rate of negative estrogen receptors (ERs) (60% in cases vs. 29% in controls), negative progesterone receptors (PgRs) (79% vs. 43%), HER2/neu over expression (44% vs. 13%) and immunostaining for Ki-67 ≥20% (84% vs. 55%), with p-value <0.001 for all four parameters in univariate analyses. On multivariate analysis, HER2/neu over expression and Ki-67 ≥20% were independent predictive factors of brain relapse (Odds Ratio (OR) 2.55, 95% confidence intervals (CI) 1.10-5.94 and OR 2.97, 95% CI 1.01-8.73, respectively). Endocrine unresponsive tumours (both ER and PgR <10%) showed an increased risk of relapse with BM of borderline significance (OR 1.91, 95% CI 0.87-4.12). Conclusion: Patients with ER and PgR negative tumours either with or without HER-2/neu over expression should be considered at higher risk of BM.*

The incidence of brain metastases (BM) is apparently rising in patients with advanced breast cancer (ABC) (1), possibly due to better therapeutic approaches for control of metastatic growth in other organs. Occurrence of relapse of BM severely affects quality of life and is associated with dire prognosis.

Median survival after the diagnosis of symptomatic central nervous system (CNS) involvement is roughly 4 months, with 2-year survival less than 2% (2, 3). Breast carcinoma is the second most common cause of BM, which occurs in approximately 10-15% of patients, although autopsy data suggest an higher prevalence in up to 30% of patients (2, 4). Furthermore, breast cancer (BC) is the most common solid tumour to exhibit leptomeningeal colonization (5, 6).

A strong correlation with paclitaxel-based chemotherapy and cerebral relapse has been observed (1). Patients with estrogen (ER) and progesterone (PgR) receptor negative tumours seem to be at increased risk of developing cerebral metastases (12), possibly due to the poorer prognosis associated with endocrine-unresponsive tumours.

Recently a higher risk of developing BM was reported for young patients with Her-2/neu over expression (7, 8) and for patients treated with trastuzumab-based therapy, with observed incidence rate from 25% to 34% (9, 10). The mechanism postulated is that trastuzumab therapy may selectively destroy non BM, prolonging overall survival, and therefore allowing a later development of cerebral relapse. Patients with Her-2/neu over expressing tumours apparently had a longer survival after the occurrence of BM, compared with patients whose cancer did not express Her-2/neu. This survival advantage may possibly be explained by better control of extra-cranial disease in this subgroup of patients, due to trastuzumab-based treatments (10). Data are not consistent in all reports and one study did not confirm the association between brain relapse and trastuzumab-based therapy (11).

Because of the relatively low incidence of cerebral metastases, standard follow-up and restaging programs do not usually include routine brain imaging unless symptoms are present. The possibility of identifying a subgroup of patients at higher risk of BM may lead to target screening and eventually prophylactic measures in order to detect early disease or to prevent cerebral relapse.

In the Unit for Medical Care of our institution, 72 patients with ABC and symptomatic BM were identified. At the time, these patients were receiving systemic treatment for their disease (endocrine therapy or chemotherapy). With the aim of better defining the features of breast cancer metastatic to the CNS, the choice of procedures for care and

Correspondence to: Giuseppina Sanna, MD, Department of Medicine, European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy. Tel: +39 02 57489 460-938, Fax: +39 02 55210169, e-mail: giuseppina.sanna@ieo.it

Key Words: Advanced breast cancer, brain metastases, risk factors.

related prognosis, we evaluated the biological characteristics of BC at diagnosis in this group of patients in comparison with BC in a matched control group of patients selected among women referred to the same institute.

Patients and Methods

Patient populations. Data on 72 consecutive patients with ABC and BM were collected. All cases were diagnosed with BC from January 1986 to October 2003 and were non-metastatic at diagnosis (M0). Cerebral relapse was diagnosed at the European Institute of Oncology from March 1999 to May 2006. The following parameters were recorded at BC diagnosis: age of patient, staging (TNM UICC), ER, PgR, Her2/neu and Ki-67 expression and treatment modality. Recorded follow-up variables were tumour recurrence and patient survival.

Patients in the control group were selected from all M0 BC patients without BM referred to the same institute and included in a centralized database. The variables used to make the randomly assigned matches were as follows: year of diagnosis (within 3 years); age at diagnosis (within 5 years); tumour size (pT1 *versus* pT2 *versus* pT3-4); nodal status (node-negative *versus* node-positive).

For 8 cases we were able to match only one control, leading to a comparison group of 136 patients. Each control patient had a follow-up time at least as long, as the time between breast cancer diagnosis and the occurrence of brain metastases of the corresponding case.

Specimen analysis and immunohistochemistry. All the included patients had pathological evaluation performed at the European Institute of Oncology. All records were reviewed retrospectively.

Immunostaining specimens for the localization of ER and PgRs, Her-2/neu protein and Ki-67 antigen were performed on consecutive tissue section biopsies obtained from the primary tumour, and in distant metastases, where available.

The expression of ER and PgR and the tumor proliferative fraction were evaluated immunohistochemically as previously reported (12). Results were recorded as the percentage of cells showing definite nuclear immunoreactivity over ≥2000 neoplastic cells in 10 randomly selected high power fields (HPF = x 400) at the periphery of the tumor. Steroid hormone receptor status was classified as negative (ER and/or PgR <10% of the cells), or positive (ER and/or PgR ≥10% of the cells).

HER-2/*neu* over expression was also investigated immunohistochemically, using a specific polyclonal antiserum (Dako A/S [Carpinteria, CA, USA], working dilution 0.05 mol/L) and the Envision detection reagent (Dako A/S), according to the manufacturer's instructions. The staining results were recorded in a four-tier scale, from 0 to 3+, according to the percentage of immunoreactive cells, and to the intensity and completeness of membrane staining as recommendend by the Food and Drug Administration (13). Only an intense and complete membrane staining >10% of the tumor cells was taken as evidence of Her-2/neu over-expression (3+). In unclear cases (moderate staining) Her-2/neu amplification was evaluated through FISH test.

Statistical methods. The primary aim of the study was to find which factors could have a significant prognostic impact on BM from BC. The Chi-square test or the Fisher exact test were used in the

Table I. *Descriptive analysis and comparisons between cases and controls.*

Classification	Cases No=72 No. (%)	Controls No=136 No. (%)	P-value*
Age†			
<35 years	13 (18)	23 (17)	
35-50 years	33 (46)	62 (46)	0.970
51+ years	26 (36)	51 (37)	
pT†			
pT1	31 (47)	60 (48)	
pT2	23 (35)	45 (36)	0.929
pT3 or pT4	12 (18)	20 (16)	
pN†			
Negative	35 (49)	63 (47)	0.756
Positive	36 (51)	71 (53)	
Estrogen receptors			
<10%	42 (60)	38 (29)	<0.001
≥10%	28 (40)	92 (71)	
Progesteron receptors			
<10%	55 (79)	56 (43)	<0.001
≥10%	15 (21)	74 (57)	
Her2/neu			
Overexpressed	30 (44)	14 (13)	<0.001
Not overexpressed	38 (56)	93 (87)	
Er and Pgr <10%			
Her2/neu			
Overexpressed	22 (55)	8 (29)	0.031
Not overexpressed	18 (45)	20 (71)	
Er and/or Pgr ≥10% Her2/neu			
Overexpressed	8 (31)	6 (8)	0.006
Not overexpressed	18 (69)	73 (92)	
Ki-67			
<20%	7 (16)	51 (45)	<0.001
≥20%	37 (84)	63 (55)	

†Matching variable; *Differences among cases and controls were tested using the Chi-square test or Fisher's exact test.

univariate analysis to assess differences between cases and controls in the distribution of prognostic variables.

A multivariate conditional logistic regression model for matched pairs was fitted to assess the independent prognostic significance of the variables differently distributed between the two groups. The secondary aim was to study case overall survival (OS), calculated from BM diagnosis to death from any cause. Survival curves were estimated using the Kaplan-Meier method and the log-rank test was used to assess survival differences between groups.

All analyses were performed with the SAS software (SAS Institute, Cary, NC, USA). All tests were two-sided.

Results

Age distribution, pT staging and nodal involvement were similar in the two groups (*p*-value of 0.97, 0.929, 0.756, respectively). Median age was 47 years for both groups (Table I).

Table II. *Multivariate analysis.*

	OR (95% C.I.)*	*P*-values*
Her2/neu Overexpressed	2.55 (1.10; 5.94)	0.029
Estrogen and Progestinic Receptors <10%	1.91 (0.87; 4.12)	0.109
Ki-67 ≥20%	2.97 (1.01; 8.73)	0.048

*From conditional logistic regression model for matched pairs.

Dots represent censored observations, overall-survival median time after brain MTS was 9 months.

Figure 1. *Overall-survival after brain metastasis.*

Focusing on biological variables, 42 patients (60%) of the study group had negative ER expression compared to 38 patients (29%) of the matched cohort (*p*-value <0.001). The same trend was observed when analyzing PgR status, with 55 patients (79%) of cases, compared with 56 (43%) of controls with negative PgR expression (*p*-value <0.001).

When considering Her2/neu, 30 patients in the study group (44%) compared to 14 patients in the control one (13%) had Her2/neu over expression (*p*<0.001). As expected, when considering ER/PgR negative with Her2/neu over-expression tumours in the two groups, the difference maintained a highly statistical significance: in the study group, Er/PgR <10% and c-erbB2 over expression was observed in 22 patients (55%), compared to 8 patients (29%) in the matched group, with a *p*-value of 0.031.

The proliferation index was significantly higher at diagnosis in the study cohort with 37 tumours (84%) with Ki 67 ≥20%, compared with 63 (55%) in the control group (*p*<0.001) (Table I).

Performing a multivariate analysis, according to a conditional logistic model for matched pairs, Her2/neu over expression was found to be an independent biological variable predictive of brain relapse, with an Odds Ratio (OR) of 2.55 (95% CI 1.10-5.94) and *p*-value of 0.029. The same result was observed when considering the proliferation index: ki-67 ≥20% translated into an OR of 2.97 (95% CI 1.01-8.73), with a *p*-value of 0.048 (Table II).

Focusing on endocrine unresponsive tumours (both ER and PgR <10%), the OR was 1.91 (95% CI 0.87-4.12), with a *p*-value of borderline statistical significance (*p*=0.109).

First events in the study group included lung metastases in 12 patients (17%). Other sites of progression were: liver (9 patients, 13%), bone (9 patients, 13%), skin (10 patients 14%) and regional nodes (9 patients, 13%). Five patients developed brain metastasis as the first site of relapse.

At a median follow-up of 55 months (range 13-200) we observed 63 deaths in the study group (88%) and at a median follow-up of 60 months (range 24-204), 8 deaths were reported in the matched cohort.
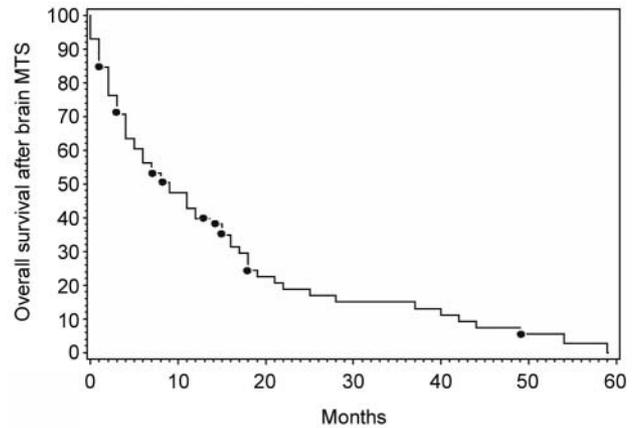
Median overall survival (OS) after brain relapse diagnosis was 9 months (Figure 1). No differences in survival were observed considering patients with Her2-neu over expressing tumours, with highly proliferating or with endocrine unresponsive cancer (log-rank *p*-value of 0.62, 0.79, 0.22, respectively).

Considering systemic treatment, among the 72 cases, 2 patients received taxane-based therapy in the neoadjuvant setting, 3 patients as adjuvant therapy and 28 patients for metastatic disease. Therefore nearly 50% of patients underwent taxane-based therapy before cerebral relapse occurred (33 patients, 46%).

Focusing on biological therapy, 19 patients with Her2-neu over expressing tumours (63%) received trastuzumab-based therapy before symptomatic BM diagnosis.

Discussion

In the present study, we retrospectively reviewed data from 72 patients with advanced breast cancer diagnosed with symptomatic brain metastases. These patients were able to receive systemic treatment for their disease. Observed median overall-survival after cerebral relapse was nine months, significantly longer than in other reports, possibly due to the selection of patients (2, 3). In the univariate analysis about 60% of patients in the study group were ER-negative compared with 29% of the matched cohort (*p*-value <0.001). The same trend was observed when analyzing PgR status, with 80% of cases, compared with 43% of controls, with negative PgR expression (*p*-value <0.001).

These data are consistent with other studies. Several authors reported a correlation between the absence of ER and PgR and the development of brain metastases (9, 14-19). Slimane *et al.* (15) underlined that the occurrence of

lung metastases may be a risk factor for brain relapse. In our report, 17% of patients had lung metastases as the first site of progression, so it does not appear as a major predictive variable.

The proliferation index was significantly higher at diagnosis in the study group with 37 tumours (84%) with Ki 67 >20%, compared with 63 (55%) in the control group ($p<0.0001$). This observation may eventually represent an epiphenomenon of increased Her-2 over expression, which is often correlated with ER/PgR negativity and poorly differentiated cancers.

Her-2/neu over expression seems to be another marker for risk of developing brain metastases.

In our report, when considering Her2/neu as a variable, 30 patients in the study group (44%) compared to 14 patients in the control one (13%) had Her2-neu over expression ($p<0.0001$). On multivariate analysis, Her2/neu over expression and ki67 ≥20% were independent predictive factors of brain relapse (OR 2.55, 95% CI 1.10-5.94 and OR 2.97, 95% CI 1.01-8.73, respectively). Endocrine unresponsive tumours (both ER and PgR <10%) reflected an increased risk of brain relapse of borderline significance (OR 1.91, 95% CI 0.87-4.12).

Many potential molecular mechanisms have been suggested to explain the tumour aggressiveness phenotype of Her-2 over expressing tumours. Increased activation of Her-2/neu signalling has dramatic effects on cell proliferation, apoptosis resistance and cell survival, migration, and invasion (20).

Our data are consistent with the results of several reports, with an observed increased incidence of brain relapse in Her-2/neu over expressing tumours or in patients under trastuzumab-based therapy, often despite visceral control of the disease (8-9, 21-22). Her-2/neu over-expression may endow tumour cells with increased metastatic potential to sites such as the lungs and may similarly increase metastatic propensity to the CNS (23, 24).The development of brain metastastic models for breast cancer may permit direct testing of this hypothesis through transfection experiments. Another theory is that trastuzumab may allow micrometastatic brain metastases to become symptomatic as a natural consequence of an extended life-span. Trastuzumab, as well as cytotoxic agents, seems to be active against systemic metastases but relatively ineffective against CNS localizations due to its poor penetration of the blood-brain-barrier (25, 26). In our report, 63% of Her2-neu over expressing tumour received trastuzumab-based therapy before symptomatic BM diagnosis; nearly 50% of patients underwent taxane-based therapy before cerebral relapse occurred. The use of trastuzumab-based therapy and taxane-based chemotherapy have been increasing in patients with breast cancer, both in the adjuvant (27, 28) and in the advanced setting (29, 30).

It would be interesting to prospectively evaluate the incidence of brain relapse in patients receiving both taxane or trastuzumab-based therapy.

Brain metastases seem to mantain Her2/neu over expression, therefore targeting Her2/neu with drugs that penetrate the blood-brain barrier may eventually lead to clinical cerebral response. There are ongoing studies with lapatinib, an oral tyrosine kinase inhibitor with potent anti-Erb1 and Erb2 activity, in patients with brain metastases and Her-2/neu over expressing tumours. Results from clinical trials are pending. Recently at ASCO 2006, Lin *et al.* presented the preliminary results of a phase II trial of lapatinib for brain metastases in patients with HER2-neu over expressing cancer. Two women achieved a partial response according to RECIST criteria. Four of the 16 women with measurable non-CNS disease achieved partial response (31). Although the study failed to demonstrate the hypothesized level of activity, there is sufficient evidence that lapatinib can penetrate the blood-brain barrier.

Conclusion

The current staging system does not include brain imaging, due to the relatively low incidence of the condition. Diagnosis of brain metastases is based on patient symptoms and neuro-imaging. In high-risk patients, the possibility of screening for brain metastases may avoid the occurrence of neurological symptoms. Whether early diagnosis would improve quality of life and prolong survival remains to be seen.

The design of therapeutic and preventive approaches to brain relapse would further benefit from an increased understanding of the blood-brain and blood-tumour barriers as well as other host-tumour interactions in the CNS. In high-risk patients, the use of adjuvant therapies potentially active against cerebral metastases should be prospectively investigated (31).

References

1 Crivellari D, Pagani O, Veronesi A, Lombardi D, Nole F, Thurlimann B, Hess D, Borner M, Bauer J, Martinelli G, Graffeo R, Sessa C and Goldhirsch A: International Breast Cancer Study Group. High incidence of central nervous system involvement in patients with metastatic or locally advanced breast cancer. Ann Oncol *12*: 353-356, 2001.

2 Di Stefano A, Yong Yap Y, Hortobagy GN and Blumenschein GR: The natural history of breast cancer patients with brain metastases. Cancer *44*: 1913-1918, 1979.

3 Zimm S, Wampler GL, Stablein D, Hazra T and Young HF: Intracerebral metastases in solid tumour patients: natural history and results of treatment. Cancer *48*: 384-394, 1981.

4 Langer CJ and Metha MP: Current management of brain metastases, with a focus on systemic options. J Clin Oncol *23(25)*: 6207-6219, 2005.

5   Sawaya R, Bindal R, Lang F and Abi-Said D: Metastatic Tumours. *In*: Brain Tumours: An Encyclopedic Approach. Kaye AH and Laws ER Jr (eds.). New York, Churchill Livingstone pp. 999-1026, 2001.

6   Weil RJ, Palmieri DC, Bronder JL, Stark AM and Steeg PS: Breast cancer metastasis to central nervous system, review. Am J Pathol *167(4)*: 913-920, 2005.

7   Altaha R, Cromwell E, Hobbs G, Higa G and Abraham J: Increased risk of brain metastases in patients with HER-2/neu-positive breast carcinoma. Cancer *103(3)*: 442-443, 2005.

8   Miller KD, Weathers L, Haney G, Timmerman R, Dickler M, Shen J and Sledge W Jr: Occult central nervous system involvement in patients with metastatic breast cancer: prevalence, predictive factors and impact on overall survival. Annals of Oncology *14*: 1072-1077, 2003.

9   Clayton A, Danson S, Jolly S, Ryder W, Burt P, Stewart A, Wilkinson P, Welch R, Magee B, Wilson G, Howell A and Wardley A: Incidence of cerebral metastases in patients treated with trastuzumab for metastatic breast cancer. Br J Cancer *91*: 639-643, 2004.

10 Kirsch DG, Ledezma CJ, Mathews CS, Bhan AK, Ancukiewicz M, Hochberg FH and Loeefler JS: Survival after brain metastases from breast cancer in the trastuzumab *hera*. J Clin Oncol *23(9)*: 2114-2116, 2005.

11 Lai R, Dang CT, Malkin MG and Abrey LE: The risk of central nervous system metastases after trastuzumab therapy in patients with breast cancer. Cancer *101(4)*: 810-816, 2004.

12 Decensi A, Robertson C, Viale G, Pigatto F, Johansson H, Kisanga ER, Veronesi P, Torrisi R, Cazzaniga M, Mora S, Sandri MT, Pelosi G, Luini A, Goldhirsch A, Lien EA and Veronesi U: A randomized trial of low dose tamoxifen on breast cancer proliferation and blood estrogenic biomarkers. J Natl Cancer Inst *95*: 779-790, 2003.

13 Jacobs TW, Gown AM and Yaziji H: Specificity of Herceptest in determining HER2/neu status of breast cancer using the United States Food and Drug Administration-approved scoring system. J Clin Oncol *17*: 1983-1987, 1999.

14 Evans AJ, James JJ, Cornford EJ, Chan SY, Burrel HC, Pinder SE, Gutteridge E, Robertson JF, Hornbuckle J and Cheung KL: Brain metastases from breast cancer: identification of high-risk group. Clin Oncol (R Coll Radiol) *16(5)*: 345-349, 2004.

15 Slimane K, Andre F, Delaloge S, Dunant A, Perez A, Grenier J, Massard C and Spielmann M: Risk Factors for brain relapse in patients with metastatic breast cancer. Annals of Oncology *15*: 1640-1644, 2004.

16 Samaan NA, Buzdar AU, Aldinger KA, Schultz PN, Yang KP, Romsdahl MM and Martin R: Estrogen receptor: a prognostic factor in breast cancer. Cancer *4*: 554-560, 1981.

17 Stewart JF, King RJ, Sexton SA, Millis RR, Rubens RD and Hayward JL: Oestrogen Receptor, sites of metastatic disease and survival in recurrent breast cancer. Eur J Cancer *17*: 449-453, 1981.

18 Gonzalez-Angulo AM, Cristofanilli M, Strom EA, Buzdar AU, Kau S-W, Broglio KR, Smith TL and Hortobagy GN: Central Nervous System Metastases in patients with high-risk breast carcinoma after multimodality treatment. Cancer *101(8)*: 1760-1766, 2004.

19 Pestalozzi BC, Zahrieh D, Price KN, Holmberg SB, Lindtner J, Collins J, Crivellari D, Fey MF, Murray E, Pagani O, Simoncini E, Castiglione-Gertsch M, Gelber RD, Coates AS and Goldhirsch A: Identifying breast cancer patients at risk for central nervous system (CNS) metastases in trials of the International Breast Cancer Study Group. Ann Oncol *17*: 935-944, 2006.

20 Yarden Y and Slikowsky M: Untangling the ErbB signalling network. Nat Rev Mol Cell Biol *2*: 127-137, 2001.

21 Bendell JC, Domchek SM, Burstein HJ, Harris L, Younger J, Kuter I, Bunnell C, Rue M, Gelman R and Winer E: Central Nervous system metastases in women who received trastuzumab-based therapy for metastatic breast carcinoma. Cancer *97*: 2972-2977, 2003.

22 Stemmler HJ, Kahlert S, Siekiera W, Untch M, Heinrich B and Heinemann V: Characteristics of patients with brain metastases receiving trastuzumab for HER2-overexpressing metastatic breast cancer. Breast *15(2)*: 219-25, 2006.

23 Yu D and Hung MC: Expression of activated rat neu oncogene is sufficient to induce experimental metastasis in 3T3 cells. Oncogene *6*: 1991-1996, 1991.

24 Yu D, Wang SS, Dulski KM, Tsai CM, Nicolson GL and Hung MC: C-erb-2/neu overexpression enhances metastatic potential of human lung cancer cells by induction of metastasis-associated properties. Cancer Res *54*: 3260-3266, 1994.

25 Lin N, Bellon J and Winer E: CNS metastases in breast cancer. J Clin Oncol *22*: 3608-3617, 2004.

26 Pestalozzi B and Brignoli S: Traztuzumab in CSF. J Clin Oncol *18(11)*: 2349-2351, 2000.

27 Colozza M, de Azambuja E, Cardoso F, Bernard C and Piccart MJ: Breast cancer: Achievements in adjuvant systemic therapies in the pre-genomic era. The Oncologist *11(2)*: 111-125, 2006.

28 Ferretti G, Papaldo P, Fabi A, Carlini P, Felici A and Cognetti F: Adjuvant trastuzumab with docetaxel or vinorelbine for HER-2-positive breast cancer. The Oncologist *11(7)*: 853-854, 2006.

29 Eniu A, Calmieri FM and Perez EA: Weekly administration of docetaxel and paclitaxel in metastatic or advanced breast cancer. The Oncologist *10(9)*: 665-685, 2005.

30 Pegram MD: Docetaxel and herceptin: foundation for future strategies. The Oncologist *6(Suppl 3)*: 22-25, 2001.

31 Lin NU, Carey LA, Liu MC, Younger J, Come SE, Bullitt E, Van Den Abbeele AD, Li X, Hochberg FH and Winer EP: Phase II trial of lapatinib for brain metastases in patients with HER2+ breast cancer. Clinical Science Symposium, ASCO, 2006.