Biomarkers, 2010; 15(2): 191–193

informa
healthcare

LETTER TO THE EDITOR

Increased HER2/neu expression in recurrent hormone receptor-positive breast cancer

Virginia G. Kaklamani[1], Mary Cianfrocca[1], Jennifer Ciccone[1], Kelly Kindy[1], Alfred Rademaker[1], Elizabeth L. Wiley[2], William Gradishar[1], and Ruth M. O'Regan[3]

[1]*Division of Hematology/Oncology, Department of Medicine and Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA,* [2]*University of Illinois, Chicago, IL, USA, and* [3]*Emory Winship Cancer Institute, Atlanta, GA, USA*

Approximately 70% of breast cancers are hormone receptor (HR)-positive (Normanno et al. 2005) and can potentially benefit from therapies targeted to the estrogen receptor, such as aromatase inhibitors (AI) or tamoxifen (Normanno et al. 2005). However, despite the use of adjuvant endocrine therapies in HR-positive breast cancer, 19.6–55.2% of patients experience recurrence of their breast cancer (Early Breast Cancer Trialists' Collaborative Group (EBCTCG) 2005). Although definitive mechanisms of endocrine resistance remain unknown, several potential mechanisms have been described, including loss and/or mutations of the estrogen receptor (ER), increased upstream growth factor signalling and changes in ER coactivator expression (Nicholson et al. 2004, Normanno 2005). Increased upstream growth factor signalling, through epidermal growth factor (EGF), has been demonstrated to play a role in endocrine resistance in preclinical models (Yee & Lee 2000). In xenograft models Creighton et al. (2008) showed that HER2 signalling was increased in endocrine-resistant models. We have previously demonstrated that MCF7 breast cancers that are resistant to selective ER modulators (SERMs) such as tamoxifen, have increased EGFR and Her-2/neu (a member of the EGFR family of receptors) expression *in vitro* and *in vivo* compared with their parental tamoxifen-sensitive cancers (O'Regan et al. 2006).

A total of 30 female patients with HR-positive infiltrating breast cancer at diagnosis who subsequently had a local or distant recurrence were retrospectively included in the study. These patients were initially seen at Northwestern University between 1996 and 2004. Patients were included if they had biopsy specimens available for both primary and recurrent disease. All patients but one had ER and/or progesterone receptor (PR)-positive disease at diagnosis and the majority were treated with endocrine therapy. None of the patients included in this analysis received adjuvant trastuzumab.

Immunohistochemistry (IHC) staining of the primary and recurrent specimens for ER, PR and Her-2/neu was performed in the Department of Pathology at Northwestern University and were scored independently by two breast pathologists, a pathologist scoring the original tissue and a study pathologist rescoring independently the tissue samples. Concordance was 100%. ER and PR scores ranged from 0 to 100% and were considered positive if the staining was ≥5%.

Statistical analysis was performed using the McNemar's test. Samples were paired for the same patient at diagnosis and recurrence. The statistical program SAS 9.1 was used.

Of the 30 patients, 26 patients had local recurrence, one patient had both local and distant recurrence and three patients had distant recurrence at the time of biopsy (Table 1). The reason for the high number of local recurrences was the fact that local recurrences were more likely to be biopsied than distant recurrences. As shown in Table 2, recurrences were associated with a decrease in ER and PR expression compared with the primary specimen. The percentage of positively stained cells for ER decreased from 79% to 59% ($p = 0.035$).

Address for Correspondence: Virginia Kaklamani, Division of Hematology/Oncology, Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, 676 N. St Clair Street, Suite 880, Chicago, IL 60611, USA. Tel: (312) 695-0320. Fax: (312) 695-0318. E-mail: v-kaklamani@northwestern.edu

(Received 02 July 2009; revised 05 September 2009; accepted 05 September 2009)

ISSN 1354-750X print/ISSN 1366-5804 online © 2010 Informa UK Ltd
DOI: 10.3109/13547500903312173

Table 1. Patient demographics.

Patient characteristics	
Number of patients	30
Age (years), median (range)	53.5 (29–96)
Stage at diagnosis, *n* (%)	
I	7 (25.9)
II	11 (40.7)
III	9 (33.3)
Time to relapse (years), median (range)	3 (1–6)
Local management at diagnosis, *n* (%)	
Mastectomy	13 (43.3)
Lumpectomy	17 (56.7)
Radiation	24 (80)
Adjuvant endocrine therapy, *n* (%)	
Tamoxifen	20 (66.7)
AI	4 (13.3)
Nothing	3 (10)
Both	1 (3.3)
Missing	2(6.7)
Adjuvant chemotherapy, *n* (%)	
Yes	21 (70)
No	8 (26.7)
Missing	1 (3.3)
Site of first recurrence, *n* (%)	
Local	26 (86.7)
Distant	3 (10)
Both	1 (3.3)
ER+/PR+/HER2-	12 (40)
ER+/PR-/HER2-	12 (40)
ER-/PR+/HER2-	0
ER+/PR+/HER2+	3 (10)
ER+/PR-/HER2+	2 (6.6)
ER-/PR+/HER2+	0
ER-/PR-/HER2-	1 (3.3)
ER-/PR-/HER2+	0

Table 2. The mean percentage of estrogen receptor (ER) and progesterone receptor (PR) positivity and Her2/neu-positive scores at diagnosis and recurrence.

	At diagnosis (%)	At recurrence (%)	*p*-Value
ER	79	59	0.035
PR	34	22	0.13
HER2/neu (2+, 3+)	27	53	0.01

The percentage of positively stained cells for PR samples decreased from 34% to 22% but this result did not reach statistical significance. The expression of Her-2/neu was significantly increased in the recurrences compared with the primary tumour. A total of 27% of tumours had an IHC stain of 2+ or 3+ upon diagnosis whereas 53% of recurrences had an IHC stain of 2+ or 3+ ($p = 0.01$). Furthermore, in ten of the patients (30%) there was an increase in Her-2/neu expression in the recurrent cancers compared with the primary cancers. Given the small number of patients we were not able to perform multivariate analysis to evaluate the influence of chemotherapy/endocrine therapy on the type of recurrence.

The current study provides supportive evidence that in women with HR-positive tumours, treated with endocrine therapy, recurrent disease is associated with a decrease in both ER and possibly PR and an increase in Her-2/neu expression by IHC. Although this is indirect evidence of the role of Her-2/neu in endocrine resistance, these data support *in vivo* data showing that breast tumours resistant to endocrine therapies, such as tamoxifen, have increased signalling through Her-2/neu (O'Regan et al. 2006), and suggest the intriguing possibility that Her-2/neu-directed therapies could inhibit hormone-resistant tumour growth. Furthermore given the fact that the majority of the patients received adjuvant tamoxifen there may still be a role for aromatase inhibitors in this setting.

Our study has several limitations. It includes a small number of patient samples and the patient group includes patients diagnosed from 1996 to 2004 during which time the treatment for breast cancer changed. Furthermore, most recurrences studied were local recurrences. However the fact that ER, PR and Her-2/neu were available in all paired samples is a strength of our current study.

A few studies have been published evaluating Her-2/neu in primary and metastatic breast cancer (Broom et al. 2009, Gancberg et al. 2002, Gong et al. 2005, Masood & Bui 2000, Zidan et al. 2005) with conflicting results. Broom et al. did not find any discordance in Her-2/neu between primary and metastatic tumours (Broom et al. 2009) and Gancberg, Masood and Gong and their coworkers found little discordance (Gancberg et al. 2002, Gong et al. 2005, Masood & Bui 2000). However Zidan et al. (2005) found 12% discordance between primary and metastatic tumour tissue with an increase in Her-2/neu expression in the metastasis. A possible explanation for these conflicting results is that these studies included HR-negative tumours. Our hypothesis based on *in vivo* data as well as our own data is that HR-positive tumours have a higher likelihood of showing increased Her-2/neu expression on recurrence in association with resistance to endocrine therapy as a result of resistance to endocrine therapy. Using serum Her-2/neu measurement, Lipton et al. (2005) showed that 26% of patients with ER-positive breast cancer treated with tamoxifen or letrozole, converted from Her-2/neu negative to positive at the time of disease progression. These data are consistent with our finding as well as findings from *in vivo* studies of increased Her-2/neu expression in endocrine-resistant breast cancer.

It is still unclear as to what degree of HER2 expression is necessary to predict benefit from trastuzumab therapy. In a recent analysis of NSABP B-31 (Perez et al.

2007), the investigators found that a subset of patients (44 patients in the trastuzumab arm and 59 in the control arm) who were included in the analysis but were later found to have a negative test by FISH and an IHC score of ≤ 2 by the central laboratory, had a non-significant benefit from the addition of trastuzumab (HR 0.51, $p = 0.13$). In this analysis, 174 patients (9.7% of cases) were found by central testing to be negative for *HER2* gene amplification as well as having 'normal' expression of the HER2 protein (IHC 1+ and 2+) (Paik et al. 2007). Despite this fact, this subset of patients had a more than 50% benefit from the addition of trastuzumab to chemotherapy, which was statistically significant. In summary, our results support the role of Her-2/neu in acquired hormone resistance. Over 50% of patients in this analysis had Her-2/neu expression of 1+ or greater. Based on preclinical data and the recent analysis of NSABP B-31 suggesting that Her-2/neu-directed therapy may be effective in tumours with minimal to moderate Her-2/neu protein expression, these patients could benefit from treatment with trastuzumab or lapatinib. We are currently evaluating this hypothesis in a clinical trial.

Acknowledgements

In memory of Leslie Diaz. This work was supported by the Lynn Sage Foundation (Chicago, IL) and the Lynn Sage Scholar Award.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Broom RJ, Tang PA, Simmons C, Bordeleau L, Mulligan AM, O'Malley FP, Miller N, Andrulis IL, Brenner DM, Clemons MJ. (2009). Changes in estrogen receptor, progesterone receptor and Her-2/neu status with time: discordance rates between primary and metastatic breast cancer. Anticancer Res 29:1557–62.

Creighton CJ, Massarweh S, Huang S, Tsimelzon A, Hilsenbeck SG, Osborne CK, Shou J, Malorni L, Schiff R. (2008). Development of resistance to targeted therapies transforms the clinically associated molecular profile subtype of breast tumor xenografts. Cancer Res 68:7493–501.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG). (2005). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687–717.

Gancberg D, Di LA, Cardoso F, Rouas G, Pedrocchi M, Paesmans M, Verhest A, Bernard-Marty C, Piccart MJ, Larsimont D. (2002). Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. Ann Oncol 13:1036–43.

Gong Y, Booser DJ, Sneige N. (2005). Comparison of HER-2 status determined by fluorescence *in situ* hybridization in primary and metastatic breast carcinoma. Cancer 103:1763–9.

Lipton A, Leitzel K, Ali SM, Demers L, Harvey HA, Chaudri-Ross HA, Evans D, Lang R, Hackl W, Hamer P, Carney W. (2005). Serum HER-2/neu conversion to positive at the time of disease progression in patients with breast carcinoma on hormone therapy. Cancer 104:257–63.

Masood S, Bui MM. (2000). Assessment of Her-2/neu overexpression in primary breast cancers and their metastatic lesions: an immunohistochemical study. Ann Clin Lab Sci 30:259–65.

Nicholson RI, Staka C, Boyns F, Hutcheson IR, Gee JM. (2004). Growth factor-driven mechanisms associated with resistance to estrogen deprivation in breast cancer: new opportunities for therapy. Endocr Relat *Cancer*11:623–41.

Normanno N, Di MM, De ME, De LA, De MA, Giordano A, Perrone F. (2005). Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. Endocr Relat Cancer 12:721–47.

O'Regan RM, Osipo C, Ariazi E, Lee ES, Meeke K, Morris C, Bertucci A, Sarker MA, Grigg R, Jordan VC. (2006). Development and therapeutic options for the treatment of raloxifene-stimulated breast cancer in athymic mice. Clin Cancer Res 12:2255–63.

Paik S, Kim C, Jeong J, Geyer CE Jr, Romond EH, Mejia-Mejia O, Mamounas EP, Wickerham D, Costantino JP, Wolmark N. (2007). Benefit from adjuvant trastuzumab may not be confined to patients with IHC 3+ and/or FISH-positive tumors: central testing results from NSABP B-31. Am Soc Clin Oncol 25 (Suppl. 18):abstr 511.

Perez EA, Romond EH, Suman VJ, Jeong J, Davidson NE, Geyer CE Jr, Martino S, Mamounas EP, Kaufman PA, Wolmark N. (2007). Updated results of the combined analysis of NCCTG N9831 and NSABP B-31 adjuvant chemotherapy with/without trastuzumab in patients with HER2-positive breast cancer. Am Soc Clin Oncol 25 (Suppl. 18S):abstr 512.

Yee D, Lee AV. (2000). Crosstalk between the insulin-like growth factors and estrogens in breast cancer. J Mammary Gland Biol Neoplasia 5:107–15.

Zidan J, Dashkovsky I, Stayerman C, Basher W, Cozacov C, Hadary A. (2005). Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. Br J Cancer 93:552–6.