HER-2/neu overexpression in invasive ductal breast cancer – an association with other prognostic and predictive factors

Tatjana Ivković-Kapicl[1], Slavica Knežević-Ušaj[1], Milana Panjković[2], Dragana Đilas-Ivanović[1], Mileta Golubović[3]

SUMMARY

Background: *HER-2/neu is a proto-oncogene that is amplified/overexpressed in 15 to 30% of invasive breast cancers. The purpose of this study was to determine if any relationship exist between HER-2/neu protein overexpression and estrogen receptor (ER), progesterone receptor (PR), grade, size, and lymph node status in female breast cancer.*

Methods: *A total of 100 cases of invasive ductal breast cancer were included in this study. The hormone receptors and HER-2/neu were studied immunohistochemically (IHC). Using the HER-2/neu DAKO scoring system, scores of 0, 1+ and 2+ were defined as negative and 3+ as positive.*

Results: *HER-2/neu protein overexpression was seen in 20 (20%) of cases. HER-2/neu protein overexpression was present in 4 of 52 T1 lesions (8%), in 11 of 37 T2 lesions (30%), in 3 of 6 T3 lesions (50%), and in 2 of 5 T4 lesions (40%), ($p<0.05$). Protein overexpression was seen in 7 of 17 grade III tumors (41%), and 13 of 61 grade II tumors (21%). Overexpression was not detected in grade I tumors ($p<0.01$). Of the 20 Her-2/neu positive cases, ER- and PR-negative status was detected in 60% and 70%, respectively.*

Conclusion: *Statistically significant correlation was found between HER-2/neu protein overexpression and large tumor size, high histological grade, and ER-, PR-negativity. There was no correlation with lymphonodal status.*

Key words: *Breast Neoplasms; Carcinoma, Ductal, Breast; Receptor, erbB-2; Neoplasm Invasiveness; Immunohistochemistry; Proto-Oncogene Proteins; Gene Expression Regulation, Neoplastic*

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[1]Oncology Institute of Vojvodina, Sremska Kamenica, Serbia, [2]Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia, [3]Clinical Center Montenegro Podgorica, Montenegro

Correspondence to: Asist. Dr. Tatjana Ivković-Kapicl, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia, Institutski put 4, 21204 Sremska Kamenica, Serbia

kapicl@Eunet.yu

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INTRODUCTION

The HER-2 gene encodes a 185 kDa transmembrane phosphoglycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family. Her-2/neu (c-erbB-2) gene amplification, which usually results in overexpression of the encoded transmembrane protein, occurs in approximately 15 to 30% of invasive breast cancers (1-6). There are several possible uses of HER2 status. Many studies have shown that HER-2/neu overexpression is an adverse prognostic factor (2,7-9). Her-2/neu overexpressing tumors were shown to increase disease recurrence and metastasis, and shorten survival. The overexpression of Her-2/neu protein and amplification of the Her-2/neu gene is also associated with poor prognostic tumor characteristics such as high histologic grade, high proliferative index, negative or lower estrogen receptor (ER) expression, lymphoid infiltration, p53 mutation, absence of bcl-2, and absence of lobular histology (10-13). Thus, HER2 status might be incorporated into a clinical decision, along with other prognostic factors, regarding whether to give any adjuvant systemic therapy.

Her-2/neu status is also predictive for several systemic therapies. In this regard, HER2 positivity appears to be associated with relative resistance to endocrine therapies in general. Although controversial, preclinical and clinical studies have suggested that this effect may be specific to selective estrogen receptor modulator therapy such as tamoxifen. HER2 status also appears to be predictive for either resistance or sensitivity to different types of chemotherapeutic agents (14,15).

Perhaps most importantly, several studies have now shown that agents that target HER2 are remarkably effective in both the metastatic and adjuvant settings. Trastuzumab, a humanized monoclonal antibody, offers an extra treatment option, in monotherapy, and also in combination with hormonal agents, and with chemotherapy in doublets and triplets in women whose tumors are strongly positive for HER-2/neu (16).

HER2 testing should be routinely performed in patients with a new diagnosis of invasive breast cancer. However, controversies still exist about the best assay in assessing HER-2/neu status. Several techniques are available for the assessment of Her-2 status in patients with breast cancer. The most practical to perform in the routine practice of pathology are immunohistochemistry (ICH) to assess HER-2 protein overexpression, fluorescence in situ hybridization (FISH) to assess gene amplification, and chromogenic in situ hybridization (CISH) which allows detection of gene amplification too. IHC is the most frequently used technique in reporting HER-2/neu status, even though it is affected by many technical variables (3,10,17,18).

The most of the prospective randomized adjuvant trials of trastuzumab, have been proposing the use of an algorithm to optimize and simplify the assessment of HER-2/neu status, suggesting that FISH or CISH should be reserved for those cases with 2+ staining by IHC (5,10,18).

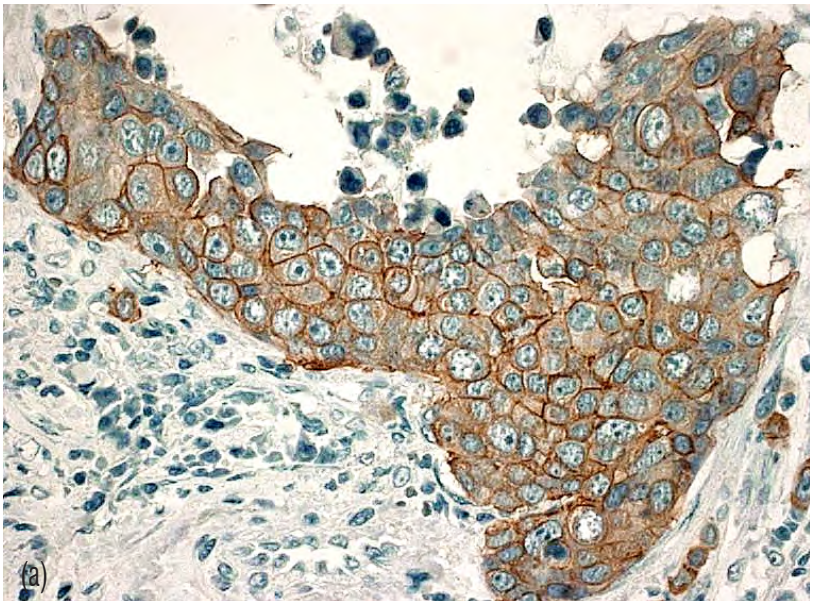
The objective of our study was to understand better the relationship between Her-2 status and ER and PR expression, histologic grade, tumor size, and lymph node status in invasive ductal breast carcinomas.

METHODS

We studied 100 patients with ductal invasive breast carcinoma who underwent total mastectomy or lumpectomy with axillary dissection between 2000 and 2003, at the Oncology Institute of Vojvodina, Sremska Kamenica. The pathology report was used to determine lymph node status, and pathologic size of the invasive tumor component. The histological grading was performed using the modified criteria of Bloom and Richardson, as described by Elston and Ellis (19,20).

All immunohistochemical studies of HER-2/neu, ER and PR, were performed

on 4-mm sections of formalin-fixed, paraffin-embedded tissues after antigen retrieval procedure, as described in our previous study (21). IHC staining for ER and PR was carried out according to the LSAB2 method using DAKO, N-series primary monoclonal antibodies. ER and PR positivity was defined as nuclear staining in more than 10% of tumor cells (10,22). The percentage of positive cells was semiquantified manually. HER2 protein expression was detected using DAKO HercepTest. Her-2/neu was scored on a 0 to 3 scale according to the criteria set by DAKO. Positive IHC staining was defined as strong membrane staining in more than 10% of the tumor cell population, whereas weak to moderate staining, and faint membrane staining in more than 10% of the tumor cells, as well as membrane staining in less than 10% of the tumor cell population and cytoplasmic staining were considered to be negative (Figure 1) (18). Fluorescence in situ hybridization (FISH) was not performed on the weak positive cases (score 2) in this study.



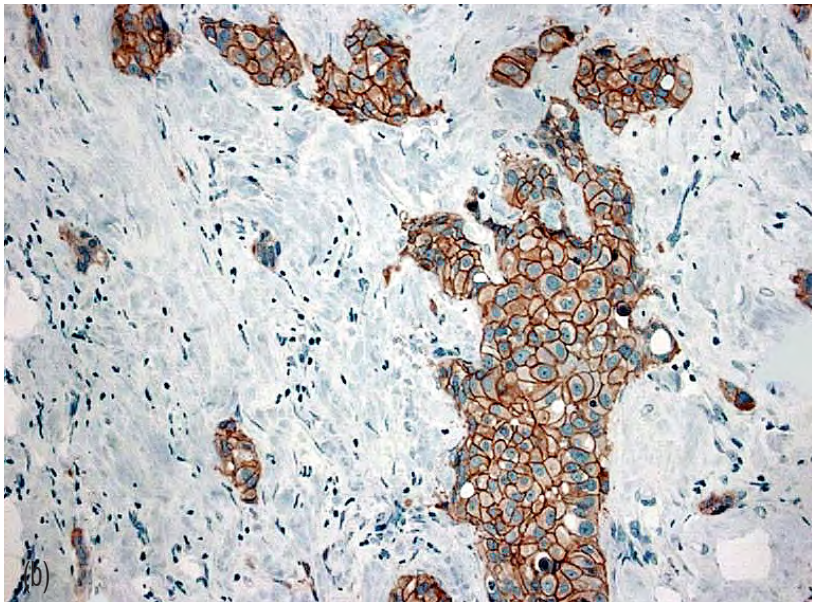


Figure 1. Microscopy pictures ilustrating the patterns of Her-2 immunostaining in breast carcinoma. (a) Intermediate (2+) pattern, showing weak to moderate complete membrane staining in more than 10% tumos cells; B-SA, x 400 (b) Strongly positive (3+) pattern shows intense membrane staining in more than 10% of the tumor cells; B-SA, x 200

For each run of staining, positive and negative control slides were also prepared.

The Student's t-test was used for comparison of mean tumor size for each category of cases. The chi square test was used to examine the categorical variables and the association between HER-2/neu status and other clinicopathological variables. The results were considered statistically significant if the p value was < 0.05. All statistical analyses were performed with SPSS software version 11.0.1 for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Table 1 summarizes the clinicopathological features of all 100 women with operable ductal invasive breast carcinoma. Table 2 shows data for DAKO score 3+ versus 0, 1+, or 2+ cases. Overall HER-2 protein overexpression was seen in 20 cases (20%). HER-2/neu protein overexpression was present in 4 of 52 T1 lesions (8%), in 11 of 37 T2 lesions (30%), in 3 of 6 T3 lesions (50%), and in 2 of 5 T4 lesions (40%), ($p<0.05$, chi-square test). Tumors with strong Her-2 expression tended to be larger than those lacking overexpression, with mean sizes of 3.48 (1.66) cm and 2.27 (1.24) cm, respectively ($p<0.01$, Student's t test). Protein overexpression was seen in 7 of 17 grade III tumors (41%), and 13 of 61 grade II tumors (21%). Overexpression was not detected in grade I tumors ($p<0.01$, chi-square test). A positive correlation was detected between the grade of the tumors and HER-2 protein overexpression ($r=0.36$, $p<0.01$). Of the 20 Her-2/neu positive cases, ER- and PR-negative status was detected in 60% and 70%, respectively. On the other hand, ER- and PR-negative carcinomas were Her-2/neu positive in 44 and 41%, respectively. In this study, Her-2/neu protein overexpression was associated with a statistically significant higher rate of ER- and PR-negative status ($p<0.01$, chi-square test). A negative correlation between Her-2 expression and ER and PR was noted ($r=-0.37$ and $r=-0.35$ respectively, $p<0.01$).

Table 1. Clinicopathological characteristics in infiltrating ductal breast carcinoma specimens

Clinicopathological features (n=100)			
		N	%
Tumor size	T1b	13	(13%)
	T1c	39	(39%)
	T2	37	(37%)
	T3	6	(6%)
	T4	5	(5%)
Tumor grade	HG 1	22	(22%)
	HG 2	61	(61%)
	HG 3	17	(17%)
Lymph node	negative	52	(52%)
	positive	48	(48%)
HER-2/neu*	negative	80	(80%)
	positive	20	(20%)
ER	negative	27	(27%)
	positive	73	(73%)
PR	negative	34	(34%)
	positive	66	(66%)

ER, estrogen receptor; PR, progesterone receptor
* HER-2/neu was defined as negative when the DAKO score was 0, 1+ or 2+, and positive when 3+

Table 2. Association between HER-2/neu protein overexpression and pathologic characteristics in infiltrating ductal breast carcinoma specimens

Clinicopathological features	HER-2/neu negative n=80	HER-2/Neu positive n=20	p Value
Lymph node			
N0	41	11	**NS**
N1+N2	39	9	
Tumor size			
T1b	11	2	**p<0.05**
T1c	37	2	
T2	26	11	
T3	3	3	
T4	3	2	
Tumor grade			
G1	22	0	**p<0.01**
G2	48	13	
G3	10	7	
ER			
negative	15	12	**p<0.01**
positive	65	8	
PR			
negative	20	14	**p<0.01**
positive	60	6	

In summary, in this study of 100 cases of infiltrating ductal breast carcinoma in which Her-2/neu protein overexpression by IHC was performed, a statistically significant association was established between Her-2/neu protein overexpression and large tumor size, high histologic grade, and absent ER and PR receptors.

DISCUSSION

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior. Clinical and pathological variables such as tumor size, histologic grade, histologic type, lymph node metastases, vascular space invasion, tumor cell proliferation, tumor necrosis, extent of ductal carcinoma in situ, and age may help in predicting prognosis and the need for adjuvant therapy. Newer prognostic factors and predictors of responses to therapy are needed, however, to distinguish subgroups with different biological features within carcinomas that otherwise appear homogenous according to classic pathological and clinical criteria. ER, PR, and HER2 represent the most acceptable factors for predicting prognosis, response or resistance to treatment, and the potential use of newer drugs such as trastuzumab in the case of HER2 overexpression (23).

In this study, we found that 20 (20%) of 100 cases were Her-2 positive. Although there is a wide variation in Her-2 overexpression and amplification, our figure appears to be within the commonly accepted rate of 15 to 30% (5,6).

Our study, as well as others, did not find a significant association between Her-2/neu overexpression and positive axillary lymph nodes (11,13,24,25).

Tumor size is one of the most useful predictors of tumor behavior in breast cancer. Our results show a tendency of Her-2 overexpression to be more associated with larger tumor size (p<0,05). The higher rates of Her-2 overexpression in larger tumors size have been also documented in some previous studies (26). On the other hand, Her-2/neu overexpression was not found to be associated with large tumor size by some other studies (11-13).

Her-2 amplification/overexpression in different histologic grades of female breast cancer has traditionally been a subject of interest. In a study of Hoff et al., higher-grade tumors were more likely to demonstrate Her-2/neu amplification than lower grade ductal carcinomas (p < 0.001) (27). Similarly, other studies have also reported that histologic high-grade tumors are associated with an increased rate of Her-2/neu positivity (11-13,24,25). Her-2/neu was also found to correlate with high nuclear grade (28). In this study of 100 cases, the majority of these Her-2/neu protein overexpressed infiltrating ductal carcinomas were high histologic grade (p<0.01).

Estrogen and progesterone receptor determination are established procedures in the routine management of patients with breast cancer, primarily as predictive factors for response to therapeutic and adjuvant hormonal therapy (17,23,29). The inverse association between HER-2/neu and hormone receptors leads to lower or absent hormone receptors in women with HER-2/neu positive breast cancer (11-13,24). This is one of the reasons why women who overexpress HER-2/neu may be resistant to tamoxifen. In our study, Her-2/neu protein overexpression was associated with a statistically significant higher rate of ER- and PR-negative status (p< 0.01). However, as also demonstrated in this study and others, ER-positive cases can have Her-2/neu overexpression/amplification. ER-positive, Her-2/neu -positive tumors have a poorer disease free and overall survival than ER-positive, Her-2/neu–negative tumors, suggesting that Her-2/neu overexpression/amplification may be a better predictor of response to tamoxifen therapy than ER status alone (10). Huang HJ et al. reported that in women with ER-positive tumors, the expression of PR affects the likelihood of HER-2/neu overexpression, and it may be that women with ER+PR- tumors should be targeted with more aggressive treatment than those with ER+PR+ tumors (13). An interesting article that analyzed the association between Her-2/neu and hormone receptor status in breast cancer showed that the amplification/overexpression of this oncogene was associated with lower ER/PR levels since they were analyzed as continuous rather than dichotomous variables. The authors reported that whenever tumors were positive for both hormone receptors and Her-2/neu, the levels of ER/PR were significantly lower than tumors with nonelevated Her-2/neu expression (30). The recent understanding of the molecular basis of breast cancer growth and progression led to the identification of tumor subtypes with potentially different biologic behavior: luminal, Her-2 overexpressing and basal-like breast carcinomas. The tumors of the basal subtype express the basal cytokeratins; they are histologically poorly differentiated and ER-negative/PR-negative/Her-2-negative. This group presents a therapeutic challenge for the oncologist. Siziopikou et al. reported that the majority of the "triple negative" patients have basal subtype tumors with high EGFR expression and that these tumors may be the subgroup of breast carcinomas that could potentially benefit the most from novel EGFR-targeted therapeutic strategies (31,32).

CONCLUSION

Her-2/neu status in breast cancer is important because it provides valuable prognostic, predictive, and therapeutic information. The association of

Her-2/neu with additional prognostic factors has always been of interest. In this study of 100 cases of infiltrating breast carcinomas in which Her-2/neu by IHC was performed, Her-2/neu protein overexpression was seen in 20 cases (20%). There was no significant association with lymphonodal status. However, there was a significantly higher rate of Her-2/neu protein overexpression in tumors with large size, high histologic grade, and negative ER/PR status.

Conflict of interest

We declare no conflicts of interest.

REFERENCES

1 Chang E, Lee A, Lee E, Lee H, Shin O, Oh S, Kang C. HER-2/neu oncogene amplification by chromogenic in situ hybridization in 130 breast cancers using tissue microarray and clinical follow-up studies. *J Korean Med Sci* 2004;19:390-6.

2 Ross SJ, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *The Oncologist* 1998;3:237-52.

3 Schnitt SJ. Breast cancer in the 21st century: neu opportunities and neu challenges. *Mod Pathol* 2001;14(3):213-8.

4 Yarden Y, Baselga J, Miles D. Molecular approach to breast cancer treatment. *Semin Oncol* 2004;31:6-13.

5 Perez E A, Pusztai L, van De Vijver M. Improving patient care through molecular diagnosis. *Semin Oncol* 2004;31:14-20.

6 Piccart MJ, Mano M, Lohrisch C, Di Leo A. Herceptin for the treatment of breast cancer: what we know-and what we have yet to learn. *Cancer Futures* 2002;1:73-9.

7 Paik S, Hazan R, Fisher ER, Sass RE, Fisher B, Redmond C, et al. Pathologic findings from the nations' surgical adjuvant breast and bowel project: prognostic significance of erb B2 protein overexpression in primary breast cancer. *J Clin Oncol* 1990;8:103-12.

8 Xing W-R, Gilchrist KW, Harris CP. FISH detection of HER-2/neu oncogene ampflication id early onset breast cancer. *Breast Cancer Res Treat* 1996;39:203-12.

9 Tetu B, Brisson J. Prognostic significance of Her-2/neu oncogene expression in node-positive breast cancer. The influence of the pattern of immunostaning and adjuvant therapy. *Cancer* 1994;73:2359-65.

10 Ferrero-Pous M, Hacene K, Bouchet C, Le Doussal V, Tubiana-Hulin M, Spyratos F. Relationship between c-erbB-2 and other tumor characteristics in breast cancer prognosis. *Clin Cancer Res* 2000;6:4745-54.

11 Ariga R, Zarif A, Korasick J, Reddy V, Siziopicou K, Gattuso P. Correlation of Her-2/neu gene ampflication with other prognostic and predictive factors in female breast carcinoma. *Breast J* 2005;11:278-80.

12 Prati R, Apple SK, He J, Gornbei JA, Chanh HR. Histopathologic characteristics predicting HER-2/neu amplification in breast cancer. *Breast J* 2005;11:433-9.

13 Huang H J, Neven P, Drijkoningen M, Paridaens R, Wildiers H, Van Limberger E, et al. Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. *J Clin Pathol* 2005;58:611-6.

14 Paik S, Brayant J, Park C, Fisher B, Tan-Chiu E, Hyams D, et al. ErbB-2 and response to doxorubicin in patients with axillary lymph-node positive, hormone receptor-negative breast cancer. *J Natl Cancer Inst* 1998;90:1361-70.

15 Carlomagno C, Perone F, Gallo C, De Laurentiis M, Lauria R, Morabito A, et al. C-erbB-2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lympph node metastasis. *J Clin Oncol* 1996;14:2702-8.

16 Bell R, Verma S, Untch M, Cameron D, Smith I. Maximizing clinical benefit with trastuzumab. *Semin Oncol* 2004;31:35-44.

17 Bast RC, Ravdin P, Hayes DF, Bates S, Fritsche H, Jessup JM, et al. 2000 update of recommendations for use of tumor markers in breast and colorectal cancer: clinical practice guidelines of American Society of Clinical Oncology. *J Clin Oncol* 2001;19:1865-78.

18 Wollf AC, Hammond EH, Schwartz JN, Hagerty KL, Allerd C, Cotte RJ. American society of clinical oncology/college of american pathologists guideline recommendations for human epidermal growth factor receptro 2 testing in breast cancer. *J Clin Oncol* 2007;25:118-45.

19 Elston C, Ellis I. Assessment of histological grade. In: Symmers W, editor. The Breast, 2nd ed. New York: Churchill Livingstone Company; 1998. p. 365-84.

20 Tot T, Tabar L, Dean P. Practical Breast Pathology. 1st ed. Thieme Medical Publishers; 2002. p. 125-35.

21 Ivković-Kapicl T, Knežević-Ušaj S, Panjković M, Mastilović K. An immunohistohemical analysis of angiogenesis in invasive ductale breast carcinoma with correlations to clinicopathologic factors. *Vojnosanit Pregl* 2006;6(1):139-46.

22 R Xu, Feiner H, Li P, Yee H, Inghirami G, Delgado Y, Perle M. Differential ampflication and overexpression of HER-2/neu, p53, MIB1, and estrogen receptor/progesteron receptor among medullary carcinoma, atypical medullary carcinoma, and high-grade invasive ductal carcinoma of the breast. *Arch Pathol Lab Med* 2003;127:1458-64.

23 Schnitt SJ. Traditional and newer pathologic factors. *J Natl Cancer Inst Monogr* 2001;30:22-6.

24 Almasri NM, Al Hamad M. Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. *Breast Cancer Res* 2005:7;598-604.

25 Bozcuk H, Uslu G, Pestereli E, Samur M, Ozdogan M, Karaveli S, et al. Predictor of distant metastasis at presentation in breast cancer: a study also evalueting associations among common biological indicators. *Breast Cancer Res Treat* 2001;68:239-48.

26 Bhatavdekar JM, Patel DD, Shah NG, Vora HH, Suthar TP, Chikhlikar PR, et al. Prognostic significance of immunohistochemically localized biomarkers in stage II and stage III breast cancer: a multivariate analysis. *Ann Surg Oncol* 2000;7:305-11.

27 Hoff ER, Tubbs RR, Myles JL, Procop GW. HER-2/neu amplification in breast cancer. Stratification by tumor type and grade. *Am J Clin Pathol* 2002;49:110-3.

28 Berger MS, Locher GW, Saurer S, Gullick WJ, Waterfiels MD, Groner B, et al. Correlation of c-erbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res* 1998;48:1238-43.

29 Donegan WL. Tumor-related prognostic factors for breast cancer. *Ca Cancer J Clin* 1997;47:28-51.

30 Konecny G, Pauletti G, Pegram M, Untch M, Dandekar S, Aguilar Z, et al. Quantitative association between HER-2/neu and steroid hormone receptrors in hormone receptor-positive primary breast cancer. *J Natl Cancer Inst* 2003;95:142-53.

31 Siziopikjou KP, Cobleigh M. The basal subtype of breast carcinomas may represent the group of breast tumors that could benefit from EGFR-targeted therapies. *Breast* 2007;16:104-7.

32 Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367-74.