

## HER-2 Gene Amplification and Overexpression as a Target for Therapy of Breast Cancer Patients

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The human epidermal growth factor receptor type 2 (HER-2), also known as c-erbB-2 or *neu*, is amplified and overexpressed in approximately 20% to 30% of breast cancers. This somatic genetic alteration is associated with shorter disease-free and overall survival (1, 2, 5, 10) and is a target for treatment with the anti-HER2 antibody trastuzumab (Herceptin)(8, 11, 12) as well as tyrosine kinase inhibitors such as lapatinib.

The importance of HER-2 amplification/overexpression as a molecular marker in the clinical management of women with breast cancer, especially in the adjuvant setting, has focused more attention on the assessment of this alteration in the pathology laboratory. Two different immunohistochemical assay methods and one fluorescence *in situ* hybridization (FISH) assay method have been approved by the U.S. Food and Drug Administration for the selection of patients for trastuzumab therapy. Although these assay methods are significantly correlated with one another in almost all published comparisons including our own (9, 10), there are some important considerations related to differences between these assay methods in selecting patients for treatment (6, 7).

Both as a single agent (8) and in combination with conventional chemotherapy, clinical trials of trastuzumab have demonstrated an improved response rate or improved overall survival of women with HER-2-positive metastatic breast cancer. Subsequent clinical trials of trastuzumab in the adjuvant setting conducted by the NSABP/Intergroup and the Breast International Group (BIG) have shown longer disease-free survival, longer time to recurrence, fewer distant metastases and longer overall survival in the trastuzumab-treated group. More recently, the Breast Cancer International Research Group 006 clinical trial of trastuzumab in the adjuvant setting compares a standard anthracycline-containing chemotherapy (AC -> T) treatment arm with a standard anthracycline-containing chemotherapy plus trastuzumab arm (AC -> TH) and with a synergistic chemotherapy plus trastuzumab (TCH) treatment arm (n=3,222)(12). An interim analysis of clinical follow-up demonstrates a disease-free survival and overall survival advantage for both trastuzumab-containing treatment arms compared to standard anthracycline-containing chemotherapy. In addition, the two trastuzumab-containing treatment arms are not significantly different from one another. The findings raise questions about the need for anthracycline-containing chemotherapy in HER-2-positive early stage invasive breast cancer, especially when cardiac toxicity and development of secondary malignancies are considered (12).

An additional issue raised in analysis of the BCIRG 006 clinical trial is the potential for a second gene, topoisomerase II-alpha, also located on the long arm of chromosome 17 to be co-amplified along with HER-2 and to be associated with responsiveness to anthracycline-containing chemotherapy. Although HER-2 gene amplification and overexpression has been suggested to predict responsiveness to anthracycline-containing chemotherapy, the protein product of the topoisomerase II-alpha gene is the known target for this drug. Therefore, topoisomerase II-alpha gene co-amplification has been compared with responsiveness to anthracycline-containing chemotherapy in the first interim analysis of BCIRG 006 clinical trial study subjects to evaluate this association. Among all women entered in the HER-2-positive BCIRG 006 clinical trial, as well as among women who were treated with anthracycline-containing chemotherapy alone, women whose breast cancers showed TOP2A gene co-amplification had a significantly longer disease-free, recurrence-free and overall survival compared to women whose breast cancers lacked TOP2A amplification. In patients treated with

chemotherapy alone the findings demonstrate that TOP2A gene co-amplification is a potentially useful predictive marker of responsiveness to anthracycline-containing chemotherapy.

Characterization of molecular genetic alterations in human breast cancers provide a target for development of therapies that specifically interfere with the function of these genetic alterations and interfere with the expression of a malignant phenotype. Given the large number of genetic alterations in human breast cancers, it is anticipated that additional targeted therapies will be developed for this disease.

### Learning Objectives

At the completion of this lecture it is expected that participants will be able to address the following learning objectives:

1. Summarize clinical utility of HER-2 gene amplification and overexpression in breast cancer.
2. Discuss advantages and disadvantages of HER-2 testing methods.
3. Describe clinical studies supporting trastuzumab as an efficacious drug for the adjuvant treatment of HER-2-positive breast cancer patients.
4. Assess preliminary results suggesting topoisomerase II-alpha gene amplification is a potentially useful predictive marker of responsiveness to anthracycline-containing chemotherapy.

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**E-Cadherin in Breast Carcinoma:  
Is It Necessary or Even Helpful?  
- Time Will Tell -**

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Lobular carcinoma in situ (LCIS) was initially described in 1941 by Foot and Stewart (AJP 1941;17:491-496), and in 1952 Godwin (Cancer 1952;259-266) described a case of LCIS evolving to invasive lobular carcinoma (ILC). Hence, lobular carcinoma has been with us in the literature for at least 6 decades and is a well-defined clinicopathologic lesion easily recognized by competent experienced surgical pathologists. Likewise, invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS) have a long history and are well-defined lesions. Yet, examples of invasive breast carcinoma and/or CIS occur that show mixed, intermediate, or overlapping features between lobular and other forms of breast carcinoma, especially ductal carcinoma. A test to help distinguish these lesions might be of benefit, but first, is it that clinically important to separate lobular from ductal carcinoma?

The distinction between ductal and lobular breast carcinoma supposes important clinicopathologic implications. Indeed, conventional wisdom states that patients with lobular carcinoma have an increased risk for bilateral disease (~1/3rd [with reported rates varying widely depending upon how thoroughly the data were collected and tabulated]), but also, ductal carcinoma has a significant risk of bilateral disease (~1/6<sup>th</sup>). The related phenomenon of "true" ipsilateral multicentricity is reported to be ~2/3rds for LCIS and up to ~1/3rd for DCIS (Blair & Copland. The Breast, Chap 35, WB Saunders, 1991). Hence, DCIS is considered amenable to local-regional therapy (surgical excision + radiation); whereas LCIS is not, because of its greater risk of multicentricity and bilaterality.

Yet, recent data reported by Ottesen (JASP 17:14-21,1993) provide additional insight into the significance of LCIS. They reported results of a follow-up study (median follow-up 61 months) of 69 patients with LCIS and 19 patients with LCIS + DCIS that had been treated with excision only. Of the pure LCIS cases, 20% showed cellular heterogeneity with a component of both small and large nuclei. The DCIS lesions showed clinging, solid, cribriform, and/or papilliferous types. This mixture prompted the authors to state that "the demonstration of LCIS in combination with DCIS in 19 of the 88 original lesions (22%) makes a theory of coincidence unlikely, but rather seems to indicate a relation between the two. Supporting this view, the components in several cases were found close together, sometimes merging, with the individual cells being hardly distinguishable in the junctional zone...". Seventeen percent (13 in the LCIS group and 2 in the LCIS + DCIS group) recurred (refinding of LCIS was not considered recurrence), and all recurrences were ipsilateral. In fact, the distribution of the recurrences within the

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breast among the LCIS and LCIS + DCIS groups were not significantly different. Roughly, 50% of the recurrences were invasive carcinomas. Furthermore, the frequency of recurrence was significantly associated with the number of lobules involved by LCIS and the nuclear size of the LCIS cells. LCIS lesions with less than 10 lobules involved by LCIS and small nuclear size had a 7% recurrence rate, whereas, there was a 41% recurrence rate when more than 10 lobules were involved by LCIS and the lesion contained large nuclei. Cases of LCIS with mixtures of small and large nuclei were included among those with large nuclei only. The authors illustrated LCIS with and without large cells, although they did not provide strict definitions or actual dimensions for separating small-cell from large-cell examples of LCIS.

Nonetheless, this presenter believes that in cases where CIS displays features of ductal and lobular types, a diagnosis of DCIS should be favored to encourage adequate local excision of the lesion. Also, mention should be made of the concomitant LCIS patterns to emphasize the possible greater risk of bilateral breast carcinoma. This will encourage adequate follow-up studies of the contralateral breast. That intermediate forms of CIS exist is no surprise, because the terminal duct lobular unit complex (lobule) is the site of origin not only of LCIS but also the bulk of DCIS.

The growth pattern of ILC is often different from that of IDC – that is, ILCs often show skip invasive lesions (multifocality) and a more diffuse (non scirrhous) patterns within the breast. Hence, ILC would seem less amenable local surgical excision, but direct comparison of the effectiveness of lumpectomy + radiation between ILC vs IDC have shown no differences in outcome (Eur J Cancer 28:660-666, 1992).

These clinicopathologic correlations are based on traditional morphologic definitions of lobular and ductal carcinoma, and introducing a new test to define these differences (i.e., presence or absence of E-cadherin staining) may or may not help, especially if the intermediate or mixed staining patterns. Our experience with differential E-cadherin staining holds true for classic examples of lobular carcinoma (E-cadherin negative) and other breast carcinoma types – that is, usually invasive ductal (E-cadherin positive) for both the invasive and in situ lesions. However, like others, we've found that tumors with intermediate or mixed features have shown intermediate or mixed staining (Am J Clin Pathol 2001;115:534-42; Am J Surg Pathol 2001; 25:229-36). Hence, E-cadherin does not eliminate the subjectivity of the lobular vs. ductal distinction.

I asked UCSD's leading breast oncologist how lobular vs ductal histology would affect her patient management. She stated that she treats both invasive breast carcinomas the same, given all other prognosticators, etc. are equal. However, she said that, given invasive lobular carcinoma, she would be more likely to do contralateral breast MRI, if financial concerns also favored it – MRI is a \$2000.00 test. But, a patient with invasive ductal carcinoma may also get contralateral MRI, if the primary was hard to detect (i.e., mammogram negative) or obscured by fibrocystic changes usually also present in the opposite breast.

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in cell-cell adhesion. The function of E-cadherin in preventing metastasis in tumour development is believed to be dependent on intracellular catenins. In a previous study, the expression of E-cadherin was examined in a series of human breast carcinomas. In that study, **down-regulation of E-cadherin failed to correlate with lymph node and/or distant metastasis**. In the present study, the expression of alpha-, beta-, and gamma-catenins were examined in a subset of the same tumours in order to evaluate their possible role in breast cancer metastasis. Tumour tissues from 90 primary breast carcinomas were immunostained for alpha-, beta-, and gamma-catenins. Reduced or absent immunoreactivity in the tumour tissue was seen in 63 (70.0 per cent) for alpha-catenin, in 50 (55.6 per cent) for beta-catenin, and in 50 (55.6 per cent) for gamma-catenin. **Reduced expression of each of the catenins failed to correlate to metastasis**. However, when all of the four proteins (E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin) were analysed as one group, a significant association was seen between reduction in immunoreactivity of at least one of these four proteins and the presence of metastases. These results indicate that if one of these proteins is down-regulated, the function of the others in suppressing metastasis is altered. A significant association was seen between lobular invasive tumours and beta-catenin expression.

**3. P-cadherin expression in breast carcinoma indicates poor survival.** Cancer 1999, 86(7) p1263-72.

The cadherin family of cell-cell adhesion molecules and their associated proteins, the catenins, are essential to embryonic development and the maintenance of adult tissues. During development, the homotypic interaction of a particular cadherin with an identical cadherin expressed on a neighboring cell results in the sorting of cells to form distinctive tissues. Cadherins are believed to be tumor suppressors, and their altered expression and function have been associated with tumor development. **METHODS: The authors examined the expression of P-cadherin, E-cadherin, and N-cadherin, and alpha-catenin and beta-catenin in 183 cases of invasive breast carcinoma** by immunohistochemistry on paraffin sections using specific antibodies and a streptavidin antigen retrieval method. **RESULTS: P-cadherin** was positive in 95 cases and negative in 88 cases of breast carcinoma. **Positive P-cadherin expression in breast carcinoma showed a strong correlation with poor patient prognosis**. Five years after surgery, 90% of the patients with P-cadherin negative tumours were alive in contrast to only 59% of patients with P-cadherin positive tumours. The difference in survival reached statistical significance (P = 0.0001) as early as 2 years after surgical treatment. Expression of N-cadherin, alpha-catenin, and beta-catenin did not correlate with patient survival. Multivariate statistical analyses of the data showed that expression of P-cadherin was independent of tumor size and lymph node metastases, but correlated inversely with estrogen/progesterone receptor status. In ductal carcinomas, positive P-cadherin expression correlated with a higher histologic grade. In contrast, expression of **E-cadherin was low in high grade ductal carcinomas but negative tumours were uncommon. Negative or low E-cadherin expression did not correlate with poor survival**. In lobular carcinomas, E-cadherin expression frequently was negative or low, and P-cadherin always was negative. **CONCLUSIONS: Expression of P-cadherin in breast carcinoma is associated strongly with poor survival and constitutes an independent prognostic predictor. P-cadherin expression is a better indicator of clinical outcome than alterations in the expression of E-cadherin, N-cadherin, alpha-catenin, or beta-catenin.**

**4. Separating favorable from unfavorable prognostic markers in breast cancer: the role of E-cadherin.** Cancer Res 2000, 60(2) p298-304

Distant metastases are the major cause of morbidity and mortality in women with breast cancer. The ability to predict the metastatic proclivity is essential in choosing the optimal treatment. Tumor size and grade, which are frequently used markers in node-negative breast cancer patients, are inadequate markers for prognosis and individualized treatment design. The steps in metastatic progression include angiogenesis, invasion, and changes in adhesion characteristics. We developed a strategy for choosing biomarkers representing these steps in malignant progression to identify patients with occult metastases who will need chemotherapy and spare the women whose tumors have not developed the capacity to spread. To evaluate the added significance of E-cadherin to that of nm23-H1 and angiogenesis in determining metastatic proclivity, we used archival material from 168 node-negative breast cancer patients who were treated with mastectomy without any adjuvant chemotherapy or hormone therapy. Immunohistochemistry was used to detect E-cadherin and nm23-H1 expression, whereas angiogenesis was determined by microvessel count (MVC) after immunohistochemical staining. The median follow-up was 14 years. **We**

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**found that E-cadherin is better in identifying the poor prognosis patients**. The 14-year disease-free survival (DFS) is 84%, 80%, and 56% in patients with high, intermediate, and low E-cadherin. The worst prognosis group using nm23-H1 and MVC as biomarkers has a 14-year DFS of 62%. In this group, if E-cadherin is low, the 14-year DFS is further decreased to 44%. **Nm23-H1 and MVC are better in identifying the good prognosis patients**. The long-term DFS is ~80% if MVC is low or E-cadherin is high. **Multivariate analysis shows that E-cadherin, nm23-H1, and MVC are more significant prognostic biomarkers than tumor size or grade.**

**5. Retention of the expression of E-cadherin and catenins is associated with shorter survival in grade III ductal carcinoma of the breast.** J Pathol 2000, 193(4) p433-41

**Many studies have investigated the relationship between the E-cadherin/catenin axis and breast cancer biology, and yet, unlike the studies in other tumour systems, which have shown a relationship between down-regulation and poor survival, no clear association has emerged in breast.** Since accumulating evidence suggests that ductal carcinoma of no special type (NST) represents a diverse group of biologies, this study has focused on grade III ductal carcinoma, in order to reduce the heterogeneity of the study population. A total of 470 breast tumours were studied. Consecutive sections were labelled with antibodies which recognize E-cadherin and the arm proteins with which it interacts: alpha-, beta-, and gamma-catenin. Membrane-bound and cytoplasmic **E-cadherin and membrane-bound alpha-catenin expression were associated with a positive oestrogen receptor (ER) status, gamma-catenin with a negative ER status, and, surprisingly, all three with poor survival**. Taken together, these findings suggest that a conserved E-cadherin/catenin axis may play a part in determining disease outcome in grade III breast carcinoma.

**6. E-cadherin relates to EGFR expression and lymph node metastasis in primary breast carcinoma.** Br J Cancer 1996, 74(8) p1237-41.

Expression of the calcium-dependent cell-cell adhesion molecule E-cadherin has been examined in 187 primary breast carcinomas using an immunohistochemical technique. The pattern and extent of reactivity has been correlated with clinicopathological data including tumour type, grade and lymph node status and with other prognostic parameters including oestrogen receptor (ER) status, expression of c-erbB-2, p52 protein and epidermal growth factor receptor (EGFR). Two patterns of E-cadherin staining were observed in carcinomas, membrane reactivity and a diffuse cytoplasmic staining. A marked difference in expression of E-cadherin was observed between infiltrating lobular carcinomas (ILC) and infiltrating ductal carcinomas (IDC), the former showing complete loss of membrane staining, whereas 93% of IDC retained some level of E-cadherin expression. **IDC reactivity was not related to tumour grade but there was a significant association between reduced membrane levels of E-cadherin and the presence of lymph node metastasis, and a highly significant correlation between the presence of cytoplasmic E-cadherin and metastasis**. A significant relationship was also demonstrated between reduced E-cadherin reactivity and expression of EGFR. These findings emphasise the complexity of control of E-cadherin in breast carcinomas and provide evidence of a link between membrane signalling pathways and modulation of E-cadherin expression.

**7. An immunohistochemical examination of the expression of E-cadherin, alpha- and beta/gamma-catenins, and alpha2- and beta1-integrins in invasive breast cancer.** J Pathol 1999, 187(5) p523-9

**This study examines the expression of the cell-cell adhesion molecules E-cadherin and its associated protein, the catenins and the matrix-cell adhesion molecules beta1- and alpha2-integrins, in primary invasive breast carcinoma.** Expression was assessed immunohistochemically on frozen sections by semi-quantitative scoring of the intensity and proportion of immunoreactivity in 55 cases. Associations with each other and with other histological and prognostic features were sought. There was a significant association between loss of E-cadherin expression and loss of alpha- and beta/gamma-catenin immunostaining. In 20 per cent of cases, membranous immunoreactivity with E-cadherin antibody was absent. Absent cytoplasmic expression of alpha- and beta/gamma-catenins was seen in 24 and 22 per cent of breast cancers, respectively. The intensity of reactivity with E-cadherin showed a significant association with histological grade (p=0.002) and tumour type (p<0.001). Lobular carcinomas frequently showed loss

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Moreover, if we are to employ a new test to define a cancer type, one should evaluate the test's ability to separate the cancers into clinically meaningful subtypes – that is, cancer types having significantly different survivals and/or therapies. On cannot automatically and arbitrarily assign the decades of morphologically derived data for lobular carcinoma to all E-cadherin negative tumours. The clinical value of E-cadherin staining itself must be evaluated (E-cadherin positive vs. E-cadherin negative carcinomas) and the findings compared to other traditional measures. This is where the added value of this test should be found. Indeed, some studies are emerging to this end. The results appear a bit confusing, more complex than initially anticipated, and contradictory. (Unfortunately, contradictory findings in this field usually auger "poor overall survival" for an ostensible prognostic factor.) I have "abstracted" a many of these abstracts for your perusal in the appendix to my handout. You can read and decide yourself, if E-cadherin adds significant prognostic information and is worth the added cost of performing the test.

Finally, if we are to use an IHC test to make important diagnostic distinctions with critical prognostic and/or therapeutic implications, we must standardize methods to get reproducible results between labs. Thus far, I know of no consensus and/or standardized method for doing E-cadherin. As with other IHC tests, different labs often obtain different results – why should this inter-laboratory variation be different for E-cadherin?

**References:**

**1. The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer.** Int J Oncol 2001;18(3) p513-20.

Loss of E-cadherin and catenin expression may be associated with distant and lymph node metastases in breast cancer. Heterogeneity of E-cadherin expression is associated with poor prognosis, suggesting that E-cadherin and catenins may serve as useful prognostic markers for invasive breast carcinoma. **Reduction or loss of expression of either E-cadherin or catenin is associated with invasion, metastasis and poor prognosis in several types of human malignancies.** We investigated the expression of E-cadherin and alpha- and beta-catenins by immunohistochemistry in 171 cases of primary invasive breast cancer, and compared the expression with clinicopathological parameters to define the relationship between expression and prognosis. E-cadherin immunoreactive protein was shown to be expressed in 97 of cases. Reduction or lack of expression of E-cadherin was associated with distant metastasis. Based on immunohistochemical heterogeneity, E-cadherin-positive tumours were classified into heterogeneous, homogeneous and intermediate types. Interestingly, although patients with heterogeneous type demonstrated the lowest incidence of distant metastasis at diagnosis, they showed a higher incidence of subsequent distant metastasis, after surgery, and a lower survival rate than those with homogeneous type (p=0.05). **E-cadherin expression was reduced or negative in metastatic axillary lymph nodes regardless of the expression in the primary tumour, suggesting that changes in E-cadherin expression are associated with not only distant metastasis but also lymph node metastasis.** Tumour reactivity for E-cadherin and beta-catenin expression demonstrated a higher incidence of distant metastasis than those expressing both catenins, suggesting that the expression of catenins is involved in breast cancer metastasis. **Reduction or loss of E-cadherin and catenin expression may be associated with distant and lymph node metastases in invasive breast cancer, and the heterogeneous type may be associated with poor prognosis.**

**2. E-cadherin and alpha-, beta-, and gamma-catenin protein expression in relation to metastasis in human breast carcinoma.** J Pathol (England), Jul 1998, 185(3) p262-6

In the metastatic process, various cell-cell adhesion molecules seem to play an important role. E-cadherin, a transmembrane protein with an extracellular and an intracellular domain, is one of the key players involved

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of expression of E-cadherin, as reported elsewhere; loss of catenin expression was also found in these tumours. alpha-catenin intensity also showed a relationship with grade (p=0.008) and with oestrogen receptor (ER) status (p=0.006). beta/gamma-catenin expression was not associated with other known prognostic factors. Forty-nine per cent and 42 per cent of cases showed no membrane immunostaining with beta- and alpha-2-integrin, respectively, and co-ordinated loss of beta- and alpha-2-integrin expression was found. Both beta-1 and alpha-2-integrin expression were associated with histological grade (p=0.003 and p=0.031, respectively) and beta1 immunoreactivity with tumour type (p=0.010). **None of the variables examined showed a statistically significant association with tumour size or lymph node stage, or with overall survival, although distant metastasis was seen (p=0.007) towards poorer survival of patients with tumours with absent or weak expression of beta1-integrin.** The expression of these markers is of biological interest, but appears to be of little additional use in predicting clinical behaviour.

**8. Re-expression of E-cadherin, alpha-catenin and beta-catenin, but not of gamma-catenin, in metastatic tissue from breast cancer patients.** J Pathol 2000, 190(1) p15-9.

Tumour cell invasion and metastasis are the processes that kill most cancer patients. Tumour cells with the greatest invasive and metastatic capacity may be those with the highest number of genetic alterations. The present study has analysed the expression of several tumour-related proteins in both primary tumours and metastatic lesions from 34 breast cancer patients. Protein expression of p53, bcl-2, p21, cyclin D1, E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin was investigated by IHC using monoclonal antibodies. Metastatic tissue showed a different expression profile from the primary tumour in most patients. **The most significant finding was the re-expression of E-cadherin, alpha-catenin, and beta-catenin, and increased down-regulation of gamma-catenin, in metastatic lesions. These results demonstrate that tumour cells, when released from the primary site and after escape with elsewhere, are capable of re-expression of adhesion molecules.** gamma-catenin may play a different role in metastatic lesions than in primary tumours, since it is selectively down-regulated in tumour tissue at the metastatic site.

**9. Clinicopathologic implications of E-cadherin reactivity in patients with lobular carcinoma in situ of the breast.** Cancer 2001, 92:738-47

The current study addressed two questions pertaining to lobular carcinoma in situ (LCIS) of the breast. First, does the risk of a subsequent carcinoma decrease over time after an LCIS biopsy and second, **what is the clinical significance of E-cadherin reactivity?** **METHODS: Eighty-two consecutive patients with a biopsy containing LCIS only, no prior history of breast carcinoma, and follow-up information available for the period 1983-1976** were reviewed. Six patients underwent mastectomy. The LCIS vs. hundred eighty-six sections were stained with E-cadherin. E-cadherin reactivity was correlated with clinicopathologic features of the LCIS and subsequent tumors. The mean number of blocks stained per case was 5.9. The mean follow-up period was 21.6 years. **RESULTS: Sixteen patients (19.5%) developed 21 subsequent invasive carcinomas (9 ipsilateral, 5 contralateral, and 5 bilateral carcinomas).** The 10-year and 20-year actuarial rates of developing subsequent carcinoma were 7.8% and 15.4%, respectively. Six of the 21 carcinomas (29%) developed after 20 years. Nine LCIS cases (10.9%) had focal E-cadherin reactivity. When compared with patients with nonreactive LCIS, patients with E-cadherin-reactive LCIS more frequently developed a subsequent ipsilateral carcinoma that had a ductal component (55.7% vs. 12.3%, P < 0.01). The subsequent carcinomas also developed after significantly shorter time periods (mean of 7.6 years vs. 19.6 years; P < 0.01). **CONCLUSIONS: LCIS appears to confer a persistent, increased risk of subsequent breast carcinoma that does not appear to decrease over time.** E-cadherin reactivity appears to identify a subset of LCIS patients with risk factors for subsequent carcinoma similar to those of patients with low-grade intraductal carcinoma.

**11. Correlation of E-cadherin expression with clinicopathological parameters in breast carcinoma.** Saudi Med J (Saudi Arabia), Aug 2004, 25(8) p1024-7

To investigate the correlation between the E-cadherin (E-CD) expression and clinicopathological parameters including tumor grade, patient age, tumor size, necrosis, peritumoral lymphovascular invasion and lymph node status in breast carcinomas. **METHODS:** The specimens were surgically obtained from 51

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female patients with breast carcinoma between 1997 and 2001 in Karadeniz Technical University Medicine Faculty Farabi Hospital, Trabzon, Turkey. Histologic grading was according to the Bloom and Richardson method. Tumors were classified as grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated). Necrosis was graded as (-), (+), (++) and (+++). RESULTS: Grade I breast carcinomas (n=17) showed greater immunoreactivity than grade 2 (n=22) and grade 3 (n=12) carcinomas. None of the infiltrating lobular carcinomas expressed E-CD. Statistically, significant difference has been noticed between E-CD expression and the histological grade. In contrast, **no association were found between E-CD expression and metastatic potential, tumor size, tumor necrosis and patients' age.** CONCLUSION: Results in the present report suggest that **E-CD expression in breast carcinoma is more related to histological type and differentiation grade than with metastatic potential, tumor size, tumor necrosis and patients' age.**

**12. E-cadherin expression in invasive non-lobular carcinoma of the breast and its prognostic significance.** Histopathology (England), Jun 2005, 46(6) p685-93

E-cadherin is a cell adhesion molecule that is expressed in normal breast tissue and is often considered useful as a phenotypic marker in breast cancer diagnosis, with absence of its expression frequently observed in tumours of lobular subtype. However, **the clinicopathological and prognostic value of E-cadherin in the more frequent non-lobular types of breast carcinoma is unclear.** METHODS AND RESULTS: E-cadherin expression was assessed immunohistochemically in a large and well-characterized series of invasive non-lobular breast carcinoma types (n=1665) with long-term clinical follow-up (median 56 months) using tissue microarray technology, to determine the relationship between its expression and primary tumour characteristics and disease outcome. Only membranous expression of E-cadherin was considered in this study and its expression was categorized as normal (H-score=100) or reduced [absent or below the median (score <=100)]. **Complete absence of its expression (score=0) was detected in 7.2% of cases.** On univariate analysis, reduced E-cadherin expression was associated with a reduced disease-free interval and overall survival and also with indicators of poor prognosis including larger tumour size, higher histological grade, development of distant metastasis and tumours negative for oestrogen receptors. No association between E-cadherin expression and lymph node status was found. On multivariate analysis, E-cadherin was an independent predictor of disease-free interval [hazard ratio (HR) 1.56, 95% confidence interval (CI) 1.23, 1.99, P<0.001] and overall survival (HR 1.53, 95% CI 1.09, 2.14, P=0.013) and there was some evidence that the prognostic value was greater in those with positive lymph nodes (P interaction=0.099). CONCLUSIONS: **These results suggest that E-cadherin loss may play a role in progression, development of distant metastasis and recurrence in non-lobular invasive carcinomas of the breast and its assessment by immunohistochemistry may help in the identification of patients with poor outcomes.**

**13. Prognostication of invasive ductal breast cancer by quantification of E-cadherin immunostaining: the methodology and clinical relevance.** Histopathology (England), Aug 2002, 41(2) p127-33

AIMS: We tried to improve the evaluation of E-cadherin immunostaining in paraffin sections, to distinguish the less aggressive variants of ductal infiltrating breast cancer from other variants. METHODS AND RESULTS: The method graded the membrane staining and estimated the fraction of area of cancer tissue stained at the respective staining grade, resulting in an immunohistochemical staining index. At the cut-point 0.35 the index divided all 157 patients (P=0.0188), and 57 node-positive patients (P=0.0006) into two groups of different survival. In multivariate analysis (all patients) **E-cadherin immunorec was inferior to mitotic index (SMI) (P=0.0002), but still significant (P=0.0031). Among node-positive patients, E-cadherin was even more powerful and superior (P=0.0001) to the still significant SMI (P=0.0023),** and E-cadherin immunostaining and the mitotic activity (SMI) combined did not need the support of other prognosticators in the Cox model. CONCLUSIONS: The study suggests that E-cadherin immunostaining can be used efficiently in finding patients with favourable outcome among node-positive patients.

**14. Retention of the expression of E-cadherin and catenins is associated with shorter survival in grade III ductal carcinoma of the breast.** J Pathol (England), Apr 2001, 193(4) p433-41

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respectively). CONCLUSIONS: **alpha-Catenin, especially its cytoplasmic expression, seems to be a more sensitive prognostic marker than the other members of the E-cadherin complex in invasive breast cancer.**

**20. E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters.** Am J Clin Pathol (United States), Mar 2006, 125(3) p377-85

Our objective was to assess the loss of E-cadherin (EC) as a diagnostic marker or a predictor of prognosis. We stained 276 breast carcinomas with monoclonal antibodies to EC (invasive lobular carcinomas [ILC] and variants, 59; invasive ductal carcinoma and ductal special types [IDC], 204; tubulolobular carcinoma [TLC], 4; and invasive carcinoma [IC], uncertain whether lobular or ductal type, 9). The results were as follows: EC-IDCs, 99.5%; EC-ILCs, 90%; EC-TLCs, 10%; EC-pleomorphic ILCs, 20%; EC-ICs, 44%. All 4 TLCs showed positive tubules while cords were negative. Statistically a correlation of EC loss with a positive diagnosis of ILC was found but there was no correlation with any prognostic tumor variables. A negative EC stain confirms the diagnosis of ILC (specificity, 97.7%, negative predictive value, 96.8%, sensitivity, 88.1%, positive predictive value, 91.2%). **EC is helpful in classifying cases with indeterminate histologic features, EC loss is uncommon in nonlobular carcinomas with no correlation to currently established prognostic variables.**

- Morphology predicts E-cadherin immunostaining, so why spend additional money to stain?
- Best to study E-cadherin positive vs. negative breast carcinomas, looking for clinical significance.
- Thus far, investigation of point 2 above is contradictory and more works needs to be done.

**Many studies have investigated the relationship between the E-cadherin/catenin axis and breast cancer biology and yet, unlike the studies in other tumour systems, which have shown a relationship between down-regulation and poor survival, no clear association has emerged in breast.** Since accumulating evidence suggests that ductal carcinoma of no special type (NST) represents a diverse group of biologies, this study has focused on grade III ductal carcinoma, in order to reduce the heterogeneity of the study population. A total of 470 breast tumours were studied. Consecutive sections were labelled with antibodies which recognize E-cadherin and the arm proteins with which it interacts: alpha-, beta-, and gamma-catenin. **Membrane-bound and cytoplasmic E-cadherin and membrane-bound alpha-catenin expression were associated with a positive oestrogen receptor (ER) status, gamma-catenin with a negative ER status, and, surprisingly, all three with poor survival.** Taken together, these findings suggest that a conserved E-cadherin/catenin axis may play a part in determining adverse outcome in grade III breast carcinoma.

**15. E-cadherin as a prognostic indicator in primary breast cancer.** Br J Cancer (Scotland), Dec 14 2001, 85(12) p1958-63

Epithelial cadherin (E-CD) is a member of the cadherin family of cell adhesion molecules and has been implicated as an invasion suppressor molecule in vitro and in vivo. **We analysed 174 breast tumours from the Nottingham/Tenovus Breast Cancer Series** immunohistochemically for E-CD expression using the mouse monoclonal antibody HECD-1 (Zymed Laboratories Inc.). In normal epithelial cells E-CD was strongly expressed at cell-cell boundaries. 66% of the breast cancers examined had reduced intensity of E-CD expression with 74% having significant reductions in the proportion of E-CD-positive tumour cells. Using a combined intensity/proportion score, significant associations were found between E-CD expression and tumour type (P<= 0.001), ER status (P= 0.026) and histological grade (P= 0.031). **Expression of E-CD was not found to be related to recurrence, distant metastases, lymph node stage, vascular invasion, primary tumour size, prognostic group or survival. This E-CD expression in human breast cancer appears to have minimal prognostic value, but may have a role as a phenotypic marker.**

**16. Aberrant P-cadherin expression: is it associated with estrogen-independent growth in breast cancer?** Pathol Res Pract (Germany), 2002, 198(12) p795-801

Breast carcinomas represent a heterogeneous group of tumors, with a diverse biologic behavior, outcome, and response to therapy. Recent studies have demonstrated that alterations in the expression of adhesion molecules in cancer cells are related to aggressiveness and poor prognosis. The aim of our study was to investigate the expression of P-cadherin in breast carcinomas and correlate it with estrogen receptor (ER) status. We selected 73 ductal carcinomas in situ (DCIS) and 149 invasive carcinomas of the breast, and assessed the expression of P-cadherin as well as other biologic markers. P-cadherin expression showed a strong inverse correlation with ER expression in both types of breast carcinoma (in situ and invasive). P-cadherin-positive and ER-negative tumors were related to a higher histologic grade, a high proliferation rate, and expression of c-erbB-2. **We demonstrated that P-cadherin identifies a subgroup of breast carcinomas that lacks ER expression, and correlates with higher proliferation rates and other predictors of aggressive behavior.** We believe that these tumors represent an advanced step in cancer progression, and our data support the hypothesis that an estrogen-independent pathway regulates P-cadherin expression.

**17. Immunophenotypic analysis of inflammatory breast cancers: identification of an 'inflammatory signature'.** J Pathol (England), Mar 2004, 202(3) p265-73

**Inflammatory breast cancer (IBC) is a rare but very aggressive form of breast cancer.** Its definition is based on clinical criteria, but a molecular definition could be useful when data are incomplete or features are missing. **Recently, the identification of overexpression of E-cadherin in IBC has improved understanding of the molecular basis of this disease.** Consequently, the aim of this study was to try to determine an immunophenotypic 'signature' of IBC. A series of 80 cases of IBC were compared with 552 non-IBC control cases and a model was elaborated to evaluate the probability of an inflammatory carcinoma being present in any clinical situation. Tissue microarrays (TMAs) were used to determine the immunohistochemical profile of eight proteins including E-cadherin, EGFR, oestrogen and progesterone

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receptor (ER and PR), MIB1, ERBB2, MUC1, and P53. All the parameters tested were differentially expressed between IBC and control cases in univariate analysis (p < 0.001). The five variables that were significantly associated with IBC in multivariate analysis were E-cadherin > or = 300 [HR = 5.64 (2.92-10.87)], ER negative [HR = 3.00 (1.67-5.31)], MIB1 > 20 [HR = 3.54 (1.87-6.71)], MUC1 cytoplasmic staining [HR = 2.72 (1.49-4.96)], and ERBB2 positive 2+ or 3+ [HR = 2.46 (1.26-4.78)]. The probability that a breast cancer with this full phenotype diagnosis was an IBC was 90.5%. If any one of the five parameters was missing, this probability dropped to 75% and was less than 50% when one, two, or three parameters were present. The 5-year overall survival (OS) and 5-year disease-free survival (DFS) of patients with IBC were not significantly different from those of the non-IBC control group that expressed four or five parameters (nIBC-1), but this nIBC-1 control group had a significantly worse outcome than the non-IBC control group (nIBC-2) with only 0-3 parameters (p = 0.0409 for OS and p < 0.0001 for DFS). In conclusion, an immunophenotypic signature was suggested for IBC. This could help to determine the worst cases, independent of clinical criteria.

**18. Role of E-cadherins in development of lymphatic tumor emboli.** Cancer (United States), May 1 2003, 97(9) p2341-7

E-cadherin (E-cad) is a cell adhesion molecule that is expressed in normal breast tissue. While loss of E-cad expression is a characteristic feature of lobular carcinoma, it also is observed in infiltrating ductal carcinoma (IDC). **The presence of peritumoral intralymphatic emboli also is a poor prognostic feature in IDC. Invasive lobular carcinoma rarely is associated with intralymphatic emboli.** In the current study, the authors assessed E-cad expression in cases of IDC with and without intralymphatic tumor emboli to examine the potential role played by these molecules in the development of lymphatic emboli. METHODS: Fifty patients with high-grade invasive ductal carcinoma—25 with prominent lymphatic invasion (LVI) and intralymphatic tumor emboli and 25 without LVI—were tested for expression of E-cad. For both groups, the intensity and frequency of E-cad expression was evaluated in tumor cells and lymphatic emboli; normal lobules were used as internal controls. RESULTS: Membranous expression of E-cad was observed in normal lobules and tumor cells in all patients, with the tumor cells exhibiting varying degrees of loss of expression. In the 25 LVI-positive patients, the majority of tumor cells (including intralymphatic emboli) expressed E-cad with an intensity and distribution similar to what was seen in normal lobules. In the LVI-negative patients, the intensity and the distribution of E-cad staining varied significantly. Tumor cells at the tumor-stroma interface showed a greater frequency and intensity of E-cad expression than did cells in the central region of the tumor. CONCLUSIONS: **Strong expression of E-cad was observed in LVI-positive patients with high-grade IDC but not in LVI-negative patients. Emboli also exhibited high-intensity expression.** These findings, taken in conjunction with the knowledge that intralymphatic tumor emboli in lobular carcinoma (which is E-cad-negative) are rare, suggest that E-cad plays an important role in tumor development and growth within the lymphatics.

**19. Abnormal alpha-catenin expression in invasive breast cancer correlates with poor patient survival.** Histopathology (England), Jun 2002, 40(6) p536-46

AIMS: alpha-Catenin is a member of the E-cadherin-catenin family of adhesion molecules whose role is essential for the function of the E-cadherin complex. In this study, we have evaluated the expression of alpha-catenin but also of the other catenins (beta-, gamma- and p120-catenin) and E-cadherin in invasive breast cancer and statistically analysed these expressions with known clinicopathological parameters, c-erbB-2 oncoprotein expression and patient survival. METHODS AND RESULTS: Abnormal E-cadherin and beta-catenin expression, especially loss of expression, was associated with lobular histological type of breast carcinomas (P=0.03 and P=0.01, respectively). Abnormal E-cadherin and alpha-catenin expression was associated with high histological grade ductal carcinomas (P=0.01 and P=0.03, respectively). Abnormal E-cadherin and beta-catenin expression was correlated with lymph node metastases (P=0.02 and P=0.05, respectively), while abnormal alpha- and beta-catenin were correlated with the advanced stage of the disease (P=0.04 and P=0.05, respectively). Abnormal p120-catenin expression was associated with loss of PR (P=0.008). Survival analysis demonstrated a statistically significant association between abnormal alpha-catenin expression and poor patient survival (P=0.02). When survival analysis was performed according to the different patterns of abnormal expression, statistically significant associations were seen between cytoplasmic alpha- and beta-catenin expression and poor survival (P=0.006 and P=0.04,

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## Multigene Expression Profiling as a Prognosticator and/or Predictor of Therapeutic Response in Breast Cancer Patients

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Clearly, not all breast cancers appear the same or behave in the same manner. The phenotypical (i.e., histopathologic) variations have been apparent to surgical pathologists for decades, but with the recent onset of gene microarrays, “different subtypes” of breast cancer have been characterized. The close link between expression genotype and observed phenotype is well established, and thus, finding a close correlation between gene microarray signatures and histopathology is to be expected (indeed, one should predict the other). For example, in one current scheme the gene microarray subtypes include the basal-cell like, the HER2+ overexpressing, and the luminal A, B and C subtypes – each apparently associated with a different clinical outcome. The basal-cell like subtype is characterized absent expression of both hormone receptors and HER2 (i.e., triple negative) and by high expression of keratins 5, 14, and 17, laminin, EGFR, and fatty acid-binding protein 7. The HER2+ overexpressing subtype is characterized by high expression of genes in the HER2 amplicon. The luminal A subtype is characterized by the highest expression of the ER alpha gene. The luminal B and C subtypes have a lower expression of the ER cluster and a possible poorer outcome. The basal-cell like and HER2+ overexpressing subtypes apparently have the worst prognosis, whereas the luminal A group has the best prognosis. However, identification of these subtypes is still not broadly clinically used.

Other strategies for evaluating tumors in the clinical setting have been developed using smaller sets of genes. One such strategy is a 21-gene assay marketed as Oncotype DX (Genomic Health, Inc., Redwood City, CA) which is currently in commercial use in the USA (\$3,460.00 per assay). One advantage of this test is the use of paraffin-embedded blocks. Oncotype DX has been reported to predict 10-year distant recurrence in patients with estrogen receptor-positive, axillary lymph node-negative breast cancer. This genomic assay has also been reported to predict chemotherapy and endocrine therapy response. More validation studies are needed and prospective, randomized clinical trials are currently underway using this test. Yet, other multigene tests are finding their way in clinical practice. A 70-gene assay, which has been developed by a group in The Netherlands, is currently being used as a tool to assign treatment in women with early stage breast cancer.

In the future, clinical decisions may be dictated by the genetic characteristics of the tumor, with the clinical characteristics becoming less important. It is hoped that tailoring treatment, based on individual tumor characteristics, will help develop better therapeutic strategies and save many of our patients from unnecessary toxic therapy.

[There is abundant literature on prognostic factors for cancer patients, but very few such factors are used in clinical practice. Prognostic factors are unlikely to be used unless they are therapeutically relevant (i.e., unless they become predictive factors), and most publications do not establish such relevance.]

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risks were 6.2%, 17.8%, and 19.9% for ER-positive patients not treated with tamoxifen. In both the tamoxifen-treated and -untreated groups, approximately 50% of patients had low risk Recurrence Score values. They concluded that the assay was strongly associated with risk of breast cancer death among ER-positive, tamoxifen-treated and -untreated patients.

(4) Habel LA, Shak S, Jacobs MK, Capra A, Alexander C, Pho M, Baker J, Walker M, Watson D, Hackett J, Blick NT, Greenberg D, Fehrenbacher L, Langholz B, Quesenberry CP. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res*. 2006;8(3):R25. Epub May 31 [See Note #4 below]

(Note #4: From the Division of Research, Kaiser Permanente, Oakland, California, USA. The following authors received support for study-related activities from Genomic Health, Inc., but have no other competing financial or nonfinancial interests: LAH, MJK, AC, NTB, DG, CPQ, BL, and LF. The remaining co-authors (SS, CA, MP, JB, MW, DW, and JH) are employees or consultants for Genomic Health, Inc.)

**Fifth**, Paik et al. (5) studied the relationship between the Oncotype DX assay and chemotherapy benefit. 651 patients were gathered from the NSABP-B20 trial. The assay was performed on tumors from the tamoxifen-treated and tamoxifen plus chemotherapy-treated patients. Cox proportional hazards models were utilized to test for interaction between chemotherapy treatment and the RS. The test for interaction between chemotherapy treatment and the Oncotype DX assay was statistically significant ( $P = .038$ ). Patients with high-RS ( $> = 31$ ) tumors (i.e., high risk of recurrence) had a large benefit from chemotherapy. Patients with low-RS ( $< 18$ ) tumors derived minimal, if any, benefit from chemotherapy treatment. Patients with intermediate-RS tumors did not appear to have a benefit.

(5) Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006 Aug 10;24(23):3726-34. May 23 [See Note #5 below.]

**Sixth**, Mina et al. (6) wanted to identify patients responding to primary chemotherapy. Patients with newly diagnosed stage II or III breast cancer were treated with sequential doxorubicin and docetaxel. Pretreatment core biopsy samples were interrogated for genes that might correlate with pathologic complete response (pCR). In addition to the individual genes, the correlation of the Oncotype DX assay with pCR was examined. Of 70 patients enrolled in the parent trial, core biopsies samples with sufficient RNA for gene analyses were available from 45 patients. Twenty-two of the 274 candidate genes assessed correlated with pCR ( $p < 0.05$ ). Genes correlating with pCR could be grouped into three large clusters: angiogenesis-related genes, proliferation related genes, and invasion-related genes. Expression of estrogen receptor (ER)-related genes and Oncotype DX Recurrence Score did not correlate with pathologic complete remission. Gene expression analysis on core biopsy samples is feasible and identifies candidate genes that correlate with pCR to primary chemotherapy.

(6) Mina L, Soule SE, Badve S, Baehner FL, Baker J, Cronin M, Watson D, Liu ML, Sledge GW Jr, Shak S, Miller KD. Predicting response to primary chemotherapy: gene expression profiling of paraffin-embedded core biopsy tissue. *Breast Cancer Res Treat*. 2006 Oct 13. [See Note #5 below.]

(Note #5: Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest in Genomic Health, which included employment, leadership, stocks, and/or honoraria: S. Shak, J. Baker, M. Cronin, F.L. Baehner, and C. Geyer.)

**Seventh**, Buysse et al. (7) developed a 70-gene signature was previously shown to have prognostic value in patients with node-negative breast cancer. Patients ( $n = 307$ , with 137 events after a median follow-up

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The field of microarray expression profiling is burdened with both unrealistic hype and excessive skepticism, but in this review, my intent is to avoid either extreme. My purpose is to share current information regarding multigene profiling in breast cancer patients, while focusing on Oncotype DX. Later, I'll offer commentary. Let's start by reviewing the published data.

I was able to find and analyze eight extant publications, the first publication from the December 2004 NEJM (1). In this study Paik and coworkers (1) investigated distant tumor recurrence rates in tamoxifen-treated patients with estrogen-receptor-positive breast cancer who had negative lymph nodes. The authors contend that predicting outcome in this group is poorly defined by traditional clinical and histopathological measures. Using a reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay (Oncotype DX from Genomic Health), the investigators measured 21 prospectively selected genes in paraffin-embedded tumor tissue selected from the breast cancers of patients who were enrolled in the NSABP clinical trial B-14. The levels of expression of 16 cancer-related genes and 5 reference genes were used in a prospectively defined algorithm to calculate a recurrence score (RS) and to determine a risk group (low, intermediate, or high) for each patient. Adequate assay profiles were obtained in 668 of 675 tumor blocks. The proportions of patients categorized as having a low, intermediate, or high risk by the RT-PCR assay were 51, 22, and 27 percent, respectively. The Kaplan-Meier estimates of the rates of distant recurrence at 10 years in the low-risk, intermediate-risk, and high-risk groups were 6.8 percent, 14.3 percent, and 30.5 percent. The rate in the low-risk group was significantly lower than that in the high-risk group ( $P < 0.001$ ). In a multivariate Cox model, the recurrence score provided significant predictive power that was independent of age and tumor size ( $P < 0.001$ ). The recurrence score was also predictive of overall survival ( $P < 0.001$ ) and could be used as a continuous function to predict distant recurrence in individual patients. Also, the multivariate Cox proportional-hazards analyses were performed to explore the relation between distant recurrence and age, tumor size, tumor grade, *HER2* amplification, amounts of estrogen- and progesterone-receptor protein. In addition to the recurrence score, poor tumor grade was a significant predictor of distant recurrence.

(1) Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004 [Dec] 30;351(27):2817-26. [See Note #1 below]

(Note #1: It should be added that this project was supported by the National Surgical Adjuvant Breast and Bowel Project and Genomic Health. Drs. Paik, Shak, Baker, Cronin, Walker, and Bryant report holding a patent for the RT-PCR - Oncotype DX - assay used in this study. Drs. Shak, Baker, Cronin, and Watson report holding equity ownership or stock options in Genomic Health and being employed by Genomic Health, the commercial entity that sponsored the study. Dr. Walker reports having received consulting fees from Genomic Health. Dr. Baehner reports having received consulting fees from Genomic Health. Dr. Paik, lecture fees from Genomic Health; and Dr. Wickerham, consulting fees from AstraZeneca.)

**Second**, Esteva et al (2) tested the ability of the Oncotype DX assay to accurately determine the risk of recurrence in patients with node-negative breast cancer who did not receive systemic therapy. A secondary objective was to determine whether the quantitative RT-PCR data correlated with immunohistochemistry assay data regarding estrogen receptor, progesterone receptor, and human hEGFR2 status. 149 eligible patients had been followed for at least 5 years (median follow-up was 18 years). Median age at diagnosis was 59 years; mean tumor diameter was 2 cm; and 69% of tumors were estrogen receptor positive. The 5-year disease-free survival rate for the group was 80%. The Oncotype DX recurrence score was not predictive of distant disease recurrence; yet, there was a high concordance between the Oncotype DX assay and immunohistochemical assays for estrogen receptor, progesterone

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of 13.6 years) from five European centers were divided into high- and low-risk groups based on the gene signature classification and on clinical risk classifications. Patients were assigned to the gene signature low-risk group if their 5-year distant metastasis-free survival probability as estimated by the gene signature was greater than 90%. Patients were assigned to the clinicopathologic low-risk group if their 10-year survival probability, as estimated by Adjuvant software, was greater than 88% (for estrogen receptor [ER]-positive patients) or 92% (for ER-negative patients). Hazard ratios (HRs) were estimated to compare time to distant metastases, disease-free survival, and overall survival in high- versus low-risk groups. The 70-gene signature outperformed the clinicopathologic risk assessment in predicting all endpoints. The 70-gene signature adds independent prognostic information to clinicopathologic risk assessment for patients with early breast cancer.

(7) Buysse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, d'Assinques MS, Bergh J, Lidereau R, Paul Ellis P, Harris A, Bogaerts J, Therasse P, Floore A, Amakrane M, Piette F, Rutgers E, Sotiriou C, Cardoso F, Piccart M. On behalf of the TRANSGO Consortium. Validation and Clinical Utility of a 70-Gene Prognostic Signature for Women With Node-Negative Breast Cancer. *JCO*. Vol. 98, No. 17, 1183-1192, September 6, 2006 [see note #6 below]

(Note #6: Affiliations of authors: International Drug Development Institute, Brussels, Belgium (MB, MA, FP); Institut Jules Bordet, Brussels, Belgium (SL, CS, FC, MJP); Netherlands Cancer Institute, Amsterdam, The Netherlands (LvV, ER); European Institute of Oncology and University of Milan School of Medicine, Milan, Italy (GV); National Center of Competence in Research Molecular Oncology, Swiss Institute of Experimental Cancer Research, Epalinges & the Swiss Institute of Bioinformatics, Lausanne, Switzerland (MD); Agendia B.V., Amsterdam, The Netherlands (LvV, AMG, AF); Institut Gustave Roussy, Villejuif, France (MSA); Karolinska Institute, Stockholm, Sweden (JB); Centre René Hufnagel and Institut National de la Santé et de la Recherche Médicale, St Cloud, France (RL); Guy's Hospital, London, UK (PE); John Radcliffe Hospital, Oxford, UK (AH); European Organization for the Research and Treatment of Cancer Data Center, Brussels, Belgium (JB, PT)

**Eighth**, Foekens, et al (8) previously identified a 76-gene prognostic signature for lymph node-negative (LNN) breast cancer patients. The aim of this study was to validate this gene signature in an independent more diverse population of LNN patients from multiple institutions. Using custom-designed DNA chips they analyzed the expression of the 76 genes in RNA of frozen tumor samples from 180 LNN patients who did not receive adjuvant systemic treatment. The 76-gene signature was highly informative in identifying patients with distant metastasis within 5 years (hazard ratio, [HR], 7.41) even when corrected for traditional prognostic factors in multivariate analysis. The actuarial 5- and 10-year distant metastasis-free survival were 96% and 94%, respectively, for the good profile group and 74% and 65%, respectively for the poor profile group. The 76-gene signature was confirmed as a strong prognostic factor in subgroups of estrogen receptor-positive patients, pre- and postmenopausal patients, and patients with tumor sizes 20 mm or smaller. The data provide a strong methodological and clinical multicenter validation of the predefined prognostic 76-gene signature in LNN breast cancer patients.

(8) Foekens JA, Atkins D, Zhang Y, Sweep FC, Harbeck N, Paradiso A, Cufur T, Siewerts AM, Talantov D, Span PN, Tjan-Hehn VC, Zito AF, Specht K, Hoefler H, Goloub R, Schittulli F, Schmitt M, Bees LV, Klijn JG, Wang Y. Multicenter validation of a gene expression-based prognostic signature in lymph node-negative primary breast cancer. *J Clin Oncol*. 2006 Apr 10;24(11):1665-71. Epub 2006 Feb 27 [See Note #7]

(Note #6: From the Department of Medical Oncology, Erasmus Medical Center, Daniel den Hoed Cancer Center, Rotterdam; Department of Chemical Endocrinology, Radboud University Nijmegen Medical Center; Department of Medical Oncology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands; Verides LLC, San Diego, CA; Fraunkelink and Institut für Allgemeine Pathologie und Pathologische Anatomie, Technische Universität, Muenchen, Germany; National Cancer Institute, Bari, Italy; and the Institute of Oncology, Ljubljana, Slovenia.)

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receptor, and hEGFR2. The investigators concluded that further work needed to be done to develop an assay to identify the likelihood of recurrent disease in patients with node-negative breast cancer who do not receive adjuvant tamoxifen or chemotherapy.

(2) Esteva FJ, Sahin AA, Cristofanilli M, Coombes K, Lee SJ, Baker J, Cronin M, Walker M, Watson D, Shak S, and Hortobagyi GN. Prognostic Role of a Multigene Reverse Transcriptase-PCR Assay in Patients with Node-Negative Breast Cancer Not Receiving Adjuvant Systemic Therapy. *Clin Cancer Res* 2005;11, 3315-3319, May [See Note #2 below]

(Note #2: Authors' Affiliations: Departments of Breast Medical Oncology, Pathology, and Biostatistics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas and Genomic Health, Inc., Redwood City, California)

**Third**, Gianni et al (3) sought to identify gene expression markers that predict the likelihood of chemotherapy response. They also tested whether chemotherapy response is correlated with the 21-gene Recurrence Score (Oncotype DX). Patients with locally advanced breast cancer received neoadjuvant paclitaxel and doxorubicin. RNA was extracted from the pretreatment formalin-fixed paraffin-embedded core biopsies. The expression of 384 genes was quantified using reverse RT-PCR and correlated with pathologic complete response (pCR). The performance of genes predicting for pCR was tested in patients from an independent neoadjuvant study where gene expression was obtained using DNA microarrays. Of 89 assessable patients, 11 (12%) had a pCR. Eighty-six genes correlated with pCR (unadjusted  $P < .05$ ); pCR was more likely with higher expression of proliferation-related genes and immune-related genes, and with lower expression of estrogen receptor (ER)-related genes. In 82 independent patients treated with neoadjuvant paclitaxel and doxorubicin, DNA microarray data were available for 79 of the 86 genes. In univariate analysis, 24 genes correlated with pCR with  $P < .05$ . The Oncotype RS was positively associated with the likelihood of pCR ( $P = .005$ ), suggesting that the patients who are at greatest recurrence risk are more likely to have chemotherapy benefit. Quantitative expression of ER-related genes, proliferation genes, and immune-related genes are strong predictors of pCR in women with locally advanced breast cancer receiving neoadjuvant anthracyclines and paclitaxel.

(3) Gianni L, Zambetti M, Clark K, Baker J, Cronin M, Wu J, Mariani G, Rodriguez J, Carcangiu M, Watson D, Valagussa P, Rouzre R, Symmans WF, Ross JS, Hortobagyi GN, Pizzuti L, Shak S. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol*. 2005 Oct 10;23(29):7265-77. Epub 2005 Sep 6 [See Note #3 below]

(Note #3: From the Istituto Nazionale Tumori, Milan, Italy; The University of Texas M.D. Anderson Cancer Center, Houston, TX; Genomic Health Inc, Redwood City, CA; and Millennium Pharmaceuticals Inc, Cambridge, MA. Kim Clark, Joffe Baker, Maureen Cronin, Jenny Wu, Drew Watson, and Steven Shak had either leadership roles, stock, employment, or stock in Genomic Health. Jeffrey S. Ross had employment, stock, and research funding from Millennium Pharmaceuticals Inc.)

**Fourth**, Habel et al. (4) evaluated the performance of the Oncotype DX assay among node-negative patients from a community hospital setting. A case-control study was conducted among 4,964 Kaiser Permanente patients diagnosed with node-negative invasive breast cancer from 1985 to 1994 and not treated with adjuvant chemotherapy. 220 cases were patients who died from breast cancer. 570 controls were breast cancer patients who were individually matched to cases with respect to age, race, adjuvant tamoxifen, medical facility and diagnosis year, and were alive at the date of death of their matched case. After adjusting for tumor size and grade, the Oncotype DX assay was associated with risk of breast cancer death in ER-positive, tamoxifen-treated and -untreated patients ( $P = 0.003$  and  $P = 0.03$ , respectively). At 10 years, the risk for breast cancer death in ER-positive, tamoxifen-treated patients were 2.8%, 10.7%, and 15.5% for those in the low, intermediate and high risk groups, respectively. The

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## COMMENTARY:

As previously noted, the field of microarray expression profiling is burdened with both unrealistic hype and excessive skepticism (9). Indeed, I want to guard against falling victim to either extreme, in spite of my being a surgical pathologist.

I believe the only change that can be predicted with 100% accuracy is that there will be change (often driven by technology), and currently molecular biology is “sexy,” especially to some clinicians who sometimes appear to relish the idea of extinguishing morphologists. Thus, if surgical pathologists are not aware of new developments in the field of molecular biology, someone else will be and make good use of relevant advances in the field. We should make those advances in molecular biology our advances and not allow our apparent intrinsic allergy to base pairs to impair our judgment. But, this does not mean we should accept like myrmidons everything “molecular,” and healthy skepticism is prudent.

Moreover, healthy skepticism is reflected in comments from our clinical colleagues. In the 2006 update of the St. Gallen International Oncology Conference on breast cancer, Thueringmann et al. (10) stated that... “gene expression profiling is now under investigation as a potential means to identify patients who are at high risk of relapse, or who may be more likely to fail endocrine therapies. The promise of gene profiling technology is that it may one day be possible to identify those patients for whom endocrine therapy is likely to have the greatest benefit. It has even been proposed that such technology may be an alternative to clinical guidelines. Nonetheless, the technology, still in the early stages of development, will require more refinement and prospective validation.”

The St. Gallen International Consensus Panel (updated 2006)(10) continues to recommend using traditional markers of breast cancer to guide prognostication and therapeutic decision making (i.e., tumor size, tumor grade, patient age, and hormone-receptor status).

Also, the comments of Reis-Filho et al (11) are relevant. He and coworkers state that... “expression profiling has been extensively applied to the study of breast cancer and undoubtedly is changing the way breast cancer is perceived. Over the past few years, several groups have described prognostic signatures (gene lists) that are purported to be more accurate prognostic factors than well established clinical and pathological features. In addition, cDNA and oligonucleotide microarrays have also been used to devise predictive signatures in the setting of neoadjuvant chemotherapy setting. However, it seems that the enthusiasm with this new technology has led most of us to turn a blind eye to some serious methodological problems which are evident in landmark papers on breast cancer expression profiling. These issues include small and biased cohorts of patients, inappropriate statistical analysis, and lack of thorough validation of the technology.”

(9) Simon R. Roadmap for developing and validating therapeutically relevant genomic classifiers. *JCO* 23:7332-41; 2005)

(10) Guidelines for the adjuvant treatment of postmenopausal women with endocrine-responsive breast cancer: Past, present and future recommendations Beat Thueringmann, Dieter Koehler, and Hans-Joerg Senn. *Eur J Cancer*. Received 1 July 2006, accepted 6 September 2006. Available online 7 November 2006.)

(11) Reis-Filho JS, Westbury C, and Pierga J-Y. The impact of expression profiling on prognostic and predictive testing in breast cancer. *Journal of Clinical Oncology*, Vol 24, No 11 (April 10), 2006; pp. 1665-1671.)

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Like the two groups cited above, I believe there are reasonable and healthy reasons to remain skeptical regarding the role of the Oncotype DX assay and other multigene expression assays in breast cancer patients. I believe (as stated by the St. Gallen Consensus Panel) that these multigene expression assays are still in the early stages of development and require more refinement and prospective validation. I believe some healthy critique is called for.

First, two studies [the Esteva et al. (2) and Mina et al. (6) publications] failed to validate the Oncotype DX assay – that is, the authors state, “the Oncotype DX recurrence score was not predictive of distant disease recurrence (2),” and the “Oncotype DX recurrence score did not correlate with pathologic complete remission (6).” Esteva and coworkers (2) further concluded that, “further work needed to be done to develop an assay to identify the likelihood of recurrent disease in patients with node-negative breast cancer who do not receive adjuvant tamoxifen or chemotherapy.” These two publications do not necessarily invalidate the positive publications, but they make one question the putative robustness of the Oncotype DX assay and its substantial cost of \$3,460.00 per assay.

Second, there is tremendous overlap in the authors of these reports, with many having clear-cut financial interests in Genomic Health, which is the company marketing Oncotype DX. This seems unavoidable when the test being evaluated is patented and only available through Genomic Health. To Genomic Health’s credit, many of these same authors appear on the two papers reporting no validation of Oncotype DX. Nonetheless, I would like to see the Oncotype DX and other multigene expression assays validated on other independent patient cohorts and by investigators with absolutely no financial ties to Genomic Health or to other private firms with financial ties to these assays.

Third, I’m not convinced the various investigators have given the less expensive (and less “sexy”) traditional prognostic or therapeutic markers an adequate or even fair evaluation. For example, in the 2004 NEJM article (1) tumor grade was found by multivariate analysis to provide additional independent prognostically useful information. I’d like to see the Oncotype DX assay compared to tumor grade performed by a panel of nationally recognized breast pathology experts. This is important, because determining a tumor grade is more readily available and much, much less expensive than \$3,460.00. Furthermore, the data from the Paik et al. (5) article suggest that an index using tumor grade and progesterone receptor status may perform as well as the Oncotype DX assay in predicting prognosis and response to chemotherapy – both traditional markers showed significant correlations with the recurrence score (both  $p < .001$ ). (Adding a third traditional factor, such as mitotic figure index or Ki-67 score, may also prove very predictive.) Also, in spite of the contention of multigene advocates, the data from the Paik et al. (5) paper show substantial inter-observer agreement between pathologist tumor grading when the pathologists used a standardized (modified Bloom-Richardson) grading scheme (71% concordance; kappa statistic = 0.59). Paik et al. (5) found that patients with high-RS ( $\geq 31$ ) tumors (i.e., high risk of recurrence) had a large benefit from chemotherapy. Patients with low-RS ( $< 18$ ) tumors derived minimal, if any, benefit from chemotherapy treatment. Likewise, patients with intermediate-RS tumors showed no apparent significant benefit. 25% of the patient population had a high-risk RS and, of these, nearly 70% had poorly differentiated (high-grade) tumors. It seems that tumor grade might serve well as a “poor-patient” multigene expression assay.

Fourth, I’m not convinced that the 21 genes selected for the Oncotype DX assay are necessarily the best group to select, nor that we need all 21 genes assayed – maybe fewer genes would provide essentially the same information at much less cost. For example, the data from the Paik et al. (5) article showed that the “proliferating gene group-TH” (5 gene profile)(i.e., HR=0.33;  $p = .039$ ) was essentially as statistically robust in predicting chemotherapy responsiveness as the full Oncotype DX assay (16 gene profile)(i.e., HR=0.32;  $p = .038$ ). Other studies have found that much of the “power” of the multi-gene signatures correlated predominantly with certain gene groups, especially the proliferation related genes (6). Why pay for all the other additional genes? Why not count mitotic figures, perform a mitotic figure index, or perform Ki-67 immunohistochemistry – all much, much less expensive. Moreover, the Europeans appear to achieve the same results while selecting different genes both numerically and qualitatively (7,8) – that is, what and/or whose multi-gene signature is best? We don’t have these answers yet.

- **Value remains to be confirmed with additional studies, especially by investigators independent of any financial interest.**
- **Added value of a \$3,460.00 test over and above traditional prognosticators remains unproven.**