National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Breast Cancer

Michael J Duffy[1*], Francisco J, Esteva[2], Nadia Harbeck[3], Rafael Molina[4], Daniel F Hayes[5]

[1]Department of Nuclear Medicine, St Vincent's University Hospital and UCD School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland, [2]Departments of Breast Medical Oncology, Molecular and Cellular Oncology, University of Texas M. D. Anderson Cancer Center, Houston Texas, USA, [3]Frauenklinik der Technischen Universität München, Klinikum rechts der Isar, Munich, Germany, [4]Laboratory of Biochemistry, Hospital Clinico Provincial, Barcelona, Spain and [5]Breast Oncology Program, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI, USA.

***Sub-Committee Chair**, to whom all comments should be addressed *via* e-mail to Michael.J.Duffy@ucd.ie, with copies to Cathie Sturgeon at C.Sturgeon@ed.ac.uk and ediamandis@mtsinai.on.ca

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Abbreviations: CMF, cyclophosphamide, methotrexate and 5-fluorouracil; ER, estrogen receptor; FISH, fluorescent *in situ* hybridization; PAI-1, plasminogen activator inhibitor 1; PR, progesterone receptor; uPA, urokinase plasminogen activator.

INTRODUCTION

Breast cancer is by far the most common cancer affecting women worldwide with approximately one million new cases diagnosed each year (1). In 2004, an estimated 217,000 women were diagnosed with breast cancer in the USA and approximately 40,000 died from the disease (2). Currently, there are more than two million women in the United States who are living with a history of breast cancer (3). While the worldwide incidence of the disease appears to be increasing, mortality rates are now declining in a number of Western countries such as the United States and the United Kingdom (4).

The main presenting features in women with symptomatic breast cancer include a lump in the breast, nipple change or discharge and skin contour changes. Definitive diagnosis requires biopsy and histopathology. Currently available blood-based biomarkers are of no value in the early diagnosis of breast cancer.

The primary treatment for localised breast cancer is either breast-conserving surgery and radiation or mastectomy. Following primary treatment, most women with invasive breast cancer receive systemic adjuvant therapy such as chemotherapy or hormone therapy. Both these forms of adjuvant therapy have been shown to reduce systemic recurrence and mortality from breast cancer (5,6). For example, a meta-analysis of approximately 18,000 women participating in 47 randomized trials comparing combined adjuvant chemotherapy *vs* no chemotherapy concluded that polychemotherapy produced a significant proportional annual reduction in mortality both for women younger than 50 years (27%) and for those aged 50 to 69 years (11%) (5). The absolute benefit for lymph node-negative patients however, was relatively small, i.e. 7% for women under 50 years and only 3% for those between 50 and 69.

A different meta-analysis involving 37,000 women enrolled in 55 randomized trials comparing adjuvant tamoxifen *vs* placebo concluded that five years of tamoxifen therapy reduced the risk of recurrence by 47% and risk of death by 26% when given to patients with estrogen receptor (ER)-positive disease (6). Patients with ER-negative tumors however, did not benefit from adjuvant tamoxifen.

Since not all patients with breast cancer may need adjuvant treatment [e.g. approximately, 70% of lymph node-negative patients are cured of their disease by surgery and radiotherapy (7)] and not all patients benefit from this treatment, rational management requires the availability of reliable prognostic and predictive markers. Recommendations regarding the use of currently available prognostic and predictive markers for breast cancer are discussed below.

Subsequent to primary therapy, patients with a diagnosis of breast cancer are usually followed-up at regular intervals. Historically, surveillance has included clinical history, physical examination, mammography, chest X-ray, biochemical testing and the use of tumor markers. This practice is based on the assumption that the early detection of recurrent disease leads to a better outcome. However, at present, the clinical benefit of close surveillance is unclear (8).

Although adjuvant therapy improves patient outcome, 25-30% of women with lymph node-negative and at least 50-60% of those with node-positive disease develop recurrent disease (9). Therapy options for metastatic breast cancer include chemotherapy (e.g. anthracycline or taxane-based), hormone therapy or Trastuzumab (Herceptin®) combined with chemotherapy (9). Currently, metastatic breast cancer is regarded as incurable and thus the goal of treatment is generally palliative. In this context, the use of serial levels of serum tumor markers is potentially useful in deciding whether to persist in using a particular type of therapy, terminate its use or switch to an alternative therapy.

Based on the above, it is clear that optimal management of patients with breast cancer requires the use of a number of tumor markers. The aim of this article is to present new National Academy of Clinical Biochemistry guidelines on the use of both tissue and serum-based tumor markers in breast cancer. A summary of guidelines published by other Expert Panels on this topic is also provided.

CURRENTLY AVAILABLE MARKERS FOR BREAST CANCER

Table 1 lists the mostly widely investigated tissue-based and serum-based tumors markers for breast cancer. Also listed, is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use. The levels of evidence grading system used is based on that described by Hayes et al (74) *[see Section 1]*.

TUMOR MARKERS IN BREAST CANCER: NACB RECOMMENDATIONS

Table 2 presents a summary of recommendations from various Expert Panels on the use of tumor markers in breast cancer. This table also summarises the National Academy of Clinical Biochemistry (NACB) guidelines for the use of markers in this malignancy. Below, we present a more detailed discussion on the most clinically useful markers listed in Table 2.

Estrogen (ER) and Progesterone Receptors (PR

Routine assay of estrogen receptors (ER) (i.e. ER-alpha) and progesterone receptors (PR) on all newly diagnosed breast cancers has been recommended by Expert Panels of the American Society of Clinical Oncology (ASCO), European Group on Tumor Markers (EGTM), European Society of Medical Oncology (ESMO) and European Society of Mastology (EUSOMA) (Table 2). The NACB Panel agrees with these recommendations. The primary purpose of determining ER and PR is to select for likely response to endocrine therapy in patients with either early or advanced breast cancer. Additionally, in combination with other factors, ER and PR may also be used for prognostic purposes. However, as predictors of patient outcome, hormone receptors are relatively weak factors and are of little clinical value in lymph node-negative patients. Hormone receptors should therefore not be used alone for determining outcome in breast cancer. However, in combination with established prognostic factors, hormone receptors may be used to predict risk of recurrence. Determination of ER-beta has no clinical application at present.

Recommended Assay for ER and PR

ER (i.e. ER-alpha) and PR can be measured by ligand-binding assay, ELISA or immunohistochemistry. The advantages and disadvantages of these different assays are summarised in Table 3. It is important to note that most of the clinical data relating to both ER and PR was derived from biochemical (ligand-binding and ELISA) assays. Some recent reports however, have shown that the immunohistochemical determination of ER provides clinical information at least as powerful as that obtained with the biochemical assays (82-87). Indeed, one report claimed that the use of immunohistochemistry to determine ER was superior to that of biochemical assays, for predicting response to therapy (82). Compared to ER, fewer data are available on the clinical value of PR, as determined by immunohistochemistry (87-89). As with ER, the predictive power of PR as determined by immunohistochemistry appears to be superior to that obtained using ligand-binding assays (89).

Because of its ease of use and application to a wider range of tumors (e.g. small as well as large tumors and paraffin-embedded as well as frozen tissue), the NACB Panel recommends the use of immunohistochemistry for the determination of both ER and PR.

The following points should be borne in mind when determining ER and PR by immunohistochemistry:

- Immunohistochemical assays used should have been shown to give values that correlate with biochemical assays and should be validated for both predictive

and prognostic purposes, e.g. 6F11 MAb (Novocastra, Burlingame, CA and Newcastle UK) or antibody ID5 (Dako, Glostrup, Denmark) for ER and antibody 1A6 (Novocastra), PR88 (Biogenex, Menarini Diagnostics, Finch-Hampstead, Berkshire, UK) or monoclonal antibody 1294 (Dako, Glostrup, Denmark) for PR (82,83,89-91).

- Internal controls should be included in each examination. A tissue control with receptor-positive cancer cells and adjacent benign epithelium has been previously recommended (91).
- Participation in an External Quality Assessment (EQA) scheme is essential (90,91).
- Scoring of stain may be based either on percentage of cells staining or on a combination of percentage of cells staining plus intensity of stain. A semi-quantitative score should be reported rather than a negative or positive value (90,91). It is important to state that patients with low ER levels (e.g. staining in 1-10% of the cells) have been reported to respond to endocrine therapy (82).
- Only nuclear staining should be evaluated.
- The report should mention source of primary antibody as well as type of tissue used (e.g. paraffin-embedded or frozen) (91).

HER-2 (c-erbB-2)

In agreement with the ASCO (76) and NCCN Panels (71), the NACB Panel also recommends determination of HER-2 on all newly diagnosed breast cancers (Table 2). The primary purpose for determining HER-2, at present, is to select patients who may be treated with Trastuzumab (Herceptin®) in either early or advanced breast cancer. In combination with other factors, HER-2 may also be used to determine prognosis. Insufficient data are currently available to recommend HER-2 for predicting response either to adjuvant endocrine therapy or to cyclophosphamide, methotrexate and 5-fluorouracil (CMF)-based adjuvant chemotherapy (18,19,76,92). HER-2 however, may be used to predict the superiority of anthracycline-based adjuvant chemotherapy over CMF (76). Insufficient data are presently available to recommend routine use of serum HER-2 testing. Preliminary findings however, suggest that serum HER-2 may be of value in monitoring patients with advanced breast cancer undergoing treatment with Trastuzumab (57).

Recommended Assays for HER-2

Two main types of assay are used to detect HER-2 in breast tumors, i.e. immunohistochemistry and fluorescent *in situ* hybridisation (FISH) (93-99). The advantages and disadvantages of these methods are summarized in Table 4 (93-99). The NACB Panel recommends 2 possible approaches for measuring HER-2:

- Use of FISH *or*
- Use of immunohistochemistry with validated antibodies and standardized methodology. For equivocal cases (i.e. those with scores of 2+), testing with FISH should be carried out.

The following points should be borne in mind when measuring HER-2:

- Only validated reagents and standardized methodology should be used.
- Internal controls should be included in every examination, e.g. a strongly positive cancer specimen as a positive control with adjacent benign epithelium as negative control (91).
- Participation in an EQA program is essential.
- Only the invasive component of the tumor should be scored (91).
- With immunohistochemistry, only membrane staining should be reported (91).
- HER-2 testing for clinical purposes should be carried out in laboratories performing large numbers of this test.
- The report should contain type and source of assay used including source of primary antibody and any other critical reagent (91).

Currently, the US Food and Drug Administration (FDA) has approved four assays for detecting HER-2 in breast cancer. Two of these assays are based on immunohistochemistry (Dako Corporation, Carpinteria, CA and Ventana Medical Systems, Inc, Tucson, AZ) and two on FISH (Ventana Medical Systems, Inc and Vysis Inc, Downers Grove, Il). Both immunohistochemistry assays have been approved for identifying women with advanced breast cancer for therapy with Trastuzumab. The FISH-based tests were originally cleared for the selection of women with node-negative disease at high risk for progression and for response to doxorubicin-based therapy. More recently, these tests have also been approved for selecting women with metastatic breast cancer for treatment with Trastuzumab.

Urokinase plasminogen activator (uPA) and Plasminogen activator inhibitor 1 (PAI-1)

Results from a pooled analysis comprising more than 8000 patients have shown that both uPA and PAI-1 are strong (relative risk >2) and independent (i.e. independent of nodal metastases, tumor size and hormone receptor status) prognostic factors in breast cancer (21). For axillary node-negative patients, the prognostic impact of these two proteins has been validated using both a randomised prospective trial (Chemo N_0 study) and a pooled analysis of small-scale retrospective and prospective studies (20,21). uPA and PAI-1 are thus the first biological factors in breast cancer to have their prognostic value validated using Level 1 evidence studies (72).

The NACB Panel therefore states that testing for uPA and PAI-1 may be carried out to identify lymph node-negative patients that do not need or are unlikely to benefit from adjuvant chemotherapy. Measurement of both proteins should be performed as the information provided by the combination is superior to that from either alone (21,24). Lymph node-negative patients with low levels of both uPA and PAI-1 have a low risk of disease relapse and thus may be spared from the toxic side effects and costs of adjuvant chemotherapy. Lymph node-negative women with high levels of either uPA or PAI-1 should be treated with adjuvant chemotherapy. Indeed, results from the Chemo N_0 trial (20) as well as data from recent large retrospective studies (24,25) suggest that patients with high levels of uPA/PAI-1 derive an enhanced benefit from adjuvant chemotherapy.

Recommended assays for uPA and PAI-1

Measurement of both uPA and PAI-1 should be carried out using a validated ELISA. A number of ELISAs have undergone technical validation (100) while some have also been evaluated in an EQA scheme (101). For determining prognosis in breast cancer, the NACB Panel recommends use of an ELISA that has been both technically and clinically validated (e.g. from American Diagnostic Inc, CT). Extraction of tumor tissue with Triton X-100 is recommended (102). It is important to note that in order to perform an ELISA for uPA or PAI-1, a small piece of fresh (i.e. not fixed in formalin) breast tumor must be stored in liquid nitrogen immediately after histological diagnosis.

Recently, a microassay using as little as 100 mg of tumor tissue was described for the measurement of uPA and PAI-1 (103,104). This assay can also use material from two or three core biopsies or five to ten 90 µm thick cryosections. Although not yet clinically validated, preliminary data showed that uPA and PAI-1 levels in core

biopsies correlated well with corresponding levels in surgically removed tissue. As immunohistochemical determination of uPA/PAI-1 has not yet been clinically validated, this methodology cannot be recommended, at present, for the routine determination of these proteins in breast cancer.

CA 15-3/BR 27.29

The CA 15-3 and BR 27.29 (also known as CA27.29) serum assays detect the same antigen, i.e. MUC1 protein and provides similar clinical information. CA 15-3 has however, been more widely investigated than BR 27.29. There are conflicting views about the value of CA 15-3 and BR 27.29 in the postoperative surveillance of patients without evidence of disease (75-81). Although increasing CA 15-3 or BR 27.29 levels can pre-clinically detect distant metastatic disease in approximately 70% of asymptomatic patients, there is no high level evidence study showing that the early diagnosis of progressive disease followed by initiation of therapy positively impacts on either patient survival or quality of life. Furthermore, there is no universally accepted or clinically validated definition of a clinically significant tumor markers increase. A confirmed increase of at least 25% however, is widely interpreted to signify a clinically significant increase.

Based on current evidence, the NACB Panel recommends against routine CA 15-3 (or BR 27.29) testing in asymptomatic patients following diagnosis of operable breast cancer. The Panel, however, would like to note that there are a number of small studies suggesting that the early initiation of therapy based on increasing serum markers levels can lead to an enhanced outcome (105-107). Although these studies do not provide high-level evidence that early treatment based on rising tumor marker levels positively impacts on patient outcome, some doctors as well as some patients may wish to have serial levels of CA 15-3 (or BR 27.29) determined following primary surgery. The ultimate decision about whether or not to use CA 15-3 (BR 27.29) in this situation must be taken by the doctor in consultation with the patient.

According to both ASCO and NCCN, CA 15-3 (or BR 27.29) should not be used alone for monitoring therapy in advanced disease (71,75,76). However, for patients with non-evaluable disease, both Panels state that a confirmed increase in marker concentrations suggests progressive disease. In contrast to ASCO and NCCN, the EGTM Panel recommends that, in patients with metastatic disease, markers should be determined prior to each course of chemotherapy and at least every 3 months for patients receiving hormone therapy (77).

The NACB Panel states that CA 15-3 or BR 27.29 may be used to monitor chemotherapy in patients with advanced breast cancer, especially in patients with non-evaluable disease. Two successive increases are likely to indicate progressive disease and may result in cessation of therapy, change in therapy or entry of patient into clinical trials evaluating new anti-cancer treatments. However, as with markers during post-operative surveillance, there is no universally accepted or clinically validated definition of a clinically significant increase in marker concentration during therapy of advanced disease.

It is important to bear in mind that following the initiation of chemotherapy, a transient increase in serum marker levels may occur (108,109). Such transient increases or spikes usually subside within 6-12 weeks after starting chemotherapy. Increases in markers levels unrelated to tumor progression might also occur as a result of certain benign diseases (42). These increases may be transient or progressive depending on whether the benign disease is short-lived or continues to deteriorate.

Recommended assays for CA 15-3/BR 27.29

The FDA has approved a number of commercially available CA 15-3 and BR 27.29 assays.

Carcinoembryonic antigen (CEA)

As for CA 15-3 and BR 27.29, the NACB Panel does not recommend routine use of CEA in the surveillance of patients with diagnosed breast cancer. For monitoring patients with advanced disease, CEA should not be used alone. For monitoring patients with non-evaluable disease, CEA may occasionally be informative when CA 15-3/BR 27.29 is not. As a marker for breast cancer, CEA is generally less sensitive than CA 15-3/BR 27.29 but on occasion, it can be informative when levels of MUC-1-related markers remain below the cut-off point.

Recommended Assay for CEA

The FDA has approved a number of commercially available CEA assays.

BRCA1 and BRCA2

According to the Task Force of the Cancer Genetics Studies Consortium (CGSC), “early breast and ovarian cancer screening are recommended for individuals with BRCA1 mutations and early breast cancer screening for those with BRCA2 mutations” (69). No recommendation however, was made for or against prophylactic

surgery (e.g., mastectomy or oophorectomy). The guidelines further stated that "these surgeries are an option for mutation carriers, but evidence of benefit is lacking, and case reports have documented the occurrence of cancer following prophylactic surgery. It is recommended that individuals considering genetic testing be counselled regarding the unknown efficacy of measures to reduce risk and that care for individuals with cancer-predisposing mutations be provided whenever possible within the context of research protocols designed to evaluate clinical outcome" (69). It is important to point out that these guidelines were based on Expert Opinion only.

In 2003, an ASCO Panel published a detailed policy statement regarding genetic testing for cancer susceptibility (70). This statement included recommendations in the following areas: indications for genetic testing, regulation of testing, insurance reimbursement, protection from discrimination, confidentiality issues associated with genetic testing, continuing educational challenges and special research issues surrounding genetic testing of human tissues.

According to the Consensus Panel of the 8th St Gallen Conference, treatment decisions for women with mutations in BRCA1 or BRCA2 genes "need to include consideration of bilateral mastectomy with plastic surgical reconstruction, prophylactic oophorectomy, chemoprevention and intensified surveillance" (92).

The NACB Panel supports the statements published by CGSC, ASCO, NCCN, US Preventive Services Task Force (USPSTF) and the St Gallen Consensus Panel (69,70,71,72,73,92).

CONCLUSION

The best-validated markers in breast cancer are all tissue based and include ER, PR, HER-2, uPA and PAI-1. Assay of ER, PR and HER-2 is now mandatory on all newly diagnosed breast cancer patients. The measurement of uPA and PAI-1, although technically and clinically validated (20,21,100,101,110), is not yet in widespread clinical use, mainly due to the requirement of a minimum amount of fresh or freshly frozen tissue. Assay of these proteins however, may be used to aid the selection of lymph node-negative breast cancer patients that do not need adjuvant chemotherapy. Although widely used in post-operative surveillance and monitoring therapy in advanced disease, the clinical value of CA 15-3 and other serum markers has not yet been validated by a Level I evidence study.

Table 1. Useful and potentially useful markers for breast cancer.

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE[1]	Ref
Tissue-Based Markers				
Estrogen receptor (ER)	For predicting response to hormone therapy in both early and advanced breast cancer	In clinical use	I	6,10
	In combination with other factors for assessing prognosis in breast cancer. ER alone is a relatively weak prognostic factor	In clinical use	III	10,11
Progesterone receptors (PR)	Usually combined with ER for predicting response to hormone therapy	In clinical use	I	12,13
HER-2	Determining prognosis, most useful in node-positive patients. Conflicting data in node-negative patients	In clinical use in some centers	II-III	14
	For selecting patients with either early or metastatic breast cancer for treatment with Trastuzumab (Herceptin)	In clinical use	I	15-17
	For predicting resistance to hormone therapy in breast cancer	Results conflicting, undergoing further evaluation	III	18,19
	For predicting resistance to CMF[4] in early breast cancer	Results conflicting, undergoing further evaluation	III	18,19
	For selecting response to high dose anthracycline-based therapy in early breast cancer	Undergoing further evaluation	III	18,19
Urokinase plasminogen activator (uPA)	For determining prognosis in breast, cancer, including the subgroup with axillary node-negative disease	Prognostic value validated in both a prospective randomised trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany.	I	20, 21
	For predicting resistance to hormone therapy in advanced breast cancer	Undergoing evaluation	III-IV	22, 23
	For predicting enhanced response to chemotherapy in early breast cancer	Undergoing evaluation	III	24-26
PAI-1	Usually assayed in combination with uPA, i.e. for determining prognosis in breast cancer including the subgroup with node-negative disease. Provides prognostic information additional to that of uPA.	Prognostic value validated in both a prospective randomised trial and a pooled-analysis. In clinical use in parts of Europe, e.g. Germany.	I	20,21
	In combination with uPA may be of value for predicting enhanced response to adjuvant chemotherapy and resistance to hormone therapy in advanced disease	Undergoing further evaluation	III	22-26
Cathepsin D	For determining prognosis in breast cancer	Results conflicting. However, using a specific ELISA, most reports show a prognostic value. Prognostic value in node-negative breast cancer validated by meta-analysis. Not in clinical use	I (Only in node-negative disease)	27-29
p53	For evaluating prognosis in breast cancer	Results conflicting when p53 protein is determined by IHC[5]. Mutations in p53 gene however, correlates with adverse outcome. Undergoing further evaluation	III (with IHC), I (with mutation testing)	30,31
	For predicting response to both chemotherapy and hormone therapy in breast cancer	Results conflicting. Undergoing further evaluation	III	31,32
DNA ploidy	For assessing prognosis in breast cancer	Results conflicting. Undergoing further evaluation	III	33,34
S-phase	For assessing prognosis in breast cancer	Most studies conclude that increased S-phase predicts poor outcome. Undergoing further evaluation	III	35-36
Angiogenesis	For assessing prognosis	Most studies conclude that increased rates of angiogenesis predicts poor outcome. However, reproducibility is poor and use as measure of angiogenesis to guide treatment decisions is not recommended	II-III	37,38
Gene expression	For assessing prognosis	Undergoing evaluation. For one of these profiles (39,40), a multicenter	III-IV	39,40

microarray		validation study is planned		
Oncotype DX™	For predicting response to adjuvant tamoxifen in lymph node-negative breast cancer	Validated in a prospectively designed study, assay can be carried out on paraffin-embedded tissue. In clinical use	I/II	41
Serum-Based Markers				
CA 15-3	Post-operative surveillance in patients with no evidence of disease	In clinical use, but value of changing therapy for patients with rising levels not validated in a high-level evidence study	III	42,43
	Monitoring therapy in advanced disease	In clinical use, but value not validated in a high-level evidence study	III	42,43
	Assessing prognosis. High preoperative levels (e.g. > 30 U/L) predict adverse outcome	Not in clinical use	III	44-47
BR 27.29	Provides similar information to CA 15-3 but not as widely investigated as CA 15-3	In clinical use, but value not validated in a high-level evidence study	III	48,49
CEA	Post-operative surveillance in patients with no evidence of disease. Overall, appears to be less sensitive than CA 15-3/BR 27.29	In clinical use, but value not validated in a high-level evidence study.	III	50-53
	Monitoring therapy in advanced disease, especially if CA 15-3/BR 27.29 is not elevated	In clinical use, but value not validated in a high-level evidence study.	III	50,-53
	Assessing prognosis. High preoperative levels predict adverse outcome	Not in clinical use	III	44,46,53
TPA[2]	Post-operative surveillance in patients with no evidence of disease	In clinical use in some countries, but value not validated in a high level evidence study.	III	50,52
	Monitoring therapy in advanced disease. May be useful if CA 15-3, BR 27. 29 or CEA are not elevated	In clinical uses in certain countries, but value not validated by a high level evidence study.		43,52
TPS[3]	As for TPA	As for TPA	III	54,55
HER-2 (shed form)	Determining prognosis; predicting response to hormone therapy, chemotherapy and Trastuzumab; post-operative surveillance and monitoring therapy in advanced disease. Less sensitive than either CA 15-3 or CEA but may be useful in monitoring if CA 15-3, BR 27.29 or CEA are not elevated. Preliminary results suggest that serum HER-2 may be of value in monitoring Trastuzumab therapy in patients with advanced breast cancer	Undergoing evaluation.	III-IV	56,57
Proteomics	Detecting early disease and monitoring	Undergoing evaluation	IV/V	58,59
Tumor Cells				
Tumor cells in bone marrow	For assessing prognosis	Prognostic value validated in a pooled analysis. Not in clinical use	I	60-62
Tumor cells in axillary nodes	For assessing prognosis	Most studies conclude that the detection of tumor cells in axillary nodes predicts adverse prognosis but magnitude of effect appears relatively weak. Undergoing further evaluation	II-III	63,64
Tumor cells in sentinel lymph nodes	For assessing prognosis	Undergoing evaluation. Two prospective trials are currently in progress	IV/V	65,66
Tumor cells in circulation	For assessing prognosis and monitoring therapy in advanced disease	Undergoing evaluation	III	67,68
Genetic Markers				
BRCA1	For identifying individuals who are at high risk of developing breast or ovarian cancer in high risk families	In clinical use in specialised centers	Expert opinion	69-73
BRCA2	As for BRCA1	In clinical use in specialised centers	Expert opinion	69-73

LOE, level of evidence (as defined in ref. 74); TPA, tissue polypeptide antigen; TPS, tissue polypeptide specific-antigen; CMF, cyclophosphamide, methotrexate, 5-fluorouracil; IHC, immunohistochemistry.

Table 2. Recommendations for use of markers in breast cancer by different Expert Groups.

Marker (s)	Application	ASCO[1] (75,76)	EGTM[2] (77)	Joint EGTM /NACB[3] 78)	ESMO[4] (79,80)	EUSOMA[5] (81)	NCCN[6] (71)	NACB 2006*
ER + PR	For predicting response to hormone therapy	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	For prognosis	**No**	**Yes**, in combination with other factors	None published	None published	None published	**Yes**	**Yes**, in combination with existing factors
HER-2	For predicting response to Trastuzumab in advanced disease	**Yes**	**Yes**	None published	**Yes**	None published	**Yes**	For predicting response to Trastuzumab in either early or advanced breast cancer
	For prognosis	None published	**Yes**, in combination with other factors	None published	None published	None published	**Yes**	**Yes**, in combination with other factors
	For predicting response to hormone therapy	**No**	**No**	None published	None published	None published	None published	**No**
	For predicting response to adjuvant CMF	**No**	**No**	None published	None published	None published	None published	**No**
	For predicting response to adjuvant anthracycline-based therapy	**Yes ?**	**Yes**	None published	None published	None published	**Yes**, for predicting superiority of anthracycline-based over non-anthracycline-based adjuvant therapy	**Yes**, as per NCCN
CA 15-3 /BR27-29	Surveillance following surgery	**No**	**Yes**	**Yes**	**No**	None published	**No**	May provide lead-time for early detection of metastasis but clinical value of lead-time unclear
CA 15-3 /BR27.29	Monitoring advanced disease	**Yes**, in selected cases, e.g., in absence of measurable disease	**Yes**	**Yes**	**Yes**, in non easily measurable disease	***Yes**, in absence of evaluable disease	None published	**Yes**, especially in patients with non-evaluable disease
CEA	Surveillance following surgery	**No**	**Yes**	None published	**No**	None published	None published	**No**
	Monitoring advanced disease	**Yes**, in selected cases, e.g., in absence of measurable disease	**Yes**	None published	**No**	***Yes**, in absence of evaluable disease	None published	**Yes**, as per ASCO and EUSOMA
uPA/PAI-1	For determining prognosis in lymph node-negative patients	None published	**Yes**	None published	None published	None published	None published	**Yes**
BRCA1 BRCA2	For identifying women at high risk of developing breast cancer	See ref. 70 for general guidelines on genetic testing for cancer susceptibility	None published	None published	None published	None published	None published	NACB supports documents of CGSC[8], ASCO, NCCN and [9]USPSTF (69-73)

[1]ASCO, American Society of Clinical Oncology; [2]EGTM, European Group on Tumor Markers; [3]NACB, National Academy of Clinical Biochemistry; [4]ESMO, European Society of Clinical Oncology; [5] EUSOMA, European Society of Mastology; [6] NCCN, National Comprehensive Cancer Network; [7]NR, no recommendation published; CGSC, [8]Cancer Genetics Studies Consortium and US Preventive Services Task Force.

*Recommendations state serum markers without referring to specific markers.

Table 3. Advantages and disadvantages of different assays for hormone receptors

Ligand-binding assay	ELISA	Immunohistochemistry
Advantages		
• Quantitative	• Quantitative	• Simple and relatively cheap
• Can determine functionality of receptor with respect to hormone binding	• No radioactivity required	• Can assess tissue architecture, distinguishing invasive, *in situ* and normal breast tissue
• Can determine Km of receptor for ligand	• Simpler than ligand binding	• Can use small amounts of tissue including fine needle aspirates and core needle biopsies
• Should be able to detect total ER, i.e. ER-α and ER-β but does not discriminate between the two forms		• Normal breast epithelial cells in adjacent tissue provide an internal positive control, at least for ER
Disadvantages		
• Time-consuming	• Requires large amount of frozen tissue	• Semi-quantitative
• Cumbersome	• Relatively time-consuming	• Interpretation subjective
• Expensive		• Difficult to standardize
• Requires large amount of tumor tissue		• Different antibodies can give different results
• Requires frozen tissue (must be rapidly frozen in liquid nitrogen and maintained at low temperature)		
• Requires radioactivity		
• May yield false negative ER values[a]		

[a] In tumors removed from patients receiving Tamoxifen, when endogenous levels of steroid ligand are high, or when insufficient breast cancer is present in the tissue mass;

Table 4. Advantages and disadvantages of different assays for HER-2 immunohistochemistry

Immunohistochemistry	FISH
Advantages	
• Low cost	• Relatively more objective scoring system and easier to standardize
• Simple	• Provides a more robust signal than immunohistochemistry
• Widely available	• Appears to be more accurate in predicting response to Trastuzumab in patients with advanced breast cancer
Disadvantages	
• Evaluation is subjective and thus difficult to standardize	• Relatively expensive
• Loss of sensitivity due to antigenic alteration due to fixation	• Less widely available than immunohistochemistry (requires fluorescent microscope)
• Wide variability in sensitivity of different antibodies and different results from the same antibody, depending on staining procedure (81)	• May sometimes be difficult to identify carcinoma in tissues with ductal carcinoma *in situ*
• Borderline values (e.g. 2+) require additional testing	• Requires longer time for scoring than immunohistochemistry
	• Unable to preserve slide for storage and review
	• Cut-off to establish critical level of amplification and clinical outcome uncertain

Data summarised from refs 93-99. FISH, fluorescence *in situ* hybridisation.

REFERENCES

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1999. Int J Cancer 1999; 80: 827-841.
2. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E. Cancer statistics, 2004. CA Cancer J Clin 2003;53:4-25.
3. Hewitt M, Breen N, Devesa S. Cancer prevalence and survivorship issues: Analysis of the 1992 National Health Interview Survey. J Natl Cancer Inst 1999;91:1480-1486.
4. Peto R, Boreham J, Clarke M, Davies C, Beral V. UK and USA breast cancer deaths down 25% in year 2000 at ages 20-69 years. Lancet 2000; 355:1822.
5. Early Breast Cancer Trialist's Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised clinical trials. Lancet 1998;352:930-942.
6. Early Breast Cancer Trialist's Collaborative Group. Tamoxifen for early breast cancer: an overview of randomized trials. Lancet 1998;351:1451-1467.
7. Morris AD, Morris RD, Wilson JF, White J, Steinberg S, Okunieff P, *et al*. Breast conserving therapy *vs* mastectomy in early stage breast cancer: a meta-analysis of 10-year survival. Cancer J Sci Am 1997; 3: 6-12.
8. Emens LA, Davidson NE. The follow-up of breast cancer. Sem Oncol 2003;30:338-348.
9. Bernard-Marty C, Cardoso F, Piccart MJ. Facts and controversies in systemic treatment of metastatic breast cancer. Oncologist 2004;9:617-632.
10. Duffy MJ. Estrogen receptor α and estrogen receptor β: role in breast cancer. Crit Rev Clin Lab Sci, 2006.
11. Mirza AN, Mirza NQ, Vlastos G, Singletary E. Prognostic factors in mode-negative breast cancer. A review of studies with sample size more than 200 and follow-up more than 5 years. Ann Surg 2002;235:10-26.
12. Ravidin PM, Green S, Dorr TM, McGuire WL, Fabian C, Pugh RP, et al, Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Study Group. J Clin Oncol 1992;10:1284-1291.
13. Bardou VJ, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in 2 large breast cancer databases. J Clin Oncol 2003;21:1973-1979.

14. Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, et al. The HER-2/neu gene and protein in breast cancer 2003; biomarker and target of therapy. Oncologist 2003;8:307-325.
15. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpressed HER2. N Eng J Med 2001;344:783-792.
16. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, et al, Trastuzumab plus adjuvant chemotherapy for operable HER-2-positive breast cancer. N Engl J Med 2005;353:1673-1684.
17. Piccart-Gebhart MJ, Proctor M, Leyland-Jones B, Goldhirch A, Untch M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in HER-2-positive breast cancer. N Engl J Med 2005;353:1659-1684.
18. Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol 2001;19:2334-2356.
19. Duffy MJ. Predictive markers in breast and other cancers. Clin Chem 2005;51:494-503.
20. Janicke F, Prechtl A, Thomssen C, Harbeck N, Meisner C, Sweep F, et al. For the German Chemo N_o Study Group. Randomized adjuvant chemotherapy trial in high-risk node-negative breast cancer patients identified by urokinase-type plasminogen activator and plasminogen activator inhibitor type 1. J Natl Cancer Inst 2001;93:913-920
21. Look M P, van Putten WLJ, Duffy MJ, Harbeck N, Christensen U, Thomssen C, et al. Pooled analysis of prognostic impact of tumor biological factors uPA and PAI-1 in 8377 breast cancer patients. J Natl Cancer Inst, 2002;94:116-128.
22. Foekens J, Look MP, Peters HA, van Putten WK, Portengen H, Klijn JG, et al. Urokinase-type plasminogen activator and its inhibitor PAI-1: predictors of poor response to tamoxifen therapy in recurrent breast cancer. J Natl Cancer Inst 1995;87:751-756.
23. Meijer-van Gelder ME, Look MP, Peters HA, Schmitt M, Brunner N, Harbeck N, et al. Urokinase-type plasminogen activator system in breast cancer: association with tamoxifen therapy in recurrent disease. Cancer Res 2004;64:4563-4568.
24. Harbeck N, Kates RE, Schmitt M. Clinical relevance of invasion factors urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 for individualised therapy in primary breast cancer is greatest when used in combination. J Clin Oncol 2002;20:1000-1007.

25. Harbeck N, Kates RE, Look MP, Meijer-van Gelder M, Klijn JGM, Kruger A, et al. Enhanced benefit from adjuvant chemotherapy in breast cancer patients classified high-risk according to urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type 1 (N=3424). Cancer Res 2002;62:4617-4622.
26. Harbeck N, Kates RE, Look MP, Foekens J, on behalf of the Pooled Analysis Study of the EORTC Receptor and Biomarker Group (RBG). J Clin Oncol, 2004 ASCO Annual Proceedings (Post-meeting Edition). Vol 22, No 14S (July 15 Supplement), 2004:523.
27. Foekens JA, Look M, Bolt-de Vries Meijer-van Gelder ME, van Putten WLJ, Klijn JGM. Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients. Br J Cancer 1999;79:300-307.
28. Ferrandina G, Scambia G, Bardell F, Benedetti P, Mancuso S, Messori A. Relationship between cathepsin-D content and disease-free survival in node-negative breast cancer patients: A meta-analysis. Br J Cancer 1997;76:661-666.
29. Ravdin PM, Tandon AK, Allred DC, Clark GM, Fuqua SAW, Hilsenbeck SM, et al. Cathepsin D by western blotting and immunohistochemistry: Failure to confirm correlation with prognosis in node-negative breast cancer. J Clin Oncol 1994;12:467-474.
30. Elledge RM, Allred DC. Prognostic and predictive value of p53 and p21 in breast cancer. Breast Cancer Res Treat 1998; 52: 79-98.
31. Pharoah PDP, Day NE, Caidas C. Somatic mutation in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer 1999;80:1968-1973.
32. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. Nature Cancer 2001;1:233-240.
33. Ravaioli A, Bagli L, Zucchini A, Monti F. Prognosis and prediction of response in breast cancer: the current role of the main biological markers. Cell Prolif 1998;31:113-126.
34. Masood S. Prediction of recurrence for advanced breast cancer. Traditional and contemporary pathologic and molecular markers. Surg Clin N Am 1995;4:601-632
35. Michels JJ, Marnay J, Delozier T, Denoux Y, Chasle J. Proliferative activity in primary breast carcinomas is a salient prognostic factor. Cancer 2004;100:455-464.
36. Hedley DW, Clark GM, Cornelisse CJ, Killander D, Kute T, Merkel D. DNA cytometry consensus conference. Consensus review of the clinical utility of

DNA cytometry in carcinomas of the breast. Breast Cancer Res Treat 1993;28:55-59.

37. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis. correlations in invasive breast cancer. N Eng J Med 1991;324:1-8.
38. Uzzan B, Nicolas P, Cucherat M, Perret G-Y. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. Cancer Res 2004;64:2941-2955.
39. Van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-536.
40. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskouil DW et al A gene expression signature as a predictor of survival in breast cancer. N Eng J Med 2002;347:1999-2009.
41. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M et al. A multi-gene assay to predict recurrence of tamoxifen-treated node-negative breast cancer. N Engl J Med 2005;351:2817-2826.
42. Duffy MJ. Serum Tumor Markers in Breast Cancer: Are They of Clinical Value?. Clin Chem, 2006, in press.
43. Cheung K, Graves CRL, Robertson JFR. Tumour marker measurements in the diagnosis and monitoring of breast cancer. Cancer Treat Rev. 2000;26:91-102.
44. Molina R, Filella X, Alicarte J, Zanon G, Pahisa J, Munoz M, et al. Prospective evaluation of CEA and CA 15.3 in patients with locoregional breast cancer. Anticancer Res 2003;23:1035-1042.
45. Gion M, Boracchi P, Dittadi R, Biganzoli E, Peloso L, Mione R, et al. Prognostic role of serum CA 15.3 in 362 node-negative breast cancer. An old player for a new game. Eur J Cancer 2002;38:1181-1188.
46. Ebeling FG, Stieber P, Untch M, Nagel D, Konecny GE, Schmitt UM, et al. Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. Br J Cancer 2002;22:1217-1222.
47. Duffy MJ, Duggan C, Keane R, Hill ADK, McDermott E, Crown J, et al. High preoperative CA 15-3 concentrations predict adverse outcome in node-negative and node-positive breast cancer: study of 600 patients with histologically confirmed breast cancer. Clin Chem 2004;50:559-563.
48. Dnistrian AM, Schwartz MK, Greenberg EJ, Schwartz DC. BR27.29 as a marker in breast cancer. J Tumor Marker Oncol 1995; 10: 91-97.
49. Chan DW, Beveridge RA, Muss H, Fritsche HA, Theriault R, Kiang D, et al. Use of Truquant BR radioimmunoassay for early detection of breast cancer

recurrence in patients with stage II and III disease. J Clin Oncol 1997;15:2322-2328.

50. Nicolini A, Anselmi L, Michelassi C, Carpi A. Prolonged survival by "early salvage treatment of breast cancer patients: a retrospective 6-year study. Br J Cancer 1997; 76: 1106-1111.
51. Molina R, Zanon G, Filella X, Moreno F, Jo J, Daniels M, et al. Use of serial carcinoembryonic antigen and CA 15-3 assays in detecting relapse in patients with breast cancer. Breast Cancer Res Treat 1995;36:41-48.
52. Soletormos G, Nielsen D, Schioler V, Mouridsen H, Dobernowsky P. Monitoring different stages of breast cancer using tumour markers CA 15-3, CEA and TPA. Eur J Cancer 2004;40:481-486
53. Molina R, Jo J, Filella X, Zanon G, Pahisa J, Munoz M, c-erbB-2 oncoprotein, CEA and CA 15.3 in patients with breast cancer: prognostic value. Breast Cancer Res Treat 1998;51:109-119.
54. van Dalen A. TPS in breast cancer, a comparative study with carcinoembryonic antigen and CA 15-3. Tumor Biol 1992;13:10-17.
55. Van Dalen A, Heering KJ, Barak V, Peretz T, Cremaschi A, Geroni P, et al. Treatment response in metastatic breast cancer: a multicenter study comparing UICC criteria and tumor marker changes. The Breast 1996;5:82-88.
56. Carney WP, Neuman R, Lipton A, Leitzel K, Ali S, Price C. Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patients with breast cancer. Clin Chem 2003;49:1579-1598.
57. Esteva FJ, Cheli CD, Fritsche H, Fornier M, Slamon D, Thiel RP, et al. Clinical utility of serum HER-2/neu in monitoring and prediction of progression-free survival in metastatic breast cancer patients treated with trastuzumab-based therapies. Breast Cancer Res 2005;7:R436-R443.
58. Li J, Zhang Z, Rosenweig J, Yang YY, Chan DW. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. Clin Chem 2002;48:1296-1304.
59. Vlahou A, Laronga C, Wilson L, Gregory B, Fournier K, McGaughey D, et al. A novel approach towards development of a rapid blood test for breast cancer. Clin Breast Cancer 2003;4:203-209.
60. Braun S, Pantel K, Muller P, Janni W, Hepp F, Kentenich CR, et al. Cytokeratin-positive cells in the bone marrow and survival in patients with stage I, II and III breast cancer. N Eng J Med 2000;342:525-533.

61. Wiedswang G, Borgen E, Karesen R, Kvalheim G, Nesland JM, Qvist H, et al. Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. J Clin Oncol 2003;21:3469-3478.
62. Braun S, Vogel FD, Naume B, Janni W, Osborne MP, Coombes RC, et al A pooled analysis of bone marrow micrometastasis in breast cancer. N Engl J Med 2005;353:793-802.
63. Mansi JL, Gogas H, Bliss JM, et al. Outcome of primary breast cancer patients with micrometastases: a long-term follow-up study. Lancet 1999;354:197-202.
64. International (LUDWIG) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancer. Lancet 1990;335:1565-1568.
65. European Working Group for Breast Cancer Screening Pathology. Pathological work-up of sentinel lymph nodes in breast cancer. Review of current data to be considered for the formulation of guidelines. Eur J Cancer 2003;39:1654-1667.
66. Wilke LG, Giuliano A. Sentinel lymph node biopsy in patients with early-stage breast cancer: Status of the National Clinical Trials. Surgical Clinics N Am 2003;83:901-910.
67. Cristofanilli M, Budd GT, Ellis M, Stopeck A, Matera J, Miller MC,et al. Circulating tumor cells predict progression free survival and overall survival in metastatic breast cancer. N Engl J Med 2004;351:781-791.
68. Cristofanilli M, Hayes DF, Budd GT, Ellis M, Stopeck A, Reuben JM, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. N Engl J Med 2005;23:1420-1430.
69. Burke W, Daly M, Garber J, Botkin J, Kahn MJ, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2 Cancer Genetics Studies Consortium. JAMA 1997;277:997-1003.
70. American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. J Clin Oncol 2003;21:1-10.
71. NCCN Practice Guidelines in Oncology http://www.nccn.org/professionals/physician_gls/f_guidelines.asp?button=I+Agree. Accessed 14 June 2005.
72. US Preventive Services Task Force. Genetic risk assessment and BRCA mutation testing for breast and ovarian susceptibility: Ann Int Med 2005;143:355-361
73. Nelson HD, Huffman LH, Fu R, Harria EL. Genetic risk assessment and BRCA mutation testing for breast and ovarian susceptibility: systematic evidence

review for the US Preventive Services Task Force. Ann Int Med 2005;143:362-379.

74. Hayes DF, Bast R, Desch CE, Fritsche H, Kemeny NE, Jessup JM, et al. A tumor marker utility grading system (TMUGS): a framework to evaluate clinical utility of tumor markers. J. Natl Cancer Inst. 1996; 88: 1456-1466.
75. Anonymous. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996; 14: 2843-2877.
76. Bast RC, Ravdin P, Hayes DF, Bates S, Fritsche H, Jessup JM, et al. 2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001;19:1865-1878.
77. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M, et al. Tumor markers in breast cancer: European Group of Tumor Markers (EGTM) recommendations. Tumor Biol 2005;26:281-293.
78. Fleisher M, Dnistrian AM, Sturgeon CM, Lamerz R, Wittliff J. Practice guidelines and recommendations for use of tumor markers in the clinic. In: Diamandis EP, Fritsche H, Schwartz MK, Chan DW, eds, Tumor markers: Physiology, pathobiology, technology and clinical applications, Chicago: AACC Press 2002:33-63.
79. Pestalozzi BC, Luporsi-Gely E, Jost LM, Bergh J. ESMO minimum clinical recommendations for diagnosis, adjuvant treatment and follow-up of primary breast cancer. Ann Oncol 2005;16 (Suppl 1):i7-i9.
80. Kataja VV, Colleoni M, Bergh J. ESMO minimum clinical recommendations for diagnosis, and follow-up of locally recurrent or metastatic breast cancer. Ann Oncol 2005;16: (Suppl 1):i10-i12.
81. Blamey RW. Guidelines on endocrine therapy of breast cancer, EUSOMA. Eur J Cancer 2002;38:615-634.
82. Harvey JM, Clark GM, Allred C. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol 1999;17:1474-1481.
83. Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, et al. Estrogen receptor (ER) and progesterone receptor (PgR) by ligand-binding assay compared with ER, PgR and pS2 by immunohistochemistry in predicting response to tamoxifen in metastatic breast cancer: a southwest oncology group study. Int J Cancer 2000;89:111-117.

84. McCarty KSJ, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analysis. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 1985;109:716-721.
85. Barnes DM, Harris WH, Smith P, Millis RR, Rubens D. Immunohistochemical determination of oestrogen receptors: Comparison of different methods of assessment of staining and correlation with clinical outcome of breast cancer patients. Br J Cancer 1996;74:1445-1451.
86. Pertschuk LP, Feldman JG, Kim YD, Braithwaite L, Schneider F, Braverman AS, et al. Estrogen receptor immunohistochemistry in paraffin embedded tissue with ER1D5 predicts breast cancer endocrine response more accurately than H222Spgamma in frozen sections or cytosol-based ligand binding assays. Cancer 1996;77:2514-2519.
87. Fisher ER, Anderson S, Dean S, Dabbs D, Fisher B, Siderits R, et al. Solving the dilemma of the immunohistochemical and other methods used for scoring estrogen receptor and progesterone receptor in patients with invasive breast cancer. Cancer 2005;103:164-173.
88. Chebil G, Bendahl PO, Ferno M, Idvall I, Ferno M. Estrogen and progesterone receptor assay in paraffin-embedded breast cancer, reproducibility of assessment. Acta Oncol 2003;42:43-47.
89. Mohsin SK, Weiss H, Havighurst T, Clark GM, Barardo M, Roanh LD, et al. Progesterone receptors by immunohistochemistry and clinical outcome in breast cancer: a validation study. Mod Pathol 2004;17:1545-1554.
90. Leake R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, et al, Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. J Pathol 2000;53:634-635.
91. Fitzgibbons PL, Page DL, Weaver D, Thor A, Allred DC, Clark GM, et al. Prognostic factors in breast cancer: College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000;124:966-978.
92. Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann, Senn H-J. Meeting Highlights: Updated International Expert Consensus Panel on the Primary Therapy of Early Breast Cancer. J Clin Oncol 2003:21:1-9.
93. Bartlett J, Mallon E, Cooke T. The clinical evaluation of HER-2 status: Which test to use? J Pathol 2003;199:411-417.
94. Pauletti G, Dandekar S, Rong HM, Ramos L, Peng HJ, Seshadri R, et al. Assessment of methods for tissue-based detection of the HER-2/*neu* alteration in human breast cancer: A direct comparison of fluorescence *in situ* hybridisation and immunohistochemistry. J Clin Oncol 2000;18:3651-3664.

95. Press M, Hung G, Godolphin W, Slamon DJ. Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of expression. Cancer Res 1994;54:2771-2777.
96. Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol 2002;20:3095-3105.
97. Bartlett JMS, Going JJ, Mallon EA, Watters AD, Reeves JR, Stanton P, et al. Evaluating HER-2 amplification and overexpression in breast cancer. J Pathol 2001;195:422-428.
98. Birner P, Oberhuber G, Stani J, Reithofer C, Samonigg H, Hausmaninger H, et al. Evaluation of the United States Food and Drug Administration-approved scoring and test system of HER-2 expression in breast cancer. Clin Cancer Res 2001;7:1669-1675.
99. Yaziji H, Goldstein LC, Barry TS, Werling R, Hwang H, Ellis GK, et al. HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004:291:1972-1977.
100. Benraad TH, Geurts-Moespot J, Grondahl-Hansen J, Schmitt M, Heuvel J, De Witte JH, et al. Immunoassays (ELISA) of urokinase-type plasminogen activator (uPA): report of an EORTC/BIOMED-1 Workshop. Eur J Cancer 1996;32:1371-1381.
101. Sweep CGJ, Geurts-Moespot J, Grebenschikov N, De Witt JH, Heuvel J, Schmitt M, et al. External quality assessment of trans-European multicenter antigen determination (enzyme-linked immunosorbent assay) of urokinase plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) in human breast cancer extracts. Br J Cancer 1998;78:1434-1441.
102. Janicke F, Pache L, Schmitt M, Ulm K, Thomssen C, Prechtl A, et al. Both the cytosol and detergent extracts of breast cancer tissues are suited to evaluate the prognostic impact of the urokinase-type plasminogen activator and its inhibitor , plasminogen activator inhibitor type-1. Cancer Res 1994;54:2527-2530.
103. Abraha RS, Thomssen C, Harbeck N, Mueller V, Baack K, Schmitt M, et al. Micromethod for determination of uPA and PAI-1 from preoperative core-needle biopsies in breast cancer. Breast Cancer Res Treat 2003;82 (Suppl 1):S144, abstract No 608.
104. Schmitt M, Lienert S, Prechtel D, Sedlaczek E, Welk A, Reuning U, et al. The urokinase protease system as a target for breast cancer prognosis and therapy:

technical considerations. Schmitt M, Eickler A, Welk A, Schnelldorfer C, Harbeck N. Procedure for the quantitative determination of urokinase (uPA) and its inhibitor PAI-1 in human breast cancer tissue extracts by ELISA. In Brookes S, Harris A, eds, Breast Cancer Protocols, 2005.

105. Jager W. The early detection of disseminated (metastasised) breast cancer by serial tumour marker measurements Eur J Cancer Prev 1993;2 (Suppl 3):133-139.
106. Nicolini A, Anselmi L, Michelassi C, Carpi A. Prolonged survival by “early salvage treatment of breast cancer patients: a retrospective 6-year study. Br J Cancer 1997; 76: 1106-1111.
107. Kovner F, Merimsky O, Hareuveni M, Wigler N, Chaitchik S. Treatment of disease-negative but mucin-like carcinoma-associated antigen-positive breast cancer patients with tamoxifen: preliminary results of a prospective controlled randomised trial. Cancer Chemother Pharmacol 1994;35:80-83.
108. Hayes DF, Kiang DT, Korzun AH, Tondini C, Wood WC, Kufe DW. CA 15-3 and CEA spikes during chemotherapy for metastatic breast cancer. Proc Am Soc Clin Oncol 1988;7:38.
109. Yasasever V, Dincer M, Camlica H, Karaloglu D, Dalay N. Utility of CA 15-3 and CEA in monitoring breast cancer patients with bone metastasis: special emphasis on “spiking “ phenomena. Clin Biochem 1997;30:53-56.
110. Urokinase plasminogen activator and its inhibitor PAI-1, as prognostic markers in breast cancer: from pilot to level I evidence studies. Clin Chem 2002;48:1194-1197.