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MINI REVIEW

The extracellular domain of Her2 in serum as a biomarker of breast cancer

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Abstract

Breast cancer is a major health problem worldwide. In ~15% of breast cancers, the epidermal growth factor receptor HER2, a transmembrane protein, is overexpressed. This HER2 overexpression is associated with an aggressive form of the disease and a poor clinical prognosis. The extracellular domain (ECD) of HER2 is released into the blood by a proteolytic mechanism known as "ECD shedding". This proteolytic shedding leaves a constitutively active truncated receptor in the membrane that is 10–100-fold more oncogenic than the full-length receptor and promotes the growth and survival of cancer cells. Shedding of the HER2 ECD is increased during metastasis: whereas 15% of primary breast cancer patients have elevated levels of serum HER2 ECD (sHER2 ECD), the levels reach 45% in patients with metastatic disease. Thus, sHER2 ECD has been proposed as a promising biomarker for cancer recurrence and for monitoring the disease status of patients overexpressing HER2. Nevertheless, in 2016, the American Society of Clinical Oncology advises clinicians not to use soluble HER2 levels to guide their choice of adjuvant therapy for patients with HER2-positive breast cancer, because the evidence was considered not strong enough. Currently, biomarkers such as carcinoembryonic antigen and cancer antigen 15-3 are widely used to monitor metastatic breast cancer disease even if the level of evidence of clinical impact of this monitoring is poor. In this article, we review the evidence that sHER2 ECD might be used in some situations as a biomarker for breast cancer. Although this serum biomarker will not replace the direct measurement of tumor HER2 status for diagnosis of early-stage tumors; it might be especially useful in metastatic disease for prognosis, as an indicator of cancer progression and of therapy response, particularly to anti-HER2 therapies. Owing to these data, sHER2 ECD should be considered as a promising biomarker to detect cancer recurrence and metastasis.

Worldwide, breast cancer is the most common cancer in women: it comprises 22.9% of invasive cancers in women (World Cancer Report, 2008) and 16% of all female cancers (World Health Organization, 2015). Moreover, it has the highest incidence rate and second highest mortality rate of all malignant tumors in women [1]. In 2012, 1.68 million cases and 522,000 deaths due to breast cancer were described according to the World Health Organization. The number of cases worldwide has increased significantly since the 1970s.

Breast cancers can be divided into different subtypes according to their expression of the human epidermal growth factor receptor-2 (HER2), estrogen receptor (ER), progesterone receptor (PR), and Ki67, a marker of cell proliferation [2]. Each subtype has a distinct prognosis and responds differently to various treatments. Thus, accurate characterization of these biomarkers in tumor biopsies is crucial for clinicians to select an appropriate treatment. These decisions have a huge impact on patient care. Most studies divide breast cancer into four major subtypes based on the molecular markers expression: luminal A; luminal B; triple-negative/basal-like; HER2 type (Table 1; [3]). Cancers that express ER (ER+) depend on estrogen for their growth, so they can be treated with drugs that block the effects of this hormone, such as selective estrogen receptor modulating (SERM) or disrupting (SERD) agents or

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Table 1 Breast tumor subtypes

Subtype	PR	ER	HER2	Ki67	Prevalence	Prognostic
Luminal A	+	+	−	−	23.7%[a]	Good
Luminal B	+	+	−	+	38.8%[a]	Intermediate
	+	+	+	+	14%[a]	Poor
HER2 overexpression	−	−	+	#	11.2%[a]	Poor
Triple-negative	−	−	−	#	12.3%[a]	Poor
Normal-like	+	+	−	−	7.8%[b]	Intermediate

PR progesterone receptor, *ER* estrogen receptor. #: not in the definition criteria

Prevalence: [a]data from ref. [38], [b]data from ref. [63]. Prognostic: data from ref. [3]

aromatase inhibitors, and they usually have a better prognosis. Cancers that overexpress HER2 (HER2+) or have an amplification of HER2/neu have been historically considered as the most aggressive [4]. However, HER2+ cancers respond to the anti-HER2 treatments and particularly to monoclonal antibodies like trastuzumab, and this have significantly improved the prognosis [5]. Cancers that have none of the three receptor types (ER−, PR−, and HER2−) are called triple-negative, although they rarely express receptors for other hormones, such as the androgen receptor and prolactin receptor.

HER2 belongs to the human epidermal growth factor (EGF) receptor family, which comprises EGFR, HER2/neu (also known as c-erbB2), HER3, and HER4. This family regulates many processes including cell proliferation, differentiation, migration, and survival. In contrast to the other members of the family, HER2 does not bind growth factors and has no known ligand. The HER2/neu gene encodes a 185 kDa transmembrane receptor (also known as glycoprotein $p185^{her2/neu}$) that has three domains: an intracellular tyrosine kinase domain, a transmembrane lipophilic segment, and an extracellular domain (ECD) of 105 kDa ($p105^{HER2}$ ECD; Fig. 1). Although HER2 does not bind a ligand, it is activated upon homodimerization or heterodimerization with another member of the HER family, or by proteolytic cleavage of its ECD. This dimerization with other members of the receptor family produces what has been called a "dimerization-ready signal amplifier" [6]. It also stabilizes the ligand binding and enhances kinase-mediated activation of downstream signaling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase. HER2 is normally expressed at low levels in the epithelial cells of many tissues in addition to breast, including lung, kidney, ovary, gastrointestinal tract, and placenta.

The importance of HER2/neu in tumorigenesis was first recognized when neu, the rat homologue of HER2/neu, was found to be the oncogene that causes neuroblastoma and was shown to transform NIH3T3 fibroblasts [7, 8]. In humans, the HER2/neu (or *ERBB2*) gene is located whitin the long arm of chromosome 17 (17q12), which is amplified and/or overexpressed in about 12–15% of breast tumors [9, 10]. Breast cancers associated with HER2/neu amplification or receptor overexpression have an accelerated growth rate and a greater rate of recurrence, and are associated with the poorer overall patient survival than breast cancers without HER2/neu amplification or overexpression [11]. Thus, HER2/neu overexpression is associated with aggressive disease and poor clinical prognosis.

Truncated forms of HER2 that have an oncogenic, constitutive kinase activity are generated either by proteolytic cleavage, known as "shedding", of the ECD [12] or by alternative initiation of translation [13]. Cleavage of the ECD from HER2 significantly increases the tyrosine kinase activity of the truncated receptor and substantially enhances its transforming potential: the truncated form of HER2, $p95^{HER2}$, is 10–100-fold more oncogenic than the full-length protein [14]. This constitutively active, truncated receptor promotes the growth and survival of cancer cells [14]. Its constitutive kinase activity may be due to the release of an inhibitory domain contained within the shed ECD, however, this remains to be demonstrated. Elevated levels of the HER2 ECD are found in the serum of patients with breast cancer and also in patients with other malignancies such as ovarian carcinoma, lung, and prostate cancers [15].

The ECD is released from the receptor into the circulation by a proteolytic mechanism, known as ECD shedding, and it can be detected in serum. HER2 shedding has been attributed to various zinc-containing metalloproteases including members of the MMP (matrix metalloproteinase) and the ADAM (a disintegrin and metalloproteinase) families [16]. The physiological roles of ADAMs include extracellular matrix restructuring, cell adhesion, and cell-surface protein processing [17]. ADAM 10 is the main HER2 ECD sheddase, especially in the triple-negative/basal-like subtype [18]. ADAM 10, ADAM 15, and ADAM 17 are often overexpressed in the breast cancer [19]. MMPs, also known as matrixins, are calcium-dependent, zinc-containing endopeptidases. MMPs degrade structural proteins of the extracellular matrix, but they also cleave cell-surface molecules and non-matrix proteins [6]. Chemical inhibitors with the excellent selectivity for MMP 1, MMP 2, MMP 3, and MMP 9 are potent inhibitors of HER2 ECD shedding [20].

Although soluble HER2 (sHER2 ECD) has been proposed as a useful biomarker for detecting the disease recurrence and for monitoring disease status in breast cancers that overexpress HER2, the American Society of Clinical Oncology (ASCO) [21], recommends that clinicians should not use soluble HER2 levels to guide their choice of adjuvant therapy for patients with HER2-positive breast cancer. This is due to the fact, at the time of writing

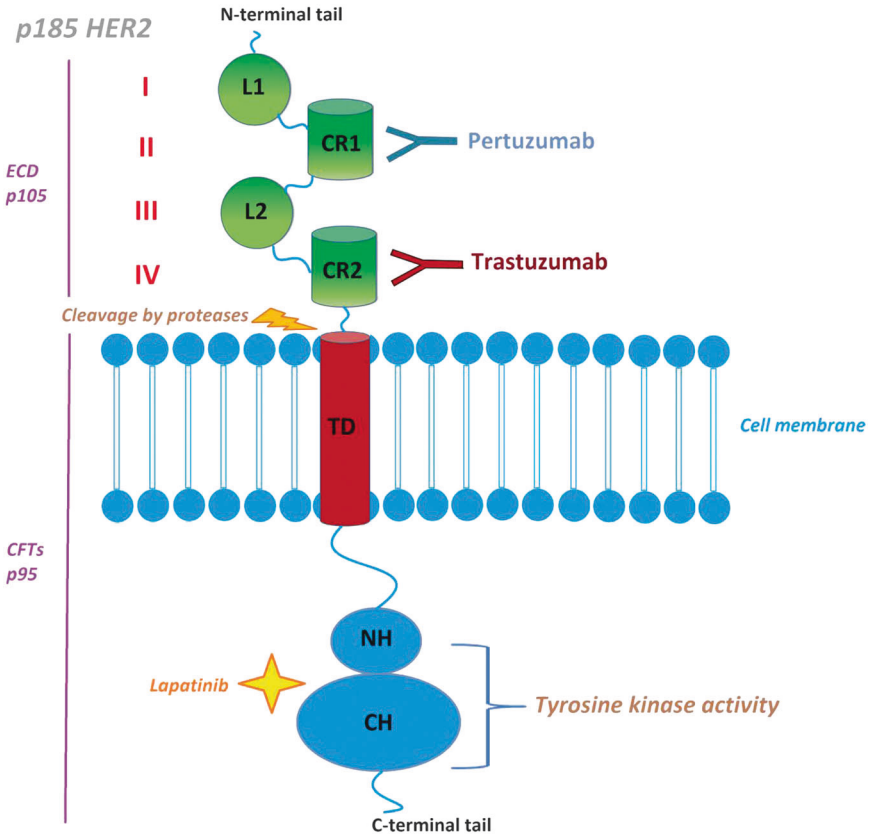

Fig. 1 The domain structure of HER2. The full-length p185 HER2 protein comprises an extracellular domain (ECD p105); a transmembrane domain (TD); a tyrosine kinase domain with amino (NH)- and carboxyl (CH)-terminal lobes and a carboxyl (C)-terminal tail. The ECD is composed of four domains: two leucine-rich domains (I/L1 and III/L2) and two cysteine-rich domains (II/CR1, IV/CR2), which contain disulfide bonds, with which the receptor dimerizes. HER2 is cleaved by proteases at a major site (residues 647–648) and a minor site (residues 644–645) in the juxtamembrane region (11 amino acid residues) to generate the soluble (s)ECD and a constitutively active, truncated, membrane-bound receptor (CFTs p95)

the guidelines, evidence was too weak. Neither does ASCO to recommend the use of sHER2 ECD levels for identifying the patients more likely to benefit from anti-HER2 therapies [21]. Yet, in one study, ~11.4% of early breast cancer patients and 36.5% of patients with metastatic disease had elevated sHER2 ECD levels [22], and another found increased sHER2 ECD levels in 18% of women with primary breast cancer and in 46% of patients with metastatic disease [23]. Currently, carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3, a fragment of MUC1/polymorphic epithelial mucin, present on all the breast cancer cells) are commonly used for monitoring cancers during and after treatment, particularly in metastatic situation. The combination of several tumor markers enhances the sensitivity for the detection of metastatic breast cancer [24]. In this article, we will review the evidence from several recent studies that indicate that sHER2 ECD can be used as a prognosis factor, as an indicator of response or resistance to therapy, and to detect the cancer recurrence and metastasis.

HER2/neu as a target for therapies

Women with breast cancer are usually eligible for therapies that target HER2 if their tumors express the HER2 protein. This status is determined by immunohistochemistry (IHC) or, if the HER2/neu gene is amplified, by fluorescence in situ hybridization (FISH). Different types of HER2-targeted therapy are currently available: trastuzumab, a humanized recombinant antibody directed against domain IV of the ECD of human HER2 (Fig. 1), which inhibits EGF-elicited intracellular signaling and marks HER2+ cells for lysis by the mechanism of antibody-dependent cellular toxicity; lapatinib, a tyrosine kinase inhibitor of both the EGFR and HER2 used in advanced breast cancer, most often when trastuzumab is no longer working; pertuzumab a monoclonal antibody directed against domain II of the ECD of HER2 used in combination with trastuzumab and docetaxel for the treatment of metastatic HER2-positive breast cancer; ado-trastuzumab emtansine (T-DM1) an antibody-drug conjugate consisting of the monoclonal antibody trastuzumab linked to the cytotoxic agent emtansine (DM1) used in advanced breast cancer in women who have already been treated with trastuzumab and chemotherapy and neratinib another dual inhibitor of HER2 and EGFR like lapatinib used to treat early-stage breast cancer after a woman has completed 1 year of trastuzumab.

Trastuzumab was the first HER2-targeted therapy approved by the U.S. Food and Drug Administration (FDA; approved in 1998) for the treatment of metastatic breast cancers that overexpress HER2. Trastuzumab can be used

with adjuvant chemotherapy (either in sequence or in combination). Trastuzumab in combination with cytotoxic anticancer agents is the gold standard for the treatment of HER2-overexpressing breast cancer. It significantly improves disease-free survival (DFS) and overall survival (OS) rates in patients with early-stage and metastatic cancers. Trastuzumab is now the foundation stone of treatment for HER2+ breast cancers. Unfortunately, however, not all patients with HER2-overexpressing breast cancers respond to trastuzumab treatment; despite this antibody therapy, metastatic breast cancer eventually progresses in the majority of cases. Cells may acquire resistance to trastuzumab by shedding the HER2 ECD, which contains the epitope for the antibody, into serum where it can bind to and neutralize the antibody; shedding also leaves the truncated form of HER2 ($p95^{HER2}$) active in the cell membrane [25].

Lapatinib was approved by the FDA in 2007 for use in combination with the fluoropyrimidine capecitabine for the treatment of patients with advanced or metastatic HER2+ breast cancers, who have failed previous therapies, including anthracyclines, taxanes, and trastuzumab. After failure of trastuzumab, improved outcomes have been observed when lapatinib is combined with capecitabine or trastuzumab [26]. We will discuss below the evidence that sHER2 ECD may be a useful biomarker for monitoring treatments with trastuzumab or lapatinib.

Methodological considerations

In 2000, the FDA recommended that sHER2 ECD should be quantified with one of two validated immunoenzymatic methods: either the sHER2 ECD assay for an automated platform (Immuno-1®, SiemensHealthcare Diagnostics) or in microtiter plate format (Oncogene Science, Siemens Healthcare Diagnostics). The immunoassay measures circulating sHER2 ECD levels in serum by using two monoclonal antibodies directed against epitopes on the ECD. The threshold level for elevated sHER2 ECD, according to the FDA guidelines, should be ≥15 ng/ml. An absolute change of ±20% or more from this threshold level has been established as a significant change by the FDA. If elevated levels of sHER2 ECD are found in patients with the suspected cancer, other pathologies should be excluded as moderately high levels of sHER2 ECD (up to 50 ng/ml) are also seen in the absence of cancer, mostly in association with the liver disease, pre-eclampsia, and chronic heart failure [6, 15]. For example, sHER2 ECD levels were reported to be elevated in 40–60% of patients with the non-malignant hepatic diseases [15].

One of the major limitations of the ELISA assay is interference due to heterophilic anti-animal immunoglobulin antibodies (HAIA), which results mostly in false positive results [27]. Many approaches have been reported to reduce this interference, including removing immunoglobulin from serum samples [28], using mouse IgG-derived blocking reagents [29], and replacing the antibody with non-IgG detection agents [30]. For example, Tchou et al. [31], used a blocking buffer called MBB to dilute serum samples, as described in their earlier studies [32, 33]. They conclude that it is possible that some sHER2 ECD levels reported in the literature could be misleading and they recommend that sHER2 ECD assays include an HAIA-preventing step such as the MBB buffer [27].

In the clinic, the amount of HER2 protein in samples of cancerous tissue is measured by IHC testing, which characterizes tissue levels of HER2 as 0 (negative), 1+ (also negative), 2+ (borderline), or 3+ (positive, i.e., HER2 protein overexpression). HER2 gene amplification in these tissues is determined by FISH or by the related technique of chromogenic in situ hybridization (CISH), the results of which are either positive (HER2 gene amplification) or negative (no HER2 gene amplification). CISH is much more practical than FISH in diagnostic laboratories because it uses bright-field microscopy rather than the more expensive and complicated fluorescence microscopy used in FISH. Both IHC and FISH have numerous limitations as diagnostic assays for tissue HER2 (tHER2) [27]. As both techniques require biopsy of the tumors, "real-time" follow-up of the progress of the disease or its response to treatment is difficult. Also, observer variability and non-standardized IHC assays and scoring systems may contribute to the finding that 12–20% of HER2/neu assays performed have erroneous results [34–38]. In many cases, there is also a significant discrepancy between the IHC and the FISH results. Cases where the IHC test is negative but the FISH test is positive (IHC−, FISH+) may be due to loss of protein antigens from samples of fixed tissues or to inaccurate observations during the IHC procedure. Cases where the IHC test is positive and the FISH test is negative (IHC+, FISH−) may be explained by false positive results in the IHC [39] or by other mechanisms that give rise to HER2 protein overexpression besides HER2 gene amplification. One such mechanism is chromosome 17 polysomy [39], which has been found in 13–46% of breast cancers, depending on the studied population and the definition of polysomy 17. As the HER2 gene is located on chromosome 17, the extra copies of the chromosome may lead to overexpression of the HER2 protein. Dysregulation of HER2 transcription may also cause overexpression of HER2 [39]. These limitations of IHC and FISH raise many concerns about our reliance on these tests as assays for HER2/neu (for a review, see ref. [27]). Thus, assays of the sHER2 ECD might prove useful to identify the patients who may be missing an opportunity to be treated with the approved HER2-targeted therapies.

Table 2 Summary of studies that investigated the correlation between tHER2 and sHER2 ECD

References	Date	Patient population	Threshold	Serum assay	Correlation
Fontana et al. [64]	1994	62 PBC	8 U/ml	Triton Diagnostic	No
Kandl et al. [65]	1994	24 MBC	10 U/ml	Triton Diagnostic	No
Kong et al. [66]	2006	86 PBC	10.2 ng/ml	ADVIA Centaur System	No
Kontani et al. [42]	2013	252 PBC and MBC	15.2 ng/ml	Advia Centaur System	No
Quaranta et al. [67]	2006	108 PBC	1368 HNU/ml	Oncogene Science	No
Reix et al. [43]	2016	334 PBC and MBC	15 ng/ml	Advia Centaur System	No
Sorensen et al. [49]	2009	826 MBC	15 ng/ml	ADVIA Centaur System	No
Tchou et al. [31]	2015	118 PBC	15 ng/ml	Oncogene Science	No
Willsher et al. [68]	1996	57 PBC and MBC	20 ng/ml	Bender MedSystem	No
Andersen et al. [69]	1995	168 PBC	1600 HNU/ml	Oncogene Science	Yes
Cheung et al. [70]	2000	20 MBC	20 ng/ml	Bayer Immuno1	Yes
Colomer et al. [71]	2000	40 MBC	450 fmol/ml	Calbiochem	Yes
Farzadnia et al. [72]	2010	74 PBC	18.4 ng/ml	Bender MedSystem	Yes
Fornier et al. [60]	2005	55 MBC	15 ng/ml	Bayer Immuno1	Yes
Garoufali et al. [73]	2008	116 MBC	12.7 ng/ml	Bayer Immuno1	Yes
Harris et al. [74]	2001	355 MBC	20 U/ml	Chiron Diagnostics	Yes
James et al. [75]	2008	100 MBC	15 ng/ml	Bender MedSystem	Yes
Kong et al. [76]	2006	195 MBC	37 ng/ml	ADVIA Centaur System	Yes
Krainer et al. [77]	1997	47 PBC	20 U/ml	Triton Diagnostic	Yes
Ludovini et al. [45]	2008	256 PBC	15 ng/ml	Oncogene Science & ADVIA Centaur	Yes
Molina et al. [15]	1996	77 MBC, 84 PBC	15 U/ml	Ciba Corning	Yes
Muller et al. [78]	2004	29 MBC	15 ng/ml	Oncogene Science	Yes
Sugano et al. [79]	2000	158 PBC	5.4 ng/ml	Nichirei	Yes
Witzel et al. [58]	2010	167 PBC	15 ng/ml	Siemens	Yes
Wang et al. [47]	2016	546 BC	15.0 ng/ml	ADVIA Centaur System	Yes (untreated tumor-bearing patients)
Pallud et al. [80]	2005	157 PBC	15 ng/ml	Bayer Immuno1	Yes for invasive
Narita et al. [81]	1992	55 PBC	20 U/ml	Triton Diagnositic	Yes for MBC, no for PBC

PBC primary breast cancer, *MBC* metastatic breast cancer

Do sHER2 ECD levels correlate with tissue HER2 levels?

Over the past 20 years, there has been a great deal of interest in the possibility of using measures of sHER2 ECD as a means to monitor tumor HER2 status without needing biopsy samples; most recent studies, however, conclude that there is no correlation between tHER2 and sHER2 ECD levels (Table 2). A study published in 2015, for example, found only 16.7% of tHER2+ early-stage breast cancer patients had elevated sHER2 ECD levels (>15 ng/ml; [31], however, in that study, sHER2 ECD levels were significantly higher in the tHER2+ subtype (45.8% had elevated sHER2 ECD levels as defined by sHER2 ECD values greater than the 75th percentile, i.e., ≥7 ng/ml) than in the tHER2- subtype (16.7%). These results are similar to those from larger clinical trials involving early-stage primary breast cancer patients. In one study, only 12% of 2318 tHER2+ patients had elevated preoperative levels of sHER2 [40]. In another recent study of 2862 cases of stage I–III primary breast cancer patients, 24% were found to be tHER2+, but only 15% of these tHER2+ patients had elevated sHER2 ECD levels [41]. Another study [42] found 23.5% of patients with tHER2+ early-stage breast cancer had elevated sHER2 ECD levels (>15 ng/ml). A later study [43] also showed that the levels of sHER2 ECD did not correlate with the level of HER2 overexpression, whether borderline (2+) or positive (3+) on the IHC testing scale described above. These authors concluded, thus, that measurements of sHER2 ECD cannot be used as a surrogate for direct determination of tumor HER2 status. In this study, only 15% of tumors overexpressing HER2 had a sHER2 ECD value above the threshold value of 15 ng/ml.

This lack of correlation between tHER2 and sHER2 ECD might be explained if only a subgroup of HER2+ tumors have a high level of HER2 ECD shedding linked to a more aggressive clinical course [44]. Consistent with this idea, some studies have found a correlation between sHER2 ECD levels and tHER2 status, disease stage, and disease-free survival time [45] (Table 2). High sHER2 ECD levels were also found to be positively associated with the tumor size, clinical stage, nodal status, histological grade, distant metastasis, and tHER2 status, and negatively associated with estrogen receptor status in a 2016 study [46]. Recently, Wang et al. [47], found in 546 breast cancer patients, a correlation between sHER2 ECD and tHER2 levels in untreated tumor-bearing patients (using a threshold level of 15 ng/ml).

The evidence described above indicates that monitoring of sHER2 ECD levels will not replace FISH and IHC testing for early-stage breast cancers but could complement these tissue assays to offer a real-time picture of HER2/neu status in patients [27]. In a population of patients with HER2− primary tumors, periodic testing for elevated sHER2 ECD levels may complement IHC and FISH testing and help to identify HER2+ patients initially classified as HER2− or for whom the HER2 status is unknown. Breast cancer patients with sHER2 ECD levels ≥15 ng/ml should be serially evaluated by IHC and FISH to determine their eligibility for HER2-targeted therapies [23].

Threshold levels of sHER2 ECD

The cut-off value above which sHER2 ECD levels are considered to be elevated may not be the same in all clinical presentations. Ethnicity, for example, may influence baseline levels of sHER2 ECD [48]. Asian descent patients had significantly higher sHER2 ECD levels than the other ethnicities [31]. Thus, it may be important to establish the cut-off values for each ethnic population. Indeed, the somewhat arbitrary threshold value for sHER2 ECD of 15 ng/ml may account for some of the variability in the comparison studies described above. For example, no correlation was found between sHER2 ECD levels and its tissue expression when a threshold value of 15 ng/ml was applied; nevertheless, when the actual sHER2 ECD levels in tHER2− patients were compared to those in tHER2+ patients, a significant difference was found [49]. This raises the question of whether the threshold value in this study was optimal. Moreover, another study [48] found that sHER2 ECD levels did correlate with tHER2 status when a higher threshold was applied. Of 195 patients with metastatic breast cancer, 76 (39%) were IHC 3+, and 19 were IHC 2+ but FISH positive. These patients had higher average sHER2 ECD levels than tHER2− patients. ROC curve analysis was used to calculate a cut-off level of 37 ng/ml with a specificity of 95% and a sensitivity 62% for the prediction of tissue HER2/neu positivity.

The prevalence of sHER2 ECD in breast cancer and its correlation with metastasis

Although elevated levels of sHER2 ECD have been documented in many studies of the breast cancer patients, they are most frequently observed in the metastatic breast cancers (for a review, see ref. [27]). Breast cancer can metastasize to anywhere in the body but the most common sites are in bone. The lungs, regional lymph nodes, liver, and brain are also often involved. In one study, levels of sHER2 ECD were elevated in 40–60% of patients with metastatic tumors in the liver [15]. The highest sHER2 ECD concentrations correlate strongly with the presence of liver metastases [6].

A large, prospective study of 334 patients [43] concluded that patients with metastases presented with more elevated sHER2 ECD levels at the time of diagnosis than did patients without metastases. In this study, elevated levels of sHER2 ECD correlated positively with parameters related to the tumor aggressiveness, such as vascular invasion, metastatic status, and the absence of estrogen receptors, but not with invaded lymph nodes and progesterone receptor-negative tumors. The lack of a correlation between high levels of sHER2 ECD and lymph node involvement in this study contradicts several previous reports in the literature [45, 48, 50]. In the Reix et al. [43] study, lymph nodes were considered to be invaded even when there was only a micrometastasis; the researchers did not score invasion intensity or the number of invaded lymph nodes. Thus, metastatic status may have been overestimated by comparison with the other studies. This probably explains the observed lack of correlation.

Most recent studies have found that the elevated sHER2 ECD levels are seen more frequently in patients with metastatic breast cancer than in those with the primary breast cancer (for a review, see ref. [23]). The presence of high sHER2 ECD levels at diagnosis in patients with breast cancers that overexpress HER2 may, therefore, be a sign of metastatic disease, indicating that the presence of metastases must be investigated. Consequently, early screening for elevated sHER2 ECD levels may improve the detection of metastatic breast cancer.

Surprisingly, Kontani et al. [42], found elevated sHER2 ECD levels in seven patients (22.6%) with metastatic or recurrent HER2− breast cancers. This might be explained if the tumors were derived from HER2-overexpressing cancer cells in the primary tumor in which HER2− cells were dominant. Alternatively, a primary cancer that does not

overexpress HER2 might transform into one which overexpresses HER2 when the patient relapses. In this study, the authors could not rule out the possibility of aberrant production of HER2 protein in the liver resulting from non-malignant hepatic disorders or metastatic tumors.

A review of the studies involving at least 50 patients, in which sHER2 ECD concentration was measured using FDA-approved tests, found elevated sHER2 ECD (>15 ng/ml) in 9–23% of patients with early breast cancer and in 22–73% of patients with metastatic breast cancer [6]. This difference between early and metastatic breast cancer might be explained by the fact that most patients with metastatic breast cancer have liver metastases and that the highest sHER2 concentrations correlate strongly with the presence of liver metastases [6].

The prognostic value of sHER2 ECD

Different parameters are used to estimate the prognostic value of a treatment. Overall survival (OS) is the percentage of people in a study or treatment group who are still alive at a certain time after they were diagnosed with or started treatment for a disease. Progression-free survival (PFS) is the length of time during and after the treatment of a disease, such as cancer, that a patient lives with the disease but it does not get worse. In a clinical trial, measuring PFS is one way to evaluate how well a new treatment actually works. In cancer, disease-free survival (DFS, also called relapse-free survival) is the length of time after primary treatment for a cancer ends that the patient survives without any cancer signs or symptoms. As for PFS, DFS is another way to evaluate a new treatment in a clinical trial.

Two recent studies indicate that sHER2 ECD results correlate with survival parameters. In the study by Reix et al. [43], DFS in patients without metastases was shorter in those with elevated sHER2 ECD levels (≥15 ng/ml) when compared to those with low sHER2 ECD levels (<15 ng/ml). Similarly, in patients with metastasis, PFS was longer in those with low sHER2 ECD levels than it was in those with high sHER2 ECD levels. OS was shorter in patients with high sHER2 ECD levels than it was in patients with low sHER2 ECD levels. Multivariate analyses adjusting for the variables grade, nodal status, vascular invasion, estrogen and progesterone receptors, HER2 status, and CA 15.3, demonstrated that sHER2 ECD was an independent prognosis factor for OS. Indeed, in breast tumors that overexpressed HER2, high sHER2 ECD levels were strongly associated with shorter OS than the OS associated with low sHER2 ECD levels. Recently, in 118 patients, DFS was significantly shorter in patients with sHER2 ECD levels ≥7 ng/ml [31]. Most recently, a study of 436 stage I–III breast cancers [51] found that high levels of sHER2 ECD were associated with short DFS times in the HR+/HER2−, HR+/HER2+, and HR−/HER2+ subtypes.

Likewise, sHER2 ECD levels appear to correlate with response to treatment. A study in 2014 measured sHER2 ECD in 190 women with metastatic breast cancer [52] and found elevated sHER2 ECD levels significantly associated with short-term response to trastuzumab treatment. The median PFS was significantly longer in patients with low levels of sHER2 ECD. Those whose sHER2 ECD levels remained low or became low after treatment had significantly longer PFS times than those whose levels remained high or converted from low to high [52]. Moreover, a study of 546 breast cancer patients at all stages [47] concluded that elevated sHER2 ECD (>15 ng/ml) was also a predictor of poor PFS after anti-HER2 therapy with trastuzumab or lapatinib. By contrast, a decrease of ≥20% in sHER2 ECD levels was associated with longer PFS and OS in patients who received anti-HER2 therapy.

The prognostic value of sHER2 ECD may be enhanced when combined with measures of the biomarker CA 15-3. In a study of early breast cancer [53], patients with both high levels of sHER2 ECD (≥15 ng/ml) and high levels of serum CA 15-3 (≥24 U/ml) had the poorest prognosis with a DFS after 3 years of 50.0%. Patients without elevated sHER2 ECD levels had a better outcome, with a DFS after 3 years of 91.2%. These researchers concluded that sHER2 ECD and CA 15-3 were strong and independent indicators of poor DFS; the use of a combination of both biomarkers proved valuable in identifying high-risk breast cancer patients. Similarly, a retrospective study of 250 patients with metastatic breast cancer [54] concluded that serum CA 15-3 and sHER2 ECD could be useful independent prognostic factors for this disease.

Evidence from the studies described above, thus, support the use of sHER2 ECD in the routine management of HER2+ breast cancer. Measurement of sHER2 ECD at the time of diagnosis can provide a useful reference value before treatment and can be used as a prognosis factor.

There are at least two possible explanations why high sHER2 ECD levels might be linked to low survival. First, the truncated $p95^{HER2}$ form that remains in the cancer cell membrane after cleavage has a higher rate of constitutive tyrosine kinase activity and is 10–100-fold more oncogenic than full-length HER2. So, shedding of the ECD may result in stronger ligand-independent growth and survival of the cancer cells, resulting in rapid growth of the tumor and low patient survival. Secondly, the binding of therapeutic anti-HER2 antibodies to sHER2 ECD may block their biological activity. So, in patients with high levels of sHER2 ECD, the amounts of active drug antibodies may be insufficient to kill the tumor cells expressing HER2. This would lead to a more aggressive clinical course for these patients.

sHER2 ECD as a biomarker to follow the efficacy of HER2-targeted therapies

The evolution of sHER2 ECD levels during neoadjuvant treatment (i.e., with trastuzumab ± chemotherapy or transtuzumab ± hormone therapy) is informative of the treatment efficacy [43]. Patients with a complete response (i.e., disappearance of all signs of cancer) as determined by IHC also showed a significant decrease in their sHER2 ECD levels, which correlates with the clinical course of the disease [43]. This effect was not observed for patients with an incomplete IHC response. However, these results were obtained from a small cohort of patients (fewer than 20 in the incomplete and complete response groups), and furthermore, pre-treatment sHER2 ECD levels were not quantified systematically before the use of trastuzumab. Thus, the utility of sHER2 ECD as a biomarker to follow the efficacy of trastuzumab therapy during neoadjuvant treatment of overexpressing HER2 invasive breast cancers remains to be addressed by complementary studies.

Few other studies have evaluated the changes in sHER2 ECD levels during neoadjuvant treatment in patients with tumors overexpressing HER2. Two small prospective studies [55, 56] reported a reduction in sHER2 ECD levels 1, 3, and 6 weeks after beginning neoadjuvant therapy. This reduction correlated with a complete response to the treatment, as determined by IHC. This effect of trastuzumab therapy on sHER2 ECD levels might be explained by the fact that the trastuzumab-binding site is located near the ECD cleavage site. The binding of trastuzumab to HER2 may interfere with HER2 cleavage by blocking protease access to the receptor, thus reducing sHER2 ECD levels. Conversely, trastuzumab might be inefficient due to the absence of trastuzumab fixation on HER2 when there are elevated levels of HER2 ECD cleavage. In such a situation, a constant or an increasing sHER2 ECD level might indicate that the treatment is not effective: consequently, sHER2 ECD could then be used as an indicator for a second-line therapy with other anti-HER2 treatment, which potentiates trastuzumab-dependent cell cytotoxicity [43].

The predictive value of sHER2 ECD for the response or resistance to HER2-targeted therapies

Resistance to cancer treatment is an important clinical problem, and a biomarker that could predict the response to anti-HER2 therapy would be very useful. Some investigators have reported that low levels of sHER2 ECD predict appropriate tumor responses to trastuzumab, whereas others have reported that high levels of sHER2 ECD predict these responses. Thus, the usefulness of sHER2 ECD as a predictor of a beneficial response to trastuzumab remains to be determined.

Levels of sHER2 ECD cannot be expected to predict certain forms of resistance to trastuzumab treatment. For example, one mechanism proposed to explain the resistance of some tumor cells to trastuzumab therapy is that the cells express a truncated form of HER2 that lacks the extracellular antibody-binding domain [43]. In this situation, the level of sHER2 ECD would be uninformative as a predictor; only IHC testing for both the intracellular and extracellular domains of the membrane-bound form of HER2, using antibodies specific for each domain, could determine whether or not cells express this truncated receptor and thus predict this form of trastuzumab resistance. One study showed that high tissue ECD status was predictive of better DFS than low tissue ECD after trastuzumab treatment, whereas intracellular domain status was not [57]. This was explained by the fact that trastuzumab binds only to the extracellular domain of HER2.

Further evidence to support the observation that sHER2 ECD levels >15 ng/ml in tHER2+ breast cancers might predict the failure of anti-HER2 therapy comes from a study by Wang et al. [47]. These authors hypothesized that if sHER2 ECD increases during therapy, fewer surface targets for trastuzumab remain on tumor cells, leading to failure of the therapy. They also found that a decrease in sHER2 ECD above or equal to 20% in HER2+ patients is an indicator of therapeutic success, regardless of whether or not the therapy is specifically directed against HER2 [47]. By contrast, a meta-analysis found no definitive relationship between baseline sHER2 ECD levels and tumor response to trastuzumab-based treatment [22]. Inconsistent with most studies, Witzel et al. [58], in the GeparQuattro trial, found a significant positive association between pathological complete remission (pCR, defined as no invasive and no in situ residuals in breast and nodes) and elevated sHER2 levels (above 15 ng/ml) with neoadjuvant transtuzumab therapy. A decrease (>20%) in sHER2 ECD levels was associated with a higher response rate (defined as the percentage of patients whose cancer shrinks or disappears after treatment) with trastuzumab-based treatment, as reported in other studies [59, 60].

These conflicting findings may be explained by differences in the methods used to assess responses, to the absence of a concurrent control arm without anti-HER2 therapy for comparing the outcome of treatment, to the heterogeneity of patient populations, to differences in anti-HER2 preparations and other companion systemic treatments, in addition to small sample sizes and inadequate statistical power [26]. In future studies, levels of sHER2 ECD may be useful to stratify and identify patients who may benefit from new targeted therapies.

In contrast to trastuzumab, the response to the tyrosine kinase inhibitor lapatinib is not affected by the levels of sHER2 ECD: a recent study based on data from three consecutive, well-conducted trials with similar designs which consider a total of 1902 patients, found that high levels of sHER2 ECD predicted long PFS times with lapatinib, independently of tHER2 status [26].

The use of sHER2 ECD to detect and predict cancer recurrence and metastasis

Rising levels of sHER2 ECD may indicate cancer recurrence and metastasis. In their cohort of 334 patients, Reix et al. [43] observed 70 events (progression, metastases, and death). An increase of sHER2 ECD with no change in CA 15-3 levels was predictive, before imaging, of relapse, disease progression, and metastasis in 18.6% of the 70 events. In 35.7% of cases, they noticed an increase of both sHER2 ECD and CA 15-3 levels before diagnosis of relapse or metastasis. In 8.6% of cases, CA 15-3 levels increased before the diagnosis of cancer recurrence, with no change in sHER2 ECD levels. In 37.1% of patients, neither sHER2 ECD nor CA 15-3 levels predicted the occurrence of metastases. Thus, in some cases, the levels of sHER2 ECD can provide useful information, alone or in combination with the levels of CA 15-3, for the prediction of early cancer recurrence. The sHER2 ECD levels increased significantly before metastasis to bone, liver, brain, and multiple organs could be detected by imaging but not before metastasis to the lungs. To consider therapeutic efficacy, they observed not only biomarker changes but also whether the level of the biomarker remained elevated or below the reference value. The change in sHER2 ECD levels was consistent with conclusions from imaging in 94% of cases, whereas CA 15-3 levels were consistent with the conclusion from imaging in only 85% of cases. The level of sHER2 ECD predicted progression to metastasis in 100% of the cases of liver and multiple organ metastases, and predicted progression to or regression of bone, lung, and brain metastasis in 90% of cases. Levels of CA 15-3 correlated less well, but discrepancies in both sHER2 ECD and CA 15-3 were never observed in a single event, again suggesting the complementarity of these biomarkers. The authors concluded that sHER2 ECD appears to be a helpful surveillance biomarker for early diagnosis of relapses and to predict metastasis of breast cancer [43].

Recent studies are consistent with the later results. One study found that in clinical stage 3 or 4 the levels of sHER2 ECD increased from 50% to 71.4% and in recurrent cancers they increased from 43.8 to 81.3% [42], confirming the conclusion that sHER2 ECD levels are useful for detecting the recurrence of the disease or monitoring disease status in breast cancers that overexpress HER2. Another study of sHER2 ECD levels in patients during and after adjuvant therapy also demonstrated that sHER2 ECD levels rise in patients who subsequently develop metastases [31] Most recently, a study [61] of 200 patients with benign breast tumors and 300 patients who had been treated for breast cancer concluded that sHER2 ECD may be a valuable, novel biomarker for recurrence and metastasis in triple-negative breast cancer.

The use of sHER2 ECD levels to detect early cancer recurrence [23] and to predict reliably the progression or regression of metastases before medical imaging [43] would allow clinicians to make necessary adjustments to drug combinations or alert them to follow-up patients more frequently. The levels of sHER2 ECD correlate better with disease recurrence and metastasis than do those of CA 15-3, but the use of both biomarkers increases the reliability of the findings [62]; these markers are not interchangeable but must be used complementary.

Conclusion

From the evidence presented above, we can see that sHER2 ECD levels can provide a useful biological index for evaluating certain aspects of breast cancers that overexpress HER2, most notably to detect cancer recurrence and metastasis. Although elevated levels of sHER2 ECD have been documented in many studies of breast cancer patients, they are most frequently observed in metastatic breast cancers; the highest sHER2 ECD concentrations correlating strongly with the presence of liver metastases. Elevated levels of sHER2 ECD correlate positively with parameters related to tumor aggressiveness. They also correlate with poor survival parameters. Moreover, sHER2 ECD appears to be a helpful surveillance biomarker for early diagnosis of relapse and to predict metastasis of breast cancer. Its utility as a biomarker to follow the efficacy of trastuzumab therapy and as a predictor of a beneficial response to this drug, however, remain unclear and demand further evidence from large, well-conducted studies. The prognostic value of sHER2 ECD may be enhanced when combined with measures of the biomarker CA 15-3. The prospect of using sHER2 ECD levels for the diagnosis of early-stage breast cancer, by contrast, is less bright. Most recent studies conclude that there is no correlation between tHER2 and sHER2 ECD levels, so measures of this serum biomarker will not replace direct measurement of tumor HER2 status by FISH and IHC testing of biopsy samples from early-stage tumors. Nevertheless, periodic testing for elevated sHER2 ECD levels may well complement these tissue assays to help identify HER2+ patients incorrectly classified as HER2−, or for whom the HER2 status is unknown,

as well as to provide a real-time picture of HER2/neu status in patients undergoing therapy.

Compilance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7–30.
2. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2013. Ann Oncol. 2013;24:2206–23.
3. Dai X, Li T, Bai Z, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5:2929–43.
4. Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. N Engl J Med. 2009;360:790–800.
5. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005;353:1673–84.
6. Tsé C, Gauchez A-S, Jacot W, Lamy P-J. HER2 shedding and serum HER2 extracellular domain: biology and clinical utility in breast cancer. Cancer Treat Rev. 2012;38:133–42.
7. Schechter AL, Stern DF, Vaidyanathan L, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature. 1984;312:513–6.
8. Shih C, Shilo BZ, Goldfarb MP, Dannenberg A, Weinberg RA. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. Proc Natl Acad Sci USA. 1979;76:5714–8.
9. Shah MV, Wiktor AE, Meyer RG, et al. Change in pattern of HER2 fluorescent in situ hybridization (FISH) results in breast cancers submitted for FISH testing: experience of a reference laboratory using US Food and Drug Administration criteria and American Society of Clinical Oncology and College of American Pathologists Guidelines. J Clin Oncol. 2016;34:3502–10.
10. Lamy P-J, Fina F, Bascoul-Mollevi C, et al. Quantification and clinical relevance of gene amplification at chromosome 17q12-q21 in human epidermal growth factor receptor-2-amplified breast cancers. Breast Cancer Res. 2011;13:R15.
11. Slamon D, Clark G, Wong S, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235:177–82.
12. Christianson TA, Doherty JK, Lin YJ, et al. NH2-terminally truncated HER-2/neu protein: relationship with shedding of the extracellular domain and with prognostic factors in breast cancer. Cancer Res. 1998;58:5123–9.
13. Anido J, Scaltriti M, Bech Serra JJ, et al. Biosynthesis of tumorigenic HER2 C-terminal fragments by alternative initiation of translation. EMBO J. 2006;25:3234–44.
14. Segatto O, King CR, Pierce JH, Di Fiore PP, Aaronson SA. Different structural alterations upregulate in vitro tyrosine kinase activity and transforming potency of the erbB-2 gene. Mol Cell Biol. 1988;8:5570–4.
15. Molina R, Jo J, Filella X, et al. Serum levels of C-erbB-2 (HER-2/neu) in patients with malignant and non-malignant diseases. Tumor Biol. 1997;18:188–96.
16. Sanderson MP, Dempsey PJ, Dunbar AJ. Control of ErbB signaling through metalloprotease mediated ectodomain shedding of EGF-like factors. Growth Factors. 2006;24:121–36.
17. Nyren-Erickson EK, Jones JM, Srivastava DK, Mallik S. A disintegrin and metalloproteinase-12 (ADAM12): function, roles in disease progression, and clinical implications. Biochim Et Biophys Acta. 2013;1830:4445–55.
18. Mullooly M, McGowan PM, Kennedy SA, et al. ADAM10: a new player in breast cancer progression? Br J Cancer. 2015;113:945–51.
19. Duffy MJ, McKiernan E, O'Donovan N, McGowan PM. Role of ADAMs in cancer formation and progression. Clin Cancer Res. 2009;15:1140–4.
20. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol. 2001;17:463–516.
21. Harris LN, Ismaila N, McShane LM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2016;34:1134–50.
22. Leary AF, Hanna WM, van de Vijver MJ, et al. Value and Limitations of measuring HER-2 extracellular domain in the serum of breast cancer patients. J Clin Oncol. 2009;27:1694–705.
23. Carney WP, Bernhardt D, Jasani B. Circulating HER2 extracellular domain: a specific and quantitative biomarker of prognostic value in all breast cancer patients? Biomark Cancer. 2013;5:31–9.
24. Pedersen AC, Sørensen PD, Jacobsen EH, Madsen JS, Brandslund I. Sensitivity of CA 15-3, CEA and serum HER2 in the early detection of recurrence of breast cancer. Clin Chem Lab Med. 2013;51:1511–9.
25. Scaltriti M, Rojo F, Ocana A, et al. Expression of p95HER2, a truncated form of the HER2 Receptor, and response to Anti-HER2 therapies in breast cancer. J Natl Cancer Inst. 2007;99:628–38.
26. Lee CK, Davies L, Gebski VJ. et al. Serum human epidermal growth factor 2 extracellular domain as a predictive biomarker for lapatinib treatment efficacy in patients with advanced breast cancer. J Clin Oncol. 2016;34:936–44.
27. Lam L, McAndrew N, Yee M, et al. Challenges in the clinical utility of the serum test for HER2 ECD. Biochim Et Biophys Acta. 2012;1826:199–208.
28. Ismail AAA. A radical approach is needed to eliminate interference from endogenous antibodies in immunoassays. Clin Chem. 2004;51:25–6.
29. Reinsberg J. Different efficacy of various blocking reagents to eliminate interferences by human antimouse antibodies with a two-site immunoassay. Clin Biochem. 1996;29:145–8.
30. Andersson M, Rönnmark J, Areström I, Nygren P-Å, Ahlborg N. Inclusion of a non-immunoglobulin binding protein in two-site ELISA for quantification of human serum proteins without interference by heterophilic serum antibodies. J Immunol Methods. 2003;283:225–34.
31. Tchou J, Lam L, Li YR, et al. Monitoring serum HER2 levels in breast cancer patients. SpringerPlus. 2015;4:237.
32. Zhang H, Fu T, McGettigan S. et al. IL8 and cathepsin B as IL8 and cathepsin B as melanoma serum biomarkers. Int J Mol Sci. 2011;12:1505–18.
33. Lam L, Czerniecki BJ, Fitzpatrick E. et al. Interference-free HER2 ECD as a serum biomarker in breast cancer. J Mol Biomark Diagn. 2014;4:151
34. Paik S, Bryant J, Tan-Chiu E, et al. Real-world performance of HER2 testing--national surgical adjuvant breast and bowel project experience. J Natl Cancer Inst. 2002;94:852–4.
35. Sui W, Ou M, Chen J, et al. Comparison of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) assessment for Her-2 status in breast cancer. World J Surg Oncol. 2009;7:83.
36. Sauter G, Lee J, Bartlett JMS, Slamon DJ, Press MF. Guidelines for human epidermal growth factor receptor-2 testing: biologic and methodologic considerations. J Clin Oncol. 2009;27:1323–33.

37. Perez EA, Suman VJ, Davidson NE, et al. HER2 testing by local, central, and reference laboratories in specimens from the north central cancer treatment group N9831 intergroup adjuvant trial. J Clin Oncol. 2006;24:3032–8.
38. Cheang MCU, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101:736–50.
39. Koo JS, Jung W, Yang WI. HER-2 protein overexpressing breast cancer without gene amplification shows higher hormone receptor expression than HER-2 protein overexpressing breast cancer with gene amplification. Int J Surg Pathol. 2009;19:425–32.
40. Moreno-Aspitia A, Hillman DW, Dyar SH, et al. Soluble human epidermal growth factor receptor-2 (HER2) levels in patients with HER2-positive breast cancer receiving chemotherapy with or without trastuzumab: results from north central cancer treatment group adjuvant trial N9831. Cancer. 2013;119:2675–82.
41. Lee HJ, Seo AN, Kim EJ. et al. HER2 heterogeneity affects trastuzumab responses and survival in patients with HER2-positive metastatic breast cancer. Am J Clin Pathol. 2014;142:755–66.
42. Kontani K, Kuroda N, Hashimoto S-i, et al. Clinical usefulness of human epidermal growth factor receptor-2 extracellular domain as a biomarker for monitoring cancer status and predicting the therapeutic efficacy in breast cancer. Cancer Biol Ther. 2013;14:20–8.
43. Reix N, Malina C, Chenard M-P, et al. A prospective study to assess the clinical utility of serum HER2 extracellular domain in breast cancer with HER2 overexpression. Breast Cancer Res Treat. 2016;160:249–59.
44. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002;2:161–74.
45. Ludovini V, Gori S, Colozza M, et al. Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival. Ann Oncol. 2008;19:883–90.
46. Shukla S, Singh B, Pathania O, Jain M. Evaluation of HER2/neu oncoprotein in serum & tissue samples of women with breast cancer. Indian J Med Res. 2016;143:52.
47. Wang T, Zhou J, Zhang S, et al. Meaningful interpretation of serum HER2 ECD levels requires clear patient clinical background, and serves several functions in the efficient management of breast cancer patients. Clin Chim Acta. 2016;458:23–9.
48. Kong Y, Dai S, Xie X, et al. High serum HER2 extracellular domain levels: correlation with a worse disease-free survival and overall survival in primary operable breast cancer patients. J Cancer Res Clin Oncol. 2012;138:275–84.
49. Sørensen PD, Jakobsen EH, Langkjer ST, et al. Serum HER-2 concentrations for monitoring women with breast cancer in a routine oncology setting. Clin Chem Lab Med. 2009;47: 1117–23.
50. Isola JJ, Holli K, Oksa H, Teramoto Y, Kallioniemi O-P. ElevatederbB-2 oncoprotein levels in preoperative and follow-up serum samples define an aggressive disease course in patients with breast cancer. Cancer. 1994;73:652–8.
51. Lee MH, Jung S-Y, Kang SH, et al. The significance of serum HER2 levels at diagnosis on intrinsic subtype-specific outcome of operable breast cancer patients. PLoS ONE. 2016;11:e0163370.
52. Shao X, Wang X, Xu X, et al. Outcome prediction values of soluble human epidermal growth factor receptor-2 extracellular domain in metastatic breast cancer. Int J Clin Exp Pathol. 2014;7:1108–13.
53. Di Gioia D, Dresse M, Mayr D, et al. Serum HER2 supports HER2-testing in tissue at the time of primary diagnosis of breast cancer. Clin Chim Acta. 2014;430:86–91.
54. Darlix A, Lamy P-J, Lopez-Crapez E, et al. Serum HER2 extracellular domain, S100ß and CA 15-3 levels are independent prognostic factors in metastatic breast cancer patients. BMC Cancer. 2016;16:428.
55. Kostler WJ, Steger GG, Soleiman A, et al. Monitoring of serum Her-2/neu predicts histopathological response to neoadjuvant trastuzumab-based therapy for breast cancer. Anticancer Res. 2004;24:1127–30.
56. Mazouni C, Hall A, Broglio K, et al. Kinetics of serum HER-2/neu changes in patients with HER-2-positive primary breast cancer after initiation of primary chemotherapy. Cancer. 2007;109:496–501.
57. Carvajal-Hausdorf DE, Schalper KA, Pusztai L, et al. Measurement of domain-specific HER2 (ERBB2) expression may classify benefit from trastuzumab in breast cancer. J Natl Cancer Inst. 2015;107:djv136.
58. Witzel I, Loibl S, von Minckwitz G, et al. Monitoring serum HER2 levels during neoadjuvant trastuzumab treatment within the geparquattro trial. Breast Cancer Res Treat. 2010;123:437–45.
59. Ali SM, Carney WP, Esteva FJ, et al. Serum HER-2/neuand relative resistance to trastuzumab-based therapy in patients with metastatic breast cancer. Cancer. 2008;113:1294–301.
60. Fornier MN, Seidman AD, Schwartz MK, et al. Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate. Ann Oncol. 2005;16:234–9.
61. Yang H, Chen L, Zhou L, et al. Serum human epidermal growth factor 2 is a novel biomarker for recurrence and metastasis in triple negative breast cancer. Clin Lab. 2017;63:53–58.
62. Esteva FJ, Cheli CD, Fritsche H, et al. Clinical utility of serum HER2/neu in monitoring and prediction of progression-free survival in metastatic breast cancer patients treated with trastuzumab-based therapies. Breast Cancer Res. 2005;7:R436–43.
63. Smid M, Wang Y, Zhang Y, et al. Subtypes of breast cancer show preferential site of relapse. Cancer Res. 2008;68:3108–14.
64. Fontana X, Ferrari P, Namer M, et al. C-erb-B2 gene amplification and serum level of c-erb-B2 oncoprotein at primary breast cancer diagnosis. Anticancer Res. 1994;14:2099–104.
65. Kandl H, Seymour L, Bezwoda WR. Soluble c-erbB-2 fragment in serum correlates with disease stage and predicts for shortened survival in patients with early-stage and advanced breast cancer. Br J Cancer. 1994;70:739–42.
66. Kong SY. Serum HER-2 concentration in patients with primary breast cancer. J Clin Pathol. 2006;59:373–736.
67. Quaranta M, Daniele A, Coviello M, et al. c-erbB-2 protein level in tissue and sera of breast cancer patients: a possibly useful clinical correlation. Tumori. 2006;92:311–7.
68. Willsher PC, Beaver J, Pinder S, et al. Prognostic significance of serum c-erbB-2 protein in breast cancer patients. Breast Cancer Res Treat. 1996;40:251–5.
69. Andersen TI, Paus E, Nesland JM, McKenzie SJ, Bø rresen A-L. Detection of C-ERBB-2 related protein in sera from breast cancer patients relationship to ERBB2 gene amplification and c-erbB-2 protein overexpression in tumour. Acta Oncol. 1995;34:499–504.
70. Cheung KL, Pinder SE, Paish C, et al. The role of blood tumor marker measurement (using a biochemical index score and c-erbB2) in directing chemotherapy in metastatic breast cancer. Int J Biol Markers. 2000;15:203–9.
71. Colomer R, Montero S, Lluch A, et al. Circulating HER2 extracellular domain and resistance to chemotherapy in advanced breast cancer. Clin Cancer Res. 2000;6:2356–62.
72. Farzadnia M, Meibodi NT, Shandiz FH, et al. Evaluation of HER2/neu oncoprotein in serum and tissue samples of women with breast cancer: correlation with clinicopathological parameters. Breast. 2010;19:489–92.
73. Garoufali A, Kyriakou F, Kountourakis P, et al. Extracellular domain of HER2: a useful marker for the initial workup and

follow-up of HER2-positive breast cancer. J Buon. 2008;13: 409–13.
74. Harris LN, Liotcheva V, Broadwater G, et al. Comparison of methods of measuring HER-2 in metastatic breast cancer patients treated with high-dose chemotherapy. J Clin Oncol. 2001;19: 1698–706.
75. James R, Thriveni K, Ramaswamy G, et al. Evaluation of immunohistochemistry and enzyme linked immunosorbent assay for HER-2/neu expression in breast carcinoma. Indian J Clin Biochem. 2008;23:345–51.
76. Kong SY, Nam BH, Lee KS, et al. Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. Clin Chem. 2006;52:1510–5.
77. Krainer M, Brodowicz T, Zeillinger R, et al. Tissue expression and serum levels of HER-2/neu in patients with breast cancer. Oncology. 1997;54:475–81.
78. Müller V, Witzel I, Lück HJ. et al. Prognostic and predictive impact of the HER-2/neu extracellular domain (ECD) in the serum of patients treated with chemotherapy for metastatic breast cancer. Breast Cancer Res Treat. 2004;86:9–18.
79. Sugano K, Ushiama M, Fukutomi T, et al. Combined measurement of the c-erbb-2 protein in breast carcinoma tissues and sera is useful as a sensitive tumor marker for monitoring tumor relapse. Int J Cancer. 2000;89:329–36.
80. Pallud C, Guinebretiere JM, Guepratte S, et al. Tissue expression and serum levels of the oncoprotein HER-2/neu in 157 primary breast tumours. Anticancer Res. 2005;25:1433–40.
81. Narita T, Funahashi H, Satoh Y, Takagi H. C-erbB-2 protein in the sera of breast cancer patients. Breast Cancer Res Treat. 1992;24:97–102.