DE GRUYTER OPEN

HeSMO • 5(1) • 2014 • 20–28
DOI: 10.2478/fco-2014-0004

Forum of Clinical **Oncology**

Central retesting of breast cancer with HER2 immunohistochemistry score of 0 or 1+ using silver-enhanced in situ hybridisation: a multicentre, prospective study in Greece

Savvas Papadopoulos[1*], Petroula Arapantoni-Dadioti[2], Konstantinos Sfikas[3], Artemis Stylianidou[3], Helen Trichia[2], Eleftheria Katsamagou[1], Vaia Baleki[4], Johannes Noe[5]

[1]*Pathology Department, Hygeia Hospital, Athens, Greece*

[2]*Pathology Department, METAXA Anticancer Hospital, Piraeus, Greece*

[3]*Pathology Department, General Maternity District Hospital "Helena Venizelou", Athens, Greece*

[4]*Roche Pharmaceuticals, Athens, Greece*

[5]*Roche Pharmaceuticals, Basel, Switzerland*

Abstract: Newly diagnosed invasive breast cancers should be evaluated for Human Epidermal Growth Factor Receptor 2 (HER2) status by immunohistochemistry (IHC) and/or in situ hybridisation (ISH) to determine eligibility for trastuzumab or other HER2-targeted therapies. Previous reports of high discordance rates between IHC and ISH have raised concerns over the accuracy of HER2 testing, especially when IHC is conducted locally. This study aimed to determine the rate of false-negative IHC results at three pathology centres (one central, two local) in Greece by central retesting of 240 prospectively collected invasive breast cancers scored as IHC 0/1+ at initial testing. All samples were from female patients (median age 58.0 years). Initial IHC tests utilised either the CB11 (159/239; 66.5%) or 4B5 (80/239; 33.5%) antibodies and were scored as 0 in 105/240 cases (43.8%) and 1+ in 135/240 cases (56.3%). All samples were centrally retested by automated silver in situ hybridisation (SISH). Of 237 samples with SISH staining suitable for assessment, 223 (94.1%; 95% confidence interval 90.3–96.5%) were classed as SISH-negative (*HER2*:chromosome enumeration probe 17 (CEP17) <1.8). Eight tested SISH-positive (*HER2*:CEP17 >2.2), providing a false-negative rate of 3.4%. A further four samples (1.7%) exhibited equivocal amplification status (*HER2*:CEP17 1.8–2.2) and two (0.8%) showed polysomy of chromosome 17. The proportion of SISH-negative results did not significantly differ between the IHC 0 and 1+ subgroups (95.2% vs. 93.2%; $p=0.505$). In conclusion, the low observed rate of false-negative IHC results in this study supports the use of IHC for initial HER2 status assessment in local or central laboratories in Greece.

Keywords: *Breast cancer • HER2 • Immunohistochemistry • Silver in situ hybridisation*

Introduction

Newly diagnosed breast cancers should undergo routine testing for overexpression of human epidermal growth factor receptor 2 (HER2) and/or amplification of the *HER2* gene (i.e., HER2-positivity) according to published recommendations [1]. For patients with HER2-positive breast cancer, anti-HER2 therapy with trastuzumab (Herceptin®, F. Hoffmann-La Roche, Basel, Switzerland) has demonstrated significant clinical benefits in both

** E-mail: Savvas.Papadopoulos@hygeia.gr*

early and metastatic disease [2–7] and is established as the standard of care [8–10]. Because HER2 status predicts trastuzumab response [2, 11], accurate HER2 testing is essential to determine which patients are most likely to benefit from this treatment. HER2 assessment is also used to determine eligibility for three other anti-HER2 therapies, pertuzumab (PERJETA®, F. Hoffmann-La Roche; Genentech Inc., South San Francisco, CA, USA) [12], T-DM1 (Kadcyla®, F. Hoffmann-La Roche; Genentech Inc.) [13] and lapatinib [14], and may additionally predict response or resistance to certain chemotherapeutic and hormonal therapies [15].

Two diagnostic techniques are routinely used for determination of HER2 status: immunohistochemistry (IHC) and in situ hybridisation (ISH) [16]. IHC is a semi-quantitative technique in which a HER2-specific antibody is used to evaluate the level of HER2 protein expression in fresh, frozen or formalin-fixed, paraffin-embedded tissue sections. Although relatively fast and inexpensive to perform, the reliability of IHC is susceptible both to inter-observer variability in interpretation and to a variety of pre-analytic and analytic factors that can influence antibody staining [1, 16]. In contrast, ISH is a quantitative assay that uses a labelled DNA probe to determine *HER2* copy number, often alongside a second probe for the chromosome 17 centromere (chromosome enumeration probe 17 (CEP17)) to control for aneusomy of chromosome 17 [16]. The first available HER2 ISH assays were fluorescence ISH (FISH) tests, which, although considered more reproducible than IHC, require a specialised fluorescence microscope for analysis and are subject to limited stability of fluorophore signals [16]. More recently, bright-field ISH techniques, including chromogenic ISH and silver-enhanced ISH (SISH), have been developed, which are analysed using conventional bright-field microscopy and permit evaluation of histological features alongside *HER2* amplification status [16]. SISH has been developed as a fully automated assay that shows low inter-observer variability and high concordance with validated FISH assays [17, 18].

Both IHC and ISH are recommended for initial HER2 testing [1], but IHC is more widely used in clinical practice [19], with ISH testing typically reserved for reflex testing of samples with an equivocal IHC (2+) score. However, previous studies have identified high rates of discordance between IHC and ISH, including both false-negative (i.e., IHC-negative/ISH-positive) and false-positive (i.e., IHC-positive/ISH-negative) IHC results [20], especially when comparing local IHC with central ISH assessments [21]. To improve the accuracy of HER2 testing in breast cancer, the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) issued guidelines in 2007 for standardisation of HER2 assessment, which recommended that HER2 tests in individual laboratories should show at least 95% concordance with a validated assay [1]. The impact of this guidance on the accuracy of HER2 testing is not fully established, but recent evidence suggests a modest decline in the incidence of false-negative results at one US centre following the publication of the ASCO/CAP document [22].

To assess the accuracy of IHC testing in current pathology practice, an international study is evaluating the rate of false-negative IHC results by central SISH retesting of approximately 1800 prospectively collected breast cancers with negative (0 or 1+) IHC scores. Here, we report results from the three Greek laboratories participating in this study, which provide an estimate of the incidence of false-negative IHC scores in routine breast cancer testing in Greece.

Materials and methods

Patient samples

Three pathology centres (two local and one central laboratory) in Greece each contributed 80 resected, prospectively collected invasive breast cancer specimens for central retesting (*n*=240). For inclusion, samples were required to have been scored as IHC 0 or 1+ at initial testing. IHC was performed using either the CB11 (Leica Biosystems Ltd, Newcastle Upon Tyne, UK) or PATHWAY 4B5 (Ventana Medical Systems Inc., Tucson, AZ) primary antibodies according to the manufacturers' instructions. Staining for HER2 scoring was interpreted according to ASCO/CAP 2007 [1] criteria as follows: Regions containing all cells with no staining or if cell membrane staining is observed in <10% of the tumour cells received a score of 0 ('negative'). Regions containing more than 10% of cells with a faint perceptible membrane staining received a score of 1+ ('negative'). Regions containing more than 10% of cells with weak-to-moderate complete membrane staining received a score of 2+ ('weakly positive'). Regions containing more than 30% of cells with a strong complete membrane staining received a score of 3+ ('positive').

Central SISH retesting

Recently cut tissue sections from the same blocks used for initial IHC analysis were provided to the central laboratory (Pathology Department, Hygeia Hospital, Athens) for SISH retesting. SISH was conducted using the INFORM HER2 Dual ISH DNA Probe Cocktail (Ventana Medical Systems Inc.), which contains a dinitrophenol (DNP)-labelled *HER2* probe and a digoxigenin (DIG)-

Table 1. Clinical characteristics of the study population.

Characteristic	Patients (*n*=240)
Age, years	
Mean (±SD)	58.5 ± 12.6
Median (IQR)	58.0 (48.0–68.0)
Type of surgery, n (%)	
Partial mastectomy	137 (57.1)
Radical mastectomy/modified radical mastectomy	94 (39.2)
Biopsy or other procedure	8 (3.3)
Unknown	1 (0.4)

IQR interquartile range; SD standard deviation

Table 2. IHC staining characteristics and classification of 240 breast cancers with score 0 or 1+ in regional laboratories.

Characteristic	Samples (*n*=240)
Duration between surgery and date of IHC examination, days[a]	
Median	6.0
IQR	2.0–11.0
Method/antibody, n (%)	
CB11 Novocastra	159 (66.3)
Ventana Pathway 4B5 iView DAB	80 (33.3)
Unknown	1 (0.4)
Measurable section, n (%)	240 (100.0)
Distribution of staining, n (%)	
Homogeneous	107 (44.6)
Heterogeneous	26 (10.8)
Focal	20 (8.3)
No staining	87 (36.5)
Membrane staining, n (%)	153 (63.5)
Proportion of cells with membrane staining	
≤10%	17 (11.1)
>10%	135 (88.2)
Unknown	1 (<0.7)
Thin ring present, n (%)[b]	153 (100.0)
Partial membrane cover, n (%)[b]	153 (100.0)
Cytoplasmic stain, n (%)	14 (5.8)
Final score	
0	105 (43.8)
1+	135 (56.2)

IQR interquartile range
[a] *Unknown for three samples*
[b] *Reported as a percentage of the 153 samples with membrane staining*

labelled CEP17. Hybridised probes were detected using the *ultra*VIEW Silver ISH DNP (SISH) and *ultra*VIEW Red ISH DIG Detection Kits (Ventana Medical Systems Inc.), which visualise *HER2* and CEP17 as black and red signals, respectively. The staining procedure was fully automated using the BenchMark ULTRA machine (Ventana Medical Systems Inc.). SISH was performed in accordance with the manufacturer's protocols by central laboratory personnel, who could access specialised support from the manufacturer if required. Prior to initiation of the study, the quality of SISH tests in the central laboratory was confirmed by the manufacturer.

For each sample, 60 nuclei were evaluated and HER2 status assigned using ASCO/CAP criteria according to the mean *HER2*:CEP17 ratio as positive (ratio >2.2), negative (ratio <1.8) or equivocal (ratio 1.8–2.2) [1]. For cases with a ratio of 1.8–2.2, an additional 20 nuclei were counted to determine the final status. In addition, the presence of polysomy of chromosome 17 was recorded where the mean number of CEP17 signals was ≥3 signals/cell.

Statistical analyses

Descriptive statistics were used to summarise patient characteristics and assay results. For continuous variables, these included measures of central tendency (median, mean) and dispersion (interquartile range (IQR), standard deviation). Categorical variables were summarised as frequencies and percentages. The distribution of *HER2*:CEP17 ratios was summarised as a histogram and kernel density estimation plot. The main study endpoint was the proportion of samples with initial IHC 0/1+ (i.e., negative) scores that were also classified as SISH-negative on central retesting. The two-sided 95% confidence interval (CI) for this endpoint was derived using the Agresti-Coull method [23]. The proportions of SISH-negative results were compared between the IHC 0 and IHC 1+ subgroups using Pearson's chi-square test of independence. All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Patient population

A total of 240 cases of invasive breast cancer with IHC score of 0 or 1+ were included in the study. Clinical characteristics of the study population are summarised in Table 1. All patients were female and the median age was 58.0 years (IQR 48.0–68.0 years). Pathological material was mostly obtained from partial (57.1%) or radical/modified radical mastectomy (39.2%), but a minority of samples were obtained via biopsy or other procedures (3.3%).

Initial assessment of HER2 status using IHC

Results of initial IHC testing of these 240 samples are summarised in Table 2. The median duration between surgery and date of IHC examination was 6.0 days (IQR 2.0–11.0 days). For the preparation of slides, the CB11 Novocastra antibody was used in 159 (66.5%) of the

Table 3. Staining characteristics of 240 IHC 0/1+ breast cancers centrally re-tested by SISH.

Characteristic	Samples (*n*=240)
Duration between surgery and date of SISH examination, months[a]	
Median	4.6
IQR	2.5–8.0
Method, n (%)	
INFORM Probe Ultra View ISH Detection Kit	240 (100.0)
Distribution of staining, n (%)	
Homogeneous	221 (92.1)
Heterogeneous	9 (3.8)
Focal	7 (2.9)
No staining	3 (1.3)

IQR interquartile range
[a] *Unknown for six samples*

Table 4. Classification of *HER2* amplification in 237 IHC 0/1+ breast cancers centrally retested by SISH.

SISH result, n (%)	All samples (n=237)	IHC score 0 (n=105)	IHC score 1+ (n=132)
HER2 status			
Negative (*HER2*:CEP17 <1.8)*	225 (94.9)	100 (95.2)	125 (94.7)
Equivocal (*HER2*:CEP17 1.8–2.2)	4 (1.7)	1 (1.0)	3 (2.3)
Positive (*HER2*:CEP17 >2.2)	8 (3.4)	4 (3.8)	4 (3.0)
**Included also polysomic samples*	*2 (0.8)*	*0*	*2 (1.5)*

cases examined, whereas the remaining cases (n=80, 33.5%) were examined with the Ventana Pathway 4B5 iView DAB. All cases examined contained a measurable section.

In all, 87 samples (36.5%) showed no IHC staining. In the remaining 153 cases (63.5%), distribution of staining in the tissue section was characterised as homogeneous (44.6%), heterogeneous (10.8%) or focal (8.3%). Membrane staining was evident in 153 samples (63.5%), which in most cases (135/150, 88.2%) affected >10% of cells. In all 153 cases, membrane coverage was partial and was described as a thin ring. Cytoplasmic staining was present in 14 cases (5.8%). Samples were scored as 0 in 105 cases (43.8%) and 1+ in 135 cases (56.2%).

Central reassessment of HER2 status using SISH

The median time between surgery and central HER2 reassessment by SISH was 4.6 months (IQR 2.5–8.0 months). Characteristics of the SISH assay are

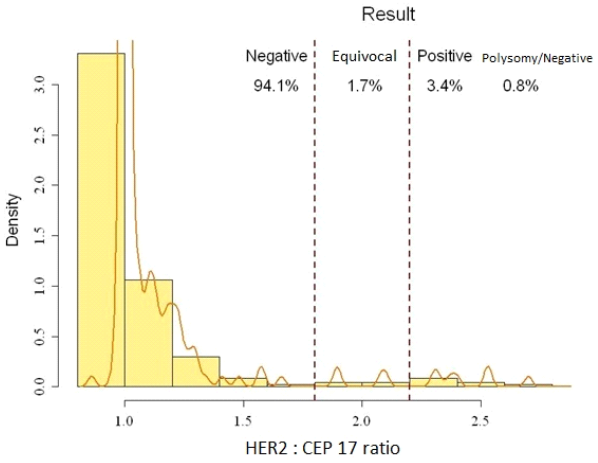

Figure 1. Distribution of HER2:CEP17 ratio in 237 cases with IHC score 0/1+ when centrally retested by SISH, shown as histogram (yellow bars) and kernel density estimation plot (orange line). Dashed lines represent the divides between negative, equivocal, and positive categories.

summarised in Table 3. Most samples analysed (n=221, 92.1%) showed homogeneous SISH staining, with a minority showing heterogeneous (3.8%) or focal (2.9%) staining patterns. Three samples showed no staining and were deemed by the investigator as unsuitable for HER2 status assessment.

Results of the SISH HER2 reassessment are summarised in Table 4 and Fig. 1. Of the 237 evaluable samples, 225 had *HER2*:CEP17 ratio <1.8; therefore, the proportion of IHC 0/1+ cases that were classed as HER2-negative when re-examined using SISH was estimated at 94.9% (95% CI 90.3–96.5%) included the two (0.8%) samples that showed polysomy of chromosome 17. Eight of the 237 samples tested positive by SISH (*HER2*:CEP17 >2.2), providing a false-negative rate of 3.4%. Four cases (1.7%) exhibited equivocal *HER2* amplification status (*HER2*:CEP17 ratio 1.8–2.2).

Comparing tumours with IHC score of 0 with those with IHC score of 1+, the distribution of samples between the SISH-positive, SISH-equivocal and SISH-negative categories appeared similar. Among the 105 IHC 0 samples, 100 (95.2%) were classed as negative, one was equivocal (1.0%) and four (3.8%) were positive by SISH. Of the 132 IHC 1+ samples, 123 (93.2%) were negative, three (2.3%) were equivocal and four (3.0%) tested SISH-positive. There was no significant difference between the proportion of cases scored as SISH-negative in the IHC 0 subgroup compared with the IHC 1+ subgroup (95.2% vs. 93.2%; p=0.505).

Discussion

This study was conducted to determine the rate of false-negative IHC results among prospectively collected invasive breast cancers by central SISH retesting of samples scored as IHC 0/1+ at initial assessment in three Greek pathology laboratories. Its main finding is that only eight of the 237 evaluable samples (3.4%) yielded a positive SISH result when retested at the central facility. *HER2* amplification among these eight tumours was mostly at the low level, with all but one having *HER2*:CEP17 ratios between 2.3 and 2.7. The low rate of false-negative IHC results across the three centres supports an approach of first-line IHC testing with reflex ISH testing being reserved for IHC 2+ cases, which is one of the strategies recommended by ASCO/CAP [1]. The ongoing international study of 1800 IHC 0/1+ cases, of which this analysis is a part, will provide further information on the 'real-world' incidence of false-negative IHC results in a larger set of samples from several different countries.

Although generally in agreement, the results of IHC and ISH assays are not fully concordant, even when performed in the same laboratory [24, 25] and both false-negative and false-positive IHC results are reported to affect substantial minorities of samples [20]. Discordance may be most pronounced between local IHC and central ISH evaluations [21, 26]. Indeed, prospective patient screening data from adjuvant trastuzumab trials highlighted a high rate of discordance between local and central assessments even when comparing results of the same technique [27, 28].

An early estimate of the rate of false-negative IHC results at local laboratories was provided by Reddy and coworkers who centrally retested all breast cancers that had been locally evaluated for HER2 status during screening for a community-based study of trastuzumab between 2001 and 2004 [29]. Of the 383 samples initially scored as IHC 0/1+, 16 (4.2%) were reassessed as FISH-positive at the central laboratory. Similarly, in a previous Greek study including 88 samples evaluated as IHC 0/1+ at initial testing in central or local laboratories between 2001 and 2005, eight (9.1%) were centrally reassessed as *HER2*-amplified by FISH [21]. The 3.4% incidence of false-negatives reported in the present study compares favourably with this previous estimate and is consistent with an improvement in the accuracy of IHC testing in Greek pathology practice over time, although there are differences in methodology and participating centres between these two studies. A US-based study has also provided evidence of a modest fall in the incidence of false-negative IHC results since the publication of the ASCO/CAP guidelines [22].

The false-negative rate in the present study is comparable with recent reports from other countries [30–33]. For example, in a study of IHC 0/1+ samples from five centres in Austria, 25 of 570 (4.4%) were centrally scored as ISH-positive when assessed using the ASCO/CAP *HER2*:CEP17 cut-off (>2.2) [31]. Using the FDA definition of ISH-positivity (*HER2*:CEP17 >2.0), Larsimont *et al* demonstrated a 3.1% false-negative rate among 456 IHC 0/1+ samples from 34 centres in Belgium [30]. Similarly, a false-negative rate of 2.9% has been reported in a single-centre retrospective study conducted in Korea [33]. The lower incidences of false-negative results recently reported in studies conducted in the US [22] and Canada [32] (1.9% and 1.0%, respectively) may reflect the centralisation of IHC testing in both of these studies.

False-negative IHC results are commonly attributed to the various pre-analytic and analytic factors that can influence the extent of immunostaining [1]. For example, formalin fixation and paraffin embedding are known to reduce the immunogenicity of the sample and so may explain a proportion of false-negative cases [34, 35]. Although antigen retrieval processes such as heat treatment can partially reverse these effects, this introduces a further potential source of variability into the assay [24]. For these reasons the ASCO/CAP guidelines emphasise the need for standardisation of sample preparation [1]. Interestingly, 17/24 (71%) of 'false-negative' samples in the Austrian retesting study acquired a 2+ or 3+ IHC score when IHC was repeated at the central facility [31]. In the present study, we did not attempt to verify the initial IHC test result, but the false-negative cases appeared to be evenly distributed between the three participating centres, one of which was the central laboratory. Although differences in antibody characteristics used might also influence the IHC staining, there was no indication that the rate of false-negative IHC results differed between the two antibodies utilised in the present study, which is consistent with previous data [36]. A further potential source of discrepant IHC/ISH results is focal loss of the chromosome 17 centromere, which can elevate the *HER2*:CEP17 ratio in the absence of *HER2* gene amplification [37]. Although a previous study found false-negative cases to be enriched for tumours with reduced CEP17 count [38], all SISH-positive cases in the present study had mean CEP17 count of ≥2.0/cell, suggesting that this was not a cause of discordance.

The clinical significance of false-negative IHC results is currently unclear. Whereas patients with false-positive results (i.e., those with a positive local HER2 assessment that was not confirmed on central testing) have been included in clinical trials of trastuzumab [39,

40], there are no efficacy data available for patients with known false-negative IHC results, who would have been excluded from trial entry based on their 'negative' IHC score. Nevertheless, these tumours are clearly ISH-positive and so the potential for this patient subgroup to benefit from trastuzumab is an important issue for future study, as is the potential prognostic relevance of IHC-negative/ISH-positive status. Interestingly, a recent study demonstrated that an equivocal (2+) IHC score, in the absence of *HER2* amplification, was associated with poorer disease-free survival compared with patients with negative (0/1+) IHC scores [41]. Future studies should also explore the potential association of false-negative IHC status with other histopathologic features such as hormone receptor status, Ki-67 or histologic grade, which could facilitate the identification of tumours most likely to benefit from confirmatory ISH even if the IHC test is negative.

A major strength of this study is its evaluation of a large number of prospectively collected IHC 0/1+ samples using validated automated SISH testing at a high-throughput central facility. One limitation is the lack of blinding of investigators at the central site to the initial IHC result, which could have biased the assessment. Furthermore, this study did not examine concordance between IHC and ISH testing for IHC 2+ or 3+ cases.

In conclusion, the present study demonstrates high concordance between initial IHC testing and central SISH retesting among IHC 0/1+ tumours in Greek pathology practice, with a low rate of false-negative IHC results, thereby supporting an approach of initial IHC testing with reflex ISH for equivocal cases. Further improvement in the accuracy of HER2 assessment may be provided by a new protein-guided ISH assay that permits simultaneous evaluation of HER2 expression and *HER2* copy number and correctly identifies false-negative cases using a single, automated assay [42].

Acknowledgements

Support for third-party writing assistance for this manuscript was provided by F. Hoffmann-La Roche Ltd.

Conflicts of interest

There are no conflicts of interest for all authors.
V.B. and J.N. are employees of Roche Pharmaceuticals.

References

[1] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF, American Society of Clinical Oncology, College of American Pathologists (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 25:118–145. doi: 10.1200/JCO.2006.09.2775

[2] Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792. doi: 10.1056/NEJM200103153441101

[3] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD (2005) Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353:1659–1672. doi: 10.1056/NEJMoa052306

[4] Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Anton A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM (2005) Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. J Clin Oncol 23:4265–4274. doi: 10.1200/JCO.2005.04.173

[5] Gianni L, Eiermann W, Semiglazov V, Manikhas A, Lluch A, Tjulandin S, Zambetti M, Vazquez F, Byakhow M, Lichinitser M, Climent MA, Ciruelos E, Ojeda B, Mansutti M, Bozhok A, Baronio R, Feyereislova A, Barton C, Valagussa P, Baselga J (2010) Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast

cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 375:377–384. doi: 10.1016/S0140-6736(09)61964-4

[6] Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639–2648

[7] Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ, Press M (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20:719–726. doi: 10.1200/JCO.20.3.719

[8] Boekhout AH, Beijnen JH, Schellens JH (2011) Trastuzumab. Oncologist 16:800–810. doi: 10.1634/theoncologist.2010-0035

[9] Jackisch C (2006) HER-2-positive metastatic breast cancer: optimizing trastuzumab-based therapy. Oncologist 11 (Suppl 1):34–41. doi: 10.1634/theoncologist.11-90001-34

[10] National Comprehensive Cancer Network (2013) NCCN Clinical Practice Guidelines in Oncology: Breast Cancer, Version 1

[11] Mass RD, Press MF, Anderson S, Cobleigh MA, Vogel CL, Dybdal N, Leiberman G, Slamon DJ (2005) Evaluation of clinical outcomes according to HER2 detection by fluorescence in situ hybridization in women with metastatic breast cancer treated with trastuzumab. Clin Breast Cancer 6:240–246. doi: 10.3816/CBC.2005.n.026

[12] Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A, Clark E, Benyunes MC, Ross G, Swain SM, CLEOPATRA Study Group (2012) Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 366:109–119. doi: 10.1056/NEJMoa1113216

[13] Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh D-Y, Diéras V, Guardino E, Fang L, Lu M, Olsen S and Blakwell K (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783-91. doi:10.1056/NEJMoa1209124

[14] Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D (2006) Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 355:2733–2743. doi: 10.1056/NEJMoa064320

[15] Yamauchi H, Stearns V, Hayes DF (2001) When is a tumor marker ready for prime time? A case study of c-*erb*B-2 as a predictive factor in breast cancer. J Clin Oncol 19:2334–2356

[16] Penault-Llorca F, Bilous M, Dowsett M, Hanna W, Osamura RY, Rüschoff J, van de Vijver M (2009) Emerging technologies for assessing HER2 amplification. Am J Clin Pathol 132:539–548. doi: 10.1309/AJCPV2I0HGPMGBSQ

[17] Dietel M, Ellis IO, Höfler H, Kreipe H, Moch H, Dankof A, Kölble K, Kristiansen G (2007) Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of *HER2* gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. Virchows Arch 451:19–25. doi: 10.1007/s00428-007-0424-5

[18] Bartlett JM, Campbell FM, Ibrahim M, Wencyk P, Ellis I, Kay E, Connolly Y, O'Grady A, Di Palma S, Starczynski J, Morgan JM, Jasani B, Miller K (2009) Chromogenic in situ hybridization: a multicenter study comparing silver in situ hybridization with FISH. Am J Clin Pathol 132:514–520. doi: 10.1309/AJCPXY3MJ6GSRCYP

[19] Ross JS (2011) Point: Fluorescence in situ hybridization is the preferred approach over immunohistochemistry for determining HER2 status. Clin Chem 57:980–982. doi: 10.1373/clinchem.2010.160762

[20] Cuadros M, Villegas R (2009) Systematic review of HER2 breast cancer testing. Appl Immunohistochem Mol Morphol 17:1–7. doi: 10.1097/PAI.0b013e318169fc1c

[21] Papadopoulos S, Kouvatseas G, Skarlos D, Malamos N, Delliou E, Saratsiotou I, Ardavanis A, Mavroudis D, Skarpidi E, Arapantoni P, Karyda I, Patakioyta F, Aravantinos G, Razis E, Fountzilas G, Kosmidis P (2007) Comparison of HER2 detection methods between central and regional laboratories in Greece. Clin Breast Cancer 7:784–790. doi: 10.3816/CBC.2007.n.040

[22] Vergara-Lluri ME, Moatamed NA, Hong E, Apple SK (2012) High concordance between HercepTest immunohistochemistry and ERBB2 fluorescence in situ hybridization before and after implementation of American Society of Clinical Oncology/College of American Pathology 2007 guidelines. Mod Pathol 25:1326–1332. doi: 10.1038/modpathol.2012.93

[23] Agresti A, Coull BA (1998) Approximate is better than “exact” for interval estimation of binomial proportions. Am Stat 52:119–126

[24] Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L (2002) 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 20:3095–3105. doi: 10.120/JCO.2002.09.094

[25] Lal P, Salazar PA, Hudis CA, Ladanyi M, Chen B (2004) HER-2 testing in breast cancer using immunohistochemical analysis and fluorescence in situ hybridization: a single-institution experience of 2,279 cases and comparison of dual-color and single-color scoring. Am J Clin Pathol 121:631–636. doi: 10.1309/VE78-62V2-646B-R6EX

[26] Perez EA, Suman VJ, Davidson NE, Martino S, Kaufman PA, Lingle WL, Flynn PJ, Ingle JN, Visscher D, Jenkins RB (2006) HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 24:3032–3038. doi: 10.1200/JCO.2005.03.4744

[27] Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, Brown A, Yothers G, Anderson S, Smith R, Wickerham DL, Wolmark N (2002) Real-world performance of HER2 testing-National Surgical Adjuvant Breast and Bowel Project experience. J Natl Cancer Inst 94:852–854. doi: 10.1093/jnci/94.11.852

[28] Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, Addo FK, Murphy B, Ingle JN, Perez EA (2002) Concordance between local and central laboratory HER2 testing in the Breast Intergroup trial N9831. J Natl Cancer Inst 94:855–857. doi: 10.1093/jnci/94.11.855

[29] Reddy JC, Reimann JD, Anderson SM, Klein PM (2006) Concordance between central and local laboratory HER2 testing from a community-based clinical study. Clin Breast Cancer 7:153–157. doi: 10.3816/CBC.2006.n.025

[30] Larsimont D, Colpaert C, Salgado R, Vermeesen N, D’hondt V, De Celle T (2011) Results of a Belgian multicenter retrospective study to determine the incidence of HER2 gene amplification in patients scored as immunohistochemistry 0 or 1+. J Clin Oncol 29:abstr 549

[31] Reiner-Concin AM, Lax S, Regitnig P, Kronberger C, Jasarevic Z, Bogner S (2011) Should HER-2 score 0/1+ breast cancer cases be retested by in-situ hybridisation? Results of a multicenter retesting study. Cancer Res 71:P5-11-15. doi: 10.1158/0008-5472.SABCS11-P5-11-15

[32] Hanna W, Barnes PJ, Chang MC, Gilks B, Magliocco A, Rees H, Robertson S, SenGupta SK, Nofech-Mozes S (2012) The incidence of false negative of HER2/Neu status in primary breast cancer in the era of standardized testing: a Canadian prospective study. Cancer Res 72:P2-10-09. doi: 10.1158/0008-5472.SABCS12-P2-10-09

[33] Lee M, Cho S, Kim D, Lee K, Lee J, Kwon H, Lee H, Lee S (2012) Concordance between immunohistochemistry and FISH (fluorescence in situ hybridization) & SISH (silver in situ hybridization) for assessment of the HER2. Cancer Res 72:PD02-06. doi: 10.1158/0008-5472.SABCS12-PD02-06

[34] Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707–712. doi: 10.1126/science.2470152

[35] Press MF, Hung G, Godolphin W, Slamon DJ (1994) Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. Cancer Res 54:2771–2777

[36] van der Vegt B, de Bock GH, Bart J, Zwartjes NG, Wesseling J (2009) Validation of the 4B5 rabbit monoclonal antibody in determining Her2/neu status in breast cancer. Mod Pathol 22:879-886. doi: 10.1038/modpathol.2009.37

[37] Marchiò C, Lambros MB, Gugliotta P, Di Cantogno LV, Botta C, Pasini B, Tan DS, Mackay A, Fenwick K, Tamber N, Bussolati G, Ashworth A, Reis-Filho JS, Sapino A (2009) Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis. J Pathol 219:16–24. doi: 10.1002/path.2574

[38] Grimm EE, Schmidt RA, Swanson PE, Dintzis SM, Allison KH (2010) Achieving 95% cross-methodological concordance in HER2 testing: causes and implications of discordant cases. Am J Clin Pathol 134:284–292. doi: 10.1309/AJCPUQB18XZOHHBJ

[39] Paik S, Kim C, Wolmark N (2008) HER2 status and benefit from adjuvant trastuzumab in breast cancer. N Engl J Med 358:1409–1411. doi: 10.1056/NEJMc0801440

[40] Perez EA, Reinholz MM, Hillman DW, Tenner KS, Schroeder MJ, Davidson NE, Martino S,

Sledge GW, Harris LN, Gralow JR, Dueck AC, Ketterling RP, Ingle JN, Lingle WL, Kaufman PA, Visscher DW, Jenkins RB (2010) HER2 and chromosome 17 effect on patient outcome in the N9831 adjuvant trastuzumab trial. J Clin Oncol 28:4307–4315. doi: 10.1200/JCO.2009.26.2154

[41] Rossi V, Sarotto I, Maggiorotto F, Berchialla P, Kubatzki F, Tomasi N, Redana S, Martinello R, Valabrega G, Aglietta M, Ponzone R, Montemurro F (2012) Moderate immunohistochemical expression of HER-2 (2+) without HER-2 gene amplification is a negative prognostic factor in early breast cancer. Oncologist 17:1418–1425. doi: 10.1634/theoncologist.2012-0194

[42] Nitta H, Kelly BD, Padilla M, Wick N, Brunhoeber P, Bai I, Singh S, Ranger-Moore J, Bieniarz C, Tsuda H, Grogan TM (2012) A gene-protein assay for human epidermal growth factor receptor 2 (HER2): brightfield tricolor visualization of HER2 protein, the HER2 gene, and chromosome 17 centromere (CEN17) in formalin-fixed, paraffin-embedded breast cancer tissue sections. Diagn Pathol 7:60-1596-7-60. doi: 10.1186/1746-1596-7-60