Curr Oncol, Vol. 19, pp. 315-323; doi: http://dx.doi.org/10.3747/co.19.1173

REVIEW ARTICLE

Testing for HER2 in breast cancer: current pathology challenges faced in Canada

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ABSTRACT

This review is designed to highlight several key challenges in the diagnosis of human epidermal growth factor receptor 2 (HER2)–positive breast cancer currently faced by pathologists in Canada:

- Pre-analysis issues affecting the accuracy of HER2 testing in non-excision sample types: core-needle biopsies, effusion samples, fine-needle aspirates, and bone metastases
- HER2 testing of core-needle biopsies compared with surgical specimens
- Criteria for retesting HER2 status upon disease recurrence

Literature searches for each topic were carried out using the MEDLINE, Embase, International Pharmaceutical Abstracts, and BIOSIS databases. In addition, the congress databases of the American Society of Clinical Oncology (2005–2011) and the San Antonio Breast Cancer Symposium (2007–2011) were searched for relevant abstracts.

All authors are expert breast pathologists with extensive experience of HER2 testing, and several participated in the development of Canadian HER2 testing guidelines. For each topic, the authors present an evaluation of the current data available for the guidance of pathology practice, with recommendations for the optimization or improvement of HER2 testing practice.

KEY WORDS

HER2 testing, breast cancer, pathology

1. INTRODUCTION

The human epidermal growth factor receptor 2 (HER2) promotes cell proliferation and angiogenesis and inhibits apoptosis via the Ras/ MAPK and PI3K/Akt pathways. The receptor is amplified or overexpressed (or both) in approximately 18%–20% of breast cancers[1].

In breast cancer, HER2-positive status is a negative prognostic factor[2]. Before the introduction of HER2-directed therapies such as trastuzumab, the life expectancy of patients with HER2-positive disease was shorter than that of patients with HER2-negative tumours[3]. For patients with HER2-positive early breast cancer, 1 year of adjuvant therapy with trastuzumab has resulted in significant improvements in overall survival and disease-free survival[4]. Furthermore, trastuzumab-based therapy for early breast cancer is associated with reduced rates of disease relapse and reduced occurrences of metastatic disease[5].

Trastuzumab-based therapy is the standard of care for HER2-positive metastatic breast cancer, and evidence is emerging that HER2-directed therapy remains a valid strategy even after disease progression[6,7]. Consequently, to accurately identify patients for whom HER2-directed therapy is appropriate, it is important that the tests and the testing procedures used to determine HER2 status both be reliable.

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have published guideline recommendations to improve the accuracy of HER2 testing in patients with invasive breast cancer[1]. The guidelines promote good general HER2 testing practice, but the information provided is insufficient in several key areas. In June 2010, an expert panel of practicing Canadian pathologists met to discuss current challenges in HER2 testing in patients with breast cancer. The aim of the discussions was to identify gaps in current HER2 testing guidelines and to provide recommendations for best practice. The outcomes of discussions relating to three key issues identified by the panel are presented here.

2. PRE-ANALYSIS ISSUES IN NON-EXCISION SAMPLE TYPES AND BONE METASTASES

2.1 Background

Surgical excisions have long been the "gold standard" sample-type for the assessment of HER2 status[8]. When

it is not possible or practical to obtain a surgical excision for HER2 testing (for example, in patients receiving neoadjuvant therapy or in patients with inoperable metastases), it may be desirable to assess HER2 status using a non-excisional sample such as a core-needle biopsy (CNB), fine-needle aspirate (FNA), or effusion specimen.

Methods currently used to test HER2 status were developed and optimized for use with formalin-fixed, paraffin-embedded breast tissue specimens derived from surgical excisions[9]. However, tissue handling and fixation methods used with non-excisional samples may vary from those used with larger tissue samples. For example, biopsies taken from bone metastases usually require decalcification before HER2 status can be assessed. Consequently, it is important to establish whether any of these non-excision sample types can reliably be used for HER2 testing, and to determine whether sample handling and fixation protocols may be modified to improve the reliability of HER2 testing.

2.2 Evidence

2.2.1 Tissue Collection and Fixative

The ASCO/CAP guidelines recommend that sample ischemia time (time from sample acquisition to fixation) should be as short as possible, because delaying fixation by more than 1 hour has been shown to negatively affect the detection of breast biomarkers[1,10]. That recommendation is underlined by the results of a recent study that compared immunohistochemistry (IHC) HER2 scores obtained for refrigerated and non-refrigerated excision samples with IHC HER2 scores obtained for corresponding CNBs (with negligible cold ischemia time). Significant reductions in IHC staining for HER2 were noted after only 2 hours of cold ischemia time for non-refrigerated samples and after 4 hours for refrigerated samples[11].

The guidelines also specify that samples should be fixed in neutral buffered formalin and that use of any alternative fixative should be validated technically against results obtained from the same samples fixed in buffered formalin[1]. Current ASCO/CAP guidelines do not provide guidance regarding sample handling or fixation techniques for use with CNBs, effusions, FNAs, or bone metastases. Neutral buffered formalin is the only fixative recommended by manufacturers of HER2 testing kits such as HercepTest[12], but FNAs and pleural effusions are often fixed in ethanol, and concerns have been expressed that that approach may have a negative impact on the reliability of HER2 testing; nonspecific (false-positive) IHC staining for HER2 protein overexpression has been observed in some studies involving ethanol-fixed samples[13–15]. Conversely, formalin fixation of FNAs appears to improve the reliability of subsequent HER2 testing; concordance rates of up to 100% have been reported between formalin-fixed FNAs or serous effusions and matched formalin-fixed tissue sections[9].

In laboratories in which samples arrive already fixed in ethanol, *in situ* hybridization (ISH) appears to be the most appropriate initial HER2-testing methodology. We identified several small studies in which liquid-based cytology samples were fixed in ethanol- or methanol-based media and tested for HER2 status using IHC and ISH (Table I). In the first of those studies, ethanol-fixed cytology samples and tissue blocks from 58 patients with invasive breast cancer were tested for HER2 status using IHC and ISH. The IHC-based HER2 testing resulted in a concordance rate of only 75.9% compared with the corresponding formalin-fixed, paraffin-embedded excision samples, but chromogenic ISH testing of cytology samples re-fixed in 4% paraformaldehyde resulted in an accuracy rate of 86.2% (50 of 58)[16]. A separate prospective study of 103 breast carcinoma samples found significant correlation between the chromogenic ISH–determined HER2 status of cytology samples post-fixed in absolute ethanol and the corresponding histology samples[17]. As with IHC, ISH-based HER2 testing of cytology samples appears most reliable when samples are fixed in formalin. A prospective study of 53 FNA cytology samples fixed in ethanol and post-fixed in formalin detected a concordance rate of 100% (compared with matched formalin-fixed tissue blocks) when HER2 status was determined using CISH[18]. More recently, Almeida and Correia[19] reported a chromogenic ISH HER2 concordance rate of 94% (34 of 36) between formalin-fixed FNA cytology samples and excision sample blocks.

2.2.2 Fixation Time

Although the ASCO/CAP guidelines recommend a fixation time of 6–48 hours for excision samples, no practical guidance is provided regarding the optimum fixation time for CNBs or other non-excision sample types[1]. Although the permeation of formalin into CNBs is generally swifter than it is into excision specimens, there is general agreement that a fixation time of at least 6 hours is required to prevent underfixation of CNBs. That period is supported by results of a study conducted by Dintzis and Allison[20], who used formalin-fixed cell pellets, in which fixation times shorter than 6 hours resulted in a decrease in IHC HER2 staining intensity and a smaller proportion of cells with membrane staining. However, because fixation entails a chemical reaction process (known as the formaldehyde clock reaction) as well as tissue permeation, there is some suggestion that a fixation time of 24 hours is ideal to complete the fixation process. Pathologists should bear in mind that short or incomplete formalin fixation results in the specimen being sequentially fixed by ethanol during standard sample processing.

Although it has been suggested that prolonged fixation may lead to false-negative HER2 test results[1], fixation time has been extended beyond 72 hours (median: approximately 79 hours)[21] and even up to 96 hours[22] without reducing IHC HER2 assay sensitivity.

TABLE I Accuracy of HER2 testing with chromogenic *in situ* hybridization using liquid-based cytology samples taken from breast tumours

	Reference			
	Sumiyoshi et al., *2006*[16]	*Sartelet* et al., *2005*[17]	*Vocaturo* et al., *2006*[18]	*Almeida and Correia, 2008*[19]
Samples (*n*)	58	103	53	36
Fixative	95% Ethanol	ThinPrep[a] Ethanol[b]	ThinPrep[a]	Not specified
Antibody	A0485 HercepTest[d]	NCL-CB11[c]	A0485 HercepTest[d]	A0485 HercepTest[d]
Sensitivity [% (95% CI)][e]	84.00	100	85 (73 to 95)	
Specificity [% (95% CI)][e]	87.90	96% (92.0 to 99.9)	100	Concordance rate 94% (cytology samples vs. histology samples)
Predictive value [% (95% CI)]				
Positive	—	86.40	100 (79 to 93)	
Negative	—	100	92 (86 to 98)	
Accuracy (%)	86.20	—	—	

[a] Contains methanol (Cytyc, Boxborough, Massachusetts, U.S.A.).
[b] Samples were post-fixed in absolute ethanol.
[c] Novocastra Laboratories, Newcastle Upon Tyne, U.K.
[d] Dake, Glostrup, Denmark.
[e] Compared with formalin-fixed, paraffin-embedded tissue sections.

2.2.3 Handling of Samples from Bone Metastases

The effect of decalcification on the reliability of HER2 testing with IHC was assessed in a prospective study of 10 randomly selected breast-tissue excision samples[23]. The authors found that membranous IHC HER2 staining was less intense and more heterogeneous in decalcified samples than in control samples. Compared with control samples, the decalcified samples showed a mean HER2 score that was reduced by 1.0 using the IHC 0 to 3+ scoring system[23]. Furthermore, fluorescence ISH (FISH) HER2 testing was unsuccessful in 2 of 2 cases. Thus, determination of HER2 status using FISH may not be possible after sample decalcification. However, controlled decalcification using EDTA and daily radiography can result in a high concordance rate between IHC and ISH. Zustin and colleagues[24] used 10% EDTA to construct a tissue microarray using 149 breast-cancer bone metastases and decalcified samples, taking daily radiographs to assess the decalcification process. The success rate for HER2 testing by FISH was 85.0% and a comparison of HER2 testing results obtained using IHC and FISH revealed a concordance rate of 76.9% for IHC 3+ samples[24]. In some centres, current practice is to isolate soft-tissue cores from a bone metastasis sample for use in HER2 testing, because decalcification of such samples is not required.

2.3 Consensus

If the appropriate precautions are observed, HER2 testing of cytology samples and bone metastases is possible.

Non-excision samples should be immediately immersed in 10% phosphate-buffered formalin and fixed for at least 6 hours. Cytology specimens should always be fixed in formalin, not ethanol.

Immunohistochemistry is not suitable for determining the HER2 status of ethanol-fixed samples. In such cases, ISH-based testing should be used.

Where soft-tissue cores can be isolated from bone metastases, those cores are preferred for determining HER2 status, because decalcification is not required. However, if such samples are not available, then use of a highly standardized decalcification protocol incorporating 10% EDTA and daily radiography is recommended, followed by HER2 testing using ISH. If EDTA-based decalcification is not possible or practical, then decalcification by other methods can be considered, although the methods must be appropriately validated. Subsequent testing of HER2 status in such cases should be performed using ISH. In the event that none of the foregoing approaches are either possible or successful, then we recommend referring to the HER2 status of the primary tumour, because

high levels of concordance have been demonstrated between the HER2 status of primary tumour samples and metastases (see Section 4, "Criteria for Retesting HER2 Status on Disease Recurrence").

In accordance with published guidelines, each laboratory should validate its HER2 testing methods wherever any one or a combination of the specimen type, handling, or fixation varies from that of the usual tissue specimen fixed in formalin.

3. HER2 TESTING IN CNBs COMPARED WITH SURGICAL SPECIMENS

3.1 Background

As already outlined, the assumption is that, when an excision specimen is available, that specimen will be the primary source of material for HER2 testing. However, in circumstances in which obtaining an excision specimen is not possible or practical, or in which concerns arise about the quality of the excision specimen, it may be necessary to consider HER2 testing using a CNB. In CNB samples, HER2 testing raises several specific analysis considerations in addition to the pre-analysis considerations discussed earlier. Those considerations include

- nonspecific staining caused by artifacts of compression or of edge and retraction; and
- the small size of CNB specimens compared with surgical specimens, resulting in methodologic inconsistency and potential for sampling errors resulting from morphologic heterogeneity.

The first of those issues is addressed by the ASCO/CAP and Canadian guidelines, which advise against HER2 testing using IHC in CNBs in which the entire core displays edge or retraction artifacts, or where crush artifacts are visible[1,25]. However, that problem is not common to all testing laboratories; some report that artifacts are not a significant feature in their analyses[26]. In practice, when such artifacts are present, the decision regarding whether and how to process the sample is left to the judgment of the individual pathologist.

The second issue, restricted sample size, also has potential implications for the reliability of HER2 testing, because equivocal IHC HER2 test results—and any consequent demand for confirmatory ISH testing—are more likely to occur with small samples than with larger specimens (unpublished data). Morphologic heterogeneity can be addressed by testing additional tissue blocks chosen to represent the varied morphology of a heterogeneous tumour. However, heterogeneity poses particular challenges in cases in which only a small volume of tissue is available for technical and morphologic assessment. For instance, HER2-positivity occurs frequently in samples of ductal carcinoma *in situ*[27]. Consequently, when small samples contain a mixture of ductal carcinoma *in situ* and invasive tumour, it may be difficult to identify invasive tumour so as to accurately gauge HER2 status. Finally, antigen retrieval and staining protocols may vary with the specimen size or type, resulting in methodologic inconsistencies compared with processing methods used for larger specimens.

Sample size is addressed by current Canadian guidelines (which specify that ISH is the preferred initial testing method for small samples and CNBs in the neoadjuvant setting), but the ASCO/CAP guidelines do not provide any specific discussion of the issue[1,25]. Consequently, there may be a perception that HER2 testing performed using CNB samples may be intrinsically less reliable than that using surgical excisions. However, if used appropriately, reliable HER2 testing is possible using CNBs[8,28].

3.2 Evidence

When IHC or FISH is used to determine HER2 status, concordance between the results obtained in CNB samples and in surgical specimens is generally excellent (about 85%–99%, Table II). However, some studies found that results obtained using IHC staining were more consistent than those obtained using FISH (99% and 94% respectively)[30].

The IHC HER2 score concordance between CNB samples and surgical excisions appears to vary according to the initial IHC score obtained for the CNB. For example, D'Alfonso *et al.*[29] reported 100% concordance between the HER2 status of CNBs scored as IHC 3+ and corresponding surgical samples tested using FISH. Concordance rates were slightly lower for CNBs scored as IHC 1+ (90.6%) and IHC 0 (85.7%). In addition, the number of cores tested can have a bearing on the concordance rate between CNB and excision samples, although testing numerous cores may not be possible in all laboratories because of the cost implications. Some studies reported concordance rates of 100% when more than 3 cores were analyzed[8,28]. Accuracy may also be improved by obtaining larger CNB samples[28,34] and by having samples evaluated by 2 or more observers[34].

Tumour heterogeneity appears not to be a significant confounding factor in studies in which the HER2 status of CNB samples was determined using ISH-based methods[29]. However, to ensure that patients whose CNBs were graded HER2-negative receive appropriate treatment, it has been suggested that ISH retesting of CNBs scored as HER2 IHC 0/1+, or IHC retesting of a corresponding surgical specimen, may be advisable[35].

The foregoing studies are published examples of HER2 testing in practice, and they reflect issues important to the accuracy and reliability of HER2 testing in CNBs. However, it should be acknowledged that it is not possible to evaluate whether the excision specimens and CNBs described in those studies were handled in a standardized manner. Certainly

TABLE II Concordance between HER2 status of core-needle biopsy (CNB) and corresponding surgical (EX) samples in recent studies

	Reference							
	Arnedos et al., *2009*[26]	*Tamaki* et al., *2010*[28]	*D'Alfonso* et al., *2010*[29]	*Apple* et al., *2009*[30]	*Apple* et al., *2009*[30]	*Park* et al., *2009*[31]	*Lebeau* et al., *2010*[32]	*Lee* et al., *2012*[33]
Samples tested (*n*)	327	353	100 (patients)	260	260	104 (patients)	500	300
Testing method	IHC	IHC	FISH	FISH	IHC	IHC	IHC	IHC/FISH
Overall concordance (%)	98.8	89.3	87	92	98	86.5	90.4	98
Concordant HER2– [*n* (%)]	283 (86.5)	182 (96.8)	12 (85.7)[a] 58 (90.6)[b]	102 (82)	83 (66)	—	411 (97.4)	261 (100)
Concordant HER2+ [*n* (%)]	40 (12.2)	12 (75.0)	100	13 (10)	6 (5)	—	27 (81.8)	33 (97.0)
Overall discordance (%)	—	10.66	13	8	2	—	—	2
Discordant (CNB+/EX–) [*n* (%)]	1	0	2 (2)	6 (5)	0 (0)	2	5 (15.2)	0 (0)
Discordant (CNB–/EX+) [*n* (%)]	3	0	2 (2)	4 (3)	3 (2)	1	0 (0)	6 (2)
Indeterminate [*n* (%)]	4 (1.2)	—	—	0 (0)	33 (26)	—	—	—

[a] Scored as IHC 0.
[b] Scored as IHC 1+.
IHC = immunohistochemistry; FISH = fluorescence *in situ* hybridization.

the pre-analysis conditions for processing CNB samples vary between studies. In some cases, fixation time was shorter than recommended by current guidelines[8]. Similarly, the antibodies or HER2 testing system used were not always specified. When defining HER2 status, half the studies did not use the current ASCO/CAP cut-off limits for HER2-positivity by IHC[26,28,31,35], instead using older cut-off limits or failing to specify the cut-off used. Current ASCO/CAP guidelines for IHC testing of HER2 status specify that, for a sample to be considered HER2-positive, more than 30% of invasive tumour cells should show intense uniform membrane staining[1]. Previously, the specified cut-off limit was 10%. Thus, as well as addressing concordance between HER2 testing in excisions and CNBs, the studies indirectly highlight the need for standardized pre-analysis and analysis measures and for adherence to current ASCO/CAP guidelines for defining HER2 status.

3.3 Consensus

Concordance between HER2 testing results in CNBs and surgical specimens is generally good, but further improvements are possible. Strict adherence to pre-analysis and analysis measures is needed, and each laboratory should evaluate HER2 status concordance rates between IHC and ISH in CNB samples before substituting one methodology for another. We recommend that reflex testing with ISH should be carried out in cases in which IHC testing does not provide a clear positive (IHC 3+) or negative (IHC 0) result.

In the neoadjuvant setting, several CNB samples of non-necrotic tumour tissue should be tested to minimize the impact of tumour heterogeneity on result interpretation and to allow sufficient tissue for retesting by IHC or ISH.

If the patient is not receiving neoadjuvant therapy, the excision specimen (where available) should be the primary specimen tested, and precedence should be given to the HER2 status of that sample unless concerns have arisen regarding its handling or preservation. If the excision specimen is of poor quality or does not contain an adequate volume of tumour, then retesting may be performed using CNB samples.

4. CRITERIA FOR RETESTING HER2 STATUS UPON DISEASE RECURRENCE

4.1 Background

Whether HER2 status can change during disease progression is a matter of controversy. Furthermore, it is unclear whether anticancer therapy, particularly HER2-directed therapy, has any effect on HER2 status[36]. A proportion of the reported discordance in HER2 status is likely to stem from variations or improvements in HER2 testing methodology rather than from a true change in HER2 status between the primary tumour and metastasis[37].

Retesting of HER2 status upon disease recurrence is not covered by current Canadian or ASCO/CAP guidelines, although a recent update to the National Comprehensive Cancer Network guidelines recommends that

the HER2 status of metastases should be tested if the HER2 status of the primary tumour is unknown or was originally negative, or if HER2 is not overexpressed[38]. We reviewed published studies addressing HER2 status in patients with metastases, including patients who received HER2-directed therapy for primary breast cancer.

4.2 Evidence

4.2.1 Retesting of Primary Tumour Tissue and Metastases in Treatment-Naïve Patients

The published data that explore the stability of HER2 status during disease progression are limited. Concordance between the HER2 status of primary tumour tissue and metastases has been investigated in several small studies. In retrospective studies in which HER2 status was compared between primary and metastatic tumour samples, discordance rates between 3.0% and 13.6% have been reported, although these examples are not exhaustive[37,39–45]. Changes in HER2 status from the primary to the metastatic setting—both from HER2-positive to HER2-negative and from HER2-negative to HER2-positive—have been reported. The discordance rates reported in prospective studies have been similar. In one small prospective study, a discordance rate of 8% was calculated based on a review of 40 patients[46]. However, only 29 pairs of samples were evaluable for HER2 status, and discordant HER2 status from the primary to the metastatic setting was detected in only 2 patients. A more recent prospective study conducted in 60 patients with primary breast cancer and metastatic lymph nodes revealed concordance rates of 95% for HER2-negative primary tumours and 83.3% for HER2-positive primary tumours[47].

4.2.2 Retesting Metastases in Patients Pretreated with Chemotherapy, With or Without Trastuzumab

There is little published evidence with which to evaluate any potential treatment effect on the HER2 status of patients who receive HER2-directed therapy for primary breast cancer and who subsequently develop metastases. A retrospective analysis by Xiao and colleagues[36] demonstrated no significant difference in the HER2 status of primary and paired metastatic tumours in trastuzumab-treated patients compared with trastuzumab-naïve patients (86.6% and 82.1% respectively, $p = 0.858$). In patients whose HER2 status was discordant between the primary tumour and metastases, no apparent link could be drawn between changes to HER2 status and chemotherapy, endocrine therapy, metastasis site, or time to relapse[36]. However, variations in testing methodologies—and equivocal HER2 testing scores—were common in tumour pairs in which the HER2 status was discordant. A separate study of 137 primary breast tumours and metachronous metastases revealed discordant HER2 status in only 10% of cases ($n = 14$). Given that some of the patients had received prior trastuzumab, it is interesting to note that a pairing of HER2-negative primary tumour with HER2-positive metastasis was significantly more common than was HER2-positive primary tumour with HER2-negative metastasis (12 cases vs. 2 cases, $p = 0.04$)[48]. However, no dedicated analysis was performed to assess the stability of HER2 status according to trastuzumab pretreatment.

The global phase II SHERSIG study (MO22004/NCT00885755) will assess changes in molecular marker expression in patients receiving HER2-directed therapy. The study will attempt to identify biomarkers associated with treatment efficacy and tumour biology from serial biopsy samples. It is expected to report during 2014.

4.3 Consensus

In accordance with the National Comprehensive Cancer Network guidelines, the HER2 status of metastases should be retested in patients whose primary tumours were HER2-negative or in whom the HER2 status of the primary tumour is unknown.

The mechanism by which HER2 status appears to change in some patients is not yet fully understood, and it is important that HER2 testing methods be standardized to ensure that the stability of HER2 status is accurately evaluated.

5. CONCLUSIONS

5.1 Pre-analysis Issues in Non-Excision Sample Types and Bone Metastases

Excision specimens and CNB samples should be transferred to the laboratory for fixation without delay (ischemia time < 1 hour) and fixed in 10% phosphate-buffered formalin for at least 6 hours. Cytology specimens should always be fixed in formalin, not ethanol. If ethanol- or methanol-fixed samples are received for HER2 testing, then ISH rather than IHC should be used as the initial testing methodology.

Soft-tissue cores are preferred for determining the HER2 status of bone metastases, because decalcification is not required. If soft-tissue cores are not available, then the following options may be considered (in order of preference):

- Decalcification using a highly standardized decalcification protocol, incorporating 10% EDTA and daily radiography, followed by HER2 testing using ISH
- Decalcification using other methods (which must be appropriately validated), followed by HER2 testing using ISH

If above approaches are not possible or successful, then refer to the HER2 status of the primary tumour.

In accordance with published guidelines, each laboratory should validate testing methods whenever any one or a combination of the specimen type, handling,

or fixation varies from that of the usual tissue specimen fixed in formalin.

5.2 HER2 Testing in CNBs

Strict adherence to pre-analysis and analysis measures is needed when performing confirmatory HER2 testing using CNBs. In CNB samples, HER2 status concordance rates between IHC and ISH should be evaluated before one methodology is substituted for another. Reflex testing with ISH should be carried out if IHC testing does not provide a clear positive (IHC 3+) or negative (IHC 0) result.

In the neoadjuvant setting, tumour samples scored as IHC 2+ or 1+ should be retested using ISH to confirm HER2 status. If the patient is not receiving neoadjuvant therapy, the excision specimen (when available) should be the primary specimen tested, and precedence should be given to the HER2 status of that sample unless there are concerns regarding its handling or preservation. If the excision specimen is of poor quality or does not contain an adequate volume of tumour, then retesting may be performed using CNB samples.

5.3 Criteria for Retesting HER2 Status on Disease Recurrence

Retesting of metastases is recommended if the HER2 status of the primary tumour is unknown or HER2-negative. The standardization of HER2 testing methods is desirable to ensure that the stability of HER2 status is accurately evaluated.

6. ACKNOWLEDGMENTS

Support for third-party writing assistance for this manuscript, furnished by Claire Cridland, was provided by F. Hoffmann–La Roche Ltd., Canada.

7. CONFLICT OF INTEREST DISCLOSURES

Funding for the National HER2 Pathology Advisory Board meeting and support for third-party writing assistance were provided by F. Hoffmann–La Roche Ltd., Canada. All advisory Board members received an honorarium to compensate them for the time taken to attend the meeting.

WH has attended other advisory board meetings at the invitation of F. Hoffmann–La Roche Ltd. and has received associated honoraria. DP has participated in other advisory board meetings and an educational program for F. Hoffmann–La Roche Ltd. and has received associated honoraria. MC has received research funding from F. Hoffmann–La Roche Ltd.

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