HER2 overexpression of breast cancers in Hong Kong: prevalence and concordance between immunohistochemistry and in-situ hybridisation assays

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Objectives To evaluate the prevalence of human epidermal growth factor receptor 2 (HER2) gene overexpression in breast cancer patients encountered in Hong Kong and the concordance of HER2 findings from primary immunohistochemistry assays and confirmatory in-situ hybridisation assays.

Design Retrospective study.

Setting Department of Clinical Oncology in a public hospital in Hong Kong.

Patients All patient referrals between July 2006 and June 2007 with newly diagnosed invasive breast cancer (for prevalence evaluation), and all patients treated at our unit with confirmatory in-situ hybridisation tests performed within the study period (for concordance evaluation).

Results There were 272 consecutive breast cancer patients eligible for prevalence evaluation. The distribution for immunohistochemistry staining in 249 cases for scores 0, 1+, 2+, and 3+ were 99 (40%), 40 (16%), 58 (23%), and 52 (21%) respectively. In the remaining 23 patients, four and 19 breast cancers were unscored and reported by immunohistochemistry to be HER2-positive and -negative, respectively. The overall HER2 overexpression rate (3+ or reported as positive) was 21%. HER2 overexpression was associated with grade 3 histology (P<0.001) and negative hormonal receptor status (P<0.001). However, it was not associated with age (P=0.525), T-classification (P=0.740), N-classification (P=0.691), nor group stages (P=0.433). Of the 37 patients with confirmatory in-situ hybridisation tests performed, 10 (71%) of 14 with immunohistochemistry staining of 3+ and 1 (4%) of 23 with immunohistochemistry staining of 2+ were found to have HER2 gene amplification.

Conclusions More than 25% of HER2 overexpression identified by immunohistochemistry assays in this Hong Kong cohort could not be verified by confirmatory in-situ hybridisation assays. Compliance with the latest guidelines for HER2 testing should improve the future accuracy and concordance.

Introduction
The human epidermal growth factor receptor 2 (HER2) gene has emerged as a very important predictor of outcome among patients having invasive breast cancers; its amplification is associated with more aggressive disease and a worse prognosis. HER2 positivity is also associated with relative resistance to endocrine therapies and non-anthracycline, non-taxane containing chemotherapy regimens. Most importantly, therapies, such as trastuzumab (Herceptin) targeting the HER2 oncoproteins, have recently been established as the standard component in the treatment of HER2 overexpressed breast cancers, in both adjuvant and metastatic settings. Accordingly, starting from 2005, HER2 status has been incorporated into the risk allocation of the St Gallen consensus to guide the use of systemic adjuvant therapies.

In Hong Kong, since the late 1990s HER2 testing has been routinely performed for all
newly diagnosed invasive breast cancers. Although in-situ hybridisation (ISH) assay (for HER2 gene amplification) is often considered the more desirable method of testing for it, the immunohistochemistry (IHC) assay (for HER2 protein overexpression) is cheaper, more widely available, and has long been the primary screening test in all laboratories. In-situ hybridisation tests are often reserved for equivocal cases only, but are not currently available in public hospitals. Despite the critical importance of HER2 status in prognosis and treatment decisions, the accuracy of the HER2 test has recently become a matter of much concern. Because of the expense and potential life-threatening cardiotoxicity of anti-HER2 therapies, the importance of accurate HER2 testing cannot be overemphasised.

Our study therefore aimed to assess the prevalence of HER2 overexpression among patients with invasive breast cancers in Hong Kong, and evaluate how well the HER2 status determined by IHC and the confirmatory ISH assay agreed.

Patients and methods
To assess the prevalence of HER2 overexpression, we retrospectively analysed the records of all newly diagnosed female breast cancer patients referred to the Department of Clinical Oncology, Pamela Youde Nethersole Eastern Hospital, Hong Kong between the period July 2006 and June 2007. Patients with pure in-situ carcinomas or inadequate tissue diagnoses were excluded from analysis. Patients presenting with metastatic disease were included so as to avoid selection bias.

For evaluation of concordance between IHC and ISH results, findings from all ISH tests performed within the study period were compared against the findings of primary IHC testing. Patients, who first presented before the study period, were included as long as they had confirmatory ISH tests within the study period, so that they could be considered for target therapies in either the metastatic or adjuvant setting.

Immunohistochemistry assay
Various commercially available anti-HER2 antibodies (eg CB11 monoclonal antibodies, Novocastra Laboratories, Newcastle-on-Tyne, UK) were used in different local laboratories to detect HER2 protein in IHC assays. The stained slides were scored on a 0 to 3+ scale—0 was given if there was no membrane staining or if staining was present in less than 10% of tumour cells; 1+ for faint, or barely perceptible membrane staining in more than 10% of cells with stain involving only part of the cell membrane; 2+ for weak-to-moderate complete staining in more than 10% of tumour cells; and 3+ for strong complete membrane staining in more than 10% of tumour cells. Respective scores of 0 and 1+ were considered HER2-negative, 2+ as HER2 equivocal, and 3+ as HER2-positive.

In-situ hybridisation assay
Although the ISH assay is not yet available in public laboratories, it can be performed in some private or university laboratories, mainly for confirmation of HER2 status in patients with IHC scores of 2+ or 3+. There were two main types of ISH tests used in our patients: fluorescence in-situ hybridisation (FISH) and chromogenic in-situ hybridisation (CISH). The latter tests were performed in some private laboratories for IHC 2+ patients before referral. For IHC 3+ patients, confirmatory ISH tests were considered unnecessary, even in laboratories with ISH facilities. However, as we preferred to have confirmatory ISH tests for IHC 3+ patients before starting them on anti-HER2 therapy, we arranged FISH tests in the University of Hong Kong for those who agreed to self-finance the confirmatory tests.
Fluorescence in-situ hybridisation tests were performed in the Molecular Pathology Laboratory, Department of Pathology, University of Hong Kong. It involved using the Food and Drug Administration (FDA)-approved PathVysion HER-2 DNA Probe Kit (Abbott Molecular Inc, Des Plaines [IL], US) for dual signal evaluation. HER2 gene amplification was defined as HER2 probe/control probe ratio of 2.2 or more for at least 40 nuclei of invasive cancer cells. An additional 20 nuclei were counted, whenever a borderline ratio (1.8-2.2) was initially found.

Chromogenic in-situ hybridisation has emerged as an alternative to FISH, since the latter requires additional analytic equipment not widely available in pathology laboratories (such as dark-field fluorescence microscopy and multiband fluorescence filters). The CISH test uses a digoxigenin-labelled DNA probe (SPPT-Light [Zymed] HER2 CISH kit) to HER2 and the hybridised HER2 probe is detected by a chromogenic reaction using mouse antидigoxigenin antibody, followed by antimouse-peroxidase and diaminobenzidine as chromogen. HER2 amplification is defined by an average of more than 10 dots or large clusters or a mixture of multiple dots and large clusters of the HER2 gene per nucleus in more than 50% of tumour cells. Although CISH has not yet been approved by the US FDA, a number of studies have shown good (>90 to 100%) concordance between CISH and FISH results.14,15

Statistical methods
The Chi squared test was used to test the association between HER2 overexpression and other clinicopathological characteristics. All statistical tests were two-sided and performed at the 0.05 level of significance (P value). The Statistical Package for the Social Sciences (Windows version 12.0; SPSS Inc, Chicago [IL], US) was used for all analyses.

Results
There were 272 consecutive patients with invasive breast cancers referred to our department between July 2006 and June 2007, who were eligible for review. The median age of the patients was 55 (range, 33-92) years. Their clinical and pathological characteristics are shown in Table 1.

Of the primary IHC tests, 39%, 21%, and 40% were performed in our own laboratory, other public hospital laboratories, and private laboratories respectively. There were 4 and 19 breast cancers reported to be HER2-positive and -negative respectively, without detailed IHC scoring. Of the 249 breast cancers with IHC scoring, the distribution for IHC staining of 0, 1+, 2+ and 3+ were 99 (40%), 40 (16%), 58 (23%), and 52 (21%) respectively. The overall HER2 overexpression rate (IHC staining of 3+...
or reported as positive) was 21%.

Table 2 shows the association of HER2 overexpression with common clinicopathological characteristics. In our study population, HER2 overexpression was significantly associated with grade 3 histology (for invasive ductal cancer) (P<0.001), and negative hormonal receptor status (P<0.001). However, there was no association with age (P=0.525), T-classification (P=0.740), N-classification (P=0.691), or group stages (P=0.433).

As many patients were reluctant to self-finance additional ISH tests, only 37 patients had confirmatory ISH tests performed between July 2006 and June 2007. Table 3 shows the results of concordance between their ISH and IHC tests. Overall, 10 (71%) of 14 tumours with IHC staining of 3+ and 1 (4%) of 23 with IHC staining of 2+ were found to have HER2 gene amplification by ISH assays.

### Discussion

Although some early studies reported HER2 overexpression in as many as 30% of breast cancers, more recent findings suggest the true frequency of HER2-positive breast cancer to be around 15 to 20%. In our prior study of 902 patients with known HER2 status presenting before 2003, we also found a relatively high HER2 overexpression rate of 30%. It was unclear whether this was related to the accuracy of HER2 tests in our community setting or due to ethnic variation. Moreover, in that study population, the adverse impact of HER2 overexpression was only evident in patients with four or more positive nodes, but not in patients with negative nodes or less than four positive nodes.
The HER2 overexpression rate of 21% in this more recent study is closer to the findings reported overseas, which is probably related to improved quality assurance of local laboratories in recent years. Similar to other studies, HER2 overexpression in our population was strongly associated with higher histological grades and negative hormonal receptor status. There were no apparent associations of HER2 overexpression with tumour size, nodal status, age or group stages, but our sample may not have been large enough to detect these associations.

In private laboratories where ISH tests are available, confirmatory ISH tests are often performed if tumours have IHC scores of 2+ (equivocal), as some patients may still have HER2 gene amplification and hence stand to benefit from target therapies. Although others have reported a 24% chance of detecting HER2 gene amplification by ISH assays for patients having tumours with IHC 2+ scores, in our series the yield was much lower (only 4%).

Outside a study setting, an IHC score of 3+ is usually accepted as proof of HER2 overexpression and no confirmatory tests are considered necessary. However, prospective substudies from two of the adjuvant randomised trials of trastuzumab versus nil else demonstrated that approximately 20% of HER2 assays performed at the primary treatment site’s pathology department were deemed incorrect, when the same specimen was re-evaluated in a high volume, central laboratory. Although ISH tests were not available in public hospitals, from late 2006, our patients with IHC-determined HER2 overexpression were offered the option of confirmatory FISH testing by the Molecular Pathology Laboratory of the University of Hong Kong. The concordance rate in these patients was only 71%, much lower than the 95% rate currently recognised as the international standard. However, as only a small proportion of our patients had self-financed confirmatory ISH testing and selection bias could not be excluded, our data should be interpreted with caution. Moreover, the concordance rate of assays does not necessarily assure accuracy, because there is no gold-standard HER2 testing that can accurately identify all patients who may or may not benefit from anti-HER2 therapy. For patients with discordant HER2 results (IHC 3+/ISH-negative or IHC 2+/ISH-positive), some of them may nevertheless benefit from anti-HER2 therapies, although mature clinical outcome data are not yet available.

Available information so far does not clearly demonstrate the superiority of either IHC or ISH assays as predictors of benefit from anti-HER2 therapy. The original IHC interpretation criteria are now considered to have less-than-desired specificity. To decrease the incidence of false positives, experts have specified that a threshold of more than 30% of tumour cells (rather than 10% as originally specified) should show strong circumferential membrane staining for a positive (3+) result. Strong circumferential staining in 30% or less of cells would then be considered equivocal and be subjected to confirmatory ISH tests (not yet available in public hospitals). Since April 2007, our own pathology laboratory has also adopted this new criterion, although the HER2 results reported here were analysed according to original criteria, which are still being used in some local laboratories.

Considering the very high cost of anti-HER2 drug therapy (often up to HK $200 000), not to mention the requirement for 12 months of intravenous therapy in an adjuvant setting and the need for regular cardiac monitoring, measures to enhance the accuracy and reproducibility of HER2 tests could well reduce medical expenses substantially. Adoption of the latest guideline and steps to reduce assay variation (specimen handling, assay exclusion, and reporting criteria) could facilitate better selection of patients for anti-HER2 therapies.

References