Disclosure/conflict of interest

The authors declare no conflict of interest.

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References

1 Debelenko LV, Raimondi SC, Daw N, *et al.* Renal cell carcinoma with novel VCL-ALK fusion: new representative of ALK-associated tumor spectrum. Mod Pathol 2011;24:430–442.
2 Mariño-Enríquez A, Ou WB, Weldon CB, *et al.* ALK rearrangement in sickle cell trait-associated renal medullary carcinoma. Genes Chromosomes Cancer 2011;50:146–153.
3 Kwak EL, Bang YJ, Camidge DR, *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–1703.
4 Sugawara E, Togashi Y, Kuroda N, *et al.* Identification of anaplastic lymphoma kinase fusions in renal cancer: large-scale immunohistochemical screening by the intercalated antibody-enhanced polymer method. Cancer 2010;118:4427–4436.
5 Sukov WR, Hodge JC, Lohse CM, *et al.* ALK alterations in adult renal cell carcinoma: frequency, clinicopathologic features and outcome in a large series of consecutively treated patients. Mod Pathol 2012;25:1516–1525.
6 Schmidt L, Junker K, Nakaigawa N, *et al.* Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene 1999;18:2343–2350.
7 George RE, Sanda T, Hanna M, *et al.* Activating mutations in ALK provide a therapeutic target in neuroblastoma. Nature 2008;455:975–978.
8 Davis CJ Jr, Mostofi FK, Sesterhenn IA. Renal medullary carcinoma. The seventh sickle cell nephropathy. Am J Surg Pathol 1995;19:1–11.
9 Perlman EJ. Pediatric renal cell carcinoma. Surg Pathol Clin 2010;3:641–651.
10 Cheng JX, Tretiakova M, Gong C, *et al.* Renal medullary carcinoma: rhabdoid features and the absence of INI1 expression as markers of aggressive behavior. Mod Pathol 2008;21:647–652.
11 Howlader N, Noone AM, Krapcho M, *et al.* SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). National Cancer Institute: Bethesda, MD, http://seer.cancer.gov/csr/1975_2009_pops09/ based on November 2011 SEER data submission, posted to the SEER web site, 2012.

HER-2 intratumoral heterogeneity

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To the editor: We read with great interest the paper published by Seol H *et al*[1] concerning HER-2 intratumoral heterogeneity (IH) in breast cancer.

Nowadays most pathologists involved in HER-2 characterization in breast cancer more or less frequently have to deal with cases of IH in the everyday routine.

While reading this article, we asked ourselves the following question: 'which could be the best way to write the report for the clinicians?'.

At the present time, our Lab-FISH report shows synthetically if the tumour sample is found to be amplified or unamplified; however, if focal-amplified neoplastic clones are seen, in accordance with what has been recently established in an Italian consensus conference,[2] the percentage of cells with overexpression of the protein or gene amplification (Her-2/CEP-17 ratio >2 or number of HER-2 copies >6 or HER-2 clusters), even if less than 10%, should be added in the report.

So far we have noted however that one 'final' question most clinicians frequently ask to us is: 'how should I consider it amplified or non-amplified?'.

This is likely to be due to the legislation regulating the administration of the drug (http://www.agenziafarmaco.gov.it/it/content/trastuzumab) according to which 'Herceptin should be used only in patients whose tumours show HER-2 overexpression or HER-2 gene amplification as determined by an accurate and validated test'.

The same situation occurs for gastric and gastroesophageal junction adenocarcinomas for which the law provides the following: 'Trastuzumab should be administered only to patients with metastatic gastric cancer whose primary tumours exhibit HER-2 overexpression, defined as IHC 3+ or IHC 2+ together with a positive SISH or FISH result'.

So they both only refer to HER-2 protein overexpression and/or gene amplification and make no mention at all of a possible IH.

We believe that a reasonable way to document HER-2 IH could be to attach the FISH count results to the report and to close the report with a final interpretation of the results obtained. In agreement with Albarracin *et al*,[3] we think that an analytical report completed with a critical evaluation of the results about HER-2 genetic heterogeneity (GH) should be worldwide promoted.

In accordance with the recently published guidelines,[4,5] FISH report should describe: (a) the number of cells analysed; (b) the HER-2 gene copy number per nucleus; (c) the CEP17 copy number per nucleus; (d) the HER-2/CEP17 ratio for each nucleus; (e) the overall average ratio and the s.d.; (f) the number and the percentage of cells, if any, with ratio >2.2, and finally (g) the average ratio in this group of cells.

But which could be the simplest and most practical way to report such analytical results?

For this purpose the use of a spreadsheet form, eg an Excel form (Microsoft Corporation, Redmond, WA, USA), could be adopted.

FISH analysis is currently accepted as the gold standard for HER-2 assessment in cases resulted borderline at conventional immunohistochemical assay.

Hence, it represents the end point for determining HER-2 status.

Therefore, when clear-cut results are missing, eg in case of ratio close to the cutoff, clinicians could have difficulties in the therapeutic management of patients.

And you could run the risk that a focal amplification is deemed sufficient to consider the patient eligible for trastuzumab therapy.

Besides the presence in the same tumour of areas with HER-2 amplification next to non-amplified tumour areas, notwithstanding all the devices for counting FISH signals can become cause of irresolution in writing the final report.

The differences in HER-2 status found in core biopsy, mastectomy and metastasis specimens, and the heterogeneous HER-2 protein expression (HER-2 phenotypic heterogeneity, PH) found in a primary tumour from mastectomy specimen in the case described by Wu *et al*,[6] from this point of view, are paradigmatic.

Moreover, regarding HER-2 GH, we cannot underestimate the fact that *in situ* hybridization (ISH, FISH, CISH, as well as SISH, dual or single signal) is a method prone, to some extent, to register heterogeneous events, as it works on tissue sections that are, *de facto*, much thinner than tumour nuclei are.

Chromosome 17 can be entirely or poorly represented in nuclear sections and proportions of HER-2 and CEP17 may vary from nucleus to nucleus and, consequently, HER-2/CEP17 ratios may vary, even significantly.

So, HER-2 assessment can become a tough task, especially in those cases with average number of HER-2 spots over CEP17 dots around cutoff value. In the recent study by Yang *et al*,[7] HER-2 GH was found in a small proportion of tumours with high-grade HER-2 amplification. In our opinion, this represents an expected finding, as these cases often exhibit an amplification pattern as HER-2 signal clusters.

For this reason, we believe that even the HER-2 GH assessment has little value in cases such as these.

Cases whose results are at or near the cutoff point and that, therefore, 'should be interpreted with caution',[3,6] can become a real interpretative nightmare. Furtheremore, we believe that also HER-2 PH should be taken into due consideration when reporting a HER-2 characterization test.

For example, we think that in a case of PH with about 10% of cells with 3+ score and the rest of the cells with incomplete, faint membrane decoration, might raise the same questions as a case of HER-2 GH.

We suggest that in such situations, as proposed for the GH, the IHC report should specify all the scores observed and the percentages of cells presenting each score (according to the DAKO Hercep Test scoring system).

The biological relevance of HER-2 PH should be established comparing therapeutic responses among patients with different types of HER-2 PH.

In fact, it would be very interesting to evaluate whether patients with focal 3+ staining may benefit from trastuzumab therapy, as recently this has been found to have beneficial effects for patients with HER-2 GH[8].

Besides, we believe that as for HER-2 IH, two scenarios should be clearly distinguished:

(1) cases showing true clonal heterogeneity, ie a well-defined group of tumour cells showing clear gene amplification, which usually corresponds with strong 3+ staining of that group on immunohistochemistry, *vs*
(2) scattered single cells showing apparent gene amplification without immunohistochemistry correlation.

While the former is likely true heterogeneity with potential clinical significance, the latter may be an artefact.

As stated above, only a fraction of the tumour cell nuclei are present on the slides, so the individual cells showing apparent, usually low-level (3:1 or 5:2) amplification are likely due to part of the nucleus missing from the slide. This latter pattern has no phenotypic correlate, ie one practically never sees individual strongly staining cells in the background of a HER-2 negative tumour, and lacks known clinical significance.

Finally, we totally agree with Seol *et al*.[1] when they state that '… the variability of HER-2 protein expression within a tumour is not simply a technical problem attributable to poor fixation, antigen retrieval, inadequate or suboptimal immunohistochemical procedures, but represents real biological heterogeneity.'

In fact, there is no doubt that preanalytical technical problems may affect the immunohistochemical determination of HER-2 status;[9,10] however, although continuous monitoring is required to minimize these issues, this should not be a 'refuge' when dealing with difficult and/or confounding cases due to an intrinsic 'biological' intratumoral variability.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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References

1 Seol H, Lee HJ, Choi Y, *et al.* Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol 2012;25: 938–948.
2 Consensus workshop e Raccomandazioni sull'impiego delle diverse metodiche per la determinazione dello stato di HER2 nel carcinoma mammario e nel carcinoma gastrico. Catania 14/15 Aprile 2010.
3 Albarracin C, Edgerton ME, Gilcrease MZ, *et al.* Is it too soon to start reporting HER-2 genetic heterogeneity? Arch Pathol Lab Med 2010;134:162–163.
4 Vance GH, Barry TS, Bloom KJ, *et al.* College of American Pathologists. Genetic heterogeneity in HER-2 testing in breast cancer: panel summary and guidelines. Arch Pathol Lab Med 2009;133: 611–612.
5 Wolff AC, Hammond ME, Schwartz JN, *et al.* 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 2007;25:118–145.
6 Wu JM, Halushka MK, Argani P. Intratumoral heterogeneity of HER-2 gene amplification and protein overexpression in breast cancer. Hum Pathol 2010;41: 914–917.
7 Yang YL, Fan Y, Lang RG, *et al.* Genetic heterogeneity of HER2 in breast cancer: impact on HER2 testing and its clinicopathologic significance. Breast Cancer Res Treat 2012;134:1095–1102.
8 Sukov WR, Miller DV, Duek AC, *et al.* Benefit of adjuvant trastuzumab in breast cancer patients with focal HER-2 amplified colonies: data from N9831 Intergroup Adjuvant Trial [abstract]. J Clin Oncol 2009;15s(suppl):520.
9 Pekmezci M, Szpaderska A, Osipo C, *et al.* The effect of cold ischemia time and/or formalin fixation on estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 results in breast carcinoma. Patholog Res Int 2012;2012:947041.
10 Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. Mod Pathol 2012;25:1098–1105.

Letter to the editor regarding 'Seol H, Lee HJ, Choi Y, *et al*. Intratumoural heterogeneity of *HER2* gene amplification in breast cancer: its clinicopathological significance'

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To the Editor: In Seol *et al*,[1] the authors provide a clinicopathologic analysis showing that intratumoral heterogeneity of *HER2* gene amplification is associated with short disease-free survival. They conclude that it is likely that intratumoral heterogeneity is a surrogate for chromosomal instability, and thus a poor prognosis. This result would appear directly to conflict with the study of Bartlett *et al*,[2] showing that patients with tumors that are uniformly *HER2*-amplified do worse than those with heterogeneity (eg, 30–50% of cells with a ratio >2.2). Seol *et al*[1] attribute this difference to a variation in study design—that they have selected their heterogeneous cases from tumors that were already classified as *HER2*-amplified on whole-tissue sections. To this reader, an alternative interpretation presents itself, which takes into account patient treatment, as well as one study[3] not cited by Seol *et al* (See Table 1).

Table 1 Prognostic significance of '*HER2* heterogeneity' accounting for treatment

Study	*Prognostic significance of 'Heterogeneous' or 'Borderline' HER2-amplification*	*Non-amplified cases Included*	*Treated with neoadjuvant/ adjuvant chemotherapy*	*Treated with trastuzumab*
Seol *et al*[1]	Poor[a]	No	93%	26%
Bartlett *et al*[2]	Favorable[b]	Yes	0%	0%
Dowsett *et al*[3]	No Difference[c]	No	100%	100%[d]

[a]Compared to uniformly *HER2*-amplified.
[b]Compared to uniformly *HER2*-amplified; intermediate between amplified and non-amplified.
[c]Borderline/low-*HER2*-amplified compared to highly *HER2*-amplified.
[d]Comparison based on single arm of study.

From Table 1, it appears that intratumoral heterogeneity, in and of itself, is not a poor prognostic marker at all.[2] Rather, high/unequivocal *HER2* amplification is a favorable predictor of response to (antracycline-based) chemotherapy—a result that has been well documented.[3,4] Moreover, patients with low-*HER2*-amplification—and heterogeneity, perhaps—still benefit from trastuzumab in addition to chemotherapy.[1,3]

Seol *et al*[1] rightly highlight the importance of determining the *HER2* amplification status accurately, both overall and taking into account intratumoral heterogeneity. Based on our own work, a fully satisfactory definition of heterogeneity has not been forthcoming. A persistent problem is how to distinguish bonafide heterogeneity from statistical artifact.[5] Both Bartlett *et al*[2] and Seol *et al*[1] raise the possibility of examining 'regional heterogeneity'. The current guidelines address this by recommending that distinct (clustered)