5 Pauletti G, Godolphin W, Press MF, *et al.* Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence *in situ* hybridization. Oncogene 1996;13:63–72.
6 Robertson KW, Reeves JR, Smith G, *et al.* Quantitative estimation of epidermal growth factor receptor and c-erbB-2 in human breast cancer. Cancer Res 1996;56:3823–3830.
7 Nagy P, Jenei A, Kirsch AK, *et al.* Activation-dependent clustering of the erbB2 receptor tyrosine kinase detected by scanning near-field optical microscopy. J Cell Sci 1999;112:1733–1741.
8 Leong AS, Formby M, Haffajee Z, *et al.* Refinement of immunohistologic parameters for HER2/neu scoring validation by FISH and CISH. Appl Immunohistochem Mol Morphol 2006;14:384–389.
9 Roche PC, Suman VJ, Jenkins RB, *et al.* Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. J Natl Cancer Inst 2002;94:855–857.
10 Paik S, Bryant J, Tan-Chiu E, *et al.* Real-world performance of HER2 testing—National Surgical Adjuvant Breast and Bowel Project Experience. J Natl Cancer Inst 2002;94:852–854.
11 Perez EA, Suman VJ, Davidson NE, *et al.* HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 2006;24:3032–3038.
12 Yaziji H, Goldstein LC, Barry TS, *et al.* HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004;291:1972–1977.
13 Vincent-Salomon A, MacGrogan C, Couturier J, *et al.* Calibration of immunohistochemistry for assessment of HER2 in breast cancer: results of the French multicentre GEFPICS study. Histopathology 2003;42:337–347.

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In reply: The ASCO-CAP Guidelines were generated in an attempt to optimize the accuracy of HER2 testing in breast cancer and to address the documented high levels of discordance between HER2 testing reported in the literature. Indeed, the identification of methods that can be employed to ensure the accuracy of HER2 testing was also the motivation of the study of HER2 IHC and FISH concordance that we have reported in this issue of *Modern Pathology*. Furthermore, one of the authors of our study (AG) was present at the *ad hoc* committee meeting that preceded the generation of the published guidelines.[1]

Hanna and co-workers express several 'concerns' about our 'proposal.' Our paper is not, in fact, a 'proposal' of an alternative to, nor is it a rejection of the ASCO-CAP Guidelines. It is, rather, a study documenting a method to improve further upon these guidelines. Our study represents a test of an hypothesis, which is that high levels of concordance of HER2 IHC and FISH results can be obtained, along with the elimination of most false-positive IHC results, through the use of a simple normalization technique. Our data overwhelmingly support this hypothesis.

We take exception to several points raised by Hanna and co-workers:

(1) Hanna and co-workers state that the ASCO-CAP Guidelines 'categorically state that staining of the normal epithelium is one of the exclusion criteria to reject a test result.' In fact, the ASCO-CAP Guidelines state that *strong* staining of the normal epithelium is an exclusion criterion (Table 5 of reference Wolff *et al*[1]). None of our specimens showed strong staining of the normal epithelium; in fact, although not stated in the paper, all staining of the normal epithelium, when present, was at the 1+ level. Indeed, Hanna and co-workers themselves acknowledge in their letter that their 'general experience' is that positive signal on normal breast epithelium is 'absent or minimal,' which would translate to either 0 or 1+, and is precisely the finding in our 6604 cases. Thus, there is nothing unique about the tissues or the level of immunostaining of the normal epithelium we have examined in our study.

(2) Hanna and co-workers state that the ASCO-CAP Guidelines '… ensure consistent and accurate results in at least 95% of cases with positive or negative HER2 status.' In fact, although the guidelines represent a noble effort to improve the accuracy of HER2 testing, they do not represent the product of 'evidence-based' analysis, as there were no data presented to or at the ASCO-CAP Panel that documented this level of concordance. Also unknown by the ASCO-CAP Panel is the contribution of the various elements (preanalytical, analytical, postanalytical) to the significant error rate in HER2 IHC testing. There have been no publications since the promulgation of the guidelines, other than our present study, that have documented 95% concordance between HER2 testing by IHC and FISH. The single study cited in the guidelines[2] was misrepresented as documenting a 4% discordance rate between IHC and FISH, when in fact the paper documents a 14% discordance rate within the 'central laboratory,' ie, cases that were IHC 3+ but FISH negative (Table 3 of reference Reddy *et al*[2]).

(3) Hanna and co-workers state that 'strict adherence to scoring criteria and calibrations of the test, plus use of internal controls and participation in external QA programs can result in excellent IHC/FISH concordance results of >90%; many labs have achieved that level according to published QA programs.' Two publications are cited to justify this comment;

the first (Yaziji *et al*[3] their reference 11) is an earlier publication from our laboratory in which we employed the identical subtraction method we describe in more detail here, and the second (Vincent-Salomon *et al*[4] their reference 12) employs a method in which HER2 IHC is titered to the FISH results, an interesting modification but one that would also go well beyond, and hence 'violate' a strict construction of the ASCO-CAP Guidelines.

(4) Hanna and co-workers contend that the use of ASR products make validation 'difficult.' In fact, there is no difference in the process used by a clinical laboratory for validation of a diagnostic antibody, whether it is an ASR or an IVD reagent. Indeed, the ASCO-CAP Guidelines make no recommendation as to the choice of anti-HER2 antibodies, nor to the ASR or IVD labeling of the reagent. In our study, we employed the same antibody, the Dako A0485 rabbit polyclonal antibody, that is present in kit form in the HercepTest™, and that antibody is run under identical conditions on a Dako autostainer. Interestingly, the published data of Press and co-workers[5] suggest that the HercepTest™ kit is inferior to other non-ASR antibodies in their accuracy of assessing HER2 status in a series of 117 well-characterized breast cancers.

(5) The authors contend that our normalization scheme 'would introduce [a] huge variable that will be a step backward in the attempts of our discipline to enhance the quality and consistency of HER2/neu testing in breast cancer,' but offer no evidence to support this allegation. In fact, we have demonstrated, in a series of 6604 cases, that the introduction of what Hanna and co-workers refer to as a 'variable' actually increases the accuracy of HER2 IHC testing.

(6) Hanna and co-workers appear to assume that the principal reason for unacceptably high levels of inaccuracy in HER2 testing are preanalytical factors such as fixation time, and if these could be controlled, as recommended by the ASCO-CAP Guidelines, this inaccuracy would disappear. This represents an unproved assumption, without supporting evidence. Attempts at controlling preanalytical factors, however laudatory, are by their very nature imperfect and incomplete (eg, they do not address the time period between specimen acquisition and immersion into formalin, and they do not address the issue of ratio of tissue to fixative volume). The relative contribution of all of these components to variations in HER2 IHC signal is unknown. But even controlling preanalytical factors such as fixation time within a broad time period may be imperfect: tissues fixed for 6 *vs* 48 h (both 'acceptable' under the Guidelines) will manifest markedly different levels of formalin-induced crosslinking, and may well show significantly different levels of HER2 immunostaining.

(7) Hanna and co-workers suggest that it is improper to criticize the ASCO-CAP Guidelines. We strongly disagree; it is dangerously naïve and scientifically invalid to suggest that the ASCO-CAP Guidelines are not immutable and beyond criticism. Indeed, the ASCO-CAP Guidelines are a work in progress. As new data are accumulated, it is expected that some of the assumptions made by the panel, despite the best of intentions, might prove to be incorrect, and the Guidelines modified appropriately. As a case in point, one of the modifications in HER2 scoring recommended by ASCO-CAP Guidelines is the change of the threshold for 3+ positivity from 10 to 30%. Although this was designed to reduce the number of false-positive cases, a recent study from the laboratory of Dr Stuart Schnitt has demonstrated that this change, in fact, has no effect upon reducing the number of 3+ cases.[6]

In summary, we are all in agreement that the ASCO-CAP Guidelines represent an important step forward in attempting to improve HER2 testing. However, legitimate criticisms of our study should be based on critiques of the *science*, not based on *faith* in the infallibility of these Guidelines. *Res ipso loquitor:* let the data speak for themselves. Despite all the concerns of Hanna and co-workers they have not provided any scientific evidence that contradicts or challenges the findings we have presented.

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References

1 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.
2 Reddy JC, Reimann JD, Anderson SM, *et al.* Concordance between central and local laboratory HER2 testing from a community-based clinical study. Clin Breast Cancer 2006;7:153–157.
3 Yaziji H, Goldstein LC, Barry TS, *et al.* HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004;291:1972–1977.
4 Vincent-Salomon A, MacGrogan G, Couturier J, *et al.* Calibration of immunohistochemistry for assessment of HER2 in breast cancer: results of the French multicentre GEFPICS study. Histopathology 2003;42:337–347.
5 Press MF, Slamon DJ, Flom KJ, *et al.* 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 2002;20:3095–3105.
6 Gilmore HL, Collins LC, Connolly JL, *et al.* Impact of the new american society of clinical oncology (ASCO)/college of american pathologists (CAP) guidelines on the determination of HER2 status. Mod Pathol 2008;21:32A.