

CORRESPONDENCE

Re: HER2 Testing in the Real World

We read with interest the recent studies by Paik et al. (1) and Roche et al. (2) that dealt with some of the problems with immunohistochemical (IHC) testing for the HER2 protein. We fully support the view expressed by Dr. Zujewski (3) in the editorial that accompanied these two studies regarding the need to "build quality in" when performing such assays. Because trastuzumab targets the HER2 protein, evaluation of HER2 protein status by IHC should be the most clinically relevant assay. However, the major problem with IHC for HER2 is the lack of a standardized assay among laboratories. On the basis of our recently completed study, we propose that the best way to improve the accuracy of IHC for HER2 is to calibrate it against a gold standard such as fluorescence *in situ* hybridization (FISH).

We performed a multicenter study to assess the value of calibrating IHC for HER2 against FISH to improve the accuracy of the IHC method. Twelve French Cancer Center laboratories participated in this study, in which 119 invasive breast carcinomas were evaluated for HER2. Each of the 12 laboratories used its own in-house IHC techniques to assess HER2 protein expression on formalin-fixed or Bouins liquid-fixed, paraffin-embedded tissue sections. All laboratories used the CB11 (Novocastra, Newcastle-upon-Tyne, U.K.) or the A0485 (Dako, Glostrup, Denmark) anti-HER2 primary antibody. To define HER2-positive cancers, we used two cutoff values: 10% and 60% of immunostained cells with moderate or strong intensity of staining. The 10% value was chosen because it was the usual cut point used to select patients for anti-HER2 therapy in clinical trials (4,5), and the 60% value was chosen because previous published data using this value reported a strong correlation between HER2-IHC and HER2-FISH (6). To avoid variations in fixation protocols, FISH for HER2 gene status was performed in a single laboratory on frozen sections from 116 of 119 cases. Results of IHC were collected at a cen-

tral institution and compared with the FISH results. Discordances between IHC and FISH were observed in six of the 12 cancer centers. These six cancer centers then calibrated their IHC on the basis of the FISH results and performed a second IHC run. The calibration process consisted of the introduction of heat-induced epitope retrieval and an increase in the dilutions of the primary antibodies. Using the FISH results as the gold standard, the accuracy of IHC was calculated, before and after calibration, by dividing the number of true-positive and true-negative cases, which were defined by concordant results between IHC and FISH results, by the total number of samples. Forty-four of 116 cancers (37.9%) showed HER2 gene amplification. Before calibration, the accuracy of IHC was 89.6% (95% confidence interval [CI] = 84.1% to 95.1%) at the 10% cutoff value and 93.0% (95% CI = 88.3% to 97.7%) at the 60% cutoff value. After calibration, the accuracy of the IHC was 93.0% (95% CI = 88.3% to 97.7%) at the 10% cutoff value and 95.0% (95% CI = 91.1% to 98.9%) at the 60% cutoff value, which corresponded to HER2 overexpression in 47 (39.5%) and 45 (37.8%) of 119 cancers, respectively.

In conclusion, our study showed that a high accuracy rate for IHC testing of HER2 could be obtained in all 12 participating laboratories with their own in-house IHC techniques, provided that a calibration process was performed. Heat-induced epitope retrieval, high dilutions of anti-HER2 antibody, and the use of specific controls were crucial for IHC calibration. A 95% accuracy rate for IHC, using FISH as the gold standard, was obtained by considering IHC results for HER2 as a continuous variable, with a cutoff value set to 60% positively stained invasive cells. These results emphasize that, to achieve optimal accuracy, laboratories that perform an IHC assay to assess HER2 status should ideally be those able to validate the IHC assay against a standard such as FISH before IHC is introduced into routine clinical practice. As suggested by Roche et al. (2), centralized HER2 testing, in laboratories with sufficient IHC activity and access to IHC calibration procedures on FISH, should be favored.

ANNE VINCENT-SALOMON
 GAËTAN MACGROGAN

JÉRÔME COUTURIER
 LAURENT ARNOULD
 SIMONE MATHOULIN-PÉLISSIER
 GROUPE D'ETUDE DES FACTEURS
 PRONOSTIQUES PAR IMMUNOHISTOCHEMIE
 DANS LES CANCERS DU SEIN
 (GEFPICS)

REFERENCES

- (1) Paik S; Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, et al. Real-world performance of HER2 testing—National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 2002;94:852-4.
- (2) Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, et al. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 2002;94:855-7.
- (3) Zujewski JA. "Build quality in"—HER2 testing in the real world. *J Natl Cancer Inst* 2002;94:788-9.
- (4) Cobleigh A, Vogel C, Tripathy D, Robert N, Scholl S, Fehrenbacher L, et al. 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* 1999;17:2639-48.
- (5) Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that over-expresses HER2. *N Engl J Med* 2001;344:783-92.
- (6) Couturier J, Vincent-Salomon A, Nicolas A, Zafrani B, Sastre-Garau X. Strong correlation between results of fluorescent *in situ* hybridization and immunohistochemistry for the assessment of ERBB2 (HER-2/neu) gene status in breast carcinoma. *Mod Pathol* 2000;13:1238-43.

NOTES

Affiliations of authors: A. Vincent-Salomon, J. Couturier, Institut Curie, Paris, France; G. MacGrogan, S. Mathoulin-Pélessier, Institut Bergonié, Bordeaux, France; L. Arnould, Institut Georges-François Leclerc, Dijon, France.

Correspondence to: Anne Vincent-Salomon, M.D., Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France (anne.salomon@curie.net).

GEFPICS is a group affiliated with the FNCLCC (Fédération Nationale des Centres de Lutte Contre le Cancer).

RESPONSE

We appreciate receiving the correspondence from Vincent-Salomon et al. and applaud their efforts to corroborate protein and gene HER2 testing in a single laboratory. The dataset they de-

scribe is consistent with our experience. Their letter essentially describes their calibration of immunohistochemical testing (IHC) by using fluorescence *in situ* hybridization (FISH) as a gold standard. Although we did not calibrate external IHC with central FISH, we think that our data—especially the most recent updates—show that one can achieve good internal agreement between FISH and IHC if there is coordination between the laboratories. However, the point we made regarding unacceptable discordance rates referred to a comparison between external and central laboratories, using either IHC or FISH, not to using two methodologies in a central laboratory facility. We continue accumulating and analyzing data from our study and will report follow-up information. We expect that increased awareness regarding the potential for discordance will lead to quality-control efforts (for example, those begun by the College of American Pathologists), which should improve concordance rates and optimize patient selection for anti-HER2 treatments.

EDITH A. PEREZ
ROBERT B. JENKINS

NOTES

Editor's note: Paik et al. declined to respond.

Affiliations of authors: E. A. Perez, Mayo Clinic, Jacksonville, FL; R. B. Jenkins, Mayo Clinic, Rochester, MN.

Correspondence to: Edith A. Perez, M.D., Mayo Clinic, Division of Hematology/Oncology, 4500 San Pablo Rd., Jacksonville, FL 32224 (e-mail: perez.edith@mayo.edu).

scribe is consistent with our experience. Their letter essentially describes their **CORRESPONDENCE** testing (IHC) by using fluorescence *in situ* hybridization (FISH) as a gold standard.

Although we did not calibrate external **Re: HER2 Testing in the Real World** think that these two studies regarding the need to

We read with interest the recent studies by Paik et al. (1) and Roche et al. (2) that dealt with some of the problems made regarding unacceptable discordance rates referred to a comparison being for the HER2 protein a fully supported the view expressed by Dr. Zujewski using either IHC or FISH, not to using (3) in the editorial that accompanied these two studies regarding the need to build quality in when performing such and analyzing data from our study and assays. Because trastuzumab targets the HER2 protein, evaluation of HER2 protein status by IHC should be the most clinically relevant assay. However, the lead to quality control efforts for HER2 assay, those begun by the College of American Pathologists, which should improve concordance rates and optimize patient selection for anti-HER2 treatment.

IHC for HER2 is to calibrate it against a gold standard such as FISH.

ROBERT B. JENKINS

We performed a multicenter study to determine the value of calibrating IHC for HER2 against FISH to improve the accuracy of the IHC method. Twelve French Cancer Center laboratories participated in this study, in which 119 invasive breast

Clinic, Rochester, MN. We evaluated for HER2. Each of the 12 laboratories used its own methods to assess HER2 protein expression on formalin-fixed or Bouins liquid-fixed, paraffin-embedded tissue sections. All laboratories used the CB11 (Novocastra, Newcastle-upon-Tyne, U.K.) or the A0485 (Dako, Glostrup, Denmark) anti-HER2 primary antibody. To define HER2-positive cancers, we used two cutoff values: 10% and 60% of immunostained cells with moderate or strong intensity of staining. The 10% value was chosen because it was the usual cut point used to select patients for anti-HER2 therapy in clinical trials (4,5), and the 60% value was chosen because previous published data using this value reported a strong correlation between HER2-IHC and HER2-FISH (6). To avoid variations in fixation protocols, FISH for HER2 gene status was performed in a single laboratory on frozen sections from 116 of 119 cases. Results of IHC were collected at a cen-

tral institution and compared with the FISH results. Discordances between IHC and FISH were observed in six of the 12 cancer centers. These six cancer centers then calibrated their IHC on the basis of the FISH results and performed a second IHC run. The calibration process consisted of the introduction of heat-induced epitope retrieval and an increase in the dilutions of the primary antibodies. Using the FISH results as the gold standard, the accuracy of IHC was calculated, before and after calibration, by dividing the number of true-positive and true-negative cases, which were defined by concordant results between IHC and FISH results, by the total number of samples. Forty-four of 116 cancers (37.9%) showed HER2 gene amplification. Before calibration, the accuracy of IHC was 89.6% (95% confidence interval [CI] = 84.1% to 95.1%) at the 10% cutoff value and 93.0% (95% CI = 88.3% to 97.7%) at the 60% cutoff value. After calibration, the accuracy of the IHC was 93.0% (95% CI = 88.3% to 97.7%) at the 10% cutoff value and 95.0% (95% CI = 91.1% to 98.9%) at the 60% cutoff value, which corresponded to HER2 overexpression in 47 (39.5%) and 45 (37.8%) of 119 cancers, respectively.

In conclusion, our study showed that a high accuracy rate for IHC testing of HER2 could be obtained in all 12 participating laboratories with their own in-house IHC techniques, provided that a calibration process was performed. Heat-induced epitope retrieval, high dilutions of anti-HER2 antibody, and the use of specific controls were crucial for IHC calibration. A 95% accuracy rate for IHC, using FISH as the gold standard, was obtained by considering IHC results for HER2 as a continuous variable, with a cutoff value set to 60% positively stained invasive cells. These results emphasize that, to achieve optimal accuracy, laboratories that perform an IHC assay to assess HER2 status should ideally be those able to validate the IHC assay against a standard such as FISH before IHC is introduced into routine clinical practice. As suggested by Roche et al. (2), centralized HER2 testing, in laboratories with sufficient IHC activity and access to IHC calibration procedures on FISH, should be favored.

ANNE VINCENT-SALOMON
 GAËTAN MACGROGAN

JÉRÔME COUTURIER
 LAURENT ARNOULD
 SIMONE MATHOULIN-PÉLISSIER
 GROUPE D'ETUDE DES FACTEURS
 PRONOSTIQUES PAR IMMUNOHISTOCHEMIE
 DANS LES CANCERS DU SEIN
 (GEFPICS)

REFERENCES

- (1) Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, et al. Real-world performance of HER2 testing—National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst* 2002;94:852–4.
- (2) Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, et al. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst* 2002;94:855–7.
- (3) Zujewski JA. "Build quality in"—HER2 testing in the real world. *J Natl Cancer Inst* 2002;94:788–9.
- (4) Cobleigh A, Vogel C, Tripathy D, Robert N, Scholl S, Fehrenbacher L, et al. 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* 1999;17:2639–48.
- (5) Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783–92.
- (6) Couturier J, Vincent-Salomon A, Nicolas A, Zafrani B, Sastre-Garau X. Strong correlation between results of fluorescent *in situ* hybridization and immunohistochemistry for the assessment of ERBB2 (HER-2/neu) gene status in breast carcinoma. *Mod Pathol* 2000;13:1238–43.

NOTES

Affiliations of authors: A. Vincent-Salomon, J. Couturier, Institut Curie, Paris, France; G. MacGrogan, S. Mathoulin-Pélessier, Institut Bergonié, Bordeaux, France; L. Arnould, Institut Georges-François Leclerc, Dijon, France.

Correspondence to: Anne Vincent-Salomon, M.D., Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France (anne.salomon@curie.net).

GEFPICS is a group affiliated with the FNCLCC (Fédération Nationale des Centres de Lutte Contre le Cancer).

RESPONSE

We appreciate receiving the correspondence from Vincent-Salomon et al. and applaud their efforts to corroborate protein and gene HER2 testing in a single laboratory. The dataset they de-