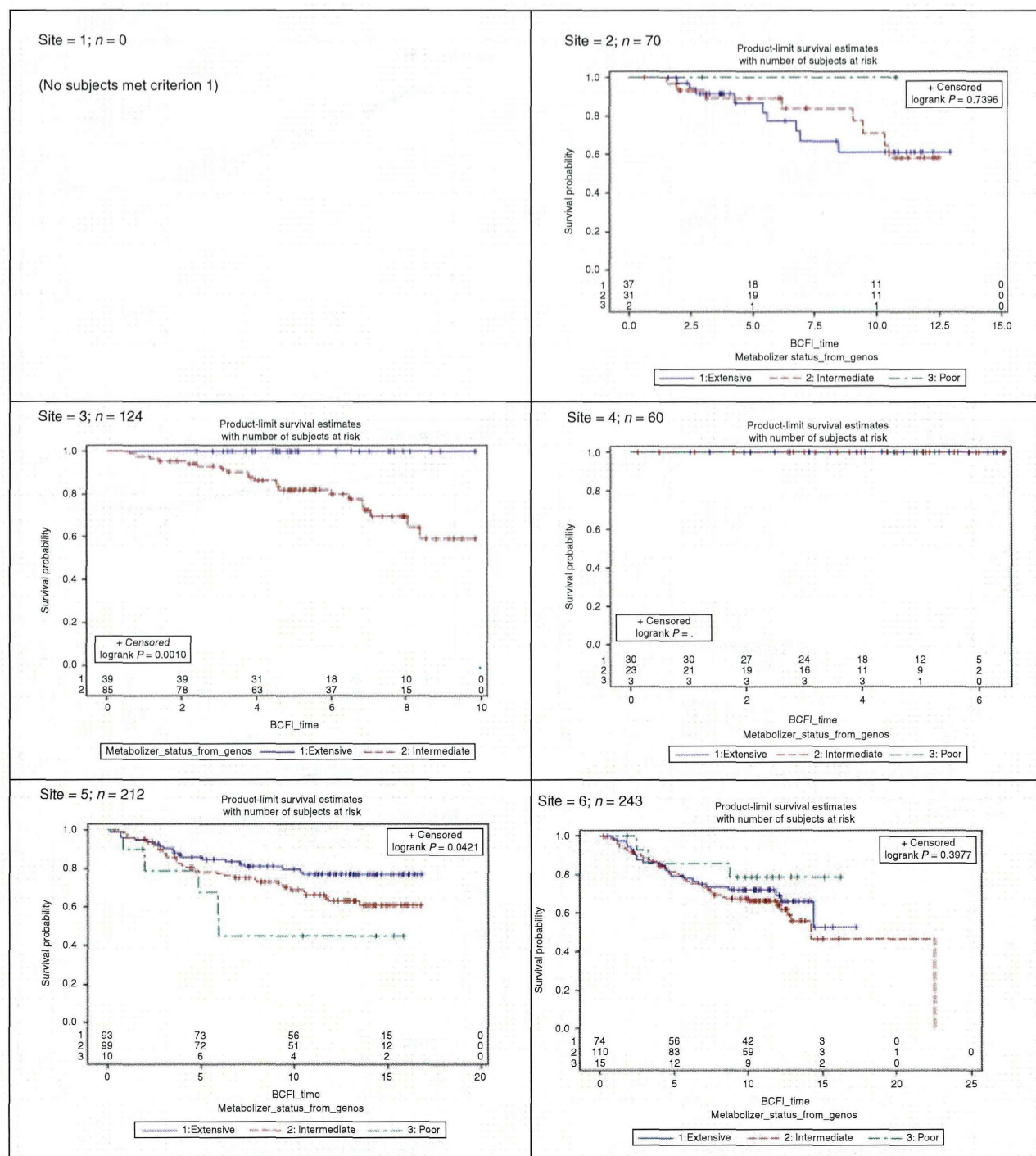


International Group 1-98 study, in which strong departures from HWE (to a magnitude of  $10^{-92}$ ) were observed, leading to a call for retraction of this article.<sup>3,12,16</sup>

For criterion 1, 49.9% of our patient DNA samples originated from blood, 21.6% from fresh-frozen tissues, and 28.5% from FFPE tissues. For criterion 2, 55.0% samples originated from blood,

20.5% were fresh-frozen tissues, and 24.5% from FFPE tissues. For criterion 3, 50.9% of DNA samples originated from blood, 31.9% from fresh-frozen tumor, 13.4% from FFPE tumor tissues, and 3.5% from FFPE normal tissue. Although we cannot exclude the presence of somatic events leading to misclassification of *CYP2D6* genotype, as evident from HWE deviation identified in data from



**Figure 3** Site-specific effects of CYP2D6 metabolizer status on clinical outcomes for subjects meeting inclusion criterion 1 (outcome = breast cancer-free interval (BCFI)).

some sites, comprehensive testing for HWE did not reveal significant violations across most sites. Moreover, the extent of deviation from HWE in the \*4 allele was not associated with sites that evinced less clinical benefit from tamoxifen in patients who were assessed to be PMs in terms of their *CYP2D6* status. This suggests that genotyping errors are unlikely to be a major issue in our analyses.

Our findings are subject to the shortcomings commonly encountered when performing retrospective “biomarker”

studies. In our study, most sites were unable to collect or control for the factors known to alter endoxifen exposure, including dose and duration of tamoxifen administration and patients’ adherence to the regimen. Although tamoxifen adherence is increasingly recognized as a critical factor for drug efficacy,<sup>23</sup> most studies evaluating tamoxifen biomarkers have not controlled for adherence. Other confounders include limited *CYP2D6* allele coverage and lack of information

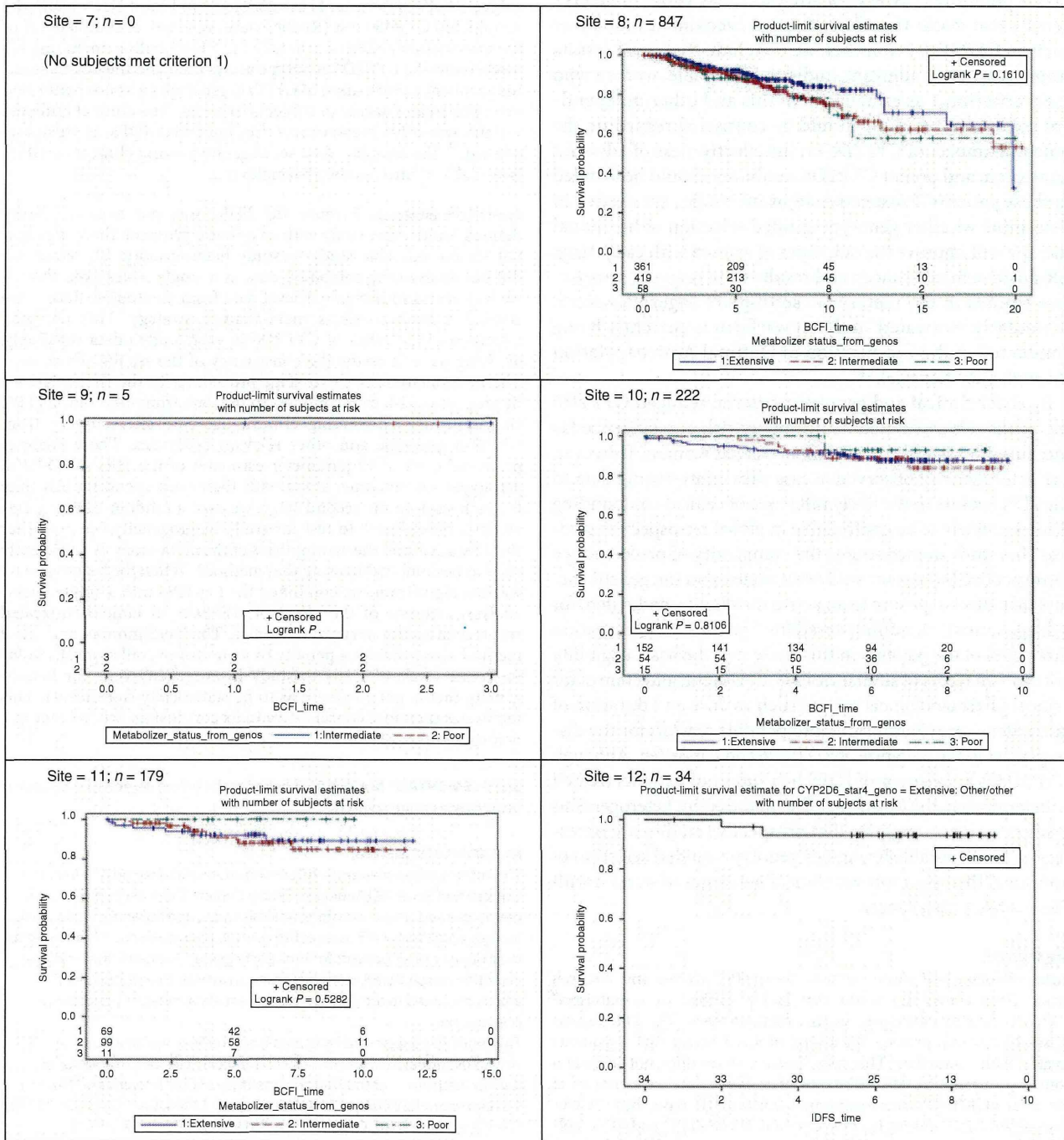


Figure 3 Continued



regarding the coadministration of CYP2D6 inhibitors, leading to potential misclassification of the CYP2D6 drug metabolism phenotype. Therefore, our meta-analysis results depend heavily on which subgroup of patients we include. If we accept that utmost precautions must be applied to avoid the distortion of results from influences derived from the aforementioned shortcomings, it follows that merely increasing the numbers of subjects without controlling the quality of input data, as done in our preliminary overview analysis,<sup>5</sup> may result in heterogeneity that masks the effect of a pharmacokinetic biomarker such as CYP2D6. From this, we conclude that until results from prospective adjuvant studies are available, women who meet criterion 1 as established in this and other independent cohorts (ABCSG 8) should be counseled regarding the potential impact of CYP2D6 on the effectiveness of adjuvant tamoxifen, and potent CYP2D6 inhibitors should be avoided in these patients. Prospective adjuvant studies are needed to determine whether genotype-guided selection of hormonal therapy will improve the outcomes of women with early-stage ER-positive breast cancer, and results from ongoing prospective studies in the metastatic setting are eagerly awaited. A similarly motivated study on warfarin is currently being conducted in the Clarification of Optimal Anticoagulation through Genetics trial.<sup>24</sup>

By strict clinical and genotype criteria, reduced CYP2D6 metabolism is associated with a higher risk of recurrence (as measured by IDFS) in tamoxifen-treated women. However, the heterogeneity observed across sites contributing data to the ITPC points to the likely influence of critical confounding factors unlikely to be controllable in global retrospective studies. This study demonstrates the complexity of performing a retrospective biomarker study that focuses on the genetic factors that affect exposure to an active metabolite, endoxifen, for a drug, tamoxifen, administered for 5 years. Our observation that <50% of the patients in this study met the basic eligibility criteria—in terms of similar disease, treatment, and control for critical pharmacological factors such as dose and duration of tamoxifen—provides insight into possible reasons for the discrepancies in the literature on CYP2D6 and tamoxifen. Although CYP2D6 is a predictor of IDFS in a subset of patients treated with tamoxifen, the lack of an effect in the entire heterogeneous study population suggests that prospective studies are necessary to finally establish whether genotype-guided selection of hormonal therapy improves clinical outcomes of women with ER-positive breast cancer.

## METHODS

**Data collection and study cohorts.** The ITPC invited any research group from across the world that had published or unpublished CYP2D6 data to participate in this meta-analysis. The ITPC comprises 12 research projects for a total of 4,973 breast cancer patients treated with tamoxifen. This retrospective study does not include a control group not treated with tamoxifen. These data were curated at the PharmGKB (Pharmacogenomics Knowledge Base, <http://www.pharmgkb.org>). Consent for participation in the ITPC and DNA collection, CYP2D6 genetic testing, and submission of data was obtained under local ethical review board permissions.

We collected information on clinical factors previously shown to be associated with breast cancer therapy and prognosis that were available from the information received from the sites. These data included demographic characteristics, cancer history, cancer recurrence, use of other therapies, use of concomitant medications known to affect CYP2D6 phenotype, ER status, and classic prognostic factors such as tumor size and number of affected lymph nodes. Information was also collected regarding the presence of CYP2D6 genetic variants (\*2, \*3, \*4, \*5, \*6, \*10, \*17, and \*41, categorized by their DNA sources), for which coverage of these alleles varied by site. For 1,635 subjects, CYP2D6 variants assessable from blood DNA using the AmpliChip CYP450 test (Roche) were collected. A complete list of the information collected is detailed in S1–S3 online, including the project-specific CYP2D6 genotype assays used and the DNA source. Independent confirmation of CYP2D6 genotypes was not performed owing to lack of access to subjects' samples. The clinical outcome variable was either breast cancer-free interval or IDFS, as previously defined.<sup>25</sup> The complete data set of genotypes and clinical variables is available at <http://www.pharmgkb.org>.

**Statistical analysis.** Because the ITPC was not a prospectively defined multicenter study with a common protocol, there is potential for considerable study-to-study heterogeneity. Therefore, we did not analyze the combined data as a single series even though we had access to individual-level data from all studies. Rather, we applied a random-effects meta-analysis strategy. This provided estimates of the effect of CYP2D6 in each study's data separately, allowing us to examine the consistency of the results across sites. The meta-analysis is a two-stage procedure. In the first stage, we fit proportional-hazards models to the data from each of the ITPC sites separately, predicting clinical outcome after surgery from CYP2D6 genotype and other relevant covariates. These analyses produced a set of 12 parameter estimates of the HRs of CYP2D6 genotypes on outcome, along with their corresponding SEs (one for each site). In the second stage, we used a random-effects meta-analysis procedure<sup>26</sup> to test for study heterogeneity (i.e., whether the 12 studies met the assumptions of the meta-analysis sufficiently so as to be combinable using that method). When the heterogeneity was not significant, we combined the log-HRs into a single, meta-analysis estimate of the effect of CYP2D6 on tamoxifen-treated recurrence and/or survival outcomes. The DerSimonian and Laird method also provides a penalty in its test of overall association for moderate levels of study-to-study heterogeneity (i.e., for heterogeneity that is not so severe as to be statistically significant). This method is therefore conservative in its conclusions when heterogeneity is a potential issue.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at <http://www.nature.com/cpt>

## ACKNOWLEDGMENTS

The authors acknowledge useful conversations with Donald A. Berry (The University of Texas MD Anderson Cancer Center). The complete data set of genotypes and clinical variables, analysis codes, and full analyses is available to registered PharmGKB users at <http://www.pharmgkb.org>. We are grateful to all breast cancer patients for their participation. We thank the physicians and other hospital staff, scientists, research assistants, and study staff who contributed to the patient recruitment, data collection, and sample preparation.

This work was supported by grants from the National Institutes of Health (National Institute of General Medical Sciences, the National Cancer Institute, and the National Institute of Child Health and Human Development R24 GM61374, CA-25224, CA-37404, CA-15083, CA-35113, CA-35269, CA-63849, CA35103, CA-35195, CA-35272, CA-35101, CA-37417, CA-35415, CA-52352, CA-35448, CA-60274, 1R01CA133049-01, U19 GM61388, CA-116201, CA-58207, CA-51008, T32 GM007546,



and CA051008; Breast Cancer Research (Scotland); Tayside Tissue Bank; California Breast Cancer Research Program grant 14OB-0166; Cancer Research UK; Deutsches Krebsforschungszentrum, Heidelberg, Germany; Robert Bosch Foundation, Stuttgart, Germany; 7FP EU Marie Curie Initial Training Network "Fighting Drug Failure" (GA 238132); Grant Stichting Emmanuel van der Schueren (scientific partner of the Vlaamse Liga tegen Kanker); and the National Project for Personalized Genomic Medicine, Ministry for Health & Welfare, Republic of Korea (A111218-PG02). W.S. is supported by grant Deutsche Forschungsgemeinschaft SCHR 1323/2-1, Germany.

The institutions in the International Tamoxifen Pharmacogenomics Consortium are listed below:

#### Writing committee:

Michael A. Province, Matthew P. Goetz, David A. Flockhart, Hiltrud Brauch, and Teri E. Klein

#### Data contributors:

*Deutsches Krebsforschungszentrum, Heidelberg, Germany:* Ute Hamann, Julia Boländer, and Hans-Ulrich Ulmer, Frauenklinik Städtisches Klinikum Karlsruhe, Germany

*Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology Stuttgart and University of Tuebingen, Tuebingen, Germany:* Hiltrud Brauch, Werner Schroth, Matthias Schwab, Stefan Winter, Michel Eichelbaum, Peter Fritz, and Wolfgang Simon

*Division of Research, Federal Institute for Drugs and Medical Devices, and University of Bonn Medical Faculty, Bonn, Germany:* Julia C. Stingl  
*Indiana University and University of Michigan, USA:* David A. Flockhart and Anne T. Nguyen

*Inje University College of Medicine and Department of Clinical Pharmacology, Busan Paik Hospital, Busan, Korea:* Jae-Gook Shin and Ji-Yeob Choi  
*Mayo Clinic in Rochester, Minnesota, USA:* Matthew P. Goetz, James N. Ingle, Vera J. Suman, and Richard M. Weinshilboum  
*Ninewells Hospital and Medical School, Dundee, UK:* Colin A. Purdie and Lee B. Jordan

*Örebro University, Sweden:* Pia Wegman

*RIKEN Center for Genomic Medicine and University of Tokyo, Japan:* Hitoshi Zembutsu, Taisei Mushirola, and Kazuma Kiyotani

*Roswell Park Cancer Institute, USA:* Christine B. Ambrosone  
*University Breast Center Franconia, University Hospital Erlangen, Erlangen, Germany:* Peter A. Fasching, Reiner Strick, and Matthias W. Beckmann  
*University Hospitals Leuven, Belgium:* Patrick Neven and Anne-Sophie Dieudonné

*University Institute of Medical and Chemical Laboratory Diagnostics, Paracelsus Private Medical University, Salzburg, Austria:* Elisabeth Haschke-Becher  
*University of California, San Francisco, San Francisco, USA:* Alan H.B. Wu, Wendy Lorizio, and Elad Ziv

*University of Dundee, UK:* Philip Quinlan, Lee B. Jordan, and Colin A. Purdie  
*Department of Gynecology and Obstetrics, University of Mainz, Mainz, Germany:* Marcus Schmidt and Heinz Koelbl

*University of Manchester, Manchester, UK:* William G. Newman, Roberta Ferraldeschi, Anthony Howell, and Ayse Latif

*Vesalius Research Center, Catholic University of Leuven, Leuven, Belgium:* Diether Lambrechts

*Yonsei University Health System, Korea:* Byeong-Woo Park

#### Statistical Analysis:

*Mayo Clinic in Rochester, MN, USA:* Vera J. Suman

*Stanford University, Stanford, CA, USA:* Teri E. Klein and Ryan Whaley

*Washington University in St. Louis, MO, USA:* Michael A. Province

#### Data Curation:

*Stanford University, Stanford, CA, USA:* Teri E. Klein, Ryan Whaley, Joan M. Hebert, and Li Gong

#### Site Principal Investigators:

Russ B. Altman and Teri E. Klein (Stanford University), Christine A. Ambrosone (Roswell Park Cancer Institute), Hiltrud Brauch (IKP Stuttgart),

Jae-Gook Shin (Inje University), Matthew P. Goetz (Mayo Clinic), William G. Newman (University of Manchester), Patrick Neven (Belgium), Alastair M. Thompson (University of Dundee), Sten Wingren (Örebro University), Hitoshi Zembutsu (University of Tokyo), and Elad Ziv (University of California, San Francisco)

#### Steering Committee:

Russ B. Altman, Hiltrud Brauch, Michel Eichelbaum, David A. Flockhart, Matthew P. Goetz, James N. Ingle, V. Craig Jordan, Teri E. Klein, Kent Osborne, Yusuke Nakamura, and Richard M. Weinshilboum

#### Participating sites:

The Christie NHS Foundation Trust, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK  
Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY, USA

Department of Clinical Medicine, Örebro University, Örebro, Sweden

Department of Genetics, Stanford University, Stanford, CA, USA

Department of Gynecology and Obstetrics, University Hospital

Erlangen, Friedrich-Alexander University Erlangen-Nuremberg,

Erlangen, Germany

Department of Gynecology and Obstetrics, University Hospitals Leuven, Leuven, Belgium

Department of Oncology, Catholic University Leuven, Leuven, Belgium

Department of Medicine/GIM and Clinical Pharmacology and

Experimental Therapeutics, and Helen Diller Family Comprehensive

Cancer Center, University of California, San Francisco, San Francisco,

CA, USA

Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA

Department of Oncology, Georgetown University, Washington, DC, USA

Department of Oncology, Mayo Clinic, Rochester, MN, USA

Department of Oncology and Pharmacology, Mayo Clinic, Rochester, MN,

USA

Department of Pharmacology; Mayo Clinic, Rochester, MN, USA

Department of Pharmacology and Pharmacogenomics Research Center, Inje

University College of Medicine and Department of Clinical Pharmacology,

Inje University, Busan Paik Hospital, Busan, Korea

Department of Surgery, Yonsei University Health System, Seoul, Korea

Deutsches Krebsforschungszentrum, Heidelberg, Germany

Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA

Division of Clinical Pharmacology, School of Medicine, Indiana University, IN, USA

Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology Stuttgart,

and University of Tuebingen, Germany

Dundee Cancer Centre, Dundee, UK

Genetic Medicine, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

Laboratory for Pharmacogenetics, RIKEN Center for Genomic Medicine, Yokohama, Japan

Laboratory of Molecular Medicine, Human Genome Center, Institute of

Medical Science, The University of Tokyo, Tokyo, Japan

Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Ninewells Hospital and Medical School, Dundee, UK

#### CONFLICT OF INTEREST

A.M.T. and W.G.N. report Roche funding for genotyping H.B. and M. Schwab report that they have initiated scientific collaborations in 2009 with Roche Molecular Diagnostics and Siemens Healthcare Diagnostics Products, respectively. P.A.F. and M.W.B. report Novartis research funding. M.-T.M.L. is a paid consultant of YongLin Health Foundation. R.B.A. is a founder, equity holder, and consultant for Personalis. The other authors declared no conflict of interest.



## Study Highlights

## WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

- ✓ There has been extensive controversy with regard to the association between *CYP2D6* genetic variants and the clinical outcomes of tamoxifen use.

## WHAT QUESTION DID THIS STUDY ADDRESS?

- ✓ The ITPC was established to address this controversy and to determine the association of *CYP2D6* status with IDFS in tamoxifen-treated early-stage, ER-positive breast cancer.

## WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

- ✓ We found that *CYP2D6* genotype was associated with a higher risk of recurrence in patients meeting the strict criterion. However, the observation of substantial heterogeneity in cohorts 2 and 3 suggests that study design factors that cannot be controlled retrospectively may obscure the predictive utility of *CYP2D6* genotype. This study demonstrates the complexity of performing a retrospective biomarker study.

## HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

- ✓ Although *CYP2D6* is a predictor of IDFS in a subset of patients treated with tamoxifen monotherapy, the lack of an effect in the entire heterogeneous study population suggests that prospective studies are necessary to fully establish the value of *CYP2D6* genotyping in tamoxifen therapy.

© 2014 American Society for Clinical Pharmacology and Therapeutics

- Jordan, V.C. Tamoxifen: a most unlikely pioneering medicine. *Nat. Rev. Drug Discov.* **2**, 205–213 (2003).
- Davies, C. *et al.* Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* **378**, 771–784 (2011).
- Johnson, M.D. *et al.* Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res. Treat.* **85**, 151–159 (2004).
- Stearns, V. *et al.* Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J. Natl. Cancer Inst.* **95**, 1758–1764 (2003).
- Goetz, M.P., Berry, D.A. & Klein, T.E. 31136 Findings from the International Tamoxifen Pharmacogenomics Consortium. Proceedings of the San Antonio Breast Cancer Symposium, San Antonio, Texas, 9–13 December 2009. Abstract 33.
- Mürdter, T.E. *et al.*; German Tamoxifen and AI Clinicians Group. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. *Clin. Pharmacol. Ther.* **89**, 708–717 (2011).
- Irvin, W.J. Jr *et al.* Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced *CYP2D6* metabolism: a multicenter study. *J. Clin. Oncol.* **29**, 3232–3239 (2011).
- Rae, J.M. *et al.*; COBRA Investigators. Cytochrome P450 2D6 activity predicts discontinuation of tamoxifen therapy in breast cancer patients. *Pharmacogenomics J.* **9**, 258–264 (2009).
- Schroth, W. *et al.* Association between *CYP2D6* polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA* **302**, 1429–1436 (2009).
- Goetz, M.P. *et al.* Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J. Clin. Oncol.* **23**, 9312–9318 (2005).
- Rae, J.M. *et al.*; ATAC trialists. *CYP2D6* and *UGT2B7* genotype and risk of recurrence in tamoxifen-treated breast cancer patients. *J. Natl. Cancer Inst.* **104**, 452–460 (2012).
- Regan, M.M. *et al.*; Breast International Group (BIG) 1-98 Collaborative Group. *CYP2D6* genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. *J. Natl. Cancer Inst.* **104**, 441–451 (2012).
- Brauch, H. *et al.* Tamoxifen use in postmenopausal breast cancer: *CYP2D6* matters. *J. Clin. Oncol.* **31**, 176–180 (2013).
- Nakamura, Y., Ratain, M.J., Cox, N.J., McLeod, H.L., Kroetz, D.L. & Flockhart, D.A. Re: *CYP2D6* genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. *J. Natl. Cancer Inst.* **104**, 1264; author reply 1266–1268 (2012).
- Stanton, V. Jr. Re: *CYP2D6* genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the Breast International Group 1-98 trial. *J. Natl. Cancer Inst.* **104**, 1265–6; author reply 1266–8 (2012).
- Goldberg, P. Experts claim errors in breast cancer study, demand retraction of practice-changing paper. *The Cancer Letter* **38**, 1–11 (2012).
- Goetz, M.P. *et al.* *CYP2D6* metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSCG) 8. *Clin. Cancer Res.* **19**, 500–507 (2013).
- Schroth, W. *et al.* *CYP2D6* polymorphisms as predictors of outcome in breast cancer patients treated with tamoxifen: expanded polymorphism coverage improves risk stratification. *Clin. Cancer Res.* **16**, 4468–4477 (2010).
- Zanger, U.M., Turpeinen, M., Klein, K. & Schwab, M. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. *Anal. Bioanal. Chem.* **392**, 1093–1108 (2008).
- Castells, A., Gusella, J.F., Ramesh, V. & Rustgi, A.K. A region of deletion on chromosome 22q13 is common to human breast and colorectal cancers. *Cancer Res.* **60**, 2836–2839 (2000).
- Hirano, A. *et al.* Allelic losses of loci at 3p25.1, 8p22, 13q12, 17p13.3, and 22q13 correlate with postoperative recurrence in breast cancer. *Clin. Cancer Res.* **7**, 876–882 (2001).
- Loo, L.W. *et al.* Differential patterns of allelic loss in estrogen receptor-positive infiltrating lobular and ductal breast cancer. *Genes. Chromosomes Cancer* **47**, 1049–1066 (2008).
- Thompson, A.M. *et al.* Comprehensive *CYP2D6* genotype and adherence affect outcome in breast cancer patients treated with tamoxifen monotherapy. *Breast Cancer Res. Treat.* **125**, 279–287 (2011).
- French, B. *et al.*; COAG (Clarification of Optimal Anticoagulation through Genetics) Investigators. Statistical design of personalized medicine interventions: the Clarification of Optimal Anticoagulation through Genetics (COAG) trial. *Trials* **11**, 108 (2010).
- Hudis, C.A. *et al.* Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. *J. Clin. Oncol.* **25**, 2127–2132 (2007).
- DerSimonian, R. & Laird, N. Meta-analysis in clinical trials. *Control. Clin. Trials* **7**, 177–188 (1986).



This work is licensed under a Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>



Open

# CYP2D6 Genotype and Adjuvant Tamoxifen: Meta-Analysis of Heterogeneous Study Populations

MA Province<sup>1</sup>, MP Goetz<sup>2</sup>, H Brauch<sup>3</sup>, DA Flockhart<sup>4</sup>, JM Hebert<sup>5</sup>, R Whaley<sup>5</sup>, VJ Suman<sup>6</sup>, W Schroth<sup>3</sup>, S Winter<sup>3</sup>, H Zembutsu<sup>7</sup>, T Mushiroda<sup>8</sup>, WG Newman<sup>9</sup>, M-TM Lee<sup>10</sup>, CB Ambrosone<sup>11</sup>, MW Beckmann<sup>12</sup>, J-Y Choi<sup>13</sup>, A-S Dieudonné<sup>14</sup>, PA Fasching<sup>12,15</sup>, R Ferraldeschi<sup>9</sup>, L Gong<sup>5</sup>, E Haschke-Becher<sup>16</sup>, A Howell<sup>17</sup>, LB Jordan<sup>18</sup>, U Hamann<sup>19</sup>, K Kiyotani<sup>8</sup>, P Krippel<sup>20</sup>, D Lambrechts<sup>21</sup>, A Latif<sup>9</sup>, U Langsenlehner<sup>20</sup>, W Lorizio<sup>22</sup>, P Neven<sup>23</sup>, AT Nguyen<sup>4</sup>, B-W Park<sup>24</sup>, CA Purdie<sup>18</sup>, P Quinlan<sup>25</sup>, W Renner<sup>20</sup>, M Schmidt<sup>3,26</sup>, M Schwab<sup>27</sup>, J-G Shin<sup>28,29</sup>, JC Stingl<sup>30</sup>, P Wegman<sup>31</sup>, S Wingren<sup>31</sup>, AHB Wu<sup>32</sup>, E Ziv<sup>22</sup>, G Zirpoli<sup>11</sup>, AM Thompson<sup>25</sup>, VC Jordan<sup>33</sup>, Y Nakamura<sup>7</sup>, RB Altman<sup>5,34</sup>, MM Ames<sup>35</sup>, RM Weinshilboum<sup>35</sup>, M Eichelbaum<sup>3</sup>, JN Ingle<sup>36</sup> and TE Klein<sup>5</sup>; on behalf of the International Tamoxifen Pharmacogenomics Consortium

The International Tamoxifen Pharmacogenomics Consortium was established to address the controversy regarding cytochrome P450 2D6 (*CYP2D6*) status and clinical outcomes in tamoxifen therapy. We performed a meta-analysis on data from 4,973 tamoxifen-treated patients (12 globally distributed sites). Using strict eligibility requirements (postmenopausal women with estrogen receptor-positive breast cancer, receiving 20 mg/day tamoxifen for 5 years, criterion 1), *CYP2D6* poor metabolizer status was associated with poorer invasive disease-free survival (IDFS: hazard ratio = 1.25; 95% confidence interval = 1.06, 1.47;  $P = 0.009$ ). However, *CYP2D6* status was not statistically significant when tamoxifen duration, menopausal status, and annual follow-up were not specified (criterion 2,  $n = 2,443$ ;  $P = 0.25$ ) or when no exclusions were applied (criterion 3,  $n = 4,935$ ;  $P = 0.38$ ). Although *CYP2D6* is a strong predictor of IDFS using strict inclusion criteria, because the results are not robust to inclusion criteria (these were not defined *a priori*), prospective studies are necessary to fully establish the value of *CYP2D6* genotyping in tamoxifen therapy.

The first two authors contributed equally to this work.

<sup>1</sup>Center for Genome Sciences and Systems Biology, Washington University School of Medicine, St. Louis, Missouri, USA; <sup>2</sup>Department of Oncology and Pharmacology, Mayo Clinic, Rochester, Minnesota, USA; <sup>3</sup>Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology and University Tuebingen, Germany; <sup>4</sup>Division of Clinical Pharmacology, School of Medicine, Indiana University, Bloomington, Indiana, USA; <sup>5</sup>Department of Genetics, Stanford University, Stanford, California, USA; <sup>6</sup>Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, Minnesota, USA; <sup>7</sup>Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; <sup>8</sup>Laboratory for Pharmacogenetics, RIKEN Center for Genomic Medicine, Yokohama, Japan; <sup>9</sup>Centre for Genetic Medicine, Manchester Academic Health Science Centre, University of Manchester and Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; <sup>10</sup>Laboratory for International Alliance, RIKEN Center for Genomic Medicine, Yokohama, Japan; <sup>11</sup>Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, New York, USA; <sup>12</sup>Department of Gynecology and Obstetrics, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; <sup>13</sup>Department of Biomedical Science, Graduate School, Seoul National University, Seoul, Korea; <sup>14</sup>Department of Oncology, Catholic University Leuven, Leuven, Belgium; <sup>15</sup>Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California, USA; <sup>16</sup>University Institute of Medical and Chemical Laboratory Diagnostics, Paracelsus Private Medical University, Salzburg, Austria; <sup>17</sup>The Christie NHS Foundation Trust, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; <sup>18</sup>Department of Pathology, Ninewells Hospital and Medical School, Dundee, UK; <sup>19</sup>Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum, Heidelberg, Germany; <sup>20</sup>Medical University Graz, Graz, Austria; <sup>21</sup>Vesalius Research Center, VIB and Laboratory of Translational Genetics, Department of Oncology, Catholic University Leuven, Leuven, Belgium; <sup>22</sup>Division of General Internal Medicine, Department of Medicine and Clinical Pharmacology and Experimental Therapeutics, and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California, USA; <sup>23</sup>Department of Gynecology and Obstetrics, University Hospitals Leuven, Leuven, Belgium; <sup>24</sup>Department of Surgery, Yonsei University Health System, Seoul, Korea; <sup>25</sup>Dundee Cancer Centre, Dundee, UK; <sup>26</sup>Department of Gynecology and Obstetrics, University of Mainz, Mainz, Germany; <sup>27</sup>Department of Clinical Pharmacology and Toxicology, University Hospital Tuebingen, Tuebingen, Germany; <sup>28</sup>Department of Pharmacology and Pharmacogenomics Research Center, Inje University College of Medicine, Busan, Korea; <sup>29</sup>Department of Clinical Pharmacology, Inje University Busan Paik Hospital, Busan, Korea; <sup>30</sup>Division of Research, Federal Institute for Drugs and Medical Devices, University of Bonn Medical Faculty, Bonn, Germany; <sup>31</sup>Department of Clinical Medicine, Örebro University, Örebro, Sweden; <sup>32</sup>Department of Laboratory Medicine, University of California, San Francisco, San Francisco, California, USA; <sup>33</sup>Department of Oncology, Georgetown University, Washington, DC, USA; <sup>34</sup>Department of Bioengineering, Stanford University, Stanford, California, USA; <sup>35</sup>Department of Pharmacology, Mayo Clinic, Rochester, Minnesota, USA; <sup>36</sup>Department of Oncology, Mayo Clinic, Rochester, Minnesota, USA. Correspondence: TE Klein (teri.klein@stanford.edu)

Received 3 June 2013; accepted 9 September 2013; advance online publication 18 December 2013. doi:10.1038/clpt.2013.186



Tamoxifen, the pioneering antiestrogenic medicine targeted to the tumor estrogen receptor (ER), is used successfully for long-term adjuvant therapy in breast cancer.<sup>1,2</sup> Extensive analyses of clinical trials demonstrate a major increase in patient survivorship in ER-positive patients. In this age of personalized medicine, any opportunity to improve response rates with tamoxifen should be rigorously investigated. Tamoxifen is considered a prodrug, given that hepatic cytochrome P450 2D6 (CYP2D6) metabolizes tamoxifen to metabolites (4-hydroxy tamoxifen and 4-hydroxy-*N*-desmethyl tamoxifen (endoxifen)) that exhibit significantly greater potency in terms of ER-binding affinity<sup>3</sup> and suppression of estradiol-stimulated cell proliferation.<sup>4</sup> CYP2D6-mediated metabolism is the rate-limiting enzymatic step for the formation of endoxifen, the most abundant active metabolite.

There has been great inconsistency among studies that have reported the association of known genetic and drug factors influencing CYP2D6 enzyme activity with tamoxifen efficacy. Therefore, the International Tamoxifen Pharmacogenomics Consortium (ITPC) was conceived, and researchers were invited to submit their data—both published and unpublished data sets regarding *CYP2D6* genetic variants and clinical outcomes in women treated with tamoxifen in the adjuvant breast cancer setting—to allow a meta-analysis of the potential associations between *CYP2D6* and clinical outcomes.

## RESULTS

The ITPC comprises 12 research projects from nine countries and three continents that contributed clinical and genetic data for a total of 4,973 breast cancer patients treated with tamoxifen. In **Table 1**, we show the sample size by site and criteria. Further details for each site are shown in **S3c and S5 online**. We reported preliminary analyses of these collected cohorts before complete curation by pooling the data from each site.<sup>5</sup> For our meta-analyses, three detailed criteria, which ranged from the most restrictive (criterion 1) to the most inclusive (criterion 3), were defined before final curation (see **S4 online**). In brief, criterion 1, derived from the NCCTG 89-30-52 clinical trial, consisted of postmenopausal women with surgically resected nonmetastatic invasive ER-positive breast cancers who received adjuvant tamoxifen monotherapy at a dose of 20 mg/day for an intended duration of 5 years, and were followed at least annually for recurrence. In addition, analysis of at least *CYP2D6*\*4 was required (detailed in **S4a online**). Criterion 2 included criterion 1 but allowed both pre- and postmenopausal patients who had received any duration of tamoxifen; moreover, annual follow-up was not required. Criterion 3 included all samples not excluded

by any exclusion test for missing data or data inconsistencies (least restrictive). Patient characteristics according to each criterion are provided in **Table 2**.

The meta-analysis results combining the hazard ratio (HR) estimates (and the corresponding standard errors (SEs)) from each site are shown for all three criteria groups and both clinical outcomes in **Table 3**. For each of the six clinical outcome/criteria groups, we give the combined meta-analysis estimate across all 12 sites, its SE, and the results of two statistical tests: a test of the significance that the meta-HR differs from 1 and a test of “homogeneity of the estimates” across sites (a significant value for the latter test indicates that there is more variability than the derSimonian and Laird random-effects model can reasonably accommodate, suggesting that the meta-estimate and its associated *P* value are suspect). As can be seen for invasive disease-free survival (IDFS), the meta-analyses for criteria 2 and 3 are nearly significantly heterogeneous, whereas there was no indication of heterogeneity for criterion 1 (*P* = 0.899). For patients meeting criterion 1, the meta-HR for IDFS was 1.25 (95% confidence interval = 1.06, 1.47), and for breast cancer-free interval, it was 1.27 (95% confidence interval = 1.01, 1.61). These are both statistically significant, at *P* = 0.009 and *P* = 0.04, respectively. However, for the criterion 2 (*P* = 0.25) and criterion 3 (*P* = 0.38) subsets, the *CYP2D6* HR was not significant for either outcome.

In **Figure 1**, we show the individual HRs for each site for subjects meeting criterion 1, assuming an additive genetic model for *CYP2D6* (coded 0 = extensive metabolizer (EM), 1 = intermediate metabolizer (IM), and 2 = poor metabolizer (PM)) as estimated from a Cox proportional-hazards model using additional risk covariates to predict clinical outcome. Corresponding figures for criteria 2 and 3 are provided in **S6 online**. (Note that the list of covariates used in the Cox models included age at primary diagnosis, menopause status at diagnosis, metastatic disease at primary diagnosis, maximum tumor dimension, number of positive nodes, grade, smoking status, ER and progesterone receptor status, intended tamoxifen dose and duration, systemic therapy before surgery, chemotherapy, radiation treatment, adjuvant aromatase inhibitor therapy, and additional hormone therapy. The specific set of covariates used for each site was chosen from this list so as to retain at least 70% of the patients from that site; hence, the exact set of covariates used differs in each site's Cox model. Moreover, several of these covariates were used as inclusion/exclusion items in the basic definitions of the three basic criteria subset groups and thus became irrelevant for those analyses.)

**Table 1** Sample size by site and criteria

Criterion	Site (N)												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
1	0	70	124	60	212	243	0	847	5	222	179	34	1,996
2	0	127	208	98	212	304	0	898	10	289	228	69	2,443
3	174	320	282	265	214	391	801	1,140	165	516	397	270	4,935
Total	174	320	282	267	214	423	801	1,140	165	519	398	279	4,973



**Table 2 Baseline patient and tumor characteristics**

Characteristic	Criterion 1 (1,996 patients)		Criterion 2 (2,443 patients)		Criterion 3 (4,935 patients)	
Age at diagnosis, years: data reported in binned ages						
Median	[65–69]		[60–64]		[60–64]	
Range	55 (ages 41–95)		75 (ages 21–95)		76 (ages 21–96)	
Menopausal status—no. (%)	Menopausal status	By age	Menopausal status	By age	Menopausal status	By age
Premenopausal (age ≤ 50)	0 (0.0%)	54 (2.7%)	241 (9.9%)	414 (16.9%)	607 (12.3%)	1,207 (24.5%)
Postmenopausal (>50)	1,688 (84.6%)	1,922 (96.3%)	1,714 (70.2%)	1,997 (81.7%)	3,267 (66.2%)	3,642 (73.8%)
Not available	308 (15.4%)	20 (1.0%)	488 (20.0%)	32 (1.3%)	1,061 (21.5%)	86 (1.7%)
Tumor size—no. (%): maximum dimension of tumor reported (if multiple tumors, largest one is ≤ 2 cm)						
≤ 2 cm	1,071 (53.7%)		1,327 (54.3%)		2,303 (46.7%)	
> 2 cm	752 (37.7%)		882 (36.1%)		2,182 (44.2%)	
Unknown	173 (8.7%)		234 (9.6%)		450 (9.1%)	
Nodal status—no. (%): number of positive nodes						
Zero nodes	1,243 (62.3%)		1,531 (62.7%)		2,423 (49.1%)	
1–3 nodes	407 (20.4%)		461 (18.9%)		1,281 (26.0%)	
4–9 nodes	103 (5.2%)		111 (4.5%)		438 (8.9%)	
> 9 nodes	43 (2.2%)		45 (1.8%)		185 (3.7%)	
Not available	200 (10.0%)		295 (12.1%)		608 (12.3%)	
Grading—no. (%): 0.5 to 1.49 considered G1, 1.5 to 2.49 G2, etc.						
G1	249 (12.5%)		317 (13%)		456 (9.2%)	
G2	1,148 (57.5%)		1,324 (54.2%)		1,965 (39.8%)	
G3	330 (16.5%)		398 (16.3%)		838 (17.0%)	
Unknown	269 (13.5%)		295 (12.1%)		1,676 (34.0%)	
ER status—no. (%)						
ER-positive	1,996 (100.0%)		2,443 (100.0%)		4,675 (94.7%)	
ER-negative	0 (0.0%)		0 (0.0%)		158 (3.2%)	
Unknown	0 (0.0%)		0 (0.0%)		102 (2.1%)	
PgR status—no. (%)						
PgR-positive	1,479 (74.1%)		1,847 (75.6%)		3,634 (73.6%)	
PgR-negative	273 (13.7%)		302 (12.4%)		665 (13.5%)	
Unknown	244 (12.2%)		294 (12.0%)		102 (2.1%)	
Radiotherapy—no. (%): radiation therapy						
Yes	1,138 (57.0%)		1,412 (57.8%)		2,868 (58.1%)	
No	720 (36.1%)		842 (34.5%)		1,507 (30.5%)	
Unknown	244 (12.2%)		189 (7.7%)		560 (11.3%)	
CYP2D6 metabolizer status						
Extensive	893 (44.7%)		1,077 (44.1%)		2,286 (46.3%)	
Intermediate	985 (49.3%)		1,230 (50.3%)		2,311 (46.8%)	
Poor	118 (5.9%)		136 (5.6%)		244 (4.9%)	
Unknown	0 (0.0%)		0 (0.0%)		94 (1.9%)	
CYP2D6 metabolizer types						
EM/UM	17 (0.9%)		23 (0.9%)		49 (1.0%)	
IM/UM	2 (0.1%)		2 (0.1%)		4 (0.1%)	
EM/EM	874 (43.8%)		1,052 (43.1%)		2,233 (45.2%)	
PM/UM	7 (0.4%)		7 (0.3%)		12 (0.2%)	

Table 2 Continued on next page



**Table 2 Continued**

Characteristic	Criterion 1 (1,996 patients)	Criterion 2 (2,443 patients)	Criterion 3 (4,935 patients)
EM/IM	327 (16.4%)	407 (16.7%)	693 (14.0%)
EM/PM	496 (24.8%)	616 (25.2%)	1,230 (25.1%)
IM/IM	64 (3.2%)	94 (3.8%)	174 (3.5%)
IM/PM	91 (4.6%)	106 (4.3%)	192 (3.9%)
PM/PM	118 (5.9%)	136 (5.6%)	244 (4.9%)
Unknown	0 (0.0%)	0 (0.0%)	94 (1.9%)
DNA source			
Blood	996 (49.9%)	1,344 (55.0%)	2,513 (50.9%)
Tumor—Frozen	431 (21.6%)	500 (20.5%)	1,575 (31.9%)
Tumor—FFPE	569 (28.5%)	598 (24.5%)	659 (13.4%)
Normal—FFPE	0 (0.0%)	0 (0.0%)	174 (3.5%)
Unknown	0 (0.0%)	1 (0.0%)	14 (0.3%)

CYP2D6, cytochrome P450 2D6; EM, extensive metabolizer; ER, estrogen receptor; FFPE, formalin-fixed–paraffin-embedded; IM, intermediate metabolizer; PgR, progesterone receptor; PM, poor metabolizer; UM, unknown metabolizer.

**Table 3 Meta-analyses of CYP2D6 HRs on clinical outcome in inclusion/exclusion criteria subsets**

	IDFS				BCFI			
	Meta-estimates		P value		Meta-estimates		P value	
	HR	95% CI	Homog <sup>a</sup>	Association <sup>b</sup>	HR	95% CI	Homog <sup>a</sup>	Association <sup>a</sup>
Criterion 1	1.25	(1.06,1.47)	0.899	0.009	1.27	(1.01,1.61)	0.858	0.041
Criterion 2	1.17	(0.90,1.52)	0.055	0.249	1.21	(0.889,1.65)	0.130	0.224
Criterion 3	1.07	(0.92,1.26)	0.099	0.382	1.10	(0.868,1.35)	0.114	0.352

BCFI, breast cancer–free interval; Homog, homogeneity; HR, hazard ratio; IDFS, invasive disease–free survival; ITPC, International Tamoxifen Pharmacogenomics Consortium.

<sup>a</sup>The homogeneity *P* value tests the hypothesis that the individual ITPC site estimates meet the statistical random-effects modeling assumptions of the meta-analysis.

A significant value indicates that there is significant heterogeneity among the sites, which casts doubt on the “combinability” of the studies for that parameter and on the validity of the corresponding association test. <sup>b</sup>The association *P* value tests the hypothesis that the combined meta-analysis estimate of the HR is significantly different from the null hypothesis value of HR = 1.

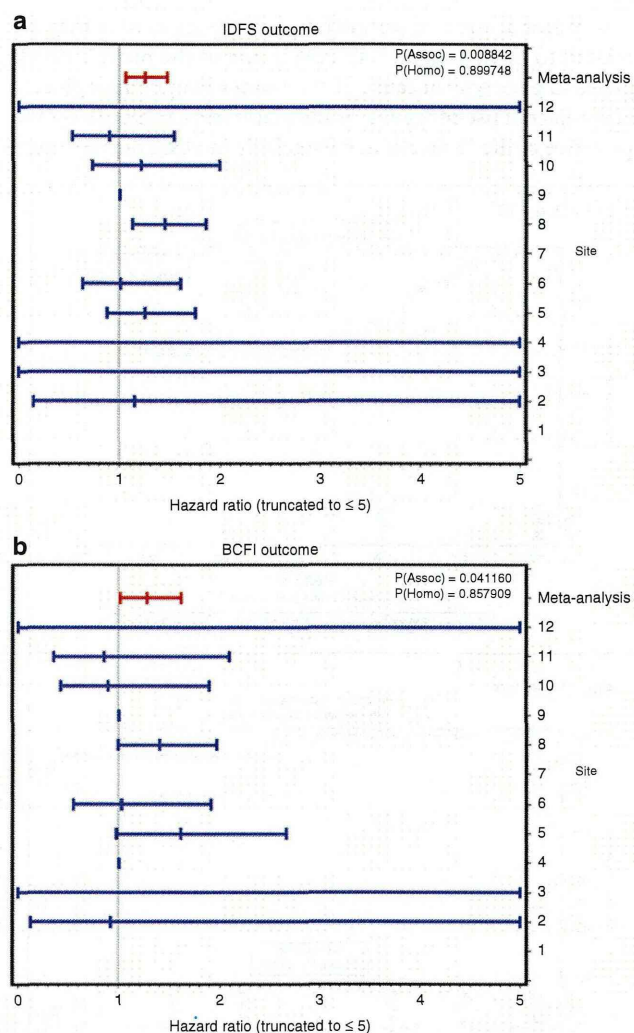
Site-specific product-limit estimates of the three CYP2D6 metabolizer status genotype groups (EM, IM, and PM) are shown in **Figures 2 and 3** for criterion 1 patients. Sites 1 and 7 had no subjects who met inclusion/exclusion for criterion 1. The corresponding figures for patients meeting criteria 2 and 3 are shown in **S6** online. As seen in **Figure 2**, for IDFS sites, 3, 5, and 8 show a strong significant effect in the direction expected by the known pharmacokinetic effects of CYP2D6 on endoxifen exposure, namely, a poorer clinical response for the IM and/or PM genotype groups. Other sites show a trend in the expected direction between the IM and EM groups, but the much smaller PM group is often inconsistent with the expectation, and the separation in the three survival curves is not strong enough to reach statistical significance (e.g., sites 6 and 12). For some sites, there is no hint of any significant difference (e.g., sites 2, 4, 10, and 11), and for one of these, site 2, the direction of effect is exactly opposite than expected. There is a danger in overinterpreting such “trends” (either in favor or against expectation) when there is no statistically significant difference, because some level of site-to-site variation is to be expected. The key question is not whether such variation exists but whether it centers over the null hypothesis or over the alternative; this is the question that the meta-analysis is designed to answer. However,

these simple product-limit survival curves show great study-to-study heterogeneity, which complicates both the analyses and the interpretation. We have similar heterogeneous results for the breast cancer–free interval outcome, shown in **Figure 3**. The corresponding figures in **S6** online show a similar pattern for the subsets of patients meeting criteria 2 and 3, although the heterogeneity seems to be even more pronounced as the exclusion criteria are loosened. This is not a surprising result, considering that the criteria themselves impose a certain level of homogeneity.

## DISCUSSION

Prospective pharmacology studies consistently demonstrate that CYP2D6 genetic variants are associated with variable plasma concentrations of endoxifen.<sup>4,6</sup> Endoxifen exposure is related to duration of tamoxifen use and dose, wherein an increase in the tamoxifen dose (from 20 to 40 mg daily) significantly increases endoxifen exposure in patients with reduced or null CYP2D6 metabolism but not in CYP2D6 EMs.<sup>7</sup> However, coadministration of CYP2D6-inhibiting drugs<sup>4</sup> reduces CYP2D6 enzyme activity, and nonadherence to tamoxifen is more commonly observed in patients with normal or increased CYP2D6 metabolism.<sup>8</sup>





**Figure 1** Individual site estimates of hazard ratios of *CYP2D6* genotype on clinical outcome, along with the meta-analyses for the criterion 1 subset. (a) Invasive disease-free survival (IDFS) outcome. (b) Breast cancer-free interval (BCFI) outcome.

Despite the consistent pharmacogenetic effects of *CYP2D6* on endoxifen exposure, there is considerable controversy regarding the validity of *CYP2D6* as a predictor of tamoxifen outcome.<sup>9,10</sup> Although recent secondary analyses from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial and the Breast International Group (BIG) 1-98 study<sup>11,12</sup> did not demonstrate an association between *CYP2D6* and tamoxifen outcome, these studies provoked criticism due to concerns regarding genotyping error and the analysis of small subsets of the main trials.<sup>13-16</sup>

By contrast, a secondary analysis from another large prospective adjuvant tamoxifen trial, the Austrian Breast and Colorectal Cancer Study Group 8 (ABCSG 8), demonstrated that for women treated with 5 years of adjuvant tamoxifen at a dose of 20 mg/day, *CYP2D6* PMs had a statistically significant higher odds of recurrence or death as compared with *CYP2D6* EMs, and *CYP2D6* PMs/IMs and PMs/EMs tended to exhibit a higher odds of recurrence as compared with patients without

the PM alleles. However, this effect was not observed for patients who had switched to anastrozole, a drug not metabolized by *CYP2D6*. These data suggest that the effects of *CYP2D6* genotype may be masked if patients receive a shorter duration of tamoxifen or other active drugs besides tamoxifen, which alter the hazard for recurrence.<sup>17</sup>

We approached the tamoxifen controversy by performing a global meta-analysis of available clinical and *CYP2D6* genetic data of tamoxifen-treated breast cancer patients. All groups from across the world with both published and unpublished *CYP2D6* data were invited to participate. We initially presented a pooled analysis of these data,<sup>5</sup> in which we found no association between *CYP2D6* and IDFS. Following this presentation, we developed a new analysis plan (not defined before the initial negative presentation), which included the following: (i) articulation of three criteria to analyze the data according to the quality of the genetic and clinical data, (ii) additional curation to obtain missing clinical and genetic data, and (iii) a new statistical analysis plan, which applied a random-effects meta-analysis strategy instead of a pooled analysis strategy. Notably, Criterion 1 is most stringent, requiring strict control for as many pharmacologic factors as possible known to affect endoxifen exposure, which include use of tamoxifen monotherapy, genotyping of multiple *CYP2D6* alleles for accurate *CYP2D6* phenotype assignment, use of one tamoxifen dose (20 mg), and intended duration of tamoxifen use for 5 years. In addition, eligibility for this cohort was restricted to women with invasive ER-positive status, postmenopausal breast cancer, and the requirement for annual follow-up, parameters required in any prospective clinical trial and that were requirements of criterion 1 (patients who were knowingly not followed were excluded from criterion 1), but not from criteria 2 and 3. These factors may have contributed to the substantial increase in heterogeneity comparing criterion 1 with criteria 2 and 3. However, it should be noted that these criteria impose a certain bias because the majority of negative studies submitted to the ITPC were observed in criteria 2 and 3.

In general, a substantial number of subjects comprising criterion 3 had misclassification of the predicted drug metabolism phenotype due to the lack of a comprehensive coverage of loss-of-function alleles.<sup>18,19</sup> More than 20 loss-of-function alleles out of 100 known *CYP2D6* genetic variants contributed to a frequency of ~8% of PMs in a population of European descent. Limiting the analysis to the most common such allele, *CYP2D6*\*4, as was frequently done in the older published literature, will result in misclassification of 35% of PMs, thereby falsely assigning the undetected PMs to the EM or IM groups. Notably, 871/1,996 patients comprising criterion 1 had optimal *CYP2D6* phenotype assessment obtained by AmpliChip genotyping, and this may have contributed to the robustness of criterion 1 results, which demonstrated an association between *CYP2D6* and tamoxifen treatment outcome (breast cancer-free interval: HR = 1.27, 95% confidence interval = 1.01-1.61).

The ITPC intended to perform a global study including several thousand patient samples; however, the majority of the subjects were not comprehensively genotyped because DNA was not of sufficient quality. We performed a subgroup analysis using patient samples for which full coverage of alleles by the