Histological Response

	CYP2D6 wt/wt	CYP2D6 wt/*V	CYP2D6 *V/*V
No (Grade 0)	13	8	5
Yes (Grade 1a or 1b (mild))	9 (40.9%)	7 (45.4%)	3 (37.5%)

$P_{fisher} = 0.925$

Breast conservative operation

	CYP2D6 wt/wt	CYP2D6 wt/*V	CYP2D6 *V/*V
No (Bt)	11	7	5
Yes (Bp)	20 (64.5%)	26 (78.8%)	9 (64.3%)

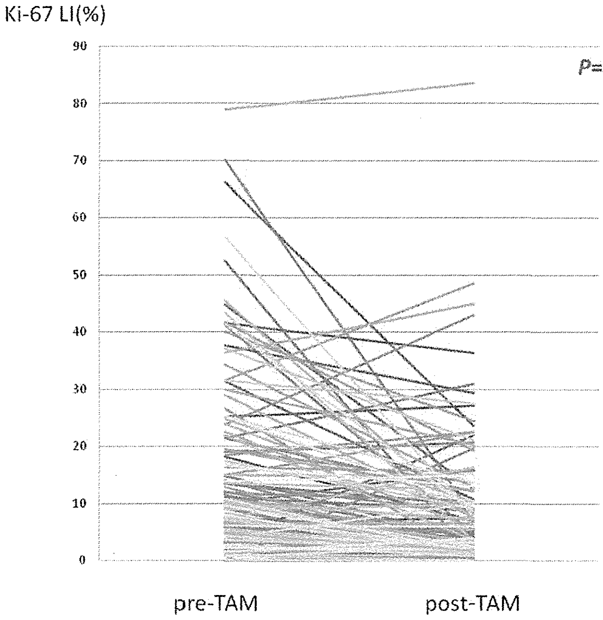
$P_{fisher} = 0.39$

Adverse event (Hot flash)

	CYP2D6 wt/wt	CYP2D6 wt/*V	CYP2D6 *V/*V
No	23	26	9
Yes (mild or moderate)	8 (25.8%)	7 (21.2%)	5 (35.7%)

$P_{fisher} = 0.56$

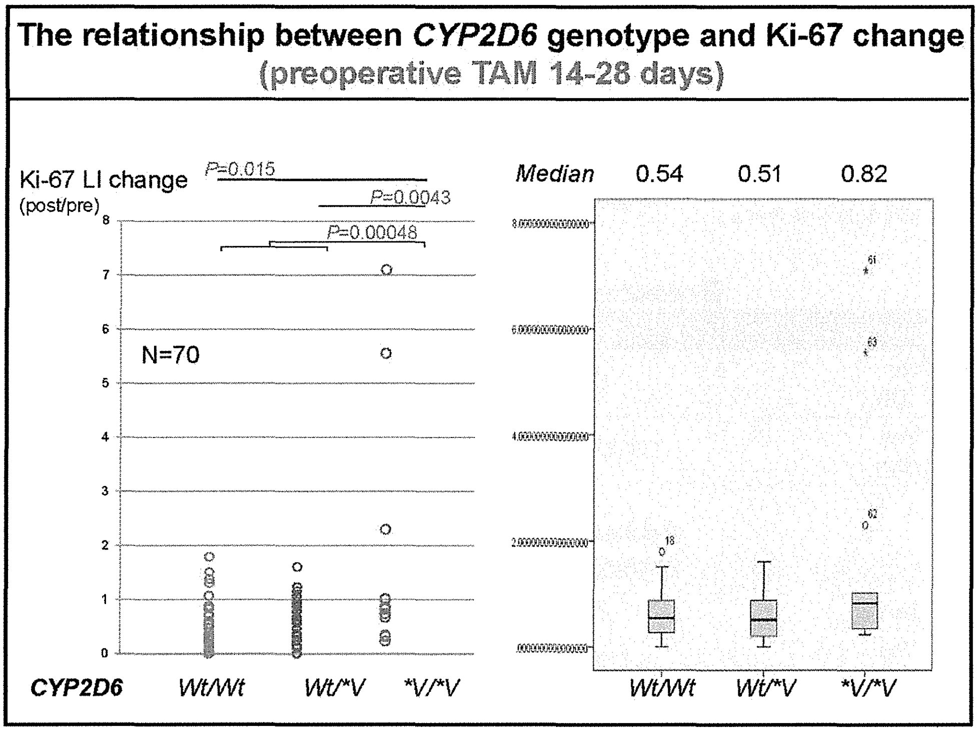
Ki-67 change after preoperative TAM in all cases



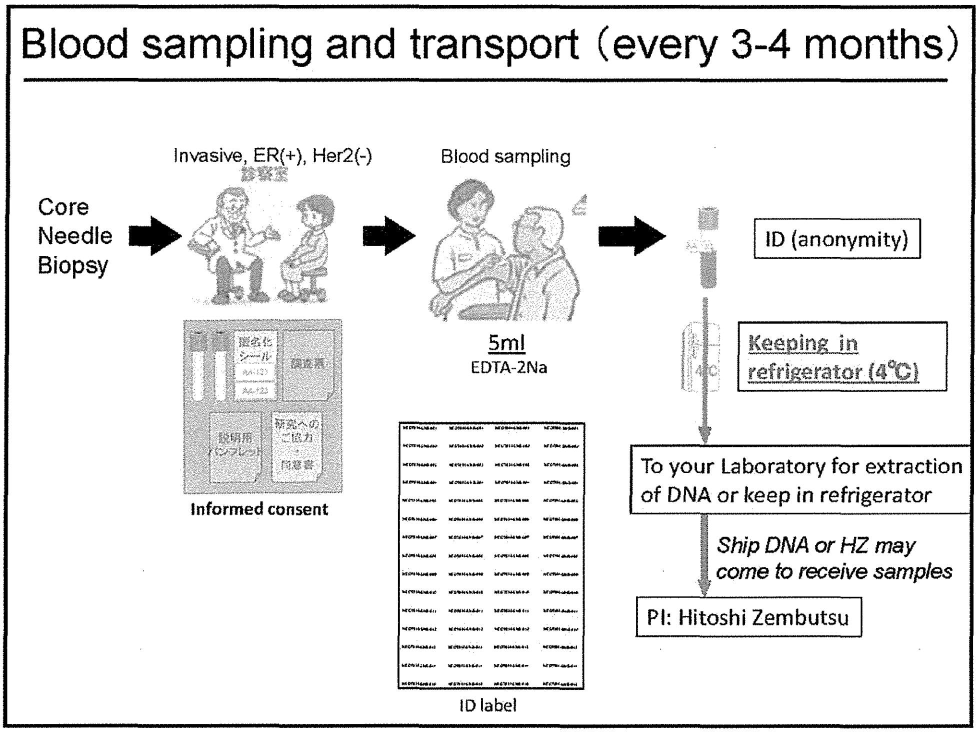
	preTAM	postTAM
Average	15.5	9.2
Median	9.6	5.3

Median Ki-67 reduction

39.1%



The relationship between *CYP2D6* genotype and Ki-67 change (preoperative TAM 14-28 days)



Blood sampling and transport（every 3-4 months）

Core Needle Biopsy → Invasive, ER(+), Her2(-) → Blood sampling → ID (anonymity)

Informed consent

5ml
EDTA-2Na

ID label

Keeping in refrigerator (4℃)

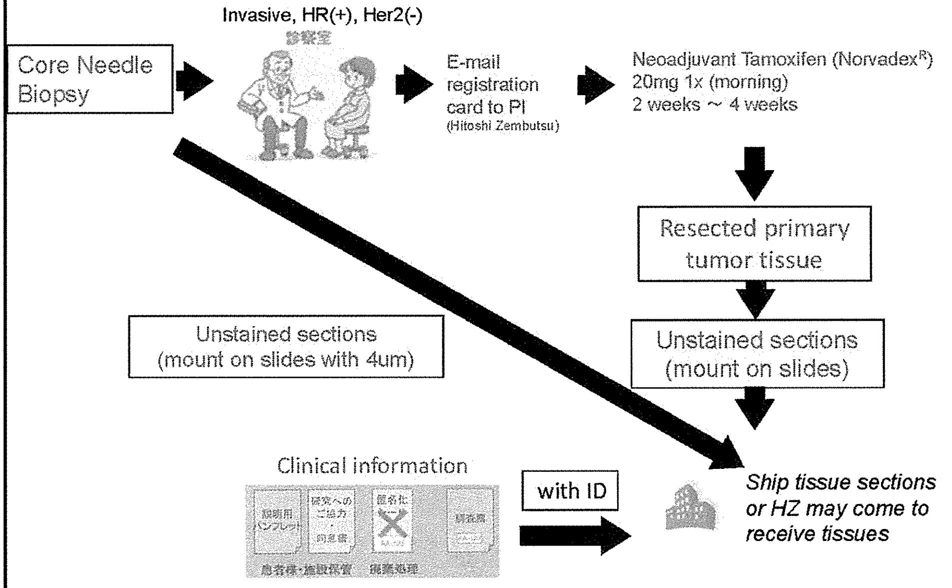
To your Laboratory for extraction of DNA or keep in refrigerator

Ship DNA or HZ may come to receive samples

PI: Hitoshi Zembutsu

Tissue sampling and clinical information

(almost every 4 - 6 months)



Invasive, HR(+), Her2(-)

Core Needle Biopsy

E-mail registration card to PI (Hitoshi Zembutsu)

Neoadjuvant Tamoxifen (Norvadex^{R})
20mg 1x (morning)
2 weeks ~ 4 weeks

Resected primary tumor tissue

Unstained sections (mount on slides)

Unstained sections (mount on slides with 4um)

Clinical information

with ID

Ship tissue sections or HZ may come to receive tissues

How to sample and submit the cancer tissues

Pretreatment tissue

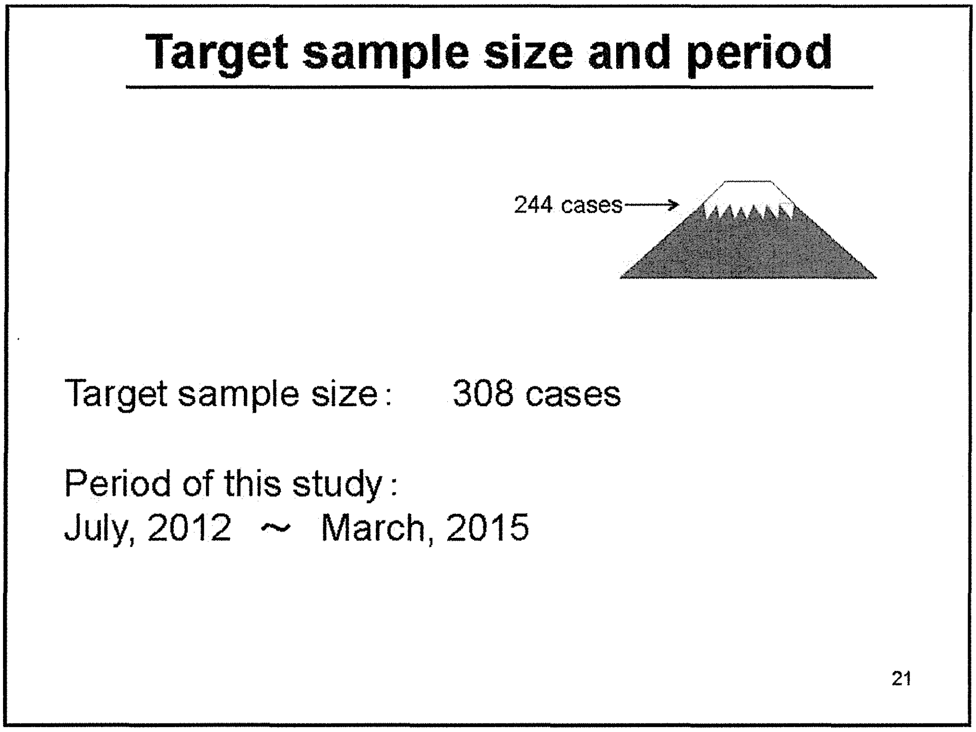
Core Needle Biopsy

1. Fixed with 10% neutral buffered formalin
2. Tissues need to be fixed within 48 hours
3. 4µm unstained sliced tissue on slide (10 slides)
4. Slide (New Silane III (5146-20F)) and box will be provided

Posttreatment tissue

Resected tissue by operation

1. Cut the tumor center (maximum diameter) by knife, and sample small tissue (3x3x3mm) for Ki-67 test
2. Tissues need to be fixed within 48 hours
3. 4µm unstained sliced tissue on slide (10 slides)
4. Slide (New Silane III (5146-20F)) and box will be provided



Target sample size and period

244 cases

Target sample size: 308 cases

Period of this study:
July, 2012 ~ March, 2015

Ⅱ．研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Hitoshi Zembutsu	Tamoxifen Pharmacogenetics		Clinical Genomics.	The McGraw-Hill Education		2013	35～37
明石　定子	治療効果判定①化学療法	位藤　俊一	乳房画像診断最前線	南江堂	東京	2013	216～221
明石　定子	乳がんの化学予防	戸井　雅和	インフォームドコンセントのための図説シリーズ　乳がん薬物療法　第2版	医薬ジャーナル社	大阪	2012	42～47

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Province MA, Goetz MP, Zembutsu H, et al.	*CYP2D6* Genotype and Adjuvant Tamoxifen: Meta-Analysis of Heterogeneous Study Populations.	Clin Pharmacol Ther.	95巻2号	216～227	2013
Chung S, Zembutsu H, Takahashi A, et al.	A genome-wide association study of chemotherapy-induced alopecia in breast cancer patients.	Breast Cancer Res.	15巻5号	R81	2013
Kiyotani K, Mushiroda T, Zembutsu H, et al.	Important and critical scientific aspects in pharmacogenomics analysis: lessons from controversial results of tamoxifen and *CYP2D6* studies.	J Hum Genet.	58巻6号	327～333	2013
Low SK, Chung S, Zembutsu H, et al.	Genome-wide association study of chemotherapeutic agent-induced severe neutropenia/leucopenia for patients in Biobank Japan.	Cancer Sci.	104巻8号	1074～1082	2013
Nosho K, Igarashi H, Hirata K, et al.	Association of microRNA-31 with *BRAF* mutation, colorectal cancer curvival and serrated pathway.	Carcinogenesis.	35巻4号	776～783	2013

Tanimizu N, Nakamura Y, Hirata K, et al.	Hepatic biliary epithelial cells acquire epithelial integrity but lose plasticity to differentiate into hepatocytes *in vitro* during development.	Journal of Cell Science.	126巻 22号	5239 ～ 5246	2013
Ichinohe N, Tanimizu N, Hirata K, et al.	Differentiation Capacity of Hepatic Stem/Progenitor Cells Isolated From D-Galactosamine-Treated Rat Livers.	HEPATOLOGY.	57巻3号	1192 ～ 1202	2013
Hashimoto K, Masumori N, Hirata K, et al.	Zoledronic acid but not somatostatin analogs exerts anti-tumor effects in a model of murine prostatic neuroendocrine carcinoma of the development of castration- resistant prostate cancer.	Prostate.	73巻5号	500 ～ 511	2013
Kuroi K, Toi M, Nakamura S, et al.	Prognostic significance of subtype and pathologic response in operable breast cancer; a pooled analysis of prospective neoadjuvant studies of JBCRG.	Breast Cancer.			2013
Yamauchi H, Nakagawa C, Nakamura S, et al.	Prospective Study of the Effect of the 21-Gene Assay on Adjuvant Clinical Decision-Making in Japanese Women With Estrogen Receptor-Positive, Node-Negative, and Node-Positive Breast Cancer.	Clin Breast Cancer.	14巻3号	191 ～ 197	2013
Yagata H, Yamauchi H, Nakamura S, et al.	Sentinel node biopsy after neoadjuvant chemotherapy in cytologically proven node-positive breast cancer.	Clin Breast Cancer.	13巻6号	471 ～ 477	2013
Nakamura S, Takahashi M, Tozaki M, et al.	Prevalence and differentiation of hereditary breast and ovarian cancers in Japan.	Breast Cancer.			2013
Mukai H, Watanabe T, Nakamura S, et al.	Final results of a safety and efficaty trial of preoperative sequential chemoratiation therapy for the nonsurgical treatment of early breast cancer; Japan Clinical Oncology Group Study.	Oncology.	85巻6号	336 ～ 341	2013
Onoda T, Yamauchi H, Nakamura S, et al.	The value of progesterone receptor expression in predicting the Recurrence Score for hormone-receptor positive invasive breast cancer patients.	Breast Cancer.			2013

Tamai N, Horii M, Nakamura S, et al.	Morphological characteristics of and factors related to moisture-associated dermatitis surrounding malignant wounds in breast cancer patients.	Oncology Nursing.	17巻5号	673 ～ 680	2013
Nakamura S	Axillary lymph node diesection in sentinel node positive breast cander; Is necessary?	Curr Opin Obstet Gyne.	13巻6号	471 ～ 477	2013
Aogi K, Saei T, Nakamura S, et al.	A multicenter, phase II study of epirubicin/cyclophosphamide followed by docetaxel and concurrent trastuzumab as primary systemic therapy for bre HER-2 positive advance ast cancer (the HER2NAT study).	International Journal of Clinical Oncology.	18巻4号	598 ～ 606	2013
Akashi-Tanaka S	Preoperative CT evaluation of intraductal spread of breast cancer and surgical treatment. Breast Cancer.	Breast Cancer.	20巻1号	21 ～ 25	2013
Iwata H, Nakamura S, et al	Analysis of Ki-67 expression with neoadjuvant anastrozole or tamoxifen in patients receiving goserelin for premenopausal breast cancer.	Cancer.	119(4)	704 ～ 713	2013
Wheeler HE, Gamazon ER, Zembutsu H, et al.	Integration of cell line and clinical trial genome-wide analyses supports a polygenic architecture of paclitaxel-induced sensory peripheral neuropathy.	Clin Cancer Res.	19巻2号	491 ～ 499	2013
Mizuguchi T, Kawamoto M, Hirata K. et al.	Prognosis and Predictors of Surgical Complications in Hepatocellular Carcinoma Patients With or Without Cirrhosis after Hepatectomy.	World J Surg.	37巻	1379 ～ 1387	2013
Hirakawa M, Sato Y, Hirata K, et al.	A phase II study of neoadjuvant combination chemotherapy with docetaxel, cisplatin, and S-1 for locally advanced resectable gastric cancer: nucleotide excision repair (NER) as potential chemoresistance marker.	Cancer Chemother Pharmacol.	71巻3号	789 ～ 797	2013
Kiriyama K, Hirohashi Y, Hirata K, et al.	Expression and function of *FERMT* genes in colon carcinoma cells.	Anticancer Res.	33巻1号	167 ～ 173	2013

Kameshima H, Tsuruma T, Hirata K. et al.	Immunotherapeutic benefit of α-interferon (IFNα) in survivin2B-derived peptide vaccination for advanced pancreatic cancer patients.	Cancer Sci.	104巻1号	124 ～ 129	2013
Akashi-Tanaka S.	Preoperative CT evaluation of intraductal spread of breast cancer and surgical treatment.	Breast Cancer.	Jan;20(1)	21 ～ 25	2013
Nakamura S.	Axillary lymph node dissection in sentinel node positive breast cancer: is it necessary?	Curr Opin Obstet Gynecol.	in press		
前佛　均、 清谷 一馬、 宇野 智子、 他８名	有害事象　ゲノムワイド関連解析によるゲムシタビン副作用関連遺伝子の同定	SURGERY FRONTIER (第 77 回)	20 巻 2 号	198 ～ 201	2013
加藤 陽一郎、 高田　亮、 岩崎 一洋、 小原　航、 前佛　均 その他２名	抗腫瘍効果　浸潤性膀胱癌に対する術前化学療法　感受性予測システムの構築	SURGERY FRONTIER (第 77 回)	20 巻 2 号	194 ～ 197	2013
宇野 智子、 前佛　均、 中村 祐輔	ゲノムワイド関連解析による疾病関連遺伝子の同定　子宮内膜症	SURGERY FRONTIER (第 77 回)	20 巻 2 号	184 ～ 187	2013
中島 光子 前佛　均	ゲノムワイド関連解析による疾病関連遺伝子の同定　ケロイド	SURGERY FRONTIER (第 77 回)	20 巻 2 号	178 ～ 183	2013
前佛　均, 清谷 一馬, 平田 公一, 中村 祐輔 他7名	ゲノムワイド関連解析によるジェムシタビン副作用関連遺伝子の同定	胆と膵	34巻2号	143 ～ 148	2013
奥田 逸子、 中島 康雄、 戸崎 光宏、 中村 清吾 他6名	ハイリスク乳癌に対する乳癌検診をどうするか “乳がん発症ハイリスクグループに対する乳房 MRI スクリーニングに関するガイドライン” の解説	日本乳癌検診学会誌	22巻2号	198 ～ 202	2013
中村 清吾	ハイリスク乳癌に対する乳癌検診をどうするか わが国におけるHBOCの現状と今後の取組み	日本乳癌検診学会誌	22巻2号	182 ～ 186	2013
中村 清吾	診療ガイドラインの社会的意義と問題点	日本内科学会雑誌	102巻9号	2285 ～ 2290	2013

桑山 隆志、 山内 英子、 中村 清吾、 他4名	術前化学療法(NAC)前 cN0 乳癌に対する NAC 後センチネルリンパ節生検(SNB)の成績	日本乳癌学会総会プログラム抄録集	第20回	322	2013
沢田 晃暢、 内田 諭子、 明石 定子、 中村 清吾 他6名	ER(+)、HER2(-)、腋下リンパ節転移陽性乳癌における Ki67 値とリンパ節浸潤の検討	日本乳癌学会総会プログラム抄録集	第20回	298	2013
Kiyotani K, Mushiroda T, Zembutsu H, et al.	A genome-wide association study identifies locus at 10q22 associated with clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients in Japanese.	Hum Mol Genet.	21巻7号	1665 ～ 1672	2012
Baldwin RM, Owzar K, Zembutsu H, et al.	A genome-wide association study identifies novel loci for paclitaxel-induced sensory peripheral neuropathy in CALGB 40101.	Clin Cancer Res.	18巻18号	5099 ～ 5109	2012
Cha PC, Zembutsu H, et al.	A genome-wide association study identifies SNP in *DCC* is associated with gallbladder cancer in the Japanese population.	J. Hum Genet.	57巻4号	235 ～ 237	2012
Kiyotani K, Uno S, Zembutsu H, et al.	A genome-wide association study identifies four genetic markers for hematological toxicities in cancer patients receiving gemcitabine therapy.	Pharmacogenet Genomics.	22巻4号	229 ～ 235	2012
Low SK, Takahashi A, Cha PC, Zembutsu H, et al.	Genome-wide association study for intracranial aneurysm in the Japanese population identifies three candidate susceptible loci and a functional genetic variant at *EDNRA*.	Hum Mol Genet.	21巻9号	2102 ～ 2110	2012
Sukawa Y, Yamamoto H, Hirata K, et al.	Alterations in the human epidermal growth factor receptor 2-phosphatidylinositol 3-kinase-v-Akt pathway in gastric cancer.	World J Gastroenterol.	18巻45号	6577 ～ 6586	2012
Ishiwatari H, Sato Y, Hirata K, et al.	Treatment of pancreatic fibrosis with siRNA against a collagen-specific chaperone in vitamin A-coupled liposomes.	Gut.	in press		2012

Hashimoto K, Masumori N, Tanaka T, MaedaT, Kobayashi K, Kitamura H, Hirata K, Tsukamoto T.	Zoledronic acid but not somatostatin analogs exerts anti-tumor effects in a model of murine prostatic neuroendocrine carcinoma of the development of castration-resistant prostate cancer.	The Prostate.	73巻	500 ～ 511	2012
Ichinohe N, Tanimizu N, Hirata K, Mitaka T. et al.	Differentiation capacity of hepatic stem/progenitor cells isolated from D-galactosamine-treated rat livers.	Hepatology.	57巻3号	1192 ～ 1202	2012
Tamura Y, Torigoe T, Hirata K, Sato N. et al.	Heat-shock proteins as endogenous ligands building a bridge between innate and adaptive immunity.	Immunotherapy.	4巻8号	841 ～ 852	2012
Takahashi A, Torigoe T, Hirata K, et al.	Heat shock enhances the expression of cytotoxic granule proteins and augments the activities of tumor-associated antigen-specific cytotoxic T lymphocytes.	Cell Stress and Chaperones.	17巻6号	757 ～ 763	2012
Shitani M, Sasaki S, Hirata K, et al.	Genome-wide analysis of DNA methylation identifies novel cancer-related genes in hepatocellular carcinoma.	Tumour Biol.	33巻5号	1307 ～ 1317	2012
Kiyotani K, Mushiroda T, Nakamura Y, Zembutsu H.	Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters.	Drug Metab Pharmacokinet.	27巻1号	122 ～ 131	2012
Kiyotani K, Mushiroda T, Imamura CK, Tanigawara Y, Hosono N, Kubo M, Sasa M, Nakamura Y, Zembutsu H.	Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes in Japanese breast cancer patients.	Breast Cancer Res Treat.	131巻1号	137 ～ 145	2012
Nyholt DR, Low SK, Zembutsu H, et al.	Genome-wide association meta-analysis identifies new endometriosis risk loci.	Nat Genet.	44巻12号	1355 ～ 1359	2012

Elgazzar S, Zembutsu H, et al.	A Genome-wide association study identifies a genetic variant in the *SIAH2* locus associated with hormonal receptor-positive breast cancer in Japanese.	J. Hum Genet.	57巻12号	766～771	2012
中村 清吾	乳癌の薬物療法（解説/特集）	クリニシアン	59巻6号	504～509	2012
笹　三徳、 清谷 一馬、 前佛　均、 中村 祐輔 他4名	CYP2D6遺伝子多型によるTAM投与量調節治療	日本乳癌学会総会プログラム抄録集	第20回	273	2012
清谷 一馬、 莚田 泰誠、 前佛　均 他7名	網羅的遺伝子多型解析による乳がんホルモン療法の治療効果の予測	薬事ニュース	12月23日		2011

Ⅲ. 研究成果の刊行物・別刷

nature publishing group ARTICLES

Open

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

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

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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.[1,2] 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[3] and suppression of estradiol-stimulated cell proliferation.[4] 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.[5] 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

	Site (*N*)												
Criterion	1	2	3	4	5	6	7	8	9	10	11	12	Total
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[a]	Association[b]	HR	95% CI	Homog[a]	Association[a]
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.

[a] 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. [b] 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.[4,6] 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.[7] However, coadministration of CYP2D6-inhibiting drugs[4] reduces CYP2D6 enzyme activity, and nonadherence to tamoxifen is more commonly observed in patients with normal or increased CYP2D6 metabolism.[8]

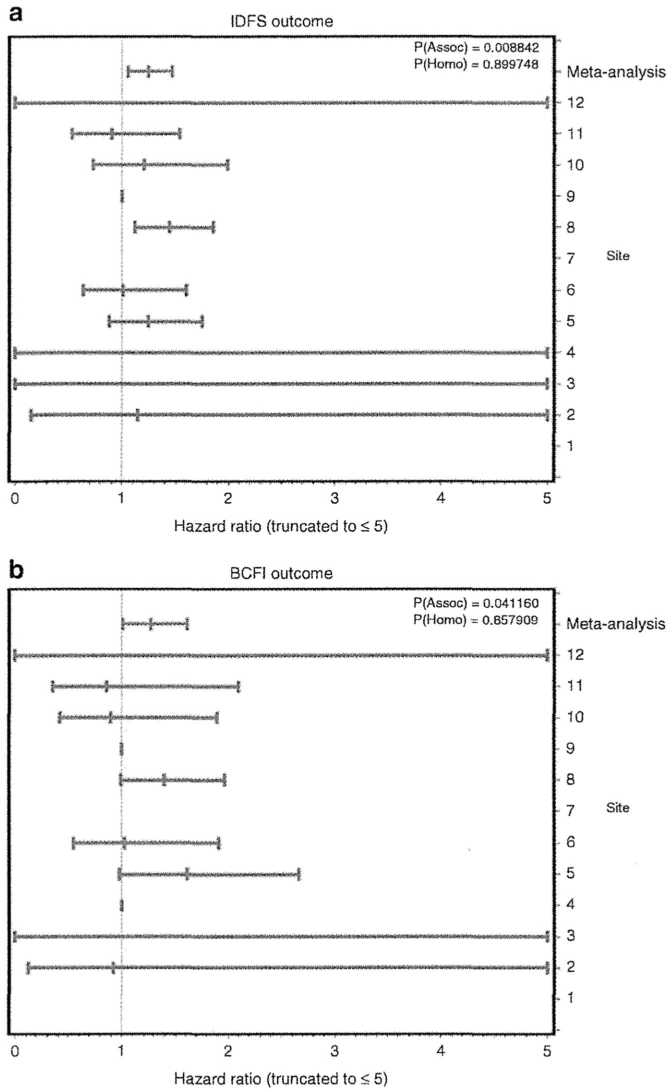

Figure 1 Individual site estimates of hazard ratios of *CYP2D6* genotype on clinical outcome, along with the meta-analyses for the criterion 1 subset. (**a**) Invasive disease–free survival (IDFS) outcome. (**b**) Breast cancer–free interval (BCFI) outcome.

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.[9,10] Although recent secondary analyses from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial and the Breast International Group (BIG) 1-98 study[11,12] 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.[13–16]

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.[17]

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,[5] 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.[18,19] 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

AmpliChip genotyping platform was available using criterion 1 (871/1,635 AmpliChip-genotyped subjects met criterion 1). When confined to the Amplichip subjects, the estimates of the pharmacodynamic HRs for *CYP2D6* were similar to what they were for the entire set of subjects meeting criterion 1.

A major source of potential genotyping errors may be related to DNA source. *CYP2D6* is one of the most difficult genes to genotype because of the numerous polymorphisms and adjacent pseudogenes. Some platforms cannot detect the presence of the *5 deletion, particularly in DNA derived from

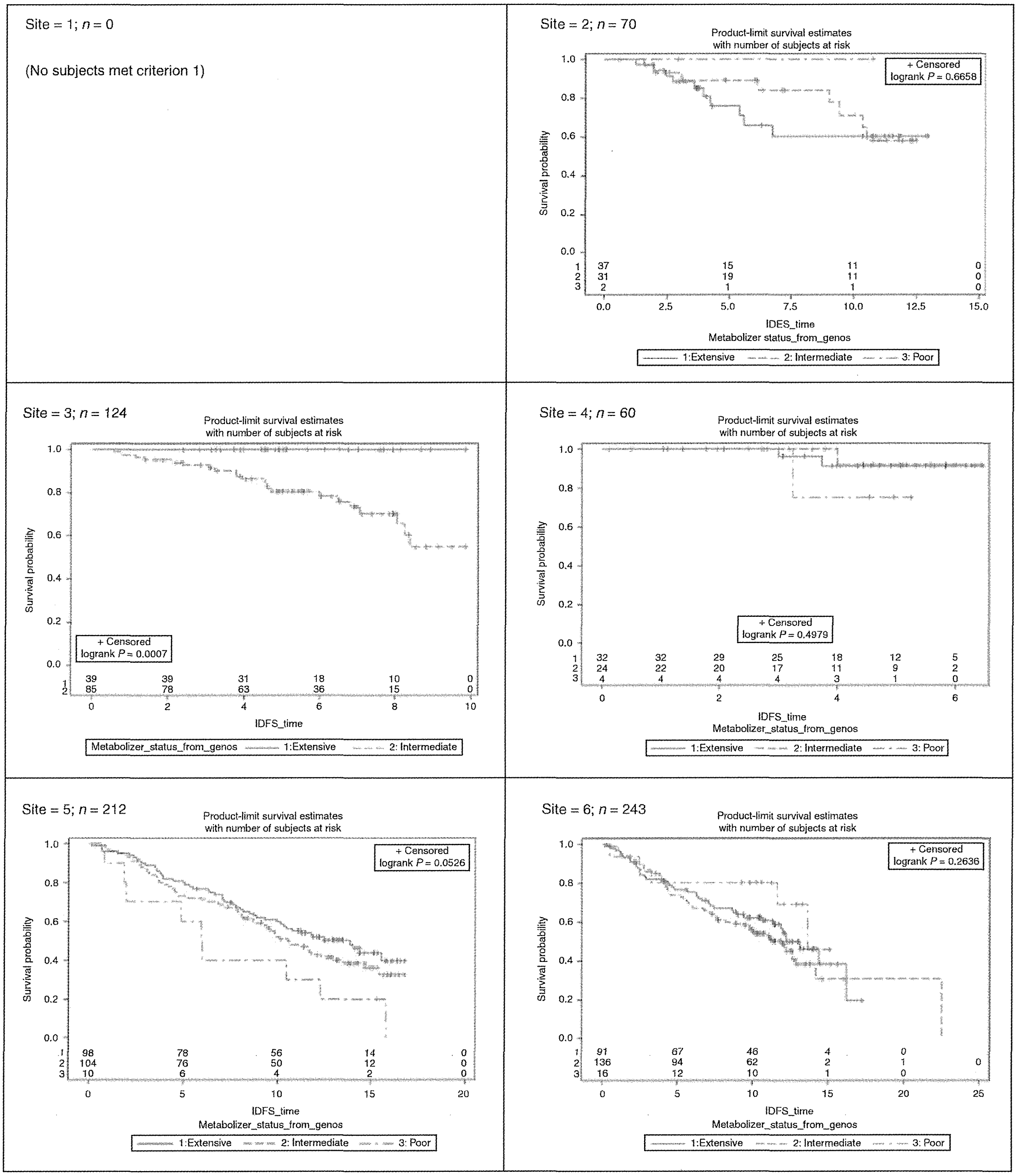

Figure 2 Site-specific effects of CYP2D6 metabolizer status on clinical outcomes for subjects meeting inclusion criterion 1 (outcome = invasive disease–free survival (IDFS)).

formalin-fixed–paraffin-embedded (FFPE) tissue. However, several sites used multiple platforms to validate their genotyping data, reducing potential genotyping errors across the entire data set. Importantly, *CYP2D6* genotypes obtained from blood-derived DNA reflect the patients' germ-line genotypes, known to influence endoxifen plasma concentrations. By contrast, *CYP2D6* genotypes from tumor-derived DNA may be subject to error due to somatic mutation by loss of heterozygosity, known to affect the *CYP2D6* locus at 22q13 in up to 30% of breast tumors.[20–22] Thus, when *CYP2D6* genotype is derived from tumor samples, an excess number of homozygotes may result as a consequence of loss of heterozygosity. This form of genotyping error is revealed by Hardy–Weinberg Equilibrium (HWE) testing, as was observed in the Breast

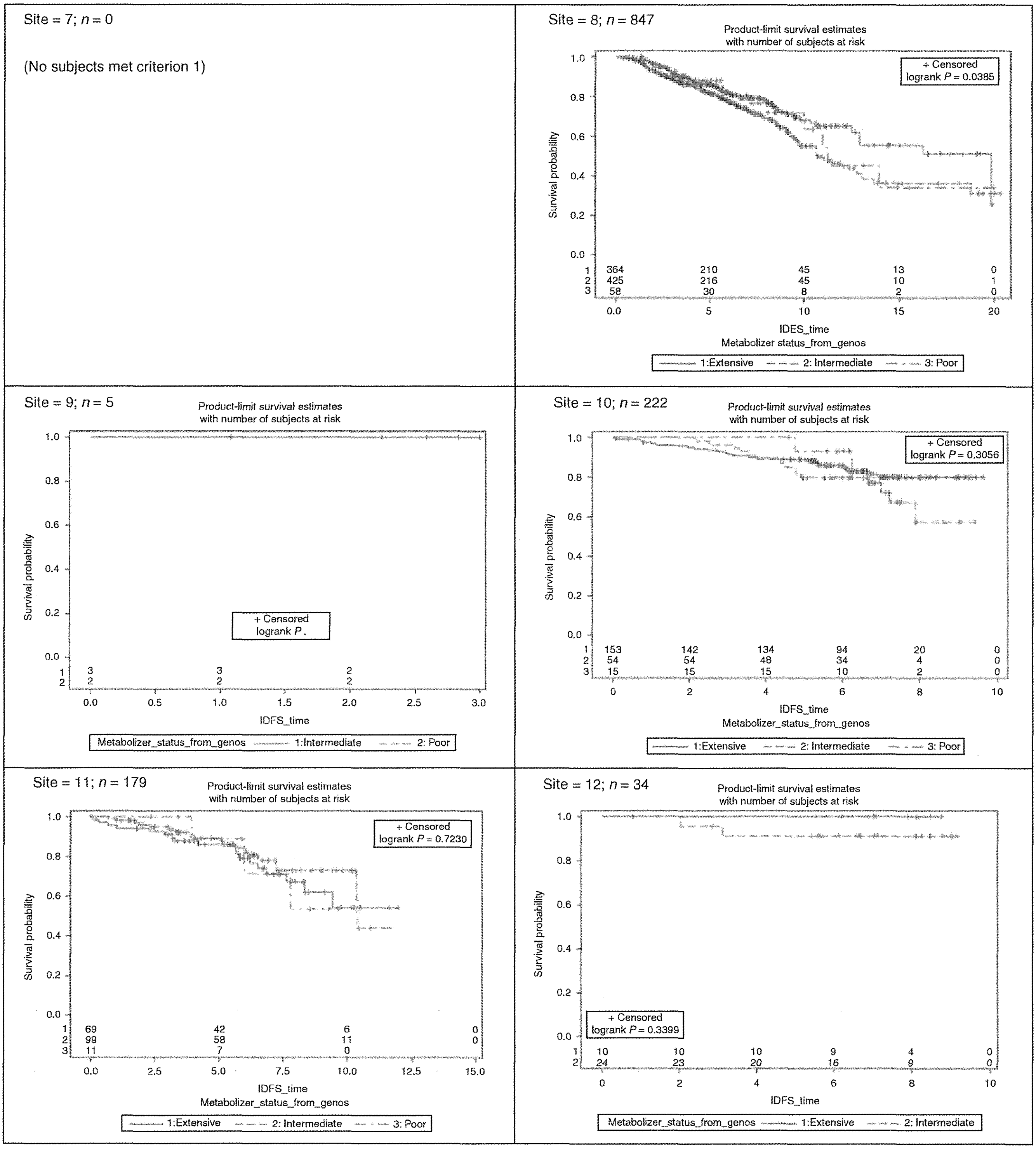

Figure 2 Continued