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P- Reviewer: Servin AL, Zhang L **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Liu XM

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ISSN 1007-9327

Lymph node shape in computed tomography imaging as a predictor for axillary lymph node metastasis in patients with breast cancer

GORO KUTOMI[1], TOUSEI OHMURA[1], FUKINO SATOMI[1], TOMOKO TAKAMARU[1], HIROAKI SHIMA[1], YASUYO SUZUKI[1], SEIKO OTOKOZAWA[2], HITOSHI ZEMBUTSU[1], MITSURU MORI[2] and KOICHI HIRATA[1]

[1]First Department of Surgery, School of Medicine, Sapporo Medical University, Sapporo, Hokkaido 060-8543; [2]Department of Public Health, School of Medicine, Sapporo Medical University, Sapporo, Hokkaido 060-8556, Japan

Received January 30, 2014; Accepted May 28, 2014

DOI: 10.3892/etm.2014.1787

Abstract. The aim of the present study was to evaluate whether preoperative computed tomography (CT) is a useful modality for the diagnosis of axillary lymph node metastasis. The axillary lymph node status was examined in patients with primary breast cancer who had undergone surgery. In total, 75 patients were analyzed with preoperative contrast CT images, following which the patients underwent an intraoperative sentinel lymph node biopsy to determine possible predictors of axillary lymph node metastasis. The lymph node shape was classified into three groups, which included fat-, clear-and obscure-types. Multivariate analysis revealed that clear-type lymph nodes in preoperative contrast CT imaging may be an independent predictor of lymph node metastasis (odds ratio, 15; P=0.003). Therefore, the results indicated that preoperative CT examination is useful to predict axillary lymph node metastasis.

Introduction

Axillary lymph node excision in breast cancer was previously the standard optimal surgical procedure for breast cancer. However, currently this procedure is not always essential since the status of axillary lymph node metastasis can be predicted by an intraoperative sentinel lymph node biopsy (SNB) (1). Despite this development, a number of institutions in Japan perform lymph node excision for cases demonstrated to be negative by intraoperative SNB. Thus, axillary lymph node dissection tends to be unnecessary, particularly in a number of patients with early stage breast cancer (2).

Axillary lymph node metastasis is a multifactorial event, and several clinicopathological factors have been reported as predictors of lymph node metastasis in breast cancer (3). However, since only a few methods exist for precisely predicting the axillary lymph node metastasis of an individual patient with breast cancer, a number of patients may not receive appropriate treatment for such metastasis.

The development of diagnostic imaging systems has facilitated the evaluation of axillary lymph node metastasis prior to surgery for breast cancer (4). Computed tomography (CT) is one of the representative modalities that can be used to evaluate the lymph node status, and is commonly used in hospitals due to its noninvasive and inexpensive characteristics. However, the number of studies investigating the clinical usefulness of CT in determining the axillary lymph node status is limited (5).

Therefore, the aim of the present retrospective study was to examine whether contrast CT imaging for the preoperative evaluation of the axillary lymph node status was a clinically useful modality.

Materials and methods

Patients. A total of 75 patients with primary breast cancer that had undergone surgical treatment at the First Department of Surgery of Sapporo Medical University (Sapporo, Japan) between 2009 and 2010 were recruited for the study. The clinical data from the Medical Records Department were retrospectively obtained. Written informed consent was required from all patients. All the patients were Japanese females that had been pathologically diagnosed with invasive ductal carcinoma without distant dissemination by whole body CT and bone scintigraphy. In this department, preoperative contrast CT is normally performed.

Data on clinical information were confirmed from the medical records of the patients and are shown in Table I. Tumor status was classified according to UCLA-integrated staging system classification with tumor, node and metastasis categories (6). The expression of the estrogen receptor or progesterone receptor was designated as positive when positive staining was observed and a total Allred score of ≥3 was achieved. Tumors that were immunohistochemically scored 2+ or 3+ and were fluorescence *in situ* hybridization-positive, were regarded as HER2-positive (7). Patients were classified into the following two groups: Group A consisted of patients

Correspondence to: Professor Koichi Hirata, First Department of Surgery, School of Medicine, Sapporo Medical University, South 1 West 16, Chuo-ku, Sapporo, Hokkaido 060-8543, Japan
E-mail: gkutomi@yahoo.co.jp

Key words: breast cancer, computed tomography, lymph node shape

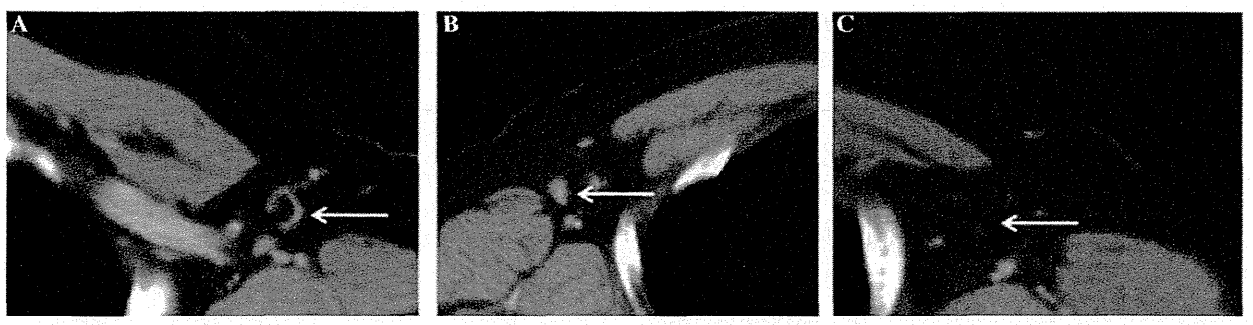

Figure 1. CT images showing (A) fat-, (B) clear-and (C) obscure-type axillary lymph nodes. CT, computed tomography.

who had been diagnosed as negative by SNB, while group B comprised patients who had been diagnosed as axillary lymph node metastasis-positive.

Evaluation of axillary lymph nodes by preoperative contrast CT. Although the axillary lymph nodes were not palpable in any patient, enhanced whole body CT (Aquilion 64; Toshiba, Tokyo, Japan) with contrast was preoperatively performed since this is the standard procedure in Japan. A helical CT unit (64-slice CT system; Light Speed VCT vision; GE Healthcare, Milwaukee, WI, USA) was used for the evaluation of the axillary lymph nodes. The patients were in a supine position and raised their arms during the CT examination. CT images of the axillary lymph nodes were obtained as 2-mm slices through the axilla. The most caudally located enhanced lymph nodes were considered to be the sentinel lymph nodes. Lymph node size and shape were evaluated, as well as the Hounsfield units (HU) of the axillary lymph nodes in the CT images. The average of the region of interest (ROI) was used to evaluate the HU as a CT score. Lymph node shapes were classified into three groups, according to a previous study (8). Nodes with an internal fat concentration were classified as the fat-type (Fig. 1A), those with a size of ≥10 mm that appeared as rounded nodes without any internal fat were classified as the clear-type (Fig. 1B), while the nodes with unclear borders were classified as the obscure-type (Fig. 1C).

SNB. Prior to the initiation of surgery, 3-5 ml indigo carmine was injected into the peritumor, as well as subcutaneous and intradermal portions of the areola. Sentinel lymph nodes were located following massaging the expected area for 2-3 min. All the sentinel lymph nodes identified were sliced into 2-mm sections and stained with hematoxylin and eosin. A surgeon conducted the SNB, while a pathologist evaluated the specimens during the surgery. Finally, SNB specimens were embedded in paraffin and evaluated.

Statistical analysis. Analysis of the continuous variables, including age, tumor size, lymph node size and the CT score, was conducted with the t-test, whereas the χ^2 test was applied for the categorical variables (Table I). For the logistic regression analysis, odds ratios and 95% confidence intervals (CIs) were calculated following adjustment for age. All the statistical analyses and corresponding P-values were two-sided, and $P<0.05$ was considered to indicate a statistically significant difference. All statistical calculations were performed using JMP version 9.0 software (SAS Institute, Cary, NC, USA).

Table I. Clinical characteristics of the 75 patients with breast cancer.

Characteristics	Patients
Mean age, years (range)	
Total (n=75)	56 (35-84)
Pre-menopause (n=28)	54 (32-60)
Post-menopause (n=47)	60 (40-82)
pT[a], n (%)	
pTis	14 (18.7)
pT1	23 (30.6)
pT2	38 (50.7)
HR status, n (%)	
ER(+), PgR(+)	40 (53.4)
ER(+), PgR(-)	19 (25.3)
ER(-), PgR(+)	7 (9.3)
ER(-), PgR(-)	9 (12.0)
HER2 status, n (%)	
Positive	11 (14.7)
Negative	64 (85.3)
pN[a], n (%)	
pN0	56 (74.7)
pN1	19 (25.3)
pN2	0 (0)
Surgery, n (%)	
Breast-conserving	28 (37.3)
Mastectomy	47 (62.7)

[a]UCLA-integrated staging system classification with tumor, node and metastasis categories (2002). HR, hormone receptor; ER, estrogen receptor; PgR, progesterone receptor.

Results

Characteristics of the patients. A total of 75 patients who had received adequate treatment for primary breast cancer were

Table II. Differences in the distributions of possible predictors for positive SNB.

Characteristics	Group A (n=56)	Group B (n=19)	P-value
Menopause (pre/post), n	17/39	11/08	0.034
Tumor size[b], cm	1.55±0.15	2.19±0.26	0.034
Axillary lymph node size[b], cm	0.56±0.05	0.92±0.09	0.0007
Axillary lymph node shape in contrast CT (fat/clear/obscure), n	17/08/31	2/14/3	<0.0001
CT score (ROI)[a,b]	0.16±21.6	31.4±31.9	<0.0001

[a]Average of the ROI. [b]Results are expressed as the mean ± standard deviation. SNB, sentinel lymph node biopsy; CT, computed tomography; ROI, region of interest.

Table III. Univariate and multivariate analyses of the predictors of SNB.

	Univariate analysis			Multivariate analysis		
Predictors	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Tumor size (≥2 cm, <2 cm)	0.84	0.29-2.39	0.74	0.45	0.10-1.8	0.26
Lymph node size (≥0.5, <0.5)	0.12	0.0062-0.64	0.01	0.16	0.0071-1.6	0.12
Shape						
Obscure	0.15	0.040-0.58	0.006	0.30	0.056-1.6	0.15
Clear	17	4.7-60	<0.001	15	2.5-89	0.003
Fat	0.27	0.56-1.3	0.102	0.16	0.025-1.1	0.06
CT score (ROI[a]; ≥0, <0)	0.22	0.047-0.74	0.013	0.95	0.15-6.0	0.95

[a]Average of the ROI. Values in brackets are the optimal cut-off point defined using a receiver operating characteristic curve. CI, confidence interval; SNB, sentinel lymph node biopsy; CT, computed tomography; ROI, region of interest.

analyzed in the study (Table I). A mastectomy was performed for 61% of the population.

Patients were classified into the following two groups according to the histological diagnosis from the SNB. Group A (n=56) patients were diagnosed as axillary lymph node metastasis-negative by SNB, while group B (n=19) patients were diagnosed as axillary lymph node metastasis-positive.

Difference in the distributions of the possible predictors of axillary lymph node metastasis. Differences in the menopausal status, histological type, tumor size, axillary lymph node size, axillary lymph node shape in contrast CT and CT scores (the average of the ROI) were analyzed between groups A and B (Table II). The menopausal status, tumor size, axillary lymph node size, axillary lymph node shape and CT score exhibited statistically significant differences when comparing the two groups (Table II). In addition, the ratio of the premenopausal group was higher in group B compared with group A (P=0.034), and the primary tumor size, axillary lymph node size and CT score (ROI) were larger in group B compared with group A (P=0.034, P=0.0007 and P<0.0001, respectively). Furthermore, of the 56 patients in group A, fat-, clear- and obscure-type lymph nodes were observed in 17 (30.4%), 8 (14.3%) and 31 cases (55.3%), respectively. By contrast, fat-, clear- and obscure-type lymph nodes were identified in two (10.5%), 14 (73.7%) and three cases (15.8%) in group B, respectively, indicating that there were statistically significant differences (P<0.0001) in the distribution of the lymph node shapes in preoperative contrast CT between the two groups (Table II).

Identification of the predictors for axillary lymph node metastasis. To identify the risk factors for axillary lymph node metastasis, logistic regression analysis of the menopausal status, tumor size, axillary lymph node size, axillary lymph node shape and CT score was conducted since the aforementioned predictors significantly differed between the groups (Table III). In univariate analysis, the menopausal status, axillary lymph node size, obscure-type lymph nodes, clear-type lymph nodes and the CT score were demonstrated to be predictors of lymph node metastasis (P=0.036, P=0.01, P=0.006, P<0.001 and P=0.013, respectively, with 95% CIs of 0.11-0.93, 0.0062-0.64, 0.04-0.58, 4.7-60 and 0.15-6.0, respectively). In addition, with regard to the multivariate analysis, clear-type axillary lymph nodes were shown to be significantly associated with axillary lymph node metastasis following adjustment for the menopausal status, axillary lymph node size, obscure-type lymph nodes and the CT

score (P=0.003; 95% CI, 2.5-89; Table III), indicating that the axillary lymph node shape in preoperative contrast CT imaging was an independent indicator of axillary lymph node metastasis (SNB-positive).

Discussion

Lymph node metastasis is an important factor that affects the prognosis and management of patients with breast cancer (9). Although the axillary lymph nodes should be dissected for patients who are considered to be axillary lymph node-positive, lymph node dissection often causes complications, including arm edema, motor disturbance of the arm and axillary numbness (10-12). Therefore, axillary lymph node dissection should be performed only following consideration of whether the procedure is essential in each patient with breast cancer. In the present study, to identify preoperative predictors for axillary lymph node metastasis, the association of possible predictors and preoperative contrast CT observations were investigated with axillary lymph node metastasis. Axillary lymph node shape in preoperative contrast CT imaging was found to be an independent predictor of metastasis. As shown in Table III, multivariate analysis indicated that clear-type axillary lymph nodes in contrast CT were likely to be a predictor of metastasis (odds ratio, 15; P=0.003; 95% CI, 2.5-89). Although soybean-shaped lymph nodes have been reported to be significantly metastatic and 'C'-shaped and ring-like lymph nodes are more likely to be nonmetastatic in contrast-enhanced CT imaging (8), the clear- and fat-type lymph nodes defined in the present study were demonstrated to correspond to the former and latter, respectively. The pathological association between the lymph node shape in contrast CT and the localization of cancer cells in lymph nodes has not yet been established. Thus, further clinicopathological investigations may clarify how the localization of cancer cells in lymph nodes influences their imaging or shape in contrast CT.

Tumor size has been reported to be one of the main predictors of axillary lymph node metastasis in several studies (13-16). Although statistically significant differences were observed in the distribution of tumor size between groups A and B (Table II), tumor size was not found to be an independent predictor for axillary lymph node metastasis in the present study (Table III). However, future studies with larger sample sizes are required to validate the association between tumor size and lymph node metastasis, since 50% of the tumors in the present study were small (<20 mm). SNB has become a standard procedure, and preoperative evaluation of the axillary lymph nodes based on imaging modalities is considered to be important for selecting appropriate breast cancer treatment (16,17). Several diagnostic imaging modalities have been used for the preoperative diagnosis of the sentinel lymph node status. Ultrasonography, magnetic resonance imaging and multidetector CT have been reported to be useful imaging systems to preoperatively evaluate the lymph node status (18-20).

Lymph node size was also shown to be associated with lymph node metastasis through univariate analysis; however, lymph node size is unlikely to be an independent predictor according to the results from the multivariate analysis (Table III). In the present study, univariate analysis demonstrated that the CT score (ROI) was a predictor of lymph node metastasis, indicating that high contrast lymph nodes on CT images, which may be a consequence of vessel development in the lymph nodes, may be associated with metastasis (Table III). These observations indicate that the evaluation of the lymph node status by preoperative contrast CT may support the intraoperative diagnosis by SNB.

In Japan, CT examinations are indispensable for the preoperative metastatic search, and are conducted in all institutions. CT is also considered to be very important for preoperative sentinel lymph node examination. The results of the present study indicate that preoperative CT examinations are useful in predicting axillary lymph node metastasis, and can provide supportive information for intraoperative sentinel lymph node diagnosis. Although further large-scale studies are required to validate these results, the observations of the present study provide useful information for identifying predictors of axillary lymph node metastasis, and may aid surgeons to determine appropriate surgical strategies for individual patients with breast cancer.

Acknowledgements

The study was supported by a grant from the Yuasa Memorial Foundation. The authors thank all the study participants.

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Int J Clin Oncol
DOI 10.1007/s10147-014-0725-z

ORIGINAL ARTICLE

A phase I study of combination therapy with nanoparticle albumin-bound paclitaxel and cyclophosphamide in patients with metastatic or recurrent breast cancer

Goro Kutomi · Tousei Ohmura · Fukino Satomi · Hideki Maeda · Hiroaki Shima · Hidekazu Kameshima · Minoru Okazaki · Hideji Masuoka · Kenichi Sasaki · Koichi Hirata

Received: 26 March 2014 / Accepted: 17 June 2014

Abstract
Background The objective of the present clinical study is to determine the maximum tolerated dose (MTD)/recommended dose (RD) of combination therapy with nanoparticle albumin-bound paclitaxel (nab-PTX) and cyclophosphamide (CPA) in patients with metastatic or recurrent breast cancer.
Methods nab-PTX and CPA were administered on the first day of each 21-day treatment cycle. The dose of CPA was fixed at 600 mg/m^2, while the dose of nab-PTX was increased from 180 mg/m^2 (Level 1) to 220 mg/m^2 (Level 2) and then to 260 mg/m^2 (Level 3).
Results A total of 11 patients from two institutions were enrolled in the present study. At Level 3, a dose-limiting toxicity (DLT) was observed in 1 patient. Considering treatment continuity and the risk of adverse events in Cycle 2 and thereafter at this level, further subject enrollment at Level 3 was discontinued after two patients had been enrolled.
Since the doses used at Level 3 were considered the MTD of nab-PTX and CPA and the doses used at Level 2 were considered the RD of nab-PTX and CPA, three additional subjects were enrolled at Level 2. No DLTs were observed at Level 2.
Conclusion The RD of combination therapy with nab-PTX and CPA was 220 mg/m^2 and 600 mg/m^2, respectively, in patients with metastatic or recurrent breast cancer.

Keywords Breast cancer · nab-paclitaxel · Cyclophosphamide · Phase I

G. Kutomi (✉) · F. Satomi · H. Maeda · H. Shima · K. Hirata
Department of Surgery, Surgical Oncology and Science, School of Medicine, Sapporo Medical University, Minami 1-Jo, Nishi 17-Chome, Chuo-ku, Sapporo, Hokkaido 060-8556, Japan
e-mail: kutomi@sapmed.ac.jp

T. Ohmura · H. Kameshima
Department of Surgery, Higashi-Sapporo Hospital, Sapporo, Japan

M. Okazaki
Sapporo Breast Surgical Clinic, Sapporo, Japan

H. Masuoka
Sapporo-Kotoni Breast Clinic, Sapporo, Japan

K. Sasaki
Department of Surgery, Muroran City General Hospital, Muroran, Japan

Introduction

Since metastatic/recurrent breast cancer is difficult to cure using existing medications, treatment objectives are to prolong patient survival and to improve patient quality of life (QOL) [1]. The 2013 Japanese Breast Cancer Guidelines recommends anthracycline- or taxane-based regimens as playing a central role in chemotherapy for metastatic/recurrent breast cancer [2]. As chemotherapy for primary breast cancer, anthracycline- or taxane-based regimens have also played a central role; however, anthracycline has been reported to cause cardiotoxicity and other unfavorable adverse events such as secondary leukemia [3]. Thus, regimens not containing anthracycline have recently been explored.

Docetaxel (DTX)/cyclophosphamide (CPA) combination therapy (hereinafter referred to as TC therapy) is a standard regimen not containing anthracycline. Basically, a synergetic effect is observed when DTX and CPA are used

Published online: 30 July 2014

in combination [4], and favorable results (i.e., response rate of 65 %, mean survival time [MST] of 22 months, and time to progression [TTP] of 6 months) were obtained from the previous phase II study of this TC therapy in metastatic/recurrent breast cancer [5]. In addition, a phase III study was conducted to compare the efficacy of postoperative TC therapy with that of postoperative doxorubicin/CPA combination therapy (hereinafter referred to as AC therapy) in patients with primary breast cancer and, as a result, TC therapy was confirmed to be superior to AC therapy in terms of 5-year disease-free survival (DFS). Consequently, TC therapy has been widely used in the clinical setting [6].

Nanoparticle albumin-bound paclitaxel (nab-PTX) is a novel nanoparticle formulation of paclitaxel (with a new dosage) bound to human serum albumin (approximately 130 nm in diameter), which does not contain absolute ethanol or any solubilizing agents [7]. Therefore, nab-PTX overcomes the aforementioned disadvantages of existing paclitaxel products, and may therefore serve as a first-line taxane.

Our hospitals and clinics are currently using TC therapy as the key postoperative chemotherapy for metastatic/recurrent breast cancer or primary breast cancer. With the aforementioned advantage of nab-PTX taken into consideration, the replacement of DTX in existing TC therapy with nab-PTX is expected to improve treatment efficacy and, at the same time, such an improvement is considered greatly beneficial to a patient's QOL. Therefore, the aim of the present phase I study was to determine the maximum tolerated dose (MTD)/recommended dose (RD) of combination therapy with nab-PTX and CPA in patients with metastatic or recurrent breast cancer.

Patients and methods

Eligibility

The eligibility criteria were (1) patients for whom invasive breast cancer was histologically confirmed; (2) patients for whom metastatic/recurrent breast cancer was clinically confirmed; (3) patients with no history of chemotherapy for their metastatic/recurrent breast cancer, or patients with a history of one regimen of preoperative or postoperative chemotherapy (for their metastatic/recurrent breast cancer) which was completed at least 6 months before participation in the present study; (4) patients for whom an immunohistochemistry (IHC) assay or a fluorescence in situ hybridization (FISH) assay did not show an overexpression of human epidermal growth factor receptor-2 (HER2) (i.e., IHC <30 % or FISH <2.2); (5) patients with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1; (6) patients with no clinically significant electrocardiographic abnormalities; (7) patients for whom each laboratory test value within 21 days before enrollment was within the following ranges (white blood cell count $\geq$4,000 cells/mm^3, neutrophil count $\geq$2,000 cells/mm^3, hemoglobin level $\geq$9.0 g/dL, platelet count $\geq$100,000 cells/mm^3, total bilirubin $\leq$1.5 mg/dL, AST/ALT $\leq$150 IU/L, and creatinine $\leq$1.5 mg/dL; (8) patients aged $\geq$20 years; (9) patients whose survival was expected to be at least 3 months from the start of treatment; and (10) patients who submitted their own written consent.

Study design

The present clinical study was an open-label phase I study conducted with the aim of determining the MTD/RD of combination therapy with nab-PTX and CPA in patients with metastatic or recurrent breast cancer.

The drugs were administered according to the protocol regimen described below. On day 1, nab-PTX was dissolved in physiological saline (100 mg/20 mL) and the necessary volume per body surface area was infused intravenously to each patient over 30 min. Then (on the same day), CPA was dissolved in 500 mL of physiological saline and the necessary volume per body surface area was infused intravenously to each patient. The protocol regimen was to be repeated in a 21-day cycle until obvious signs of disease progression or adverse events that would preclude continuation of the treatment were observed.

Blood biochemistry was performed on days 1, 8, and 15 of Cycle 1, and on day 1 of the subsequent cycles. Signs/symptoms and adverse events were also observed.

The present study was conducted after being approved by the Ethics Committee for Clinical Studies at each participating hospital or clinic, and is registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000009046).

Dose escalation

The dose of CPA was fixed at 600 mg/m^2, while the dose of nab-PTX was increased from 180 mg/m^2 (Level 1) to 220 mg/m^2 (Level 2) and then to 260 mg/m^2 (Level 3) (Table 1). The present phase I study was conducted using a '3 + 3 phase-I design' which only enabled a shift to the next level (i.e., increase in the dose of nab-PTX to the dose

Table 1 Dose levels of the regimen

Dose levels	nab-PTX (mg/m^2)	CPA (mg/m^2)
Level 1	180	600
Level 2	220	600
Level 3	260	600

for the next level) when the incidence of dose-limiting toxicities (DLTs) was ≤33 %.

Definition of DLT

A DLT was defined as any of the following symptoms occurring during Cycle 1 of the protocol regimen—(1) grade 3 or 4 thrombocytopenia, which required platelet transfusion; (2) grade 4 neutropenia, which persisted for at least 4 days; (3) grade 3 or higher febrile neutropenia; (4) grade 3 or higher non-hematologic toxicity (excluding nausea/vomiting); and (5) other adverse events that led to at least a 21-day delay in the start of Cycle 2.

Adverse events were assessed based on the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0).

Defnition of MTD and RD

Based on the occurrence of DLTs during Cycle 1 of the protocol regimen, the MTD/RD of nab-PTX and CPA was determined. In principle, the RD was defined as a dose one level lower than the MTD; however, considering the treatment continuation rate of patients in Cycle 2 or thereafter, the RD of nab-PTX and CPA was to be determined through a discussion with the Data and Safety Monitoring Committee. At the level corresponding to the estimated RD, 3 additional patients were to be enrolled and were to receive the protocol regimen for further evaluation of safety.

Results

Patient demographics

A total of 11 patients were enrolled in the present study from November 2012 to June 2013. Table 2 shows the baseline characteristics of all 11 patients enrolled. Ultimately, 3 patients were enrolled at Level 1, 6 patients were enrolled at Level 2, and 2 patients were enrolled at Level 3. For all enrolled patients, the median age was 59 years (range 51–65 years) and the ECOG PS was 0. Of the 11 patients with metastatic/recurrent breast cancer, 3 (27.3 %) had undergone chemotherapy, with 3 (27.3 %) being treated with hormones, 6 (54.5 %) with taxanes, and 7 (63.6 %) with anthracyclines.

Table 2 Patient demographics and tumor characteristics

Dose levels	Level 1	Level 2	Level 3	All
No. of patients (*n*)	3	6	2	11
Age (median)	54	61	55	59
Menopausal status				
Pre	0	1	0	1
Post	3	5	2	10
ECOG PS				
0	3	6	2	11
1	0	0	0	0
ER, PgR status				
ER +, PgR+	1	4	0	5
ER +, PgR−	0	1	1	2
ER −, PgR +	0	0	0	0
ER −, PgR−	2	1	1	4
Metastatic site				
Lung	2	2	0	4
Liver	1	1	1	3
Bone	0	1	0	1
Lymph	0	0	2	2
No. of prior metastatic regimens				
0	2	4	2	8
1	1	2	0	3
Prior endocrine therapy				
−	3	3	2	8
+	0	3	0	3
Prior taxane therapy				
−	1	3	1	5
+	2	3	1	6
Prior anthracycline therapy				
−	0	3	1	4
+	3	3	1	7

Toxicity

The median number of cycles the 11 patients underwent was 5 (range 2–6). In the 11 patients, toxicities were investigated during Cycle 1. Table 3 lists hematologic toxicities observed during Cycle 1, while Table 4 lists non-hematologic toxicities observed during Cycle 1. The major hematologic toxicities observed were leukopenia and neutropenia, and the major non-hematologic toxicities observed were peripheral neuropathy and myalgia. Grade 3 or higher leukopenia was observed in 7 out of the 11 patients (63.6 %), and grade 3 or higher neutropenia was observed in 3 out of the 11 patients (27.3 %); the latter included grade 4 neutropenia (which corresponds to a DLT) in 1 patient at Level 3. The incidence of non-hematologic toxicities at all grades was 63.6 % (7/11) for peripheral neuropathy and 36.4 % (4/11) for myalgia; the former included grade 3 peripheral neuropathy (which corresponds to a DLT) in 1 patient at Level 3.

Therefore, based on the occurrence of DLTs at Level 3 and in consideration of treatment continuity in Cycle 2 or thereafter at Level 3, the Data and Safety Monitoring Committee decided to discontinue further subject

Table 3 Hematologic toxicities (first cycle)

Dose levels	Level 1			Level 2			Level 3			All		
No. of patients (*n*)	3			6			2			11		
CTCAE grade	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Leukopenia	1	1	0	0	5	0	1	0	1	2	6	1
Neutropenia	3	0	0	3	2	0	1	0	1	7	2	1
Anemia	1	0	0	0	1	0	0	0	0	1	1	0
AST increase	1	0	0	0	0	0	0	0	0	1	0	0
ALT increase	1	0	0	0	0	0	0	0	0	1	0	0

Table 4 Non-hematologic toxicities (first cycle)

Dose levels	Level 1			Level 2			Level 3			All		
No. of patients (*n*)	3			6			2			11		
CTCAE grade	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Sensory neuropathy	1	0	0	4	0	0	1	1	0	6	1	0
AST increase	1	0	0	0	0	0	0	0	0	1	0	0
ALT increase	1	0	0	0	0	0	0	0	0	1	0	0
Myalgia	1	0	0	2	0	0	1	0	0	4	0	0
Sensory neuropathy	1	0	0	4	0	0	1	1	0	6	1	0
Myalgia	1	0	0	2	0	0	1	0	0	4	0	0
Arthralgia	1	0	0	1	0	0	0	0	0	2	0	0
Nausea	0	0	0	1	0	0	0	0	0	1	0	0
Fatigue	0	0	0	1	0	0	0	0	0	1	0	0
Anorexia	0	0	0	1	0	0	0	0	0	1	0	0
Gastrointestinal pain	0	0	0	1	0	0	0	0	0	1	0	0

Table 5 Best overall response in patients having measurable lesions

Dose level	Level 1	Level 2	Level 3	All
Number of evaluable patients (*n*)	2	5	1	8
Complete response	0	0	0	0
Partial response	0	2	1	3
Stable disease	0	2	0	2
Progressive disease	2	1	0	3

enrollment at Level 3 after two patients had been enrolled. The doses used at Level 3 were then considered the MTD of nab-PTX and CPA while the doses used at Level 2 were considered the RD of nab-PTX and CPA. At Level 2 (corresponds to the estimated RD), 3 additional patients were enrolled and safety evaluation was performed in a total of 6 patients. No DLTs were observed at Level 2.

Efficacy

In accordance with the eligibility criteria, patients were not required to have measurable lesions; therefore, the efficacy of the combination therapy was not evaluated in the 11 patients and was evaluated in 8 patients who had measurable lesions. Complete response was not seen in any patient, with partial response in 3, stable disease in 2, and progressive disease in 3 patients. Response rates to the combination therapy was seen in 3 patients (37.5 %), and the usefulness of the combination therapy was seen in 5 patients (62.5 %) (Table 5).

Discussion

In the USA and Europe, a phase I study of nab-PTX was conducted in 1998 [8] and a phase II study of nab-PTX in patients with metastatic breast cancer was conducted in 1999 [9]. In 2001, a phase III study (Study CA-012) to compare the efficacy of nab-PTX (260 mg/m^2 once every 3 weeks [q3w]) with that of standard paclitaxel (175 mg/m^2 q3w) in patients with metastatic/recurrent breast cancer was conducted [10]. In this phase III study, the response rates for target lesions were found to be 24.0 % in patients receiving nab-PTX (300 mg/m^2 q3w) and 11.1 % in

patients receiving standard paclitaxel (175 mg/m^2 q3w), demonstrating non-inferiority and superiority of nab-PTX to standard paclitaxel. Similarly, a randomized phase II study in patients with metastatic/recurrent breast cancer (Study CA-024) was conducted to compare the treatment efficacy among three nab-PTX dose groups (i.e., 300 mg/m^2 q3w, 100 mg/m^2 once every week [qw], and 150 mg/m^2 qw) and one DTX dose group (i.e., 100 mg/m^2 q3w) [11]. As a result, the response rates observed were 37, 45, 49, and 35 % for the nab-PTX 300 mg/m^2 q3w, nab-PTX 100 mg/m^2 qw, nab-PTX 150 mg/m^2 qw, and DTX 100 mg/m^2 q3w groups, respectively. The progression-free-survival (PFS) was 11, 12.8, 12.9, and 7.5 months for the nab-PTX 300 mg/m^2 q3w, nab-PTX 100 mg/m^2 qw, nab-PTX 150 mg/m^2 qw, and DTX 100 mg/m^2 q3w groups, respectively. These findings demonstrated the comparable efficacy of nab-PTX with that of DTX, irrespective of different regimens.

The higher efficacy observed with nab-PTX compared to existing paclitaxel products can be attributed to the higher probability that nab-PTX reaches and penetrates into the tumor, which was demonstrated in basic experiments [12]. The following three reasons can be considered when attempting to explain the higher intratumor concentration of nab-PTX—(a) paclitaxel is captured by Cremophor®-EL micelles originating from Taxol® in plasma, which reduces the bioavailability of paclitaxel [13]; (b) transport of nab-PTX through the epithelium is facilitated by the gp-60 albumin receptor [14]; and (c) accumulation of nab-PTX is enhanced by the action of albumin-binding secreted protein acidic and rich in cysteine (SPARC) [15].

The present phase I study demonstrated that the combination of nab-PTX (220 mg/m^2 q3w) with CPA (600 mg/m^2 q3w) would be a safe chemotherapy regimen for metastatic/recurrent breast cancer. From the previous pilot study of nab-PTX/CPA combination therapy in patients with early stage breast cancer, a high tolerability of the two drugs was reported [16], although the nab-PTX regimen used in this pilot study (i.e., 100 mg/m^2 qw) differs from that used in the present phase I study. In the previous pilot study in patients with early stage breast cancer, grade 3 or 4 neutropenia was observed in 53 % of the patients; however, only 1 episode of febrile neutropenia occurred during a total of 249 treatment cycles. In the present phase I study, grade 3 or higher leukopenia was observed in 7 out of the 11 patients (63.6 %), grade 3 or higher neutropenia was observed in 3 out of the 11 patients (27.3 %); the latter included grade 4 neutropenia (which corresponds to a DLT) in 1 patient at Level 3. No febrile neutropenia was reported in the present study. However, since these adverse events were only collected from Cycle 1 of the protocol regimen, it is considered necessary to further examine the long-term safety of the protocol regimen in a phase II study.

Because patients were not required to have measurable lesions, it was difficult to evaluate the efficacy of the combination therapy. However, the response to the combination therapy was seen in 3 (37.5 %) of the 8 patients evaluated, and the usefulness of the combination therapy was seen in 5 (62.5 %) of the 8 patients. Furthermore, the disappearance of pleural effusion and a marked reduction of liver metastases were seen. Thus, it is considered that the combination therapy was effective in treatment for metastatic/recurrent breast cancer.

From these findings, we conclude that the present nab-PTX/CPA combination therapy is effective in treating metastatic/recurrent breast cancer. Therefore, we plan to further implement a phase II study by setting response rate as the primary efficacy endpoint, where the two drugs will be used at doses corresponding to the RD (i.e., 220 mg/m^2 for nab-PTX and 600 mg/m^2 for CPA) determined from the present phase I study.

Conflict of interest All authors declare no conflicts of interest.

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Review

Pharmacogenomics

Pharmacogenomics toward personalized tamoxifen therapy for breast cancer

Hitoshi Zembutsu
Division of Genetics, National Cancer Center, Research Institute 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
Tel.: +81 3 3542 2511
Fax: +81 3 6737 1221
hzenbuts@ncc.go.jp

Tamoxifen has been used not only for the treatment or prevention of recurrence in patients with estrogen receptor positive breast cancers but also for recurrent breast cancer. Because CYP2D6 is known to be an important enzyme responsible for the generation of the potent tamoxifen metabolite, 'endoxifen', lots of studies reported that genetic variation which reduced its enzyme activity were associated with poor clinical outcome of breast cancer patients treated with tamoxifen. However, there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy. Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes provides the evidence that dose adjustment is useful for the patients carrying reduced or null allele of *CYP2D6* to maintain the effective endoxifen level. This review describes critical issues in pharmacogenomic studies as well as summarizes the results of the association of *CYP2D6* genotype with tamoxifen efficacy.

Keywords: ABCC2 • C10orf11 • CYP2D6 • dose adjustment • endoxifen • pharmacogenomics • tamoxifen

Tamoxifen has been widely used for the treatment of patients with estrogen receptor (ER) positive breast cancer mainly in adjuvant setting. The clinical benefit of this drug for the treatment of ER-positive early breast cancer is evident because recurrence and mortality rates are reduced by this drug [1]. Tamoxifen therapy for 5 years was reported to improve the risk of its relapse at least for 15 years, particularly ER-positive invasive tumors in premenopausal women [1]. However, in the result of Adjuvant Tamoxifen Longer Against the Shorter (ATLAS), the risk of recurrence during years 5–14 was greater than 20% in the patients treated with adjuvant tamoxifen therapy [2]. Although results of many studies are accumulated, the mechanisms underlying the resistance to this drug in a subset of the patients are not fully identified. Tamoxifen is, in a sense, a prodrug that requires metabolic activation to induce pharmacological activity. 4-hydroxytamoxifen and endoxifen (4-hydroxy-N-desmethyltamoxifen), which are representative metabolites of tamoxifen, are reported to be active therapeutic moieties [3]. These two metabolites have 100-fold greater affinity to ER and 30- to 100-fold greater potency in inhibiting estrogen-dependent cell growth compared with tamoxifen [3–5]. The differences in the formation of these active metabolites could affect the interindividual variability in the response to tamoxifen. CYP2D6 is well known to be one of the important enzymes for the generation of 4-hydroxytamoxifen and endoxifen. Lots of genetic polymorphisms of *CYP2D6* including alleles that alter the function and/or amount of the gene product have been reported [6]. CYP2D6 is usually classified into three groups according to the phenotypes: poor metabolizers (PMs), intermediate metabolizers (IMs) and extensive metabolizers (EMs). Patients with two null alleles are classified as PMs of drugs that are metabolized by CYP2D6 [7–9]. The major null alleles that cause the PMs in Cau-

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casians are *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6*, and account for nearly 95% of the PMs [10]. Patients classified into PMs have been reported to have lower plasma levels of endoxifen and poorer clinical outcomes when treated with tamoxifen [11]. On the other hand, although the frequency of PMs in Asians is much lower (<1%), the *CYP2D6*10* allele, which causes amino acid substitutions and reduce the enzymatic activity, has been observed in 40–50% of Asians [12,13]. The influence of *CYP2D6*10* to clinical outcome after tamoxifen therapy in adjuvant setting has been investigated [12]. Several research groups reported that the association of genetic polymorphisms of *CYP2D6* with clinical efficacy of tamoxifen [14–17], indicating the association with the plasma concentrations of the active metabolites [14,18]. Genotype-guided dose-adjustment studies for tamoxifen, which showed steady-state plasma concentrations of tamoxifen and its metabolites, have been reported to investigate the optimal dosage for each patient with breast cancer [9,19,20]. However, some studies showed negative results for the association between *CYP2D6* genotype and response to tamoxifen [21–23]. There may be several reasons for these discrepant results showing the positive and negative associations. The mechanism which causes interindividual differences in responsiveness to tamoxifen is not yet fully clarified even if the effects of genetic variation of *CYP2D6* were considered. This review comments on several factors that may have influenced the conclusions of CYP2D6–tamoxifen association studies. This process may inform some general interpretive rules around the literature of the association between *CYP2D6* genotype and tamoxifen responsiveness [24].

Metabolic pathway of tamoxifen

Tamoxifen has been known to be metabolized in liver and gut wall in humans to primary and secondary metabolites that exhibit a range of pharmacologic activity [25,26]. Therefore, the differences in systemic exposure of the active metabolites should contribute to the variable response of tamoxifen observed in patients with breast cancer [27]. Tamoxifen can be considered a prodrug because the parent drug itself does not have strong affinity for the ER but undergoes extensive biotransformation into active and inactive metabolites. CYP450 enzymes such as CYP2C9, CYP2C19, CYP2D6, CYP2B6 and CYP3A are active in this metabolic process (Figure 1). 4-hydroxytamoxifen (4-OH tamoxifen) had been reported to play an important role as active metabolite of tamoxifen. It has high affinity for ERs and 30- to 100-fold greater potency than tamoxifen in inhibiting estrogen-dependent breast cancer cell proliferation [26,28]. However, another metabolite of tamoxifen, endoxifen (4-hydroxy-N-desmethyltamoxifen), was identified (Figure 1) [29]. In the course of the metabolism of tamoxifen to 4-OH tamoxifen by multiple enzymes, endoxifen is formed preponderantly by the CYP2D6-mediated oxidation of N-desmethyl tamoxifen, which is N-desmethylated by the CYP3A enzyme (Figure 1) [30]. Endoxifen was reported in the 1980s in humans [29], however, its role had remained unknown. Endoxifen, which has high affinity for ERs and greater potency than tamoxifen, reaches greater than sixfold higher plasma concentrations than 4-OH tamoxifen in patients taking tamoxifen [26]. The hydroxylated metabolites undergo conjugation by sulfotransferases (SULTs) and/or uridine diphosphate glucuronosyltransferases (UGTs) (Figure 1) [31–35].

Association of *CYP2D6* genotype with its enzyme activity

The *CYP2D6* gene, which is located on chromosome 22, has many genetic variations with greater than 80 major alleles currently known [36]. A subset of the variations affect the gene product and result in wide interindividual and ethnic differences in CYP2D6 activity (Table 1) [6,36]. CYP2D6 plays a key role in the biotransformation of many drugs including selective serotonin reuptake inhibitors, antidepressants, antiarrythmics and neuroleptics [37]. Some of these alleles are associated with decreased enzyme activity (e.g., **9*, **10*, **17*, **41*) or null enzyme activity (e.g., **3*, **4*, **5*, **6*) as shown in Table 1. Large interindividual and ethnic variability in the metabolism of drugs by CYP2D6 suggests the genetic polymorphisms affecting the enzyme activity and gene expression [38]. The concentrations of endoxifen could vary significantly in patients treated with tamoxifen due to *CYP2D6* genetic variation [14,27]. An *in vitro* study, in which breast cancer cells are exposed to clinically equivalent concentrations of tamoxifen and its metabolites, reported that ER-α degradation and transcription caused by endoxifen was concentration dependent, with minimal effect at lower endoxifen concentrations observed in PMs, but significantly greater effects occurring at concentrations observed in IMs and EMs [22,39]. These data are considered to support the theory that endoxifen is one of the most important tamoxifen metabolites [18]. Moreover, a pharmacogenomic study reported that endoxifen concentration varied according to the number of functional *CYP2D6* alleles [40].

Effect of CYP2D6 inhibitors on the response to tamoxifen

Medical drugs, which are metabolized by CYP2D6, also affect plasma endoxifen level. Concomitant CYP2D6 inhibitor use during tamoxifen therapy has

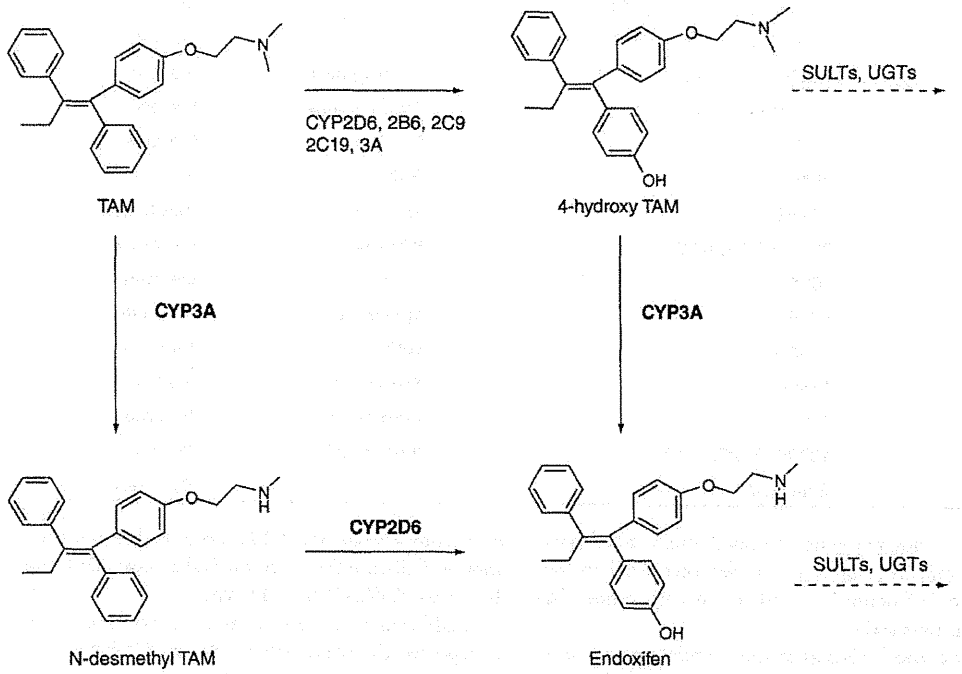

Figure 1. Metabolism of tamoxifen by the CYP450 system. The hydroxylated metabolites undergo conjugation by SULTs and UGTs.
SULT: Sulfotransferase; TAM: Tamoxifen; UGT: Uridine diphosphate glucuronosyltransferase.

been seen in about 30% of patients with breast cancer [27]. Many studies have investigated CYP2D6 inhibitory potential of medications. Generally, CYP2D6 inhibitors were classified into weak or moderate (sertraline, citalopram, fluvoxamine, etc), and strong inhibitors (paroxetine, fluoxetine, bupropion, duloxetine, etc) [39]. According to the results reported by Flockhart and colleagues, the SSRI and the selective norepinephrine reuptake inhibitors, especially strong inhibitors (fluoxetine and paroxetine), would be critical in tamoxifen pharmacogenetics because they are used in patients with breast cancer to treat hot flashes and depression [41–44]. However, the drugs classified as weak inhibitors, including sertraline and citalopram, are considered to have little or no impacts on the tamoxifen treatment [45]. Several groups reported the effects of concurrent use of CYP2D6 inhibitors with tamoxifen on the risk of the recurrence [46–50]. Fifteen drugs inhibiting CYP2D6 were investigated by Ahern *et al.*, and a null association on breast cancer recurrence was observed in the patients treated with tamoxifen [46]. However, the patients co-administrated with paroxetine were likely to show higher odds ratio [43]. Kelly *et al.* reported that absolute increases of the period for concomitant use of paroxetine with tamoxifen were significantly associated with increases in the risk of death from breast cancer, however, the other SSRIs did not [47]. Goetz *et al.* reported the significant effects of both of *CYP2D6* genotype and CYP2D6 inhibitors; however, questions remain in the contribution of CYP2D6 inhibitors to the results [15]. Further investigation considering these issues are required, however, these lines of evidence suggest that concurrent use of strong CYP2D6 inhibitors, especially paroxetine and possibly the others, should be avoided in the breast cancer patients treated with tamoxifen [51].

CYP2D6 genotype & clinical outcome after tamoxifen therapy

Endoxifen has antiestrogen effect in breast cancer cells that functions by targeting ER-α for degradation by the proteasome, blocking ER-α transcriptional activity and reducing estrogen-induced breast cancer cell growth [39]. Recently, an explosion of interest has been seen in the effect of *CYP2D6* genotype on clinical outcomes for breast cancer patients treated with tamoxifen [52]. There has been hypothesis that women with a reduced CYP2D6 enzyme activity, and thus presum-

Table 1. Genetic variation of *CYP2D6* and its enzyme activity.

CYP2D6 allele	Variant	Effect	Enzyme activity
**1*	Wild-type		Normal
**2*	-1584C>G, 1661G>C, 2850C>T, 4180G>C		Normal
**3*	2549del A	Frameshift	Inactive
**4*	1846G>A; 100C>T	Splicing defect	Inactive
**5*	CYP2D6 deletion	CYP2D6 deleted	Inactive
**6*	1707delT	Frameshift	Inactive
**7*	2935A>C	H324P	Inactive
**8*	1758G>T	Stop codon	Inactive
**9*	2613_2615 delAGA	K281del	Decrease
**10*	100C>T	P34S	Decrease
**11*	883G>C	Splicing defect	Inactive
**12*	124G>A	G42R	Inactive
**14*	1758G>A	Stop codon	Inactive
**15*	138_139InsT	Frameshift	Inactive
**17*	1023C>T, 2850C>T	T107I; R296C	Decrease
**41*	2988G>A		Decrease

ably low endoxifen concentrations, might have worse clinical outcomes after tamoxifen therapy; however, generation of definitive proof of this hypothesis has been controversial [52].

Many clinical trials as to this association have been reported until today. One of the first studies was reported by researchers in Mayo Clinic who determined *CYP2D6* genotype by extraction of DNA from postmenopausal women treated with 5-year-tamoxifen [15]. They reported that carriers of a *CYP2D6*4* (null of enzymatic activity) had a significantly shorter time-to-recurrence and relapse-free survival (HR: 1.74; p = 0.017) than those in EMs [15]. Kiyotani *et al.* reported that *CYP2D6* variants which reduce or lack enzymatic activity were significantly associated with shorter recurrence-free survival (RFS) in 282 Japanese patients receiving adjuvant tamoxifen monotherapy (HR: 9.52; 95% CI: 2.79–32.45; p = 0.000036 in patients with two variant alleles vs patients without variant alleles) [14]. However, two retrospective studies reported that no association was found between *CYP2D6* genotype and the clinical outcomes in 2005 [22,53]. Nowell *et al.* carried out a retrospective study and reported a trend toward longer overall survival in a cohort of adjuvant tamoxifen-treated breast cancer patients with *CYP2D6*4* allele [22]. A Swedish study also reported the improved outcomes in patients with at least one *CYP2D6*4* allele who were treated with tamoxifen for 2 years after surgical operation [53]. The other larger cohort by the same group also suggested that women with ER-positive breast cancer who were homozygous for *CYP2D6*4* had significantly improved disease-free survival (DFS) compared with those with *CYP2D6*1* (wild-type) [23,52].

In addition to the above trial, many clinical studies reported the relationship between *CYP2D6* genotype and clinical outcome of patients treated with tamoxifen in adjuvant or metastatic setting [16,18,54–68] (Table 2). In 2009, Schroth *et al.* subsequently published a retrospective analysis of 1325 German and North American patients with early-stage breast cancer treated with tamoxifen in adjuvant setting [16]. With a median follow-up of 6.3 years, the authors observed that women with reduced CYP2D6 activity (heterozygous for either a reduced activity or null activity allele: IMs or PMs) had a significantly poor clinical outcome (HR: 1.4; 95% CI: 1.04–1.9) compared with EMs [16]. Although PMs were at increased risk of recurrence compared with their EM counterparts with a time to recurrence HR of 1.9 (95% CI: 1.1–3.28), a significant difference in overall survival was not observed [16]. In contrast, two large studies, the Arimidex, Tamoxifen, Alone or in Combination (ATAC) and Breast International Group (BIG) 1–98 trials, for the relationship between *CYP2D6* genotype and clinical outcome after tamoxifen therapy recently reported that the relationship has not been confirmed [21,69]. However, all of the above studies including ATAC and BIG 1–98 were retrospective studies and lack for the uniformity in genotyping method, coverage of genotyped alleles, DNA source and dose of tamoxifen [70,71]. Some authors do not recommend routine use of the *CYP2D6*

test until robust confirmatory data are available from adequately powered prospective trials [72,73].

Recently, as a result of meta-analysis on data from 4973 tamoxifen-treated patients, the International Tamoxifen Pharmacogenomics Consortium (12 globally distributed sites) reported that CYP2D6 poor metabolizer status was associated with poorer invasive DFS using strict inclusion criteria (IDFS: HR: 1.25; 95% CI: 1.06, 1.47; p = 0.009) [17]. The potential role of *CYP2D6* genotype assessment in determining if the patients with ER-positive breast cancer should receive tamoxifen is still controversial. Prospective studies are necessary to establish if genotype-guided personalized tamoxifen therapy improves clinical outcomes of the patients with ER-positive breast cancer [17].

Dose-adjustment study of tamoxifen based on *CYP2D6* genotypes

The breast cancer patients who are heterozygous and homozygous for decreased function and null alleles of *CYP2D6* are reported to show lower plasma concentrations of endoxifen and 4-hydroxytamoxifen compared with patients with homozygous wild-type allele [27], resulting in worse clinical outcome in tamoxifen therapy. Kiyotani *et al.* reported tamoxifen dose adjustment study using 98 Japanese breast cancer patients, who had been taking 20 mg of tamoxifen daily as adjuvant setting [19]. In their study, dosages of tamoxifen were increased to 30 and 40 mg/day for the patients who have one or no normal allele of *CYP2D6*, respectively. In the patients with *CYP2D6*1/*10* and *CYP2D6*10/*10*, the plasma endoxifen levels after dose increase were 1.4- and 1.7-fold higher, respectively, than those before the increase ($p < 0.001$) [19]. These plasma concentrations of endoxifen achieved similar level of those in the *CYP2D6* wild-type patients receiving 20 mg/day of tamoxifen. In addition, they showed that the incidence of adverse events was not significantly different between before and after dose adjustment, and concluded that their study provided the evidence that dose adjustment could be useful for the patients carrying *CYP2D6*10* allele to maintain the effective endoxifen level. Similar genotype-guided tamoxifen dosing study was reported [9,20]. Irvin *et al.* also showed the similar results, and the feasibility of genotype-driven tamoxifen dosing and demonstrates that doubling the tamoxifen dose can increase endoxifen concentrations in IM and PM patients [9].

Possible genetic markers for clinical response to tamoxifen

As shown in Figure 1, UGTs, SULTs and the other CYPs are involved in the metabolism of tamoxifen. Some reports suggest that genetic variations in these genes may affect the efficacy or toxicity of tamoxifen therapy [14,22,27,76–79]. Several genetic polymorphisms are reported in *SULT1A1*, and some investigations on *SULT1A1*2*, which causes decreased SULT1A1 activity, failed to find association with tamoxifen efficacy [23,80]. Genetic polymorphisms in the *CYP3A4* have been reported, however, their contribution to influence the tamoxifen metabolism might be small because of their low allelic frequencies. On the other hand, *CYP3A5*3* allele is known to influence to CYP3A5 expression level [81]. Several studies investigated the association of *CYP3A5*3* with tamoxifen metabolism or clinical outcome of tamoxifen therapy, however, none of them report their significant association [27,74–76,81–83]. *CYP2C19*2* and *CYP2C19*3* are known to be null allele, and *CYP2C19*17*, which is recently identified genetic variation and located in promoter region of this gene, is associated with increased CYP2C19 activity (UM phenotype) [77,78]. The significant association with clinical outcome after tamoxifen treatment was found in *CYP2C19*17* carriers, but not in *CYP2C19*2* nor **3* carriers [77,78]. ABCC2 plays an important role in the biliary excretion of conjugated drugs and xenobiotics [84,85]. Tamoxifen and its metabolites are excreted into the biliary tract in liver as glucuronides or sulfates [86]. In a recent study, an intronic SNP in *ABCC2* was found to be significantly associated with the clinical outcome of breast cancer patients treated with tamoxifen, however, this SNP was not associated with plasma concentration of endoxifen or other metabolites [14]. This suggests that the contribution of ABCC2 to biliary excretion of tamoxifen and its metabolites might be limited. A genome-wide association study for clinical outcome of the breast cancer patients treated with tamoxifen was reported [79]. In this study, 240 patients were analyzed by genome-wide genotyping, and 105 and 117 cases were used for replication studies as independent cohorts, respectively. Out of 15 SNPs which showed significant associations with recurrence-free survival in genome-wide association study stage, rs10509373 in *C10orf11* gene on 10q22 was significantly associated with tamoxifen efficacy in the two independent replication stages [79]. Although further validation studies and functional analysis would be required to verify their results, *C10orf11* could be a promising genetic marker to predict the clinical outcomes of patients receiving tamoxifen therapy [79].

Conclusion

There have been several reports on the association between *CYP2D6* genotype and clinical outcome or tamoxifen metabolism in breast cancer patients treated with tamoxifen. The results of the association studies

Table 2. Studies evaluating association of *CYP2D6* genotype with response to tamoxifen therapy.

Study findings	Studies	n	Ratio of monotherapy (%)	Tamoxifen dose and duration	Outcome[†]	HR (95% CI)	p-value	Ref.
Positive	Goetz *et al.*	190	–	20 mg/day for 5 years	DFS	2.44 (1.22–4.90)	0.012	[74]
	Goetz *et al.*	180	100	20 mg/day for 5 years	RFS	2.69 (1.34–5.37)	0.005	[15]
	Schroth *et al.*	206	100	–	RFS	2.24 (1.16–4.33)	0.02	[75]
	Newman *et al.*	115	63.5	20 mg/day, median duration >4 years	RFS	1.9 (0.8–4.8)	0.19	[58]
	Kiyotani *et al.*	58	100	20 mg/day for 5 years	RFS	8.67 (1.06–71.09)	0.044	[12]
	Xu *et al.*	152	100	–	DFS	4.7 (1.1–20.0)	0.04	[54]
	Schroth *et al.*	1325	100	For 5 years	RFS	2.12 (1.28–3.50)	0.003	[16]
	Kiyotani *et al.*	282	100	20 mg/day for 5 years	RFS	9.52 (2.79–32.45)	0.0032	[14]
	Ramon *et al.*	91	39.8	–	DFS		0.016	[55]
	Park *et al.*	110	21.80	20 mg/day, median duration 3.9 years	RFS	5.59 (0.93–33.5)	0.05	[67]
	Thompson *et al.*	542	100	20 mg/day for 5 years	RFS	1.52 (0.98–2.36)	0.06	[68]
	Teh *et al.*	95	–	20 mg/day	RFS	13.14 (1.54–109.9)	0.004	[59]
	Sirachainan *et al.*	39	100	–	DFS		0.036	[60]
	Damodaran *et al.*	132	6.80	For 5 years	RFS	7.15 (1.77–28.89)	0.006	[61]
	Goetz *et al.*	453	100	20 mg/day for 5 years	Disease event	2.45 (1.05–5.73)	0.04	[57]
	Province *et al.*	4973 (1996)	100	20 mg/day for 5 years	IDFS	1.25 (1.06–1.47)	0.009	[17]
Negative	Wegman *et al.*	76	50	40 mg/day for 2 years	RFS	<1.0[‡]	–	[53]
	Nowell *et al.*	160	14.2	Not reported	DFS	0.67 (0.33–1.35)	0.19	[22]
	Wegman *et al.*	103	–	40 mg/day for 2 years	RFS	0.87 (0.38–1.97)	0.74	[23]
		111	–	40 mg/day for 5 years	RFS	0.33 (0.08–1.43)	0.14	
	Okishiro *et al.*	173	42.2	20 mg/day, median 52 months	RFS	0.94 (0.34–2.60)	0.95	[62]
	Stingl *et al.*	493	58	20 mg/day	TTP	–	0.10	[63]
	Kiyotani *et al.*	167	0	20 mg/day for 5 years	RFS	0.64 (0.20–1.99)	0.44	[64]
	Abraham *et al.*	3155	48.4	20 mg/day	RFS	1.57 (0.64–3.84)	0.32	[56]
	Lash *et al.*	340	–	–	Disease event	1.3 (0.60–2.9)	0.88	[65]
	Park *et al.*	130	18.2	–	RFS	1.34 (0.42–4.28)	0.63	[66]
	Rae *et al.*	588	95.7	20 mg/day for 5 years	RFS	1.22 (0.76–1.96)	0.44	[69]
	Regan *et al.*	973	100	20 mg/day for 5 years	RFS	0.58 (0.28–1.21)	0.35	[21]

All reports were retrospective studies.
[†]RFS was defined as time from surgery or randomization to diagnosis of the recurrence of breast cancer (locoregional, distant metastasis and contralateral breast events). DFS was defined as time from surgery or randomization to diagnosis of the recurrence of breast cancer or death. IDFS specifically excludes all *in situ* cancer events (ipsilateral or contralateral DCIS, ipsilateral or contralateral LCIS and all *in situ* cancers of nonbreast sites).
[‡]Not calculated HR according to *CYP2D6* genotypes.
DFS: Disease-free survival; HR: Hazard ratio; IDFS: Invasive DFS; RFS: Recurrence-free survival.

of tamoxifen metabolism with *CYP2D6* genotype are consistent in most of the studies, however, the results of the association studies of tamoxifen efficacy with *CYP2D6* genotype are still controversial. Although there might be several reasons for these controversial results, well-designed prospective studies will clarify if *CYP2D6* genotype test could improve the outcomes of women with ER-positive breast cancer. Moreover, the combined genetic test of *CYP2D6* with a few predictive genetic markers may provide new insights into personalized selection of hormonal therapy for the patients with breast cancer. The potent CYP2D6 inhibitors including paroxetine should be avoided in the breast cancer patients receiving tamoxifen as alternative treatment should be available in most cases.

Future perspective

The dose-adjustment studies based on the *CYP2D6* genotypes showed that the increase of tamoxifen dose was able to increase the plasma endoxifen level, and expected to improve the prognosis of the tamoxifen-treated patients with reduced *CYP2D6* genotype [9,19]. A large-scale prospective study will clarify whether the dose-adjustment strategy could improve tamoxifen therapy in breast cancer patients. Although there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy, one of the largest meta-analysis performed by International Tamoxifen Pharmacogenomics Consortium reported that *CYP2D6* could be a strong predictor of invasive DFS using strict inclusion criteria (postmenopausal women with ER-positive breast cancer receiving 20 mg/day tamoxifen for 5 years). In either case, prospective studies are essential to finally conclude if genotype-guided selection of tamoxifen therapy improves clinical outcomes of women with ER-positive breast cancer. If the results will show the positive association of *CYP2D6* genotype with clinical outcome of tamoxifen-treated patients, US FDA may approve and recommend routine use of the *CYP2D6* genotype test for personalized tamoxifen therapy in adjuvant or metastatic breast cancer setting.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistant was utilized in the production of the manuscript.

Executive summary

- Tamoxifen treatment reduced the risk of breast cancer relapse for at least 15 years, particularly estrogen receptor positive invasive tumors in premenopausal women.
- CYP2D6 is known to be a key enzyme to generate one of the potent tamoxifen metabolites, endoxifen.
- Although there are some discrepant reports questioning the association between *CYP2D6* genotype and clinical outcome after tamoxifen therapy, the highest level of evidence to test the CYP2D6–tamoxifen hypothesis will come from larger scale prospective clinical trials.
- Combined analysis of newly identified genetic marker(s) with previously identified ones, *CYP2D6*, *ABCC2* and so on, might be useful to predict the clinical outcome of patients receiving tamoxifen therapy.

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