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

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Received: 26 March 2014 / Accepted: 17 June 2014
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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

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

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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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Carcinogenesis Advance Access published December 10, 2013

Carcinogenesis vol.00 no.00 p.1 of 8, 2013
doi:10.1093/carcin/bgt374
Advance Access publication November 15, 2013

Association of microRNA-31 with *BRAF* mutation, colorectal cancer survival and serrated pathway

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BRAF* is an important gene in colorectal cancers (CRCs) that is associated with molecular characterization and resistance to targeted therapy. Although microRNAs (miRNAs) are useful biomarkers of various cancers, the association between miRNA and *BRAF* in CRCs is undefined. Therefore, this study was conducted to identify a relationship between specific miRNA molecules and *BRAF* mutation in CRCs and serrated lesions. miRNA array was used for the measurement of 760 miRNAs in 29 CRCs. To assess the identified miRNAs, quantitative reverse transcription–PCR was performed on 721 CRCs, 381 serrated lesions and 251 non-serrated adenomas. Moreover, proliferation and invasion assays were conducted using cell lines. miRNA array analysis revealed that microRNA-31 (miR-31)-5p was the most up-regulated miRNA in CRCs with mutated *BRAF* (*V600E*) compared with CRCs possessing wild-type *BRAF* (including cases with *KRAS* mutation). High miR-31 expression was associated with *BRAF* and *KRAS* mutations and proximal location ($P < 0.0001$). High miR-31 expression was related to cancer-specific mortality [multivariate hazard ratio = 2.06, 95% confidence interval: 1.36–3.09, $P = 0.0008$]. Functional analysis demonstrated that miR-31 inhibitor decreased cell invasion and proliferation. With regard to serrated lesions, high miR-31 expression was less frequently detected in hyperplastic polyps compared with other serrated lesions. In conclusion, associations were identified between miR-31, *BRAF* and prognosis in CRC. Transfection of miR-31 inhibitor had an antitumour effect. Thus, miR-31 may be a promising diagnostic biomarker and therapeutic target in colon cancers. Moreover, high miR-31 expression in serrated lesions suggested that miR-31 may be a key molecule in serrated pathway.

Abbreviations: CI, confidence interval; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; HP, hyperplastic polyp; HR, hazard ratio; miRNA, microRNA; miR-31, microRNA-31; MSI, microsatellite instability; MSS, microsatellite stability; OR, odds ratio; qRT–PCR, quantitative reverse transcription–PCR; SSA/P, sessile serrated adenoma/polyp; TSA, traditional serrated adenoma.

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Introduction

BRAF, a member of the RAF gene family, which encodes a serine–threonine protein kinase and plays an important role in the activation of RAS–RAF–MEK–ERK signalling pathway, is one of the targeted genes in colorectal cancers (CRCs) (1–5). With regard to patient survival and chemoresistance, previous studies have shown an association between *BRAF* (*V600E*) mutation and high cancer-specific mortality rates among patients with CRC (2,5). In addition, *BRAF* has been associated with resistance to monoclonal antibodies against the epidermal growth factor receptor (EGFR) in patients with *KRAS* wild-type metastatic CRC refractory to chemotherapy (2,3). Therefore, these results suggest that further analysis of *BRAF* may enable us to identify the molecular characterization and the potential therapeutic target in CRCs.

MicroRNAs (miRNAs) constitute a class of small non-coding RNA molecules (21–25 nucleotides) that function as post-transcriptional gene regulators. miRNAs can function as oncogenes or tumour suppressors. Therefore, they have been increasingly recognized as useful biomarkers for various human cancers (6–15). In CRCs, several miRNAs are known to be deregulated (16–39) and target genes in the downstream effectors of EGFR (25,29,31–33). However, miRNAs specific to *BRAF* or its activation remain largely unknown.

The serrated pathway has attracted considerable attention as an alternative route to CRC. Approximately 30% of CRCs are hypothesized to arise from serrated lesions (40). Accumulating evidence suggests an association between CRCs with mutated *BRAF* and serrated lesions in many cases, as indicated by the high frequency of *BRAF* mutation in serrated lesions (40,41). Of these lesions, sessile serrated adenoma/polyp (SSA/P) has been identified as the precursor lesion of microsatellite instability (MSI)-high CRC with *BRAF* mutation in the proximal colon (40–42). However, the role of miRNAs in the development of CRC via the serrated pathway has not been examined in large samples of serrated lesions to date.

Therefore, we hypothesized that some specific miRNA molecules may regulate *BRAF* activation in CRCs. They may also play an important role in the progression of serrated lesions. To test this hypothesis, we conducted miRNA array analysis to detect miRNA molecules that are potentially associated with *BRAF* mutation using a database of 1353 colorectal tumours.

Materials and methods

Patients and tissue specimens

Formalin-fixed, paraffin-embedded (FFPE) tissues of 735 CRCs (stages I–IV), 391 serrated lesions and 259 non-serrated adenomas (i.e. tubular or tubulovillous adenomas) of patients who underwent endoscopic resection or other surgical treatment at Sapporo Medical University Hospital, Keiyukai Sapporo Hospital and JR Sapporo Hospital between 1997 and 2012 were collected. To avoid selection bias as far as possible, we consecutively collected FFPE specimens of CRC tissues, serrated lesions and non-serrated adenomas. The criterion for diagnosis of CRC was invasion of malignant cells beyond the muscularis mucosa. Intramucosal carcinoma and carcinoma *in situ* were classified as adenoma. Colorectal tumours were classified by location as follows: the proximal colon (caecum, ascending and transverse colon), distal colon (splenic flexure, descending and sigmoid colon) and rectum.

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