**TABLE 2.** Observed Dose-Limiting Toxicities in Treatment Cycle 1 at Each Nintedanib Dose Level Among Evaluable Patients with BSA <1.5 $m^2$ or BSA ≥1.5 $m^2$

| Cohort | Nintedanib Dose (mg bid) | Docetaxel Dose ($mg/m^2$) | No. of DLTs/Patients[a] | Nature of DLT |
|---|---|---|---|---|
| — | 100 | 60 | 0/3[b] | — |
| BSA <1.5$m^2$ | 150 | 60 | 2/6 | (1) ALT and AST elevation; (2) ALT elevation |
| | | 75 | 1/6 | (1) ALT and AST elevation |
| | 200 | 60 | 3/3 | (1) ALT, AST, and γ-GT elevation; (2) ALT, AST, and γ-GT elevation; (3) ALT and AST elevation |
| BSA ≥1.5$m^2$ | 150 | 60 | 0/3 | — |
| | | 75 | 2/6 | (1) ALT and γ-GT elevation; (2) ALT elevation |
| | 200 | 60 | 2/6 | (1) ALT, AST and γ-GT elevation; (2) ALT and γ-GT elevation |
| | | 75 | 2/6 | (1) ALT, AST, and γ-GT elevation; (2) ALT elevation |

[a]Patients eligible for evaluation of dose-limiting toxicity.
[b]BSA in 100 mg bid group: <1.5 $m^2$, $n = 1$; ≥1.5 $m^2$, $n = 2$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; bid, twice daily; BSA, body surface area; DLT, dose-limiting toxicity; γ-GT, gamma glutamyltransferase.

the N150/D60 dose in patients with BSA less than 1.5, and for subsequent dose escalations to be performed separately for cohorts with BSA less than 1.5 and BSA greater than or equal to 1.5, respectively.

As shown in Table 2, of 12 patients with BSA less than 1.5 treated in the N150/D60 or the nintedanib 150 mg bid plus docetaxel 75 $mg/m^2$ (N150/D75) cohorts, three patients experienced DLTs (liver enzyme elevations that were reversible with dose reduction or discontinuation); two in the N150/D60 cohort and one in the N150/D75 cohort. Of 12 patients with BSA greater than or equal to 1.5 treated in the N200/D60 and the nintedanib 200 mg bid plus docetaxel 75 $mg/m^2$ (N200/D75) cohorts, respectively, two of six patients in each cohort experienced DLTs (reversible liver enzyme elevations). In eight of 12 patients who developed DLTs, nintedanib was reintroduced with dose reduction following rapid recovery of liver enzyme levels; one patient required a second dose reduction (Fig. 2). The MTD of nintedanib was thus 150 and 200 mg bid combined with 75 $mg/m^2$ of docetaxel in the BSA less than 1.5 and BSA greater than or equal to 1.5 cohorts, respectively.

## Safety Profile of Nintedanib

Of the 42 patients who received combination treatment, the most frequent drug-related AEs (all CTCAE grades) were neutropenia, leukopenia, fatigue, alopecia, decreased appetite, ALT/AST elevations, diarrhea, and γ-GT elevations (Table 3). The only grade 4 AEs were neutropenia ($n = 37$) and leukopenia ($n = 9$). Liver enzyme elevations were asymptomatic, and manageable with dose reduction or discontinuation. Among drug-related AEs commonly observed with other VEGF-targeted tyrosine kinase inhibitors, grade 1 or 2 rash was observed in 17 patients, grade 2 proteinuria in one patient, and grade 1 bleeding in seven patients; hypertension, perforation, and thromboembolism were not observed in this study.

Two patients died during the study period. One of these deaths occurred in a male patient (53 years of age; BSA = 1.92 $m^2$), who was previously treated concurrently with radiation to the mediastinum and systemic chemotherapy (vinorelbine plus cisplatin) until 19 months before beginning the present study treatment (N200/D60) for metastatic disease in mediastinal lymph nodes and an abdominal para-aortic lymph node. He responded to the study treatment

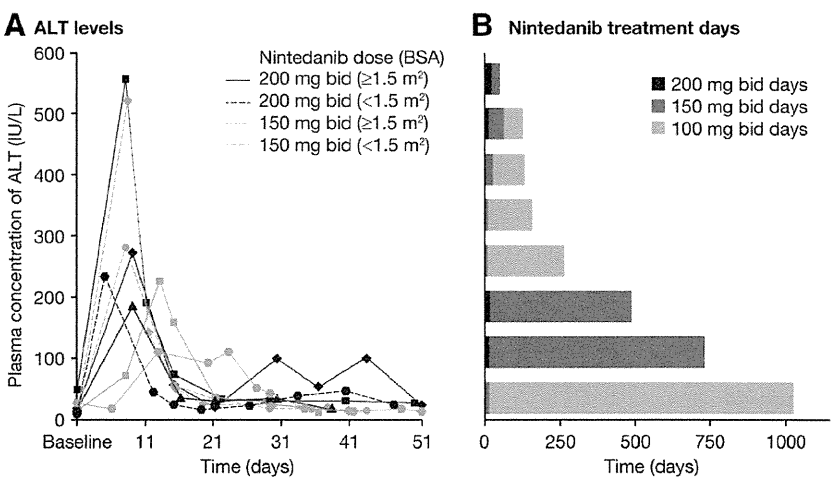


**FIGURE 2.** Change in alanine aminotransferase (ALT) values in all eight patients with dose reduction on nintedanib by dose-limiting toxicity (DLT) during first treatment course and nintedanib treatment days. BSA, body surface area.

ALT, alanine aminotransferase; bid, twice daily; DLT, dose-limiting toxicity

**TABLE 3.** Frequency of Patients with Drug-Related AEs (≥20% Incidence) Across all Dose Groups in all Treatment Courses by Body Surface Area

| | Patients with BSA <1.5 m² (*n* = 17) | | Patients with BSA ≥1.5 m² (*n* = 25) | | All patients (*n* = 42) | |
|---|---|---|---|---|---|---|
| *n* (%) | CTCAE grade 3–4 | All CTCAE grades | CTCAE grade 3–4 | All CTCAE grades | CTCAE grade 3–4 | All CTCAE grades |
| Hematologic | | | | | | |
| Neutropenia | 17 (100) | 17 (100) | 23 (92) | 23 (92) | 40 (95) | 40 (95) |
| Leukopenia | 10 (59) | 14 (82) | 17 (68) | 21 (84) | 27 (64) | 35 (83) |
| Anemia | 0 | 4 (24) | 0 | 6 (24) | 0 | 10 (24) |
| Nonhematologic | | | | | | |
| Fatigue | 0 | 15 (88) | 0 | 17 (68) | 0 | 32 (76) |
| Alopecia | 0 | 12 (71) | 0 | 18 (72) | 0 | 30 (71) |
| Decreased appetite | 1 (6) | 13 (76) | 0 | 15 (60) | 1 (2) | 28 (67) |
| Diarrhea | 0 | 6 (35) | 1 (4) | 16 (64) | 1 (2) | 22 (52) |
| Dysgeusia | 0 | 6 (35) | 0 | 11 (44) | 0 | 17 (40) |
| Rash | 0 | 8 (47) | 0 | 9 (36) | 0 | 17 (40) |
| Nausea | 0 | 7 (41) | 0 | 8 (32) | 0 | 15 (36) |
| Vomiting | 0 | 9 (53) | 0 | 5 (20) | 0 | 14 (33) |
| Stomatitis | 0 | 4 (23) | 0 | 8 (32) | 0 | 12 (29) |
| Peripheral sensory neuropathy | 1 (6) | 3 (18) | 0 | 7 (28) | 1 (2) | 10 (24) |
| Edema | 0 | 5 (29) | 0 | 4 (16) | 0 | 9 (21) |
| Laboratory abnormalities | | | | | | |
| ALT increased | 6 (35) | 13 (76) | 6 (24) | 14 (56) | 12 (29) | 27 (64) |
| AST increased | 5 (29) | 13 (76) | 2 (8) | 14 (56) | 7 (17) | 27 (64) |
| γ-GT increased | 3 (18) | 10 (59) | 4 (16) | 12 (48) | 7 (17) | 22 (52) |
| ALP increased | 1 (6) | 9 (53) | 0 | 9 (36) | 1 (2) | 18 (43) |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, body surface area; CTCAE, Common Terminology Criteria for Adverse Events; γ-GT, gamma glutamyltransferase.

(partial response), and the combination treatment was continued until cycle 27. Notable on-treatment AEs were grade 3 to 4 neutropenia and grade 1 fatigue, with no AEs of bleeding observed before the fatal event. On day 12 of cycle 27, the patient died with bleeding suggestive of hemoptysis. The second death occurred in a woman (69 years; BSA = 1.29 m²) who had progressed after first-line platinum-based chemotherapy, and received a total of three cycles of N150/D75 in the present study. On the planned day 1 of cycle 4, a grade 1 AST elevation was observed, docetaxel administration was postponed, and nintedanib treatment was interrupted. Eight days after nintedanib interruption, the study treatment was postponed again because of a grade 1 AST elevation despite no abnormalities in any other vital signs. Fourteen days after nintedanib interruption, the patient died. Based on the details available, the most probable reason for death for both patients was underlying advanced progressive lung cancer. However, the information was not sufficient to clarify the reasons for their events.

## Pharmacokinetics

Despite interpatient variability, nintedanib AUC and $C_{max}$ increased in an almost dose-proportional manner following single-dose administration (Supplemental Table S1, SDC 1, http://links.lww.com/JTO/A737). Plasma concentrations of nintedanib reached maximum levels 2 to 3 hours postadministration and then declined, with a half-life of 8 to 9 hours.

PK analysis revealed no apparent interactions between nintedanib and docetaxel. The AUC and $C_{max}$ for nintedanib (non-dose-normalized) in this study were similar to those observed in a previous Japanese phase I study of single-agent nintedanib.[11] Similarly, coadministration of nintedanib did not affect docetaxel PKs (Supplemental Table S2, SDC 1, http://links.lww.com/JTO/A737; Supplemental Figure S1, SDC 2, http://links.lww.com/JTO/A738).

Dose-normalized PK parameters ($C_{max,norm}$, $AUC_{0-12,norm}$, and $AUC_{0-\infty,norm}$) were compared among patients with BSA less than 1.5 and BSA greater than or equal to 1.5 patients. Although geometric mean values of nintedanib $C_{max,norm}$, $AUC_{0-12,norm}$, and $AUC_{0-\infty,norm}$ were slightly higher in patients with BSA less than 1.5 than in patients with BSA greater than or equal to 1.5, the wide overlap of individual patient values indicated no significant differences in nintedanib exposure between the two patient cohorts (Figure 3).

## Efficacy

Four of 42 patients were excluded from the efficacy evaluation for objective response according to RECIST because they had no post-treated tumor measurement due to treatment discontinuation during cycle 1; discontinuation

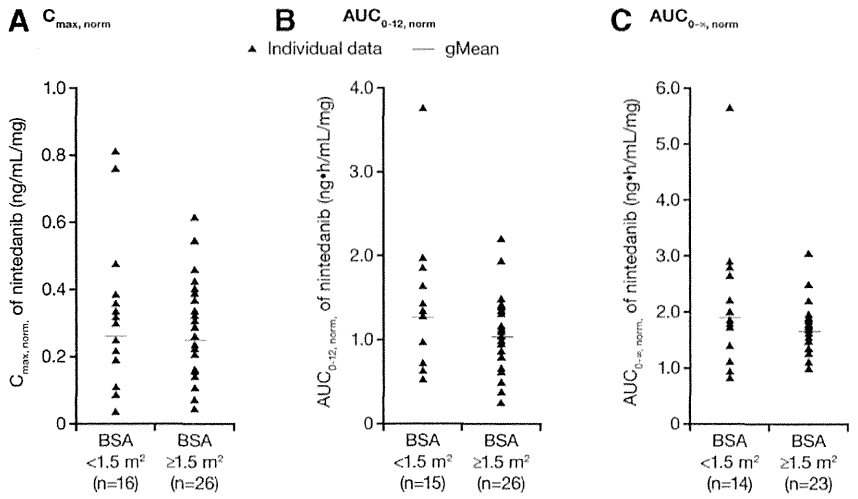


FIGURE 3. $C_{max, norm}$ (*A*), $AUC_{0-12, norm}$ (*B*), and $AUC_{0-\infty, norm}$ (*C*) of nintedanib following single oral administration of nintedanib 100, 150, or 200 mg in patients with a body surface area <1.5 m² or ≥1.5 m². $AUC_{0-\infty, norm}$, dose-normalized area under the concentration–time curve (0–∞ hours); $AUC_{0-12, norm}$, dose-normalized area under the concentration–time curve (0–12 hours); $C_{max, norm}$, dose-normalized peak concentration; gMean, geometric mean; BSA, body surface area.

was related to DLTs in three patients and early withdrawal of consent in one patient. Among 38 assessable patients, 10 had a partial response (two patients in the N150/D60 cohort, five in the N150/D75 cohort, two in the N200/D60 cohort, and one in the N200/D75 cohort), yielding an overall response rate of 26.3% (95% confidence interval [CI]: 13.4–43.1%) (Supplemental Table S3, SDC 1, http://links.lww.com/JTO/A737). All 10 responders had nonsquamous histology: nine with adenocarcinoma and one with large-cell carcinoma. A further 18 patients (47.4%) had stable disease, yielding a disease control rate of 73.7%. Median PFS was 5.7 months [95% CI: 4.3–8.3 months].

## DISCUSSION

This phase I trial was conducted to determine the MTD of nintedanib in combination with docetaxel in Japanese patients with advanced NSCLC who had previously received platinum-based chemotherapy. The MTD of nintedanib was 150 and 200 mg bid in patients with BSA less than 1.5 and BSA greater than or equal to 1.5 in combination with 75 mg/m² of docetaxel, respectively. The protocol was amended so that patients were divided according to BSA (<1.5 m² and ≥1.5 m²) due to the occurrence of an unexpectedly high number of DLTs in patients with a lower BSA (i.e., <1.5 m²). All DLTs were grade 3 liver enzyme elevations (ALT, AST, or γ-GT), and were completely reversible with dose reduction or discontinuation. A reduced dose of nintedanib was successfully reintroduced following rapid recovery of enzyme levels for eight of 12 patients who had developed liver enzyme level-related DLTs.

All three patients with BSA less than 1.5 treated with nintedanib 200 mg experienced DLTs, whereas only four of 12 patients with BSA greater than or equal to 1.5 treated at the same dose developed DLTs. This is consistent with our previous phase I study of nintedanib monotherapy, in which three of four patients with BSA less than 1.5 in the 200 mg bid cohort developed DLTs (grade 3 hepatic enzyme elevations), whereas DLTs were not reported in eight patients with BSA greater than or equal to 1.5 treated at the same dose.[11]

Studies with other small-molecule tyrosine kinase inhibitors also suggest that dosing according to BSA might be meaningful. For example, a low BSA has been associated with a high incidence of severe toxicities and DLTs in patients treated with sunitinib.[16,17] Furthermore, a reduced dose of 300 mg/day imatinib in low-BSA patients with chronic myeloid leukemia showed equivalent efficacy to the standard dose.[18,19] A large-scale PK analysis of imatinib identified a weak inverse correlation between trough concentration of imatinib and BSA.[20] Based on these observations, larger-scale investigations are warranted to identify optimal initial dosing of nintedanib, especially in low-BSA patients.

In addition to liver enzyme elevations, common drug-related AEs included hematologic toxicities, alopecia, and gastrointestinal AEs. Many of these toxicities are commonly observed during docetaxel administration.[15] These AEs were reversible and could usually be managed effectively with supportive therapies (except for alopecia). The mild-to-moderate gastrointestinal AEs and asymptomatic, reversible liver enzyme increases are consistent with the established safety/tolerability profile of nintedanib in NSCLC and other tumor types.[10,11,21–25] AEs associated with many other VEGF-targeted tyrosine kinase inhibitors, such as grade 3–4 skin toxicities, hypertension, bleeding, perforation, thromboembolism, and proteinuria,[26] were either absent or infrequent in this study.

The PK profile of nintedanib following docetaxel administration was very similar to that seen in our phase I nintedanib monotherapy study.[11] This suggests that docetaxel has no clinically relevant effect on the PK of nintedanib. Analyses of blood samples taken on day 1 of cycle 1 with docetaxel alone, and day 1 of cycle 2 of docetaxel/nintedanib showed that coadministration of nintedanib did not affect the PK of docetaxel. This is consistent with findings from a phase I study of nintedanib/docetaxel in patients with prostate cancer.[25] In the present study, we found no clear differences in PK data from patients with BSA less than 1.5 and BSA greater than or equal to 1.5. This could be due to the small sample size, so population-based PK analyses of nintedanib are needed.

Our study showed that 26% of patients achieved an objective response to nintedanib/docetaxel, with a median PFS of 5.7 months. This high level of antitumor activity is

consistent with data from the global LUME-Lung 1 trial of nintedanib/docetaxel in NSCLC, where a statistically significant improvement in PFS was observed in all patients, and a significant extension in overall survival was seen in patients with adenocarcinoma.[10]

In conclusion, the MTD for continuous daily treatment with nintedanib plus docetaxel (75 mg/m$^2$) was 150 and 200 mg bid in patients with BSA less than 1.5 and BSA greater than or equal to 1.5, respectively. There were no clinically relevant PK interactions between nintedanib and docetaxel. DLTs were observed in one-third of enrolled patients, and there were two fatal events including hemoptysis; therefore, careful observation of patients receiving nintedanib in combination with docetaxel is required in future investigations.

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FULL PAPER

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Keywords: breast cancer; triple negative; combination therapy

# A phase I dose-escalation study of eribulin and S-1 for metastatic breast cancer

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**Background:** We evaluated the safety, maximum-tolerated dose (MTD), pharmacokinetics, recommended dose for phase II (P2RD), and preliminary anticancer activity of a combination eribulin and S-1 therapeutic in metastatic breast cancer patients pretreated with anthracycline and taxane.

**Method:** Patients aged 20–74 years were recruited. In level 1, patients received S-1 (65 mg m$^{-2}$) from day 1 to 14, and eribulin (1.1 mg m$^{-2}$) on day 1 and 8 in a 21-day cycle. In level 2, eribulin was increased to 1.4 mg m$^{-2}$. In level 3, S-1 was increased to 80 mg m$^{-2}$.

**Results:** Twelve patients were enrolled into three cohorts. Planned dose escalation was completed, with one case exhibiting dose-limiting toxicity (grade 3 hypokalaemia) at level 3, without reaching the MTD. The P2RD was determined to be level 2 (eribulin 1.4 mg m$^{-2}$ and S-1 65 mg m$^{-2}$). The most common grade 3 or 4 toxicity was neutropenia (83.3%), followed by febrile neutropenia (25.0%). Five of eleven patients (41.7%) with measurable disease had a partial response. Pharmacokinetics were characterised by dose-dependent elimination and nonlinear exposure.

**Conclusion:** Dose level 3 was not tolerated owing to febrile neutropenia development. Thus, intermediate dose level 2 was recommended for further evaluation. Preliminary antitumour activity warrants further investigation in this setting.

Breast cancer is a leading cause of death among women worldwide (Jemal *et al*, 2011). Breast cancer mortality has declined in western countries because of multidisciplinary efforts over the last decade, including improved detection through screening, increased specialisation of care (Kingsmore *et al*, 2003), and better access to more effective treatments, such as improved surgical techniques, targeted radiotherapy, and adjuvant therapies, including tamoxifen (Autier *et al*, 2010). Nevertheless, clinical outcomes in metastatic breast cancer (MBC) remain poor, and identification of therapeutics to improve treatment is necessary. As MBC remains an incurable disease, the main treatment objectives are to prolong survival time and provide palliative care. The standard first-line chemotherapy for MBC utilises anthracyclines or taxanes (Mincey and Perez, 2004; Hamilton and Hortobagyi, 2005), which are the mainstays of adjuvant therapy for breast cancer. However, tumour cells often develop resistance to these drugs. Thus, novel treatments that improve overall survival (OS) but minimise toxicity and maintain a good quality of life for women with heavily pretreated MBC are necessary.

Eribulin mesylate (Halaven), an analogue of the marine sponge-derived compound halichondrin B, is a non-taxane microtubule dynamics inhibitor with a distinct mechanism of action from other tubulin-targeted drugs (Jain and Vahdat, 2011). Eribulin inhibits tumour growth in taxane-resistant human ovarian cells harbouring $\beta$-tubulin mutations, suggesting the potential to overcoming taxane resistance because of gene alterations (Kuznetsov *et al*, 2009). The side effects of this drug are reported to be manageable, with notable occurrence of neutropenia and fatigue, and a relatively low

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incidence of peripheral neuropathy (Jain and Vahdat, 2011). Further, eribulin significantly increased OS (median OS for the eribulin-treated group was 13.1 months *vs* 10.6 months for the group treated by investigator's choice) in MBC patients who were refractory to both anthracyclines and taxanes (Cortes *et al*, 2011). Recently, a large-scale phase III trial comparing eribulin to capecitabine, one of the best MBC therapeutics, revealed that the drugs had comparable efficacies. Furthermore, treatment of triple negative breast cancer (TNBC) with eribulin showed a slightly better outcome than capecitabine (Kaufman *et al*, 2012). Thus, eribulin has become a standard care for MBC.

S-1, an oral fluoropyrimidine capsule formulation that consists of 1 M tegafur (a prodrug of 5-fluorouracil (5-FU)), 0.4 M 5-chloro-2,4-dihydroxypyridine, and 1 M potassium oxonate, has efficient antitumour activity and low gastrointestinal toxicity (Okamoto and Fukuoka, 2009). It has been widely used in Asian countries, including Japan, and is accepted as a standard care for gastric (Sakuramoto *et al*, 2007; Koizumi *et al*, 2008; Boku *et al*, 2009), colorectal (Yamada *et al*, 2013), non-small-cell lung (Okamoto *et al*, 2010), and pancreatic cancer (Ueno *et al*, 2013). S-1 is also recommended as an option for third-line or later MBC treatment in Japan (JapanBreastCancerSociety, 2013), based on phase II studies that showed a response rate of 40.7 and 42.0% as first- or second-line treatment (Saek *et al*, 2004), and of 21.8% as a salvage treatment (Saeki *et al*, 2004).

The combination of eribulin and S-1 has not yet been investigated. We recently found that combination of S-1 and eribulin has a synergistic effect *in vitro* and *in vivo* (Terashima *et al*, 2014), supporting the evaluation of this combination in clinical trials. Thus, we conducted a phase I dose-escalation study using combined eribulin and S-1 to evaluate the safety and pharmacokinetic profiles of each drug. Furthermore, we determined a recommended drug dose for phase II trials (phase II trial recommended dose, P2RD).

## PATIENTS AND METHODS

**Patient eligibility.** Eligible patients were 20–74 years of age with a confirmed diagnosis of MBC. They were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ function. Previous treatments, including chemotherapy, radiotherapy, and surgery, were allowed if they had been completed at least 4 weeks before registration. However, previous administration of both anthracycline and taxanes was required. Patients with other serious illnesses or medical conditions, such as uncontrolled infection, other malignancies, or central nervous system metastases that were still symptomatic, were also not eligible for participation. All participants received information about the nature and purpose of the study, and provided written informed consent in accordance with the institutional guidelines. The consent was given before any study procedures. Having measurable disease was not a requirement to participate in this study.

**Study design and patient selection.** The study was designed as a single-centre, open-label, dose-escalation phase I trial. The primary objectives were to determine the maximum-tolerated dose (MTD) and the P2RD for the combination of eribulin and S-1, and to collect overall safety data. Secondary objectives included the determination of pharmacokinetic variables, as well as a preliminary assessment of antitumour activity in the treatment population. The study was reviewed and approved by the Institutional Review Board of Kinki University, Faculty of Medicine. This study has been registered with the UMIN Clinical Trials Registry (UMIN-CTR, UMIN 000009716). The study was performed according to the International Conference on Harmonisation Good Clinical Practices.

**Treatment schedule.** S-1 was administered orally with approximately 200 ml water and within 30 min after a meal (ideally after breakfast and evening meal, 12 h apart). S-1 was administered in two doses, 65 mg m$^{-2}$ (25 mg for body surface area (BSA) less than 1.25 m$^2$, 40 mg for BSA from 1.25 to 1.49 m$^2$, 50 mg for BSA greater than 1.5 m$^2$, twice daily) or 80 mg m$^{-2}$ (40 mg for BSA less than 1.25 m$^2$, 50 mg for BSA from 1.25 to 1.49 m$^2$, 60 mg for BSA greater than 1.5 m$^2$, twice daily) on days 1–14 every 3 weeks, in combination with two doses of eribulin (1.1 or 1.4 mg m$^{-2}$), given intravenously on days 1 and 8 every 3 weeks. In addition, for the prevention of nausea and vomiting, patients received dexamethasone 12 mg i.v. 30 min before the start of intravenous chemotherapy. Three patients were recruited at each S-1 and eribulin dose level, and at least three patients received at least one cycle and were observed for toxicity for at least 3 weeks before dose escalation was permitted. If no patients experienced a DLT, the dose was escalated to the next level in subsequent patients. If one of the three patients developed a DLT, then that dose level was expanded to six patients. If an additional patient in the six-patient cohort experienced a DLT, no further dose escalation was allowed, and the previous dose level was identified as the MTD. If none of patients at dose level 3 experienced DLTs, dose level 3 was expanded to six patients. If DLTs were exhibited in less than one of six patients at dose level 3, then the level was not escalated further, as the dosages of both drugs corresponded to those approved in Japan, and the trial would not reach a MTD. The MTD was the highest dose level at which no more than one of six patients treated exhibited DLTs. The occurrence of one or more of the following toxicities during the first cycle of chemotherapy was considered dose limiting: any National Cancer Institute Common Toxicity Criteria (NCI CTC) grade 3 or 4 non-haematological toxicity (excluding grade 3 alopecia, grade 3 nausea or vo miting, or grade 3 stomatitis persisting for $<3$ days), platelet count $<25\,000$ cells per $\mu$l or $<50\,000$ cells per $\mu$l accompanied by bleeding requiring blood transfusion, neutropenia (absolute neutrophil count $<500$ cells per $\mu$l for $>7$ days), or grade 4 febrile neutropenia (absolute neutrophil count $<500$ cells per $\mu$l accompanied by a fever $\geqslant 38.5\,^{\circ}$C (single evaluation), or a fever $>38\,^{\circ}$C for $>12$ h and unable to have S-1 for more than 6 days in the first cycle due to any toxicity).

The dose intensity was calculated as follows: the sum of the actual given dose (mg m$^{-2}$)/the actual treatment weeks $\times$ patient number. Dose delay was incorporated in actual weeks.

**Patient evaluation.** The safety and tolerability of the eribulin and S-1 combination were assessed according to the NCI CTC version 4.0. Radiological tumour assessment was performed every two cycles to confirm the response until progression. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) was calculated as the time from the first day of treatment to the first day of documented progression or death. OS was calculated from the first day of the combination therapy until death from any cause or the date of last contact. The probability of survival as a function of time was estimated with the Kaplan–Meier method. Analyses were performed with STATA version 13.1 (StataCorp, College Station, TX, USA).

**Pharmacokinetics.** The plasma pharmacokinetics of the combination treatment were investigated in order to assess the potential interactions between eribulin and S-1 at each dose level. The pharmacokinetics of eribulin were evaluated on day 1 immediately before and 0.1, 0.5, 1, 2, 4, 6, 12, and 168 h after administration during cycle 1. The pharmacokinetics of S-1 were evaluated on day 1 immediately before and 1, 2, 4, 6, and 12 h after administration during cycle 1. The plasma concentrations of eribulin and S-1 were measured by Shin Nippon Biomedical Laboratories, LTD (Tokyo, Japan) and FALCO Biosystems (Kyoto, Japan), respectively. All

concentrations were determined using liquid chromatography and tandem mass spectrometry (Matsushima *et al*, 1997). Differences in pharmacokinetic parameters were evaluated using the Student's *t*-test, and a *P* value $<0.05$ was considered statistically significant.

## RESULTS

**Patient characteristics.** From October 2012 to December 2013, 12 patients were enrolled in this phase I trial. The date of data cutoff was 20 March 2014. The characteristics of the 12 study patients are summarised in Table 1. The median age was 64 years, with a range of 49–70 years. Ten individuals had recurrent disease, whereas two had stage 4 disease. All patients had previously received two to seven chemotherapies, including anthracycline and taxane.

**Dose escalation and determination of MTD and P2RD.** The dose-escalation scheme, including the number of cycles, patients, and DLTs by dose level, is shown in Table 2. Given that no DLTs were observed at dose level 1 and 2, the dose of S-1 and eribulin was escalated to level 3. Because no DLTs were observed in the initial three patients at dose level 3, an additional three patients were assigned to dose level 3, according to the protocol. Among these three additional patients at dose level 3, one patient experienced a DLT in cycle 1, exhibiting grade 3 hypokalaemia.

**Table 1. Patient characteristics**

| Baseline characteristics | $n=12$ |
|---|---|
| Median age, years (range) | 64 (49–70) |
| **ECOG performance status** | |
| PS = 0 | 4 |
| PS = 1 | 8 |
| **Type** | |
| ER positive and PgR positive | 8 |
| HER-2 positive | 0 |
| ER negative/PgR negative/HER-2 negative | 4 |
| **Site of metastases** | |
| Liver | 6 |
| Lung | 8 |
| Bone | 9 |
| Lymph node | 3 |
| **No. of prior chemotherapy regimens** | |
| 2 Regimens | 2 |
| 4 Regimens | 6 |
| 5 Regimens | 3 |
| 7 Regimens | 1 |
| **Prior chemotherapy agents** | |
| Anthracycline | 12 (100%) |
| Taxane | 12 (100%) |
| Capecitabine | 6 (50%) |
| Vinorelbine | 2 (13%) |

Abbreviations: ER = oestrogen receptor; ECOG = European Cooperative Oncology Group; HER-2 = human epidermal growth factor receptor 2; PgR = progesterone receptor; PS = performance status.

Short episodes of febrile neutropenia that responded to treatment with oral antibiotics were observed in two of three additional patients during the assessment period. Although a pre-specified DLT was experienced in only a single patient at level 3, and the MTD of eribulin/S-1 combination therapy was not reached, three of six patients at level 3 exhibited grade 3 febrile neutropenia (two patients in cycle 1 and one in cycle 3). Although this was not defined as a DLT, in view of non-DLT grade 3 febrile neutropenia in three patients at 80 mg m$^{-2}$, DL2 was chosen as the P2RD.

**Treatment administered.** Sixty-seven cycles of chemotherapy were administered, with a median of five treatment cycles per patient (range 1–11). The mean relative dose intensities of S-1 and eribulin were 68.7% and 67.3%, respectively. Dose reductions were observed in 13 (19.4% of total cycles) cycles in six patients because of neutropenia, followed by skipping a dose in four patients (33.3%), febrile neutropenia in three patients (25.0%), and prolongation of grade 2 peripheral neuropathy in one patient. Treatment delay occurred in 19 cycles (28.3% of total cycles) in nine patients, primarily due to neutropenia.

**Toxicities.** The toxicity profiles observed over the entire treatment period are shown in Table 3. All patients who received the combination therapy were assessable for toxicity. The most common grade 3 or 4 toxicity was neutropenia in 10 (83.3%) cases, followed by grade 3 febrile neutropenia in 3 (25.0%) cases, both of which were clinically manageable. In contrast, grade 3 or 4 non-haematological toxicities were not observed throughout the study period, except for grade 3 hypokalaemia in one case (8.3%), which was defined as a DLT. Onset of the grade 3 hypokalaemia occurred in cycle 1 on day 11, and the serum potassium level was normalised within 7 days. The possible occurrence of this event was attributable to grade 2 diarrhoea along with fever, followed by decreased dietary intake. Diarrhoea was deemed an adverse event caused by S-1. The patient was receiving concomitant medications, including thiamazole 5 mg per day and pravastatin 10 mg per day, for comorbidities. The other major grade 1 or 2 non-haematological toxicities were fatigue ($n=4$, 33.3%), diarrhoea ($n=2$, 16.6%), peripheral neuropathy ($n=2$, 16.6%), and oral mucositis ($n=2$, 16.6%). There were no treatment-related deaths. The most frequent reason for discontinuation of therapy was disease progression ($n=5$, 41.7%), followed by the patients' refusal ($n=2$, 16.6%).

**Pharmacokinetics.** All 12 patients in the dose-escalation phase of the study were evaluated for pharmacokinetic analysis. The plasma concentration *vs* time curves of 5-FU and eribulin on day 1 of the first treatment cycle are shown in Figure 1A and B, respectively. The plasma concentration of S-1 peaked at 2 h, and declined from the maximum concentration ($C_{max}$) rapidly. In contrast, plasma concentrations of eribulin peaked when the infusion finished, and declined from $C_{max}$ rapidly. Both the AUC and $C_{max}$ for total 5-FU and eribulin increased proportionally with increasing dose (Table 4).

**Efficacy.** Eleven of the eleven patients were assessable for antitumour response, with a patient at dose level 3 having no measurable lesions. Five patients showed a partial response, yielding an overall response rate of 41.7% (95% confidence interval (CI): 8.9–74.4), and five other patients had stable disease, giving an

**Table 2. Dose-escalation scheme**

| | | | S-1 dose | | | | No. of cycles | | |
|---|---|---|---|---|---|---|---|---|---|
| Level | No. of patients | Eribulin dose (mg m$^{-2}$) | (mg m$^{-2}$) | BSA $<1.25$ m$^2$ | 1.25 m$^2$ $\leqslant$ BSA $<1.5$ m$^2$ | 1.5 m$^2$ $\leqslant$ BSA | Median | Range | No. of patients with DLT |
| 1 | 3 | 1.1 | 65 | 25 mg | 40 mg | 50 mg | 2 | 2–4 | 0 |
| 2 | 3 | 1.4 | 65 | 25 mg | 40 mg | 50 mg | 5 | 5–9 | 0 |
| 3 | 6 | 1.4 | 80 | 40 mg | 50 mg | 60 mg | 7 | 1–11 | 1 |

Abbreviations: BSA = body surface area; DLT = dose-limiting toxicity.

**Table 3. Profile of major toxicities during the DLT period (cycle 1)**

| | Level 1 ($n=3$) | | Level 2 ($n=3$) | | Level 3 ($n=6$) | | Total ($n=12$) (%) | |
|---|---|---|---|---|---|---|---|---|
| | Grade 1/2 | Grade ⩾3 | Grade 1/2 | Grade ⩾3 | Grade ½ | Grade ⩾3 | Grade 1/2 | Grade ⩾3 |
| Neutropenia | 1 | 2 | 0 | 3 | 1 | 5 | 2 (16.6) | 10 (83.3) |
| Febrile neutropenia | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 (25) |
| Oedema | 1 | 0 | 0 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Conjunctivitis | 0 | 0 | 1 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Blurred vision | 0 | 0 | 1 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Hypotension | 1 | 0 | 0 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Dysgeusia | 0 | 0 | 1 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Fatigue | 0 | 0 | 2 | 0 | 2 | 0 | 4 (33.3) | 0 |
| Diarrhoea | 0 | 0 | 0 | 0 | 2 | 0 | 2 (16.6) | 0 |
| Constipation | 1 | 0 | 0 | 0 | 0 | 0 | 1 (8.3) | 0 |
| Peripheral neuropathy | 0 | 0 | 0 | 0 | 2 | 0 | 2 (16.6) | 0 |
| Vomiting | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| Nausea | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| Mucositis oral | 0 | 0 | 0 | 0 | 2 | 0 | 2 (16.6) | 0 |
| Fever | 0 | 0 | 0 | 0 | 2 | 0 | 2 (16.6) | 0 |
| Anorexia | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| Rash acneiform | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| Hypokalaemia | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) |
| AST | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| ALT | 0 | 0 | 0 | 0 | 1 | 0 | 1 (8.3) | 0 |
| Bilirubin increased | 0 | 0 | 1 | 0 | 0 | 0 | 1 (8.3) | 0 |

Abbreviations: ALT = alanine transaminase; AST = aspartate aminotransferase; DLT = dose-limiting toxicity.

overall disease control rate of 91.7% (95% CI: 73.3–NA). At data cutoff, seven patients were alive and one patient remained on the combination therapy. The median PFS for all 12 treated patients was 7.6 months (95% CI: 1.3–NA) and the OS was not reached.

## DISCUSSION

Breast cancer is not a single disease, but a combination of many diseases. Regardless of disease stage, therapeutic management for patients with breast cancer should be optimised and individualised based on tumour biology, as well as many other factors surrounding them. TNBC is a distinct subset of disease, and its prognosis is worse than other subtypes, mainly due to a lack of effective targeted medicines. Recently, tremendous efforts have been made to elucidate the tumour biology of this disease, and targeted therapeutics are undergoing evaluation for patients with TNBC, including PARP and mTOR inhibitors. Nevertheless, specific targets for TNBC remain unclear. Thus, cytotoxic agents still have an important role in TNBC treatment. Chemotherapeutic combinations may be useful in patients with rapidly progressing cancer or that previously did not respond to chemotherapy. Indeed, MBC treatment guidelines recommend combination chemotherapy in these cases (Cardoso *et al*, 2014; Partridge *et al*, 2014). Thus, we sought to identify an efficacious combination with a favourable toxicity profile.

The most common grade 3 or higher toxicity observed in the current study was neutropenia (83.3%). This toxicity was also seen in previous trials evaluating 1.4 mg m$^{-2}$ of eribulin monotherapy in the same setting (Cortes *et al*, 2011; Aogi *et al*, 2012) and was mild and manageable. Further, incidence of febrile neutropenia following monotherapy was ~10%. However, we observed 25%

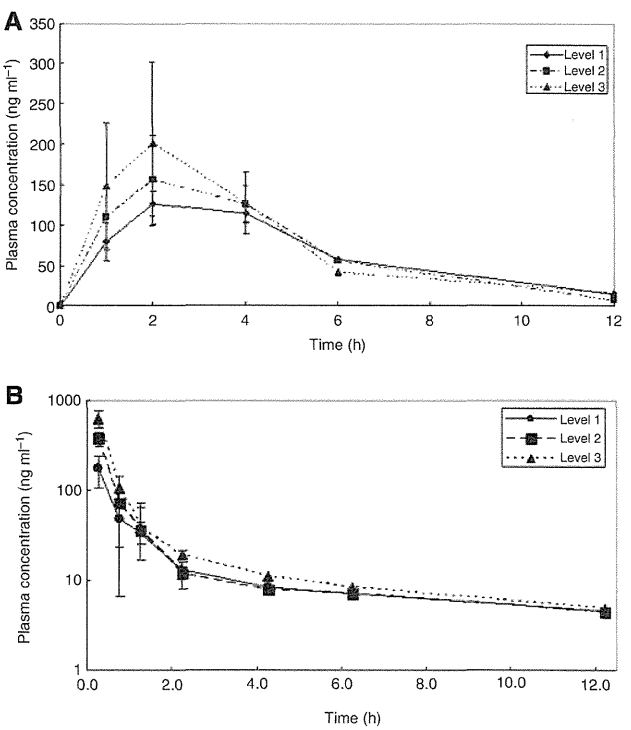


Figure 1. PK analysis of enrolled patient population: a relationship between plasma concentration *vs* time profiles for each (**A**) S-1 and (**B**) eribulin dose level.

**Table 4. Pharmacokinetic parameters for S-1 and eribulin**

| | Level 1 | | Level 2 | | Level 3 | |
|---|---|---|---|---|---|---|
| Parameter | 5-FU | Eribulin | 5-FU | Eribulin | 5-FU | Eribulin |
| $C_{max}$ (ng ml$^{-1}$) | 127.3 ± 19.1 | 186.7 ± 83.6 | 141.7 ± 67.4 | 386.1 ± 77.9 | 219.2 ± 110.8 | 593.8 ± 139.7 |
| $t_{1/2}$ (h) | 3.4 ± 2.1 | 33.3 ± 24.0 | 2.6 ± 0.7 | 33.7 ± 22.7 | 2.7 ± 1.0 | 12.9 ± 13.8 |
| $AUC_{0-inf}$ (ng ml$^{-1}$ h) | 902.6 ± 157.1 | 479.4 ± 281.5 | 753.6 ± 387.3 | 621.2 ± 454.5 | 1145 ± 694.4 | 444.9 ± 146.3 |
| CL (L h$^{-1}$ m$^{-2}$) | 64.9 ± 13.3 | 3.5 ± 3.1 | 86.2 ± 34.2 | 4.3 ± 4.5 | 76.0 ± 29.0 | 3.4 ± 0.9 |
| Vz (l m$^{-2}$) | 294.2 ± 144.7 | 99.1 ± 33.4 | 330.3 ± 189.2 | 111.9 ± 27.7 | 283.8 ± 138.2 | 51.7 ± 33.2 |
| MRT (h) | 6.1 ± 2.6 | 24.2 ± 17.9 | 4.6 ± 0.5 | 22.1 ± 15.4 | 4.7 ± 1.5 | 7.5 ± 7.3 |

Abbreviations: $C_{max}$ = Maximal plasma concentration; $t_{1/2}$ = final elimination half-life; $AUC_{0-inf}$ = area under the plasma S-1 and eribulin concentration *vs* time curve from 0 to infinity; CL = total clearance; MRT = mean residence time; Vz = volume of distribution at terminal phase; 5-FU = 5-fluorouracil. Data are mean ± s.d.

febrile neutropenia in the current study. Furthermore, after treatment with 1.4 mg m$^{-2}$ eribulin and 80 mg m$^{-2}$ S-1 in this study, all six patients experienced grade 3–4 neutropenia, and three patients developed grade 3 febrile neutropenia. These findings suggest that eribulin-induced neutropenia may be enhanced by S-1, although the PKs of eribulin were not influenced by S-1. Given that MBC patients in this setting often have insufficient bone marrow function because of prior treatments, including several lines of chemotherapy and radiation, haematological toxicities can be prolonged and exacerbated. We thus considered level 2 as the P2RD for future studies, although the MTD was not reached according to the pre-specified DLTs and the protocol. In contrast, non-haematologic DLTs were mild (grade 1 or 2), including fatigue (33.3%), diarrhoea (16.7%), and peripheral neuropathy (16.7%). One patient developed moderate hypokalaemia (grade 3), likely as a consequence of grade 2 diarrhoea, suggesting the importance of appropriate management, even for mild toxicities, in this subset of patients.

In the present study, we investigated the plasma pharmacokinetics of combination eribulin and S-1 treatment to assess potential interactions between the two drugs at each dose level. As compared with previous studies using eribulin monotherapy (Goel *et al*, 2009; Tan *et al*, 2009; Mukohara *et al*, 2012), the plasma concentration profile and pharmacokinetic parameters for eribulin did not appear to be affected by S-1 co-administration. This was consistent with previous observations that eribulin and capecitabine, another oral fluoropyrimidine prodrug used in a phase Ib trial, were not significantly different than eribulin alone (Nasim *et al*, 2012). These findings suggest that oral fluoropyrimidines, such as S-1 and capecitabine, do not interact with eribulin in terms of plasma concentration profile and pharmacokinetic parameters.

A growing amount of evidence suggests that eribulin has some off-target effects in addition to tubulin disruption. Recent preclinical studies have revealed that eribulin has the ability to convert the epithelial–mesenchymal transition (EMT) state to the mesenchymal–epithelial transition state (Yoshida *et al*, 2014). EMT has been reported to play a role in the invasive and metastatic potential of cancer progression (Hugo *et al*, 2007; Peinado *et al*, 2007; Tsuji *et al*, 2009), and the acquisition of resistance to several anti-cancer agents, including 5-FU (Thomson *et al*, 2005; Arumugam *et al*, 2009; Wang *et al*, 2009; Singh and Settleman, 2010). We recently observed that 5-FU induced EMT in TNBC, leading to 5-FU resistance, whereas eribulin reversed EMT and sensitised cells to 5-FU (Terashima *et al*, 2014). Indeed, both *in vitro* and *in vivo*, the combination of eribulin and S-1 resulted in significantly higher antitumour activity than eribulin or S-1 alone. These finding suggest that the action of eribulin on mesenchymal–epithelial transition improves 5-FU resistance, resulting in a synergistic effect. Although we are unable to reach any firm conclusion regarding the efficacy of this regimen because of the small size of our phase I trial, the promising antitumour activity of this combination in the current study, with an overall response rate of 41.7% and median PFS of 7.6 months, may reflect this mechanism of action. Thus, we plan to conduct a phase II study of this combination to further evaluate its safety and efficacy.

In conclusion, the MTD of the combination therapy was not reached in this study, and P2RD was set as 1.4 mg m$^{-2}$ eribulin intravenously injected on days 1 and 8 of a 21-day cycle in combination with 65 mg m$^{-2}$ oral S-1 for 14 days, followed by 1 week of rest. A further clinical study evaluating the safety and efficacy of this combination is warranted.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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