respectively) and no requirement of premedication for solvent-based hypersensitivity reactions. Additionally, in a preclinical study, *nab*-paclitaxel showed increased PTX transport across endothelial cells and greater antitumor activity, compared to standard PTX.[19] In phase III trials, *nab*-paclitaxel significantly increased the ORR and time to progression, compared to conventional PTX, in patients with metastatic breast cancer,[20] and significantly improved the ORR in advanced NSCLC patients, thus achieving the primary endpoint.[21]

We carried out the first phase II clinical trial to evaluate the efficacy and safety of *nab*-paclitaxel when given every 3 weeks to patients with unresectable or recurrent gastric cancer in whom treatment with one prior fluoropyrimidine-containing chemotherapeutic regimen failed.

## Materials and Methods

**Study objectives and design.** This was a non-randomized, open-label, multicenter phase II registration trial of patients with unresectable or recurrent gastric cancer who had failed treatment with first-line chemotherapy (ClinicalTrials.gov, no. NCT00661167). The primary objective was the ORR, which was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, version 1.0.[22] The definition to confirmation of complete response (CR) and partial response (PR) required 4 weeks irrespective of study endpoints. The secondary objectives were PFS, OS, the disease control rate, and safety. This trial was carried out in accordance with Japanese guidelines on Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the institutional review boards of all participating institutions.

**Patients.** Eligibility criteria for the study were: histologically confirmed adenocarcinoma of the stomach (regardless of human epidermal growth factor receptor 2 overexpression status); an age of 20–74 years; an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; a history of progression or recurrence after one prior fluoropyrimidine-containing regimen (except for taxanes such as PTX and docetaxel); a life expectancy of ≥12 weeks; and adequate bone marrow (hemoglobin level ≥8.0 g/dL, white blood cell count ≤12 000/mm$^3$ or neutrophil count ≥1500/mm$^3$, and platelet count ≥100 000/mm$^3$), liver, and renal function (serum bilirubin level ≤1.5 times the upper limit of normal; aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels ≤2.5 times the upper limit of normal; and serum creatinine level ≤1.5 mg/dL). Presence of one or more measurable lesions, according to the RECIST criteria, was also a criterion. Patients were excluded if they had brain or wide-ranging bone metastases, malignant ascites, pleural or pericardial effusion that required drainage, peripheral neuropathy of grade 2 severity or worse according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (National Cancer Institute at the National Institutes of Health, Bethesda, MD, USA), a history of drug hypersensitivity, or severe complications such as uncontrolled infection, intestinal obstruction, or pulmonary fibrosis. Patients who required continuous steroid treatment and pregnant or nursing women were also excluded. Patients were not allowed to receive concomitant radiotherapy, other chemotherapy, immunotherapy, or targeted therapy during the trial. Written informed consent was obtained from all patients before enrolment.

**Treatment.** The baseline evaluations included imaging studies (computed tomography or MRI), a complete physical examination, pregnancy testing for female patients, an assessment of the ECOG PS, a complete blood count, serum chemical and electrolyte analyses, and urinalysis.

Nanoparticle albumin-bound paclitaxel was administered on an outpatient basis by a 30-min i.v. infusion at a PTX dose of 260 mg/m$^2$ on day 1 of each 21-day cycle; no steroid or antihistamine premedication or colony-stimulating factor support was given. Treatment was continued until disease progression, unacceptable toxicity, or consent withdrawal. Three dose reduction levels (220, 180, and 150 mg/m$^2$) were implemented under the dose reduction criteria. Complete blood counts, serum chemical analyses, and urinalyses were carried out weekly during the study.

**Study assessment.** The objective disease status was assessed according to the RECIST guidelines, version 1.0.[22] Imaging studies were repeated at least every 6 weeks after treatment initiation. Safety assessments, including serial history taking and physical examinations, and laboratory assessments were carried out throughout the study. The severity of adverse drug reactions (ADR) was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. An independent review committee that comprised radiologists and medical oncologists objectively confirmed treatment responses and drug-related adverse events.

**Statistics.** The primary measure of efficacy was the ORR. The ORR in previous phase II studies of PTX as second-line treatment for metastatic gastric cancer were 24%[15] and 27%.[16] The significant ORR threshold under the null hypothesis was defined as 10%, and the expected ORR under the alternative hypothesis was defined as 25%, based on a previous PTX report. If the ORR for *nab*-paclitaxel was 25%, a sample size of 53 patients would ensure a power of at least 80% for a one-sided significance level of 2.5% in order to reject the null hypothesis that the ORR was <10%. If the lower limit of the exact two-sided 95% confidence interval (CI), based on the ORR distribution, exceeded the 10% threshold, a response rate of 11 out of 53 patients would be met.

The disease control rate was defined as the sum of the percentages of CR, PR, and stable disease (SD) for ≥6 weeks. Overall survival was defined as the time between registration and death from any cause; PFS was defined as the time between registration and disease progression or death from any cause. Both OS and PFS were estimated using Kaplan–Meier curves.

All data obtained until the completion of the study period were included in the safety analyses. The primary efficacy analysis was based on the full analysis set of the patients. The safety analysis included all treated patients who received at least one dose of the experimental drug. The clinical cut-off date for this study was May 25, 2011.

## Results

Fifty-six patients were enrolled at 10 centers in Japan between April 2008 and July 2010. One patient was ineligible because of inadequate prior treatment. Another patient was excluded from response evaluation because the initial treatment had been skipped due to rapid disease progression after registration. Fifty-five patients received the study treatment, and 55 and 54 patients were evaluable for safety and clinical response, respectively. Most of the patients were male (76.8%), and the median age was 63.5 years (Table 1). All treated patients had an ECOG PS of 0 or 1 (PS 0 = 58.9%; PS 1 = 41.1%). Thirty-five patients underwent gastrectomy. Twenty-one patients (37.5%)

**Table 1. Baseline demographic and clinical characteristics of patients with unresectable or recurrent gastric cancer receiving nanoparticle albumin-bound paclitaxel as second-line therapy**

| | No. of patients ($n$ = 56) | % |
|---|---|---|
| Gender | | |
| Male | 43 | 76.8 |
| Female | 13 | 23.2 |
| Age, years | | |
| Median | 63.5 | |
| Range | 34–74 | |
| ECOG PS | | |
| 0 | 33 | 58.9 |
| 1 | 23 | 41.1 |
| Primary lesion | | |
| Absent | 35 | 62.5 |
| Present | 21 | 37.5 |
| Type of treatment failure | | |
| First line | 40 | 71.4 |
| Adjuvant | 16 | 28.6 |
| Number of metastatic organs | | |
| 1 | 19 | 33.9 |
| 2 | 22 | 39.3 |
| ≥3 | 15 | 26.8 |
| Peritoneal metastasis | | |
| Absent | 35 | 62.5 |
| Present | 21 | 37.5 |
| Metastatic organs (overlapping) | | |
| Liver | 30 | 53.6 |
| Lung | 8 | 14.3 |
| Lymph node | 37 | 66.1 |
| Other | 23 | 41.1 |
| Adjuvant chemotherapy | | |
| S-1 | 14 | 25.0 |
| Others | 3 | 5.4 |
| First-line chemotherapy | | |
| S-1-based | 34 | 60.7 |
| Capecitabine-based | 5 | 8.9 |
| Others | 2 | 3.6 |

ECOG PS, Eastern Cooperative Oncology Group performance status; S-1, tegafur plus gimeracil plus oteracil potassium.

had peritoneal metastases. The most commonly prescribed prior chemotherapeutic agents were S-1 monotherapy as adjuvant treatment (25.0%) or S-1 in combination with cisplatin as first-line chemotherapy (35.7%). The total number of treatment cycles in the full analysis set population was 254. The median number of treatment cycles and relative dose intensity received per patient were 4 (range, 1–18), and 93.4% (range, 63.6–100.0%), respectively.

Overall responses in the 54 patients were reviewed and confirmed by the independent review committee (Table 2). One patient had a CR, 14 had PR, 17 had SD, and 21 had progressive disease. The ORR was 27.8% (95% CI, 16.5–41.6%), which exceeded the threshold response of 10% (Fig. 1). The median time to response was 36 days (range, 29–57 days).

The median PFS was 2.9 months (95% CI, 2.4–3.6 months), with a median follow-up time of 280 days (range, 46–1030 days; Fig. 2). The median survival time was 9.2 months (95% CI, 6.9–11.4 months) (Fig. 3). The median duration of treatment was 79.5 days (range, 22–477 days), with a median cumulative dose of 1574.5 mg (range, 387–6319 mg). Although 19 (34.5%) and 20 (36.4%) patients required dose

**Table 2. Clinical responses of patients with unresectable or recurrent gastric cancer receiving nanoparticle albumin-bound paclitaxel as second-line therapy**

| | No. of patients ($n$ = 54) | % |
|---|---|---|
| Complete response | 1 | 1.9 |
| Partial response | 14 | 25.9 |
| Stable disease | 17 | 31.5 |
| Progressive disease | 21 | 38.9 |
| Not evaluable | 1 | 1.9 |
| Overall response rate,% | 27.8 | |
| 95% CI | 16.5–41.6 | |
| Disease control rate,% | 59.3 | |
| 95% CI | 45.0–72.4 | |
| Progression-free survival, months | | |
| Median | 2.9 | |
| 95% CI | 2.4–3.6 | |
| Overall survival, months | | |
| Median | 9.2 | |
| 95% CI | 6.9–11.4 | |

CI, confidence interval.

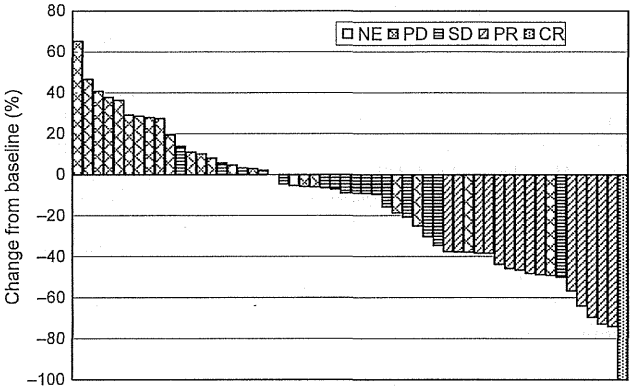


**Fig. 1.** Waterfall plot of the best overall response to nanoparticle albumin-bound paclitaxel as second-line therapy in the full analysis set of patients with unresectable or recurrent gastric cancer. CR, complete response; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

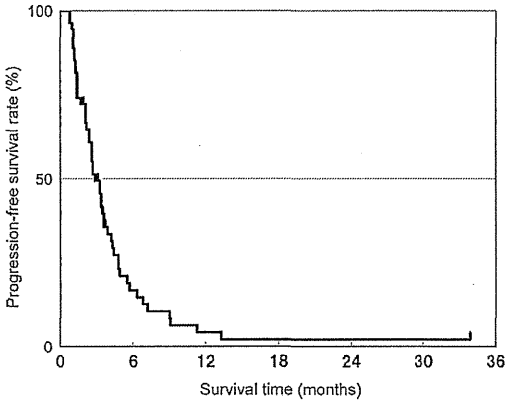


**Fig. 2.** Kaplan–Meier plots of progression-free survival in the full analysis set of patients with unresectable or recurrent gastric cancer receiving nanoparticle albumin-bound paclitaxel as second-line therapy.

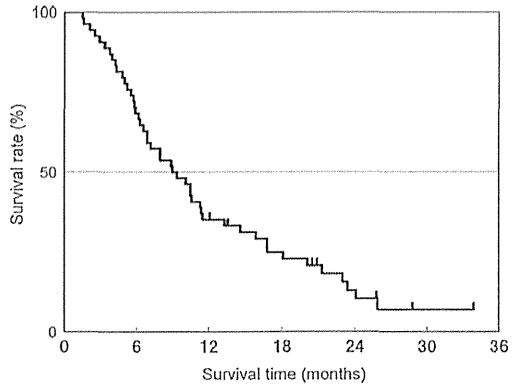


**Fig. 3.** Kaplan–Meier plots of overall survival in the full analysis set of patients with unresectable or recurrent gastric cancer receiving nanoparticle albumin-bound paclitaxel as second-line therapy.

reductions and delays, respectively, the mean relative dose intensity was 93.4% (range, 63.6–100.0%). Additional chemotherapy was given to the 44 (81.5%) patients in whom treatment with *nab*-paclitaxel failed, of whom, 37 (68.5%) received irinotecan-based chemotherapy (Table 3).

All patients were treated on an outpatient basis, and *nab*-paclitaxel was generally well tolerated. Safety was evaluated in the 55 patients who had received at least one dose of *nab*-paclitaxel. All patients reported at least one drug-related adverse event, but most adverse events were mild to moderate and well managed (Table 4). Although *nab*-paclitaxel was given without any premedication, no patients experienced hypersensitivity or acute infusion reactions. Grade 3 or 4 ADRs with incidence rates of >10% included neutropenia (49.1%), leucopenia (20.0%), lymphopenia (10.9%), and peripheral neuropathy (23.6%). No patients experienced febrile neutropenia in this study. The reasons for treatment withdrawal were mainly disease progression (87.0%) and toxicities (9.3%). There were no treatment-related deaths.

## Discussion

Paclitaxel, a microtubule-stabilizing agent, is widely used to treat breast, lung, gastric, and ovarian cancers. However, the Cremophor-containing PTX formulation has been approved and prescribed worldwide because PTX is only slightly soluble in water. Premedication with steroids, antihistamines, and $H_2$ receptor blockers before the administration of Cremophor-based PTX is essential to reduce allergic, hypersensitivity, and anaphylactic reactions in the clinical setting. *Nab*-paclitaxel is a 130-nm nanoparticle albumin-bound paclitaxel formulation that is devoid of any solvents or ethanol. *Nab*-paclitaxel thus reduces the risk of hypersensitivity reactions and does not require steroid and antihistamine premedication; in fact, hypersensitivity reactions did not occur in this study. Additionally, because the *nab*-paclitaxel formulation does not contain alcohol, it can be administered to poor metabolizers of alcohol[23] and can prevent alcohol-induced hypersensitivity reactions. Furthermore, *nab*-paclitaxel can be given over a shorter time period (30 min) and without special i.v. tubing; therefore, polyethylene-lined i.v. bags composed of polyvinyl chloride can be used for its administration.[24,25] A comparative pharmacokinetic study of *nab*-paclitaxel and conventional PTX injections was carried out.[26] Patients with advanced solid tumors were randomly assigned to receive *nab*-paclitaxel (260 mg/m$^2$ i.v. over a 30-min period) or the conventional PTX injection (175 mg/m$^2$ i.v. over a 3-h period) every 3 weeks. The PTX clearance and distribution volumes were significantly higher in patients who received *nab*-paclitaxel than in those who received conventional PTX. Furthermore, Gardner *et al.* reported that the mean fraction of unbound PTX was consider-

**Table 3. Subsequent treatment after the study chemotherapy (30-min i.v. infusion of 260 mg/m$^2$ nanoparticle albumin-bound paclitaxel every 3 weeks) in patients with unresectable or recurrent gastric cancer**

| | *n* = 54 | % |
|---|---|---|
| Any | 44 | 81.5 |
| Irinotecan | 29 | 53.7 |
| Irinotecan + Cisplatin | 8 | 14.8 |
| Paclitaxel | 3 | 5.6 |
| Others† | 4 | 7.4 |
| None | 10 | 18.5 |

†Other subsequent treatments include 5-fluorouracil/methotrexate (*n* = 2), everolimus or placebo (*n* = 1), and radiation (*n* = 1).

**Table 4. Adverse events related to nanoparticle albumin-bound paclitaxel occurring in ≥10% of patients treated for unresectable or recurrent gastric cancer**

| Type | Grade | | | | Grade 1–4 | Grade 3–4 |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | *n* (%) | *n* (%) |
| Hematologic | | | | | | |
| Anemia | 3 | 12 | 3 | 1 | 19 (34.5) | 4 (7.3) |
| Leukopenia | 13 | 23 | 11 | 0 | 47 (85.5) | 11 (20.0) |
| Neutropenia | 0 | 16 | 18 | 9 | 43 (78.2) | 27 (49.1) |
| Lymphopenia | 2 | 13 | 5 | 1 | 21 (38.2) | 6 (10.9) |
| Thrombocytopenia | 9 | 0 | 0 | 0 | 9 (16.4) | 0 (0.0) |
| Laboratory test abnormalities | | | | | | |
| AST elevation | 16 | 2 | 1 | 0 | 19 (34.5) | 1 (1.8) |
| ALT elevation | 17 | 3 | 0 | 0 | 20 (36.4) | 0 (0.0) |
| ALP elevation | 9 | 2 | 0 | 0 | 11 (20.0) | 0 (0.0) |
| Hypoalbuminemia | 10 | 3 | 0 | 0 | 13 (23.6) | 0 (0.0) |
| Protein urine | 4 | 4 | 0 | 0 | 8 (14.5) | 0 (0.0) |
| Non-hematologic | | | | | | |
| Constipation | 5 | 1 | 1 | 0 | 7 (12.7) | 1 (1.8) |
| Diarrhea | 13 | 1 | 0 | 0 | 14 (25.5) | 0 (0.0) |
| Nausea | 19 | 1 | 1 | 0 | 21 (38.2) | 1 (1.8) |
| Stomatitis | 15 | 3 | 0 | 0 | 18 (32.7) | 0 (0.0) |
| Vomiting | 4 | 1 | 1 | 0 | 6 (10.9) | 1 (1.8) |
| Asthenia | 10 | 6 | 0 | 0 | 16 (29.1) | 0 (0.0) |
| Fatigue | 1 | 8 | 1 | 0 | 10 (18.2) | 1 (1.8) |
| Malaise | 7 | 3 | 0 | 0 | 10 (18.2) | 0 (0.0) |
| Pyrexia | 7 | 3 | 0 | 0 | 10 (18.2) | 0 (0.0) |
| Weight decreased | 4 | 1 | 1 | 0 | 6 (10.9) | 1 (1.8) |
| Anorexia | 19 | 9 | 1 | 0 | 29 (52.7) | 1 (1.8) |
| Arthralgia | 16 | 1 | 3 | 0 | 36 (65.5) | 3 (5.5) |
| Myalgia | 16 | 16 | 3 | 0 | 35 (63.6) | 3 (5.5) |
| Peripheral motor neuropathy | 6 | 3 | 1 | 0 | 10 (18.2) | 1 (1.8) |
| Peripheral sensory neuropathy | 20 | 18 | 13 | 0 | 51 (92.7) | 13 (23.6) |
| Alopecia | 37 | 15 | NA | NA | 52 (94.5) | NA |
| Pruritus | 11 | 1 | 0 | NA | 12 (21.8) | 0 (0.0) |
| Rash | 10 | 1 | 0 | 0 | 11 (24.4) | 0 (0.0) |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable.

ably higher with *nab*-paclitaxel than with conventional PTX.[27] This pharmacokinetic property of *nab*-paclitaxel might be associated with higher PTX distribution to the tumor. Additionally, in preclinical studies, PTX transport across the endothelium was enhanced by albumin receptor-mediated transcytosis, and PTX delivery to tumors might be enhanced by the binding of albumin-bound PTX to interstitial albumin-binding proteins such as secreted protein acidic and rich in cysteine.[28] In a preclinical model and at equitoxic doses, the *nab*-paclitaxel-treated groups showed more complete regression, a longer time to recurrence, a longer doubling time, and prolonged survival, compared to the Cremophor-containing PTX-treated group.[19] *Nab*-paclitaxel without premedication showed significantly higher response rates and a longer time to tumor progression than PTX or docetaxel in advanced or recurrent breast cancer patients.[20,29] Additionally, weekly *nab*-paclitaxel plus carboplatin-based therapy resulted in a significantly improved ORR in advanced NSCLC patients, compared to that associated with PTX plus carboplatin, with a trend toward improved OS and PFS.[21] And in patients with metastatic pancreatic adenocarcinoma, *nab*-paclitaxel plus gemcitabine significantly improved OS, PFS, and ORR without life-threatening toxicities, which could make this treatment the standard treatment.[30]

Gastric cancer remains one of the most important malignancies, especially in Asian countries. Several phase III studies demonstrated a significantly prolonged OS in patients with advanced or recurrent gastric cancer in response to first-line fluoropyrimidine-based chemotherapies.[7,10,31] Paclitaxel at a dose of 210 mg/m$^2$, repeated every 3 weeks, was initially evaluated in Japan and yielded an objective PR rate of 28% in a registration trial of untreated or minimally treated gastric cancer patients. Several small-scale phase II studies of weekly-administered PTX reported response rates ranging from 16% to 24%[15,17] for gastric cancer patients in a second-line setting (Table 5). Furthermore, as it resulted in a better survival benefit than irinotecan in the West Japan Oncology Group WJOG4007 trial, weekly PTX could be adopted as a control arm in future phase III trials of second-line chemotherapy for gastric cancer.[32] Based on these clinical trials, weekly PTX has become the most frequently prescribed second-line drug in Japan.

This phase II study of *nab*-paclitaxel is the first phase II trial for the treatment of advanced or recurrent gastric cancer. No significant hypersensitivity or anaphylactic reactions were induced by *nab*-paclitaxel without premedication. The main reason for treatment discontinuation was disease progression, and two patients discontinued the study treatment because of adverse events, which included thrombosis and peripheral sensory neuropathy. No new safety concerns related to *nab*-paclitaxel or conventional PTX were identified, and there were no treatment-related deaths in this study. Although grade 3/4 toxicities such as neutropenia, leucopenia, and lymphopenia were observed, these ADRs were clinically well managed. Grade 3 peripheral sensory neuropathy remains an important problem that might be controlled by dose reductions and delays before the symptoms worsen. The clinical responses and PFS with *nab*-paclitaxel as second-line treatment seem comparable to those obtained in prior PTX trials, although no direct comparison data with PTX are available (Table 5). Recently, survival advantages were reported for irinotecan versus BSC and for irinotecan or docetaxel versus BSC as second-line treatment for gastric cancer patients.[13,14] Weekly PTX failed to show a survival advantage over irinotecan in a phase III trial.[32]

In conclusion, *nab*-paclitaxel, when given every 3 weeks, shows promising activity and well-tolerated toxicities in patients with previously treated unresectable or recurrent gastric cancer. A phase III trial is ongoing to evaluate the clinical benefit of *nab*-paclitaxel as second-line chemotherapy for advanced or recurrent gastric cancer (JapicCTI-132059).

**Table 5. Second-line treatments for gastric cancer**

| Regimen | No. of patients | RR (%) | MST (days) | PFS (days) | Reference |
|---|---|---|---|---|---|
| Weekly paclitaxel (80 mg/m$^2$) | 25 | 24 | 151 | 64 | 15 |
| Weekly paclitaxel (80 mg/m$^2$) | 44 | 16 | 237 | 79 | 17 |
| Biweekly paclitaxel (140 mg/m$^2$) | 40 | 17.5 | 254 | 111 | 34 |
| Triweekly paclitaxel (210 mg/m$^2$) | 26 | 27 | 319 | NA | 16 |
| Triweekly paclitaxel (210 mg/m$^2$) | 15 | 20.0 | NA | NA | 18 |
| Triweekly docetaxel (75 mg/m$^2$) | 49 | 16.3 | 252 | 76 | 33 |
| This trial | 54 | 27.8 | 279 | 88 | NA |

MST, median survival time; NA, not applicable; PFS, progression-free survival; RR, response rate.

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# Serum HER2 levels and HER2 status in tumor cells in advanced gastric cancer patients

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## Abstract

**Objective:** Increased serum human epidermal growth factor receptor 2 levels have been found in metastatic breast cancer patients and are correlated with human epidermal growth factor receptor 2 overexpression in tumor cells. However, the prevalence of serum human epidermal growth factor receptor 2 in gastric cancer patients has not been elucidated.

**Methods:** We retrospectively analyzed formalin-fixed paraffin-embedded tumor tissues and serum samples from 96 advanced gastric cancer patients. Human epidermal growth factor receptor 2 expression and gene amplification in tumor cells were determined by immunohistochemistry and fluorescence *in situ* hybridization. Serum human epidermal growth factor receptor 2 levels were measured using a chemiluminescent immunoassay. Human epidermal growth factor receptor 2 positivity in tumor cells was defined as immunohistochemistry 2+ with fluorescence *in situ* hybridization positive or immunohistochemistry 3+ with any fluorescence *in situ* hybridization results.

**Results:** All tissue samples and serum samples were successfully measured. Nineteen patients (20%) were human epidermal growth factor receptor 2-positive in tumor cells. The median serum human epidermal growth factor receptor 2 level was 9.3 ng/ml (range, 5.0–332.4 ng/ml), and serum human epidermal growth factor receptor 2 levels were significantly separated according to human epidermal growth factor receptor 2 status in tumor cells ($P < 0.0001$, Wilcoxon's rank sum test); median serum human epidermal growth factor receptor 2 levels in human epidermal growth factor receptor 2-negative patients and -positive patients were 8.9 (range, 5.0–20.5) and 24.0 (range, 9.7–332.4), respectively. There were 15 serum human epidermal growth factor receptor 2-positive patients (16%) using a cutoff value of 15 ng/ml. The sensitivity and the specificity of serum human epidermal growth factor receptor 2 with respect to human epidermal growth factor receptor 2 positivity in tumor cells were 53 and 94%, respectively.

**Conclusions:** Serum human epidermal growth factor receptor 2 measurements cannot be substituted for tissue human epidermal growth factor receptor 2 diagnosis in advanced gastric cancer

patients. However, serum human epidermal growth factor receptor 2 levels are associated with human epidermal growth factor receptor 2 overexpression in tumor cells. Further investigations of clinical significance of serum human epidermal growth factor receptor 2 as a predictive marker and a therapy-monitoring marker are warranted.

**Key words:** diagnostic marker, gastric cancer, HER2, tumor marker

## Introduction

The human epidermal growth factor receptor 2 (HER2) oncogene (also called HER2/neu or ErbB2) encodes a 185 kDa glycoprotein receptor that is a member of the epidermal growth factor receptor family. HER2 signaling promotes cell proliferation through the RAS-mitogen-activated protein kinase pathway and inhibits cell death through the phosphatidylinositol 3′-kinase-AKT-mammalian target of the rapamycin pathway (**1**). HER2 consists of an extracellular binding domain, a transmembrane lipophilic segment and a functional intracellular tyrosine kinase domain (**2**). The HER2 extracellular domain excreted in serum can be quantitatively measured using an enzyme-linked immunosorbent assay or chemiluminescence immunoassay (**3**,**4**).

HER2 overexpression (protein overexpression and/or gene amplification) has been observed in various types of cancer (e.g. breast, colorectal, bladder, ovarian, endometrial, lung, uterine cervix, head and neck, esophageal and gastric cancer). The clinical significance of HER2 overexpression has been extensively investigated in breast cancer patients. HER2 overexpression is detected in 10–34% of invasive breast cancers, it is correlated with poor prognosis, and constitutes a predictive factor of poor response to chemotherapy and endocrine therapy (**5**). HER2-targeted therapy, such as trastuzumab and lapatinib with standard treatment, is beneficial in HER2-positive breast cancer (**6**). Elevated serum HER2 levels are detected in 9–23% of patients with early breast cancer and in 22–73% of those with metastatic breast cancer (**7**). The concordance between serum HER2 levels and HER2 status in tumor cells (tissue HER2 status) has been investigated. Tse et al. (**8**) reported a high concordance between serum HER2 and tissue HER2 status; the sensitivity of serum HER2 for tissue HER2 status was 90%, and specificity was 77–83% at the cutoff level of 16 ng/ml. In contrast, other groups have reported that the sensitivity of serum HER2 for tissue HER2 status was 47–88% and specificity was 55–82% at the cutoff level of 15 ng/ml (**9**–**13**).

HER2 overexpression was observed in 22% of patients with advanced gastric and gastroesophageal junction cancer, and trastuzumab in combination with fluoropyrimidine plus cisplatin has shown significant improvement in survival and tumor response (**14**). However, the prevalence of serum HER2 in gastric cancer patients is unknown, and the utility of measuring serum HER2 levels has not been determined. Therefore, we investigated the correlation between serum HER2 levels and tissue HER2 status, and the diagnostic role of serum HER2 in advanced/recurrent gastric cancer patients.

## Patients and methods

### Patients

To be eligible for this study, patients were required to have histologically confirmed advanced or recurrent stomach adenocarcinoma, to have started chemotherapy at the National Cancer Center Hospital East during July 2009 to February 2011, and to have serum and paraffin-embedded tumor tissue samples from the primary tumor site collected and stored before the start of first-line chemotherapy. Patients who had any other active malignancies were excluded. Among 155 advanced or recurrent gastric adenocarcinoma patients who started chemotherapy at the National Cancer Center Hospital East during the study period, 100 were eligible. The reasons for exclusion were no stored serum samples for 34 patients, no stored tissue samples for 16 and other active malignancies for five.

### Tissue HER2 assay

We investigated tissue HER2 status using immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH). Tissue samples from eligible patients were collected as biopsy samples by upper endoscopy or surgical specimens before chemotherapy started. If patients had both biopsy and surgical samples, the latter were preferentially tested. The tissue samples were fixed in 10% buffered formaldehyde and embedded in paraffin. All tissue samples were derived from the primary tumor.

IHC analysis was performed using I-VIEW Pathway HER2 4B5 kit (rabbit antihuman monoclonal antibody: Ventana Medical System Inc., Tucson, AZ, USA). The intensity of the membrane staining was evaluated according to the HER2 scoring system for gastric cancer as reported previously (**14**). Briefly, surgical specimen staining patterns were scored as follows: score 0, no reactivity or membranous reactivity in <10% of cells; score 1+, faint/barely perceptible membranous reactivity in >10% of cells or cells reactive only in part of their membrane; score 2+, weak-to-moderate complete or basolateral membranous reactivity in >10% of tumor cells; and score 3+, moderate-to-strong complete or basolateral membranous reactivity in >10% of tumor cells. For biopsy specimen staining patterns, they were considered positive if the staining reactivity of each score was identified, irrespective of the percentage of tumor cells stained. The IHC score was primarily diagnosed by a pathologist of SRL Inc. (Tokyo, Japan), and the results were confirmed by T.S. and T.K.

FISH analysis was performed using the PathVysion HER2 DNA Probe Kit (Vysis Inc., Downers Grove, IL, USA), according to the manufacturer's instructions. When the ratio of HER2 signal to chromosome 17 centromere signal was ≥2.0, the gene was considered as amplified (i.e. FISH positive).

IHC and FISH analyses were performed in SRL Inc. independently from the serum HER2 assay. Tissue HER2-positive status was defined as IHC 2+ with FISH positive or IHC 3+ with any FISH result.

### Serum HER2 assay

Serum samples were provided by the National Cancer Center Biobank, Japan. Serum was collected and stored at −80°C. Serum samples were obtained just before surgery in patients who received palliative resection of the primary tumor, and after recurrence and just before starting chemotherapy in recurrent patients. Serum HER2 levels were measured using the ADIVA Centaur-HER2/neu

test on the ADIVA Centaur XP fully automated analyzer (Siemens Healthcare Diagnostics Inc., Tokyo, Japan), as previously reported (15). Serum HER2 analysis was performed by Siemens Healthcare Diagnostics Inc. independently from the tissue HER2 assay.

### Statistical analysis

We assessed whether serum HER2 levels as a continuous variable stratified by the IHC score were homogeneous or whether they were correlated with FISH. One-way analysis of variance (ANOVA) was performed for the former possibility and the coefficient of determination calculated by a general linear model was evaluated for the latter possibility. We also evaluated the association between serum HER2 levels and tissue HER2 status using Wilcoxon's rank sum test. Differences in patient characteristics were tested by Fisher's exact test.

To evaluate the predictability of HER2 status in tissue samples as the gold standard using the serum HER2 test, we used several summary statistics, such as sensitivity and specificity. First, using the cutoff value, 15 ng/ml, which is most widely used in breast cancer (7,9,10,16–25), the evaluable 100 patients were stratified into serum HER2-positive (≥15 ng/ml) or -negative (<15 ng/ml) groups, as well as. Sensitivity and specificity were defined as the proportion of the true-positive (TP)/true-negative (TN) patients among tissue HER2-positive/negative patients. TP and TN were determined based on tissue HER2 status. In TP patients, serum HER2 values were ≥15 ng/ml and the tissue HER2 status was positive. In TN patients, serum HER2 values were <15 ng/ml and the tissue HER2 status was negative. Likewise, the positive predictive value (PPV) and negative predictive value (NPV) were calculated as the proportion of TP/TN patients among serum of positive/negative patients. Accuracy was determined as the proportion of non-misspecified patients out of the total patients. Second, a receiver operating characteristic (ROC) curve was estimated by plotting the sensitivity against 1 – specificity. The area under the ROC curve was calculated as the concordance probability. Statistical analyses were performed using either SAS 9.3 (SAS Institute Inc., Cary, NC, USA) or IBM® SPSS® Statistics version 21 (IBM Corporation, Armonk, NY, USA).

### Ethical consideration

This study complied with Japanese ethical guidelines for epidemiological research and was approved by the Institutional Review Board of the National Cancer Center.

## Results

The number of eligible patients is 100, including four recurrent gastric cancer patients. However, in the recurrent gastric cancer patients, the tumor tissue from the recurrent site could not be obtained, and available tumor tissues were only surgical specimens of the primary surgery. There was temporal divergence between tumor and serum sample collection. Therefore, we report analyses in 96 advanced gastric cancer patients, excluding four recurrent cancer patients (Table 1). All tissue samples were successfully evaluated by IHC and FISH, and the results are summarized in Table 2. There were 19 tissue HER2-positive status patients, all of whom were IHC 3+.

Serum HER2 from all samples was successfully measured. The median serum HER2 level in all eligible patients was 9.3 ng/ml (range, 5.0–332.4 ng/ml). Serum HER2 levels stratified by HER2 IHC score and tissue HER2 status are plotted in Fig. 1A and B. Although serum HER2 levels were not correlated with the ratio of HER2 signal to chromosome 17 centromere signal by FISH analysis ($R^2 = 0.2651$), they were significantly different among HER2 IHC scores ($P < 0.0001$, one-way ANOVA; Fig. 1A), and separated according to tissue HER2 status ($P < 0.0001$, Wilcoxon's rank sum test; Fig. 1B); median serum HER2 levels in tissue HER2-negative status patients and -positive status patients were 8.9 (range, 5.0–20.5) and 24.0 (range, 9.7–332.4), respectively.

A total of 15 (16%) patients were serum HER2-positive using a cutoff level of 15 ng/ml. There was a significant difference in serum HER2 positivity depending on the presence of liver metastasis (11/37 with liver metastasis vs. 4/59 without liver metastasis, $P = 0.004$). The relationship between serum HER2 and HER2 status in tumor cells is summarized in Table 3. The sensitivity, specificity, PPV, NPV and accuracy of serum HER2 were 53, 94, 67, 89 and 85%, respectively. There were five false-positive patients (i.e. although serum HER2 values were ≥15 ng/ml, the tissue HER2 status was negative) and nine false-negative patients (i.e. although serum HER2 values were <15 ng/ml, the tissue HER2 status was positive). There was no significant difference in patient characteristics between TP and false-negative patients. False-positive patients had liver metastasis more frequently than TN patients (4/5 vs. 23/72, $P = 0.048$). The area under the ROC curve was 0.892 (95% confidence interval, 0.824–0.960) (Fig. 2). Elevated serum HER2 level using a cutoff level of 15 ng/ml was not a significant prognostic factor in terms of overall survival

**Table 1.** Patient characteristics of advanced gastric cancer patients

| Characteristics | |
|---|---|
| Total no. of patients | 96 |
| Sex | |
| Male | 63 |
| Female | 33 |
| Age, years | |
| Median (range) | 65.5 (29–84) |
| ECOG performance status | |
| 0 | 56 |
| ≥1 | 40 |
| Location of primary tumor | |
| Esophagogastric junction | 10 |
| Stomach | 86 |
| Number of metastatic sites | |
| 0–1 | 21 |
| ≥2 | 75 |
| Lauren's classification | |
| Intestinal type | 35 |
| Diffuse type | 40 |
| Mixed type | 21 |
| Tissue sample | |
| Biopsy | 86 |
| Surgical specimen | 10 |

ECOG, Eastern Cooperative Oncology Group.

**Table 2.** Comparison of HER2 IHC score and FISH in advanced gastric cancer patients ($n = 96$)

| | IHC 0 | IHC 1+ | IHC 2+ | IHC 3+ | Total |
|---|---|---|---|---|---|
| FISH positive | 2 | 5 | 0 | 19 | 26 |
| FISH negative | 38 | 27 | 5 | 0 | 70 |
| Total | 40 | 32 | 5 | 19 | 96 |

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

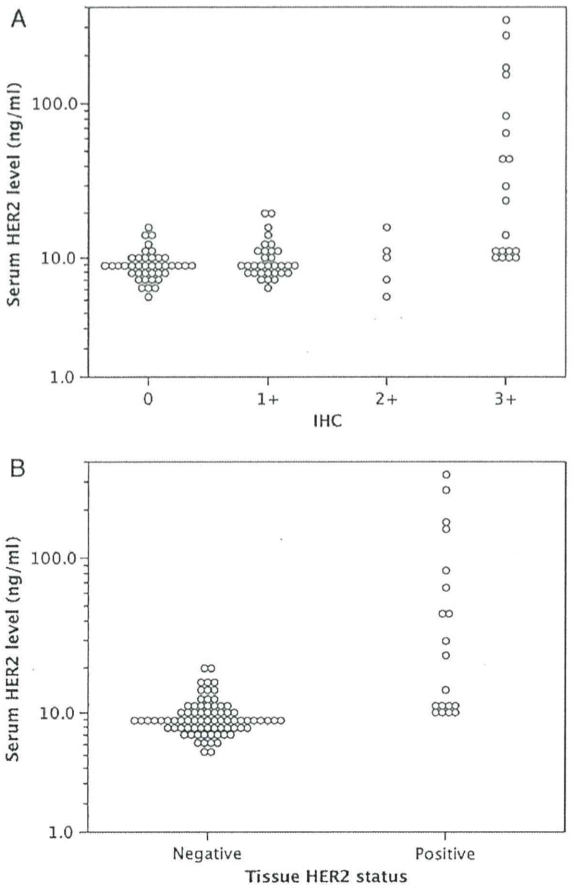


**Figure 1.** Scatter plot of serum human epidermal growth factor receptor 2 (HER2) levels stratified by immunohistochemistry (IHC) and tissue HER2 status in advanced gastric cancer patients ($n=96$). (A) Serum HER2 levels were significantly different among HER2 IHC scores ($P<0.0001$, one-way ANOVA). (B) Serum HER2 levels were significantly separated according to tissue HER2 status ($P<0.0001$, Wilcoxon's rank sum test). HER2 positivity was defined as IHC score 2+ with a ratio of HER2 signal to chromosome 17 centromere signal ≥2.0 by fluorescence *in situ* hybridization (FISH) analysis or IHC score 3+ with any FISH results in tumor cells.

**Table 3.** Comparison of serum HER2 level and tissue HER2 status in advanced gastric cancer patients ($n=96$)

| | Tissue HER2 positive | Tissue HER2 negative | Total |
|---|---|---|---|
| Serum HER2 ≥ 15 ng/ml | 10 | 5 | 15 |
| Serum HER2 < 15 ng/ml | 9 | 72 | 81 |
| Total | 19 | 77 | 96 |

HER2, human epidermal growth factor receptor 2.

(data not shown). Based on our ROC analysis, a cutoff value between 10.0 and 15.0 ng/ml appeared to be reasonable, which corresponded to a sensitivity of 84 and 53%, and a specificity of 69 and 94%, respectively. However, regardless of the cutoff value used, use of serum HER2 as a surrogate marker for tissue HER2 diagnosis is difficult.

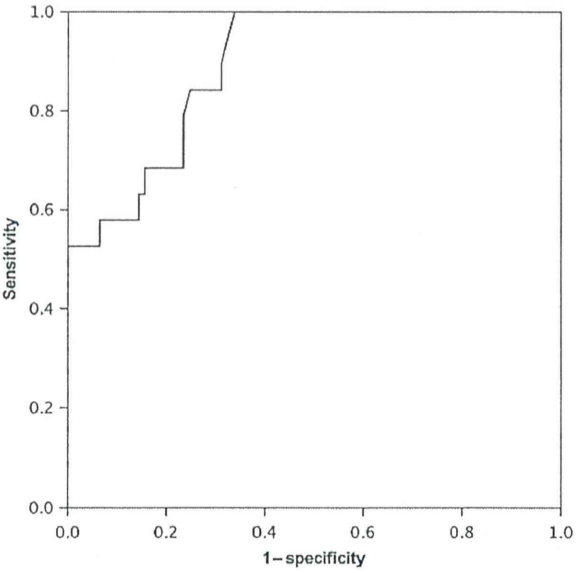


**Figure 2.** Receiver operating characteristic curve in advanced gastric cancer patients ($n=96$). Sensitivity and specificity were calculated using various cutoff levels of serum HER2 to HER2 status in tumor cells as the gold standard. The area under the curve was 0.892 (95% confidence interval, 0.824–0.960).

## Discussion

Most of the advanced gastric cancer patients who receive chemotherapy do not require tumor resection and their tissue HER2 statuses are determined by biopsy samples. In our study, only 13 patients had their HER2 status determined by surgically resected tumors and 87 patients were determined by biopsy samples. Determination of HER2 status using a small biopsy sample may have the risk of a false-negative result because gastric cancer tissue has intratumoral heterogeneous HER2 expression (26). In our previous investigation, the concordance probability for HER2 overexpression determined by IHC in surgically resected tumors and biopsy specimens was 89%, and 13 biopsy specimens from 46 patients in whom a surgically resected tumor was evaluated as HER2-positive by IHC were evaluated as negative, indicating a sensitivity of 72% (27). The sensitivity of HER2 diagnosis with biopsy specimens is insufficient. Therefore, alternative, more sensitive methods of HER2 determination should be investigated. Serum is a homogeneous material, and serum HER2 levels can be easily and quickly measured by automated methods, and can be measured objectively and repeatedly. Collecting tumor samples is invasive and occasionally cannot be carried out. Serum samples can be collected in a less invasive manner, and serum HER2 levels can be measured in a less labor-intensive way, which might be a potential useful alternative to tissue HER2 measurement.

We enrolled only advanced or recurrent gastric cancer patients who received chemotherapy. The aims of this study were to investigate the correlation between serum HER2 levels and tissue HER2 status, and to determine the diagnostic role of serum HER2. Diagnosis for HER2 status is essential when physicians determine whether anti-HER2 targeted therapy is indicated. The target patients of this study were advanced or recurrent gastric cancer patients who received chemotherapy. We did not include those who received curative surgery or best supportive care without chemotherapy because trastuzumab,

which is the only commercially available anti-HER2 targeted agent for gastric cancer patients, is currently indicated for HER2-positive advanced or recurrent patients in combination with chemotherapy.

We found that some HER2-positive gastric cancer patients had high serum HER2 levels. Serum HER2 with a cutoff level of 15 ng/ml was detected in HER2-positive advanced gastric cancer patients with a sensitivity of 53%, specificity of 94%, PPV of 67%, NPV of 89% and accuracy of 85%. In our study, serum HER2 sensitivity against HER2-positive gastric cancer was specific but not very sensitive. If HER2 positivity was determined only by serum HER2 levels, it would miss almost half of tissue HER2-positive status gastric cancer patients. Use of serum HER2 levels with a cutoff level of 15 ng/ml as a substitute for IHC and FISH analysis in tumor cells is not appropriate. A HER2 test with the combination of IHC and FISH are still regarded as the gold standard in determining HER2 positivity in gastric cancer patients.

In a previous study, the cutoff level for serum HER2 was derived from the mean + 2 SD, which was 14.78 ng/ml in a population of 241 healthy women, and 15 ng/ml was defined as the normal cutoff value (16). This cutoff value was not determined based on clinical utility to distinguish HER2-positive status in gastric cancer patients. Serum HER2 has little impact in terms of a diagnostic marker as expected from breast cancer studies. However, serum HER2 levels are increased in some advanced gastric cancer patients and are associated with HER2 overexpression in tumor cells. Therefore, these results warrant investigations of the clinical utility of serum HER2 as a predictive marker and a therapy-monitoring marker in a large and independent gastric cancer patient cohort, as in breast cancer patients (4,7,9,21,24,25,28,29).

Our study has some limitations. First, there might be bias in patient selection. We selected patients whose serum and tissue samples had been stored in our institution. There were only four recurrent gastric cancer patients included in this study. We usually store serum samples before the first treatment. With regard to the patients with recurrent gastric cancer, their serum samples were collected before surgery, but rarely at the time of recurrence, and only four recurrent patients had stored serum samples which had been collected at the time of recurrence. Furthermore, there was temporal divergence between tumor and serum sample collection. Therefore, we report analyses in 96 advanced gastric cancer patients, excluding four recurrent cancer patients. Second, for the same reason, we did not collect serum samples during treatment. Therefore, we could not discuss the utility of serum HER2 levels as a surrogate marker for treatment efficacy and disease progression. Third, although serum HER2 levels did not appear to be a prognostic factor in terms of overall survival, this study included only 19 HER2-positive status patients and no patients received trastuzumab as a first-line treatment. The clinical utility of serum HER2 as a prognostic marker and a predictive marker for anti-HER2 targeted therapy in gastric cancer patients needs to be investigated in ongoing or future prospective clinical trials.

In conclusion, serum HER2 measurements cannot be substituted for tissue HER2 diagnosis. However, serum HER2 levels are increased in some advanced gastric cancer patients and are moderately associated with HER2 overexpression in tumor tissue. Further investigations of clinical significance of serum HER2 as a predictive marker and a therapy-monitoring marker are warranted.

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## Conflict of interest statement

None of the authors has financial or personal conflicts of interest.

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