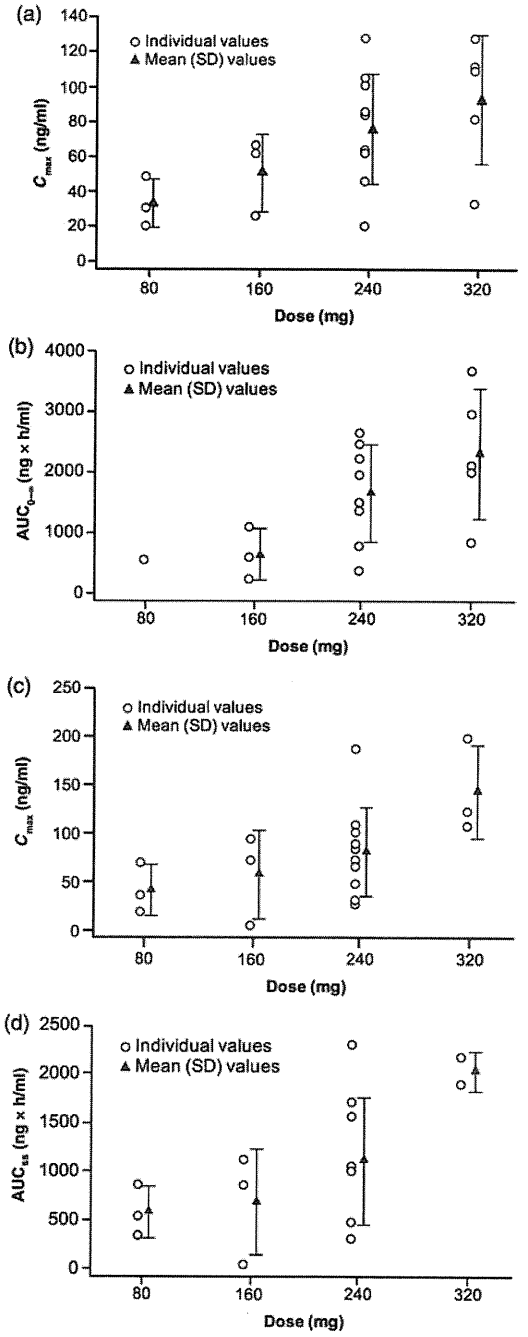

Figure 1. Individual and mean (SD) plasma neratinib exposures versus dose on study day 1 (a) C_{max} versus dose and (b) $AUC_{0-\infty}$ versus dose, and study day 21 (c) C_{max} versus dose and (d) $AUC_{0-\infty}$ versus dose. Patients with advanced solid tumors received single ascending oral doses of neratinib once daily. SD, standard deviation; C_{max}, peak concentration; $AUC_{0-\infty}$, area under the concentration–time curve from time zero extrapolated to infinite time.

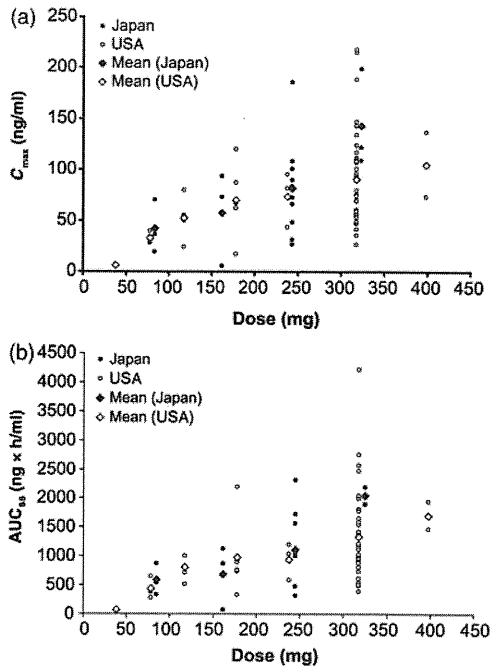

Figure 2. Comparison of neratinib exposures on study day 21: Japan versus US studies. Patients with advanced solid tumors received single ascending oral doses of neratinib once daily; (a) C_{max} versus dose and (b) AUC_{ss} versus dose. C_{max}, peak concentration; AUC_{ss}, area under the concentration–time curve at steady state.

study of neratinib that was conducted in the USA in 72 patients (92% white, 6% black or Hispanic, 1% Asian and 1% Middle Eastern) with advanced solid tumors; the DLT was Grade 3 diarrhea [1 (17%) patient in the neratinib 180 mg dose group and 5 (83%) patients in the 400 mg dose group] (17). However, due to gastrointestinal AEs, the recommended dose in ongoing Phase 3 studies is 240 mg once daily. Although diarrhea was expected in this study and was reported in 20 (95%) patients, no patients were withdrawn from the study or had a serious AE of diarrhea. Diarrhea was managed by dose interruption, dose reduction and appropriate anti-diarrhea medication.

Neratinib demonstrated promising efficacy results in Japanese patients with advanced solid tumors: PR was observed in two (10%) patients with breast cancer; three (14%) patients had SD $\geq$24 weeks and seven (33%) patients had SD $\geq$16 weeks.

PK analyses revealed that after single and multiple oral doses of neratinib, exposures (C_{max}, $AUC_{0-\infty}$ and AUC_{ss}) increased in a dose-dependent manner from 80 to 320 mg. Multiple-dose exposures were 1.2- to 1.5-fold greater than single-dose exposures across the entire dose range, thus suggesting that there was no major accumulation of neratinib after repeated daily administration of neratinib in cancer patients. The mean elimination $t_{1/2}$ on day 1 at the recommended dose of 240 mg was 14.3 h and supports a once-daily dosing regimen. Our PK data are also consistent with that reported for the US Phase 1 study of neratinib and suggest that there are no relevant differences in the PK profiles between Japanese and white patients with cancer.

This study investigated doses of neratinib from 80 to 320 mg daily. The starting dose was chosen based on information from Phase 1 study conducted in the USA (17). In the US study, diarrhea was the main DLT, with five patients in the 400 mg cohort reporting Grade 3 diarrhea. The MTD

in the US study was, therefore, established as 320 mg. In the US study, neratinib-related Grade 3 AEs were not reported at doses $\leq$80 mg. Therefore, a starting dose level of 80 mg was chosen for the current study. Based on the results of pre-clinical toxicity studies, this starting dose (80 mg/body = 48 mg/m^2 based on 1.65 m^2 human body surface area) is one-fifth of the highest non-severely toxic dosage of 45 mg/kg/day (266 mg/m^2/day, with conversion factor of 5.9), which was the highest dose used in a 4-week rat study (data on file). This dose did not elicit severe or life-threatening toxicity. This clinical dose is also supported by dosages [up to 6 mg/kg/day or 107 mg/m^2 (conversion factor of 17.9)] that did not elicit severe or life-threatening toxicity in a 4-week study in dogs (data on file).

The mean steady-state exposure of the doses at which two patients achieved PR were above the minimum efficacious dose exposure (431 ng$\times$h/ml) in nude mice. In addition, the mean steady-state exposure at the therapeutic dose of 240 mg was ~2.6-fold higher than the minimum efficacious dose exposure. However, there was no clear correlation between the dose or exposure and the severity of major AEs (i.e. diarrhea, fatigue, nausea or abdominal pain) because of the small number of patients in this study.

Irreversible inhibition of the EGFR kinase is desirable because such inhibition can occur in the presence of ATP within the cell and can only be overcome by new synthesis of EGFR. Several ATP-competitive EGFR tyrosine kinase inhibitors have been developed and investigated in clinical trials for the treatment of cancer. First-generation irreversible inhibitors include agents such as pelitinib (EKB-569). A US Phase I study showed no major antitumor responses at the MTD of pelitinib (25), although two patients in a Japanese Phase 1 study with advanced non-small cell lung cancer with EGFR mutations and acquired gefitinib resistance showed radiographic tumor regression (26). However, as pelitinib showed limited activity in Her2-dependent tumor models, the development of irreversible inhibitors with improved activity toward Her2-expressing tumors continued (16). It was discovered that attaching a large lipophilic group to the molecule resulted in improved potency for Her2 kinase inhibition (16). Thus, the structure of the second-generation irreversible pan-Her inhibitor neratinib is similar to the structure of pelitinib, but with this different aniline headpiece. The binding model for neratinib at the ATP site of Her2 indicates that the aniline portion of the molecule fits into a long lipophilic pocket. The nature and placement of these groups most likely gives neratinib its improved Her2 activity compared with pelitinib.

In conclusion, the MTD of oral neratinib was determined to be 240 mg once daily in Japanese patients with advanced solid tumors. Neratinib 240 mg was safe and well tolerated, and demonstrated encouraging antitumor activity in this patient population. We therefore recommend that this dose is used for subsequent studies in Japanese patients. The results of this Phase 1 study are consistent with those observed in white patients and warrant further investigation of neratinib in Japanese patients with solid tumors.

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Conflict of interest statement

Yoshinori Ito is a member of the Scientific Advisory Board for Pfizer Inc and has received research support from Bristol-Myers Squibb, Chugai, Novartis, and Wyeth Research (now Pfizer Inc). Masahiro Yokoyama is a consultant for Chugai. Kentaro Yamazaki has received contract research support from Amgen, Chugai, Daiichi-Sankyo, Taiho and Yakult. Kiyoshi Hashigami and Nobuko Takenaka are employees of Pfizer Inc; Hirotaka Hasegawa is a former employee of Pfizer Inc. Narikazu Boku has received research support from Taiho and has received honoraria from Daiichi-Sankyo, Ono, Taiho and Takeda.

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Phase I study of anti-CD22 immunoconjugate inotuzumab ozogamicin plus rituximab in relapsed/refractory B-cell non-Hodgkin lymphoma

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Inotuzumab ozogamicin (CMC-544), a humanized anti-CD22 antibody conjugated to the potent cytotoxic antibiotic calicheamicin, targets the CD22 antigen expressed on the majority of B-cell non-Hodgkin lymphomas. This phase I study assessed the tolerability, safety, pharmacokinetics, and preliminary efficacy of inotuzumab ozogamicin administered intravenously in combination with rituximab in Japanese patients with relapsed or refractory B-cell non-Hodgkin lymphoma. Ten patients were administered rituximab 375 mg/m^2 followed by inotuzumab ozogamicin at the maximum tolerated dose (1.8 mg/m^2). Treatment was repeated every 28 days up to eight cycles, or until occurrence of disease progression or intolerable toxicity. The safety profile was similar to that of inotuzumab ozogamicin monotherapy, with hematologic adverse events occurring most frequently. The most common grade three or higher adverse events were thrombocytopenia (70%), neutropenia (50%), leukopenia (30%), and lymphopenia (30%). The overall response rate was 80% (8/10; 95% CI, 44–98%). Drug exposure increased with successive doses, similar to the pharmacokinetic profiles observed in previous phase I monotherapy studies. Efficacy results suggested promising antitumor activity, and the overall findings support the continued clinical development of this therapeutic regimen in patients with relapsed or refractory B-cell non-Hodgkin lymphoma. This trial was registered at www.ClinicalTrials.gov as NCT00724971. (*Cancer Sci* 2012; 103: 933–938)

CD22, a B-cell antigen expressed on >90% of B-lymphoid malignancies,[1] represents an attractive therapeutic target for treatment of B-cell non-Hodgkin lymphoma (NHL). CD22 is not routinely shed into the extracellular environment;[2] rather, CD22 is rapidly internalized upon binding with a ligand or antibody, allowing efficient delivery of targeted cytotoxic agents.[3] Inotuzumab ozogamicin (CMC-544) is a targeted chemotherapy agent composed of a humanized anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic. *In vitro*, inotuzumab ozogamicin has demonstrated enhanced cytotoxic activity compared with untargeted uptake of calicheamicin.[2] Additionally, CD22 is expressed primarily on mature B-lymphocytes, with limited expression on lymphocyte precursor cells and no expression on memory B cells; therefore, minimal impact of inotuzumab ozogamicin on long-term immune function is expected.

Inotuzumab ozogamicin demonstrated promising cytotoxic activity both as a single agent and in combination with rituximab in xenograft models and *in vitro* studies.[2,4,5] Rituximab, an anti-CD20 monoclonal antibody, is indicated for single-agent use or in combination with chemotherapy for treatment of low-grade or follicular, CD20-positive, B-cell NHL, and in combination with chemotherapy for treatment of diffuse large B-cell, CD20-positive NHL.[6] Although rituximab has been effectively used in combination with chemotherapy for indolent and aggressive B-cell NHLs, some patients are not responsive, while those who do respond often experience relapse.[7] Mechanisms of rituximab resistance may include downregulation of CD20 and increased expression of complement inhibitory proteins.[8] Newer monoclonal antibodies that target B-cell antigens other than CD20 may be effective in rituximab-resistant B-cell NHL or work in synergistic fashion with rituximab to improve B-cell NHL treatment efficacy.[9] As both CD22 and CD20 are expressed in most patients with B-cell NHL,[1,7] inotuzumab ozogamicin and rituximab combination therapy in B-cell NHL may enhance the therapeutic advantage of each agent.[5]

Clinical activity was observed with inotuzumab ozogamicin monotherapy at the maximum tolerated dose (MTD) of 1.8 mg/m^2 i.v. every 28 days.[10,11] Results of inotuzumab ozogamicin at the MTD in combination with rituximab in non-Japanese patients with relapsed or refractory B-cell NHL has shown promising efficacy with a safety profile similar to that reported for inotuzumab ozogamicin alone.[12] The current study assessed the tolerability and initial safety profile of inotuzumab ozogamicin plus rituximab in Japanese patients with relapsed or refractory B-cell NHL. Secondary objectives included evaluating the pharmacokinetics and preliminary efficacy of this drug combination.

Materials and Methods

Patients. Eligible patients were aged 20–74 years, with a diagnosis of CD20- and CD22-positive B-cell NHL according to the World Health Organization classification.[13] The disease must have progressed after one or two prior therapies, and prior treatment must have included one or more doses of rituximab therapy (monotherapy or combined with chemotherapy). Maintenance therapy with rituximab was considered part of the preceding induction regimen, and patients could not be refractory to rituximab (i.e. progressive disease [PD] under treatment or <6 months of protocol therapy initiation). Other inclusion criteria included an Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 1; life expectancy

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$\geq$12 weeks; adequate organ function (absolute neutrophil count [ANC] $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L; serum creatinine $\leq 1.5 \times$ upper limit of normal [ULN] and urine protein to creatinine ratio of ≤ 0.2; total bilirubin $\leq 1.5 \times$ ULN, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $\leq 2.5 \times$ ULN); and ≥ 1 measurable lesion $\geq 1.5 \times 1.5$ cm by computed tomography (CT) scan. Patients who had received radioimmunotherapy or prior treatment with anti-CD22 antibodies were excluded. Prior allogeneic hematopoietic stem cell transplant was not allowed, and patients with prior autologous transplant were eligible if it occurred >6 months before the first study dose. No chemotherapy, anti-lymphoma immunosuppressive therapy, growth factors (except erythropoietin), or investigational agents <28 days before the first study dose (<6 weeks for nitrosoureas or mitomycin C) was allowed.

The study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent, and the protocol was approved by institutional review board at each site. This trial was registered at www.ClinicalTrials.gov (Identifier: NCT00724971).

Study design. This phase I, open-label, single-arm study evaluated the tolerability, safety, pharmacokinetics, and preliminary efficacy of inotuzumab ozogamicin administered i.v. with rituximab to Japanese patients with B-cell NHL. Screening procedures were performed within 21 days of study treatment initiation and included: medical history and physical examination; ECOG Performance Status; CD20/CD22 immunophenotyping of the B-cell lymphoma; electrocardiogram (ECG) and echocardiogram; complete chemistry panel; complete blood count (CBC) with differential; chest radiograph; CT scans of the chest, abdomen, and pelvis; clinical disease and tumor site assessments; bone marrow aspiration and/or biopsy; urinalysis; and testing for serum antibodies to inotuzumab ozogamicin and rituximab.

A fixed standard dose of rituximab 375 mg/m^2 was administered i.v. on day 1 of a 28-day (±2 days) cycle followed by inotuzumab ozogamicin 1.8 mg/m^2 i.v. on day 2 of the cycle. Treatment was repeated for up to eight cycles or until the occurrence of PD, intolerable toxicity, or patient refusal. Patients were followed for a minimum period of 1 year from the first dosing to assess progression-free survival (PFS). Six patients were to be enrolled in the first cohort; if two or fewer patients experienced a dose-limiting toxicity (DLT) within 28 days after the first dose, the dose and administration schedule would be considered reasonable and an additional four patients would be enrolled in an expanded cohort (10 patients total). If more than two of six patients experienced a DLT in the first 28 days, the tolerability of lower doses of inotuzumab ozogamicin (e.g. 1.3 mg/m^2) would be explored.

A DLT was defined as any of the following that were at least possibly related to study treatment: any grade 3/4 nonhematologic toxicity (except grade 3 nausea or vomiting, unless the patient had received optimal supportive therapy); febrile neutropenia (grade 4 ANC and temperature $\geq$38.0°C); grade 4 ANC $\geq$7 days duration; grade 4 thrombocytopenia $\geq$3 days duration or grade 3/4 thrombocytopenia associated with bleeding tendency requiring platelet transfusion; and delayed recovery (to grade $\leq$1 or baseline, except alopecia) from toxicity related to the study drug that delayed initiation of the next dose by > 2 weeks.

Safety assessment. All patients who received one or more doses of inotuzumab ozogamicin or rituximab were included in the safety analysis (safety population). Safety was monitored through physical examinations, interim history, and laboratory tests. Interim physical examinations, including liver and spleen assessments, occurred every cycle before treatment administration if deemed necessary. Vital signs were measured on each day of inotuzumab ozogamicin administration. Complete chemistry panel and CBC with differential were assessed weekly during the first four cycles and biweekly during the last four cycles. Complete spot urinalysis was performed on day 1 of every other cycle beginning with cycle 2. ECG and chest radiograph were conducted at treatment completion. Adverse events (AEs) were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Patients were monitored for AEs for 28–42 days after the last dose of inotuzumab ozogamicin, regardless of causality, and patients with evidence of treatment-related AEs at the end of treatment visit were followed until the AEs resolved or were determined to be irreversible.

Efficacy evaluation. Efficacy analyses were based on the intention-to-treat (ITT) and evaluable populations. The ITT population included all patients who were enrolled into the intended dose scheme, and the evaluable population included all patients who received two or more cycles of the study treatment, had a baseline tumor CT scan, and had undergone one or more post-baseline tumor assessments. Tumor responses were evaluated by investigator's assessment according to the International Response Criteria for NHL.[14] Overall response rate (ORR) was defined as the proportion of patients achieving complete response (CR), unconfirmed complete response (CRu), or partial response (PR). Duration of overall response was defined as the interval between first CR, CRu, or PR, until relapse or PD. PFS was defined as the interval between the first study dose and relapse, PD, or death, censored at the last tumor evaluation date. PFS rate at 1 year, defined as the proportion of patients without an event (relapse, PD, or death), was used as an early determination of PFS. Overall survival (OS) was defined as the interval between the first study dose and death, censored at the last date known alive.

Tumor responses were determined from CT scans, clinical information (e.g. liver and spleen size), "B-symptoms" (e.g. fever, night sweats, and weight loss), laboratory assessments (e.g. bone marrow biopsies), and biochemical markers of disease activity (e.g. lactate dehydrogenase). Tumor sites and clinical disease measurements were assessed: approximately every 8 weeks during treatment (or earlier with clinical evidence of tumor response), at the end of treatment visit, and approximately every 12 weeks during follow-up visits. If clinically indicated, CT scans were performed earlier than scheduled, and a confirmatory assessment was performed within 4 weeks of a documented tumor response. Tumor assessments continued until PD or death occurred, or subsequent anti-lymphoma treatment was administered.

Pharmacokinetic analyses. Serum concentrations of inotuzumab ozogamicin, total calicheamicin, and free (unconjugated) calicheamicin were determined using a validated ELISA.[10] The quantitation ranges for inotuzumab ozogamicin, total calicheamicin, and free calicheamicin were 52.2–1400, 5–100, and 1.25–150 ng/mL, respectively. Timed blood samples for pharmacokinetic analyses were collected from cycles 1 to 3, at 0 (pre-dose), 1 (before the end of infusion), 4, 48, 144, 192, 312, 480, and 648 h relative to the start of inotuzumab ozogamicin infusion; sample collection for pharmacokinetics was not collected beyond cycle 3. Pharmacokinetic parameters of inotuzumab ozogamicin and total calicheamicin were estimated by a non-compartmental method using WinNonlin, version 5.1 (Pharsight, Mountain View, CA, USA). Validated ELISAs were used for the detection of antibodies to inotuzumab ozogamicin and rituximab. The pharmacokinetic profile of rituximab was not evaluated.

Statistics. The safety and antitumor activity of inotuzumab ozogamicin plus rituximab were evaluated on an exploratory basis and summarized using descriptive statistics. Clopper-Pearson methodology was used to estimate the confidence interval

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(CI) for the ORR, and Kaplan–Meier methodology was used to analyze the duration of overall response, PFS, and OS.

Results

Patients. Ten patients (five males, five females) were enrolled; patient characteristics are summarized in Table 1. Clinical stages at screening were IIIA in five patients, IVA in three patients, and IA and IIA in one patient each. Five patients had experienced one prior lymphoma regimen and five patients had two prior regimens (including two patients who had single-agent rituximab as the second regimen); two patients had prior radiotherapy. All 10 patients received one or more cycles of both inotuzumab ozogamicin and rituximab, with a median number of four cycles (range, 1–8 cycles), and were included in the safety and ITT populations. Eight patients were included in the evaluable population for efficacy analyses, as two patients did not complete two or more cycles of the study treatment.

Safety. In the initial cohort of six patients, two patients experienced DLTs (grade 4 thrombocytopenia persisting $\geq$3 days and grade 3 increased AST). Since two or fewer patients experienced DLTs during the first cycle, an additional four patients were enrolled at the same dose level (inotuzumab ozogamicin 1.8 mg/m^2 and rituximab 375 mg/m^2). Three of the four patients in the expanded cohort experienced DLTs (grade 4 thrombocytopenia persisting $\geq$3 days).

Of the four patients who experienced thrombocytopenia qualifying as a DLT, the patient in the initial cohort had grade 4 thrombocytopenia persisting for 5 days that required a subsequent dose reduction; the patient experienced persistent grade 1–3 thrombocytopenia thereafter but continued treatment until cycle 7. Two patients in the expansion cohort experienced grade 4 thrombocytopenia persisting for 4 days and 5 days; both experienced recovery after discontinuing treatment after cycle 1 due to neutropenia. The remaining patient experienced grade 4 thrombocytopenia persisting for 3 days that required platelet transfusion and subsequent dose reduction; this patient experienced persistent grade 1–3 thrombocytopenia thereafter but remained on therapy until cycle 5. Although an additional three patients experienced grade $\geq$3 thrombocytopenia during the study, no dose delays, dose reductions, platelet transfusions, or treatment discontinuations due to thrombocytopenia were required. In addition, no grade $\geq$3 bleeding events were reported.

All 10 patients experienced one or more treatment-emergent AE, and nine patients experienced grade three or higher treatment-emergent AEs (Table 2). The most commonly reported grade $\geq$3 treatment-emergent AEs were hematologic abnormalities; other grade $\geq$3 events included hypophosphatemia (n = 2) and increased AST (n = 1; Table 3). Neutropenia led to dose delays in two patients. AEs leading to dose reductions (to inotuzumab ozogamicin 1.3 mg/m^2) included thrombocytopenia (n = 2) and increased AST (n = 1). Five patients had AEs (neutropenia [n = 3] and hyperbilirubinemia [n = 2]) that did not recover to grade $\leq$1 within 21 days of the scheduled dosing day and were discontinued from treatment. No serious AEs or deaths occurred during the study.

Efficacy. Eight of 10 patients were followed for more than 52 weeks; one patient with mantle cell lymphoma progressed during the study, and one patient with diffuse large B-cell lymphoma discontinued due to lack of efficacy. OS at 1 year (52 weeks) was 100%, as no deaths were observed during the study. In the ITT population, the ORR was 80% (95% CI, 44–98%; Table 4). In the eight evaluable patients who received

Table 1. Patient characteristics

Characteristic	Inotuzumab ozogamicin 1.8 mg/m^2 + rituximab 375 mg/m^2 (N = 10)
Median age (range), years	60.5 (46–74)
Male sex, n (%)	5 (50)
ECOG Performance Status, n (%)	
0	10 (100)
Histologic subtype, n (%)	
Follicular lymphoma	6 (60)
Mantle cell lymphoma	2 (20)
Diffuse large B-cell lymphoma	1 (10)
Mucosa-associated lymphoid tissue lymphoma	1 (10)
Stage IIIA/IVA disease	8 (80)
No. prior anti-lymphoma regimens, n (%)	
1	5 (50)
2	5 (50)
Prior radiotherapy, n (%)	2 (20)

ECOG, Eastern Cooperative Oncology Group.

Table 2. Summary of adverse events, safety population

Event, n (%)	Inotuzumab ozogamicin 1.8 mg/m^2 + rituximab 375 mg/m^2 (N = 10)
Any TEAE	10 (100)
Grade $\geq$3 TEAE	9 (90)
AE leading to dose delays	2 (20)
AE leading to dose reduction	3 (30)
AE leading to treatment discontinuation	5 (50)
Serious AE	0
Death within 28 days from last dose	0

AE, adverse event; TEAE, treatment-emergent adverse event.

Table 3. Treatment-emergent adverse events in $\geq$30% of patients (all grades) and all grade 3/4 treatment-emergent adverse events, safety population

Event, n (%)	Inotuzumab ozogamicin 1.8 mg/m^2 + rituximab 375 mg/m^2 (N = 10)	
	All grades	Grade 3/4
Thrombocytopenia	10 (100)	7 (70)
Increased AST	9 (90)	1 (10)
Leukopenia	8 (80)	3 (30)
Nausea	8 (80)	0
Increased ALT	8 (80)	0
Neutropenia	7 (70)	5 (50)
Lymphopenia	6 (60)	3 (30)
Increased LDH	6 (60)	0
Fatigue	5 (50)	0
Increased alkaline phosphatase	5 (50)	0
Decreased appetite	5 (50)	0
Hyperbilirubinemia	4 (40)	0
Headache	4 (40)	0
Decreased hemoglobin	3 (30)	0
Increased GGT	3 (30)	0
Nasopharyngitis	3 (30)	0
Pyrexia	3 (30)	0
Stomatitis	3 (30)	0
Hypophosphatemia	2 (20)	2 (20)

Adverse events (AEs) were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (Bethesda, MD, USA). Patients were monitored for AEs for 28–42 days after the last dose of inotuzumab ozogamicin. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase.

two or more cycles of study treatment and had one or more post-baseline tumor assessment, ORR was 88% (95% CI, 47–>99%). The duration of response ranged from 346 to 540 days; median duration of response could not be estimated, as no relapse or PD was observed among responders. In the ITT population, the best overall responses (from the start of treatment until PD) were CR in six patients, CRu and PR in one patient each, and stable disease (SD) in two patients. In the evaluable population, CR was achieved in six patients and CRu and SD were achieved in one patient each. Median PFS and OS could not be estimated because the number of events observed was limited during the study. The PFS rate at 1 year was 89% (95% CI, 43–98%) in the ITT population and 88% (95% CI, 39–98%) in the evaluable population.

Pharmacokinetics. Pharmacokinetic data were collected from all 10 patients. Three patients who received AE-related dose reductions of inotuzumab ozogamicin after cycle 1 and 2 patients who discontinued treatment after cycle 1 were excluded from pharmacokinetic analyses for cycles 2 and 3. Two patients who discontinued treatment after cycle 2 were also excluded from analyses for cycle 3. Drug exposure for inotuzumab ozogamicin and total calicheamicin (peak observed concentration [C_{max}] and area under the concentration-time curve [AUC]) increased with the number of doses, coinciding with a prolonged terminal half-life ($t_{1/2}$) and a commensurate decrease in apparent clearance (Table 5). The C_{max} of inotuzumab ozogamicin was typically observed at termination or shortly after completion of infusion. The C_{max} of total calicheamicin was usually observed within 4 h after the initiation of inotuzumab ozogamicin. Mean concentrations and standard deviations of inotuzumab ozogamicin and total calicheamicin in serum over time are shown in Figure 1. Concentrations of free calicheamicin were much lower than other analytes, and pharmacokinetic parameters could not be calculated. No antibodies to inotuzumab ozogamicin or rituximab were detected during the course of the study.

Discussion

This is the first full paper to report on clinical results of inotuzumab ozogamicin therapy in combination with rituximab. The different modes of action between inotuzumab ozogamicin and rituximab may potentially provide synergistic cytotoxicity when used in combination against B-cell NHL. Upon internalization of CD22-bound inotuzumab ozogamicin, calicheamicin diffuses into the nucleus and causes cell death.[3] By contrast, CD20-bound rituximab does not undergo constitutive endocytosis, but rather induces cytotoxic mechanisms that occur at the cell surface: complement-dependent cytotoxicity and antibody-dependent cell mediated cytotoxicity.[9,15] Thus, in addition to targeting different antigens, inotuzumab ozogamicin and rituximab use non-overlapping and perhaps complementary mechanisms of action.

The safety profile of this drug combination was similar to that observed with inotuzumab ozogamicin alone;[10,11] this is consistent with the fact that safety profiles of rituximab and chemotherapy versus chemotherapy alone are similar.[16] The major treatment-related AEs in a phase I study of Japanese patients with follicular lymphoma pretreated with rituximab and administered inotuzumab ozogamicin monotherapy at the MTD of 1.8 mg/m^2 were thrombocytopenia, leukopenia, lymphopenia, neutropenia, increased AST, anorexia, and nausea.[11] A similar toxicity profile was observed during a phase I study of non-Japanese patients with B-cell NHL (predominately

Table 4. Best overall response, intention-to-treat population

Best overall response, *n* (%)	FL (*n* = 6)	DLBCL (*n* = 1)	MCL (*n* = 2)	MALT (*n* = 1)	Total (*N* = 10)
Overall response	6 (100)	0	1 (50)	1 (100)	8 (80)
Complete response (confirmed)	5 (83)	0	0	1 (100)	6 (60)
Complete response (unconfirmed)	0	0	1 (50)	0	1 (10)
Partial response	1 (17)	0	0	0	1 (10)
Stable disease	0	1 (100)	1 (50)	0	2 (20)

Tumor responses were determined by the investigator according to the International Response Criteria for Non-Hodgkin Lymphoma. Tumor assessments occurred approximately every eight weeks during treatment (or sooner), at the end of treatment visit, and every 12 weeks during follow-up visits. Overall response included complete confirmed, complete unconfirmed and partial response. DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MALT, mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma.

Table 5. Pharmacokinetic parameters† of inotuzumab ozogamicin and total calicheamicin

Treatment period (day)	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC_T (ng·h/mL)	AUC (ng·h/mL)	CL (L/h)	V_{ss} (L)
Inotuzumab ozogamicin						
Cycle 1 (day 1/2)	559 (24%)	18.8 (6%)	12 300 (51%)	22 300 (13%)	0.120 (15%)	2.33 (3%)
	(*n* = 10)	(*n* = 2)	(*n* = 10)	(*n* = 2)	(*n* = 2)	(*n* = 2)
Cycle 2 (day 29/30)	822 (19%)	29.1 (75%)	27 000 (28%)	34 800 (35%)	0.078 (25%)	2.26 (77%)
	(*n* = 5)	(*n* = 2)	(*n* = 5)	(*n* = 2)	(*n* = 2)	(*n* = 2)
Cycle 3 (day 57/58)	958 (7%)	51.7 (40%)	50 100 (13%)	54 800 (13%)	0.050 (2%)	3.02 (41%)
	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)
Total calicheamicin						
Cycle 1 (day 1/2)	67.7 (22%)	61.2 (57%)	2850 (49%)	4060 (27%)	0.746 (28%)	47.6 (24%)
	(*n* = 10)	(*n* = 7)	(*n* = 10)	(*n* = 7)	(*n* = 7)	(*n* = 7)
Cycle 2 (day 29/30)	80.2 (14%)	96.4 (32%)	6490 (35%)	7360 (33%)	0.424 (31%)	45.6 (15%)
	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)	(*n* = 5)
Cycle 3 (day 57/58)	96.6 (3%)	167.9 (43%)	10 700 (44%)	11 600 (35%)	0.249 (28%)	47.8 (14%)
	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)

†Data are shown as mean values at the time points indicated (CV%); the numbers of patients evaluable for each parameter or time point are also provided. AUC, area under the concentration-time curve evaluated to infinity (cycle 1) or dosing interval (672 h; cycles 2 and 3); AUC_T, area under the concentration-time curve evaluated to the last measurable observation; CL, apparent clearance; C_{max}, peak observed concentration; CV, coefficient of variation; $t_{1/2}$, terminal half-life; V_{ss}, apparent steady-state volume of distribution.

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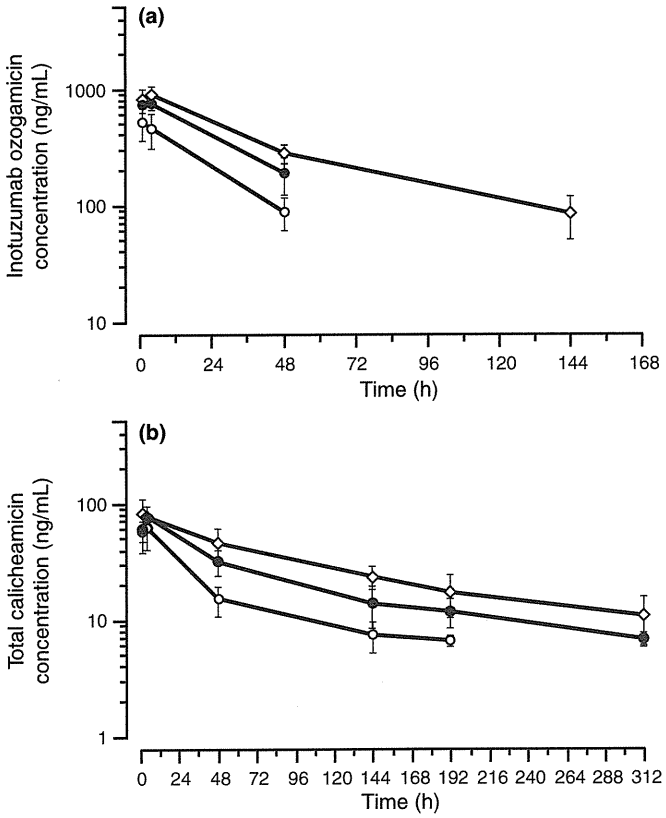

Fig. 1. Mean concentrations of inotuzumab ozogamicin (a) and total calicheamicin (b) in serum after i.v. treatment with inotuzumab ozogamicin 1.8 mg/m^2 and rituximab 375 mg/m^2, 28-day cycle. Error bars denote standard deviations. Cycle 1 (-○-); cycle 2 (-●-); cycle 3 (-◇-).

follicular lymphoma or diffuse large B-cell lymphoma) treated with inotuzumab ozogamicin 1.8 mg/m^2.[10] The most common toxicities in the current study included thrombocytopenia, leukopenia, nausea, and elevated liver function tests. Five of 10 patients had AEs that met the criteria for DLTs. However, these events were all transient laboratory abnormalities without other associated clinical sequelae. Therefore, the independent data monitoring committee considered inotuzumab ozogamicin at 1.8 mg/m^2 plus rituximab to be tolerable and safe and recommended continued clinical development with careful attention for thrombocytopenia in subsequent studies.

Patients in this study had relatively few prior treatments (one to two regimens), but included three patients with aggressive lymphoma diagnoses: two with mantle cell lymphoma and one with diffuse large B-cell lymphoma. In efficacy analyses, most patients achieved CR/CRu (7/10) or PR (1/10) and remained progression-free at 52 weeks. The clinical response in this study compares favorably to responses observed with inotuzumab ozogamicin or rituximab monotherapy. In previous phase I studies of patients with B-cell NHL administered inotuzumab ozogamicin monotherapy at the MTD, the ORRs were 39%[10] and 80%,[11] while rituximab monotherapy in patients with relapsed or refractory, low-grade or follicular, B-cell NHL was associated with an ORR of 48%.[17] The clinical activity demonstrated in this trial is consistent with the robust antitumor activity of this drug combination observed in preclinical models. *In vitro*, inotuzumab ozogamicin plus rituximab suppressed the growth of B-cell lymphoma xenografts by >90%; this effect was additive compared with either agent alone.[5]

The efficacy results were also comparable with reported results of epratuzumab, a humanized anti-CD22 monoclonal antibody, plus rituximab in patients with post-chemotherapy, relapsed or refractory, indolent B-cell NHL (ORR of 54% in 41 patients with follicular lymphoma [24% achieving a CR/CRu], and 57% in seven patients with small lymphocytic lymphoma [43% with CR/CRu]).[18] Although a definite comparison of this study with our study cannot be made due to the limited number of patients in our phase I study, the combination use of inotuzumab ozogamicin plus rituximab may have increased efficacy over combination use of two monoclonal antibodies due to the addition of a targeted chemotherapy agent.

Pharmacokinetic analyses revealed that drug exposure (C_{max}, AUC) to inotuzumab ozogamicin increased with the number of doses of combination therapy, displaying a nonlinear disposition similar to the pharmacokinetic profile observed in phase I studies of inotuzumab ozogamicin monotherapy.[10,11] No effect of rituximab on the pharmacokinetic profile of inotuzumab ozogamicin was apparent. Serum concentration increases may be partially attributable to accumulation; such nonlinearities in drug disposition are common for antibodies.[19]

Inotuzumab ozogamicin in combination with rituximab showed an acceptable safety profile in Japanese patients with relapsed or refractory B-cell NHL that is similar to the observed single-agent profile. Preliminary but encouraging evidence of clinical activity of inotuzumab ozogamicin plus rituximab was also demonstrated, and the findings support the continued clinical development of this therapeutic regimen.

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Disclosure Statement

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ORIGINAL ARTICLE

Evaluation of safety, pharmacokinetics, and efficacy of vorinostat, a histone deacetylase inhibitor, in the treatment of gastrointestinal (GI) cancer in a phase I clinical trial

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Abstract
Background Control of epigenetic changes using histone deacetylase inhibitors (HDACi) is thought to be a promising target in therapy of gastrointestinal (GI) cancer. In this study, we evaluated the safety, pharmacokinetics, and efficacy of two dosing regimens of vorinostat, an oral HDACi, in patients with GI tumors.
Methods Patients received either vorinostat 300 mg bid for 3 consecutive days followed by 4 rest days per cycle ($n = 10$) or vorinostat 400 mg qd for 21 consecutive days per cycle ($n = 6$). Pharmacokinetic parameters were assessed for the first treatment cycle. Efficacy was determined through evaluation of tumors and assessment of treatment response.
Results The median treatment duration of 300 mg bid was 52.0 days and of 400 mg qd was 51.5 days. The most common drug-related adverse events were anorexia, nausea, fatigue, and hyperglycemia. Two patients taking 400 mg qd had dose-limiting toxicities (DLTs) of thrombocytopenia. No patients taking 300 mg bid experienced DLT. Five patients taking 300 mg bid and 2 patients taking 400 mg qd maintained stable disease for >8 weeks, with the maximum duration of 245 days. Mean drug exposure (±SD) was generally higher with 400 mg qd (area under the curve [$AUC_{0-\infty}$] of 7.75 ± 2.79 μM h on Day 1 post-dose) compared with 300 mg bid ($AUC_{0-\infty}$ of 3.94 ± 1.56 μM h on Day 1 post-dose).
Conclusions Vorinostat 300 mg bid for 3 consecutive days followed by 4 days of rest was better tolerated in patients with GI cancer than a higher once daily dose. Additionally, there were patients in both groups who achieved stable disease, most maintaining it for longer than 8 weeks, suggesting vorinostat as a possible active agent in the treatment of GI cancer.

Keywords Histone deacetylase inhibitor (HDACi) · Gastrointestinal cancer · Vorinostat · Suberoylanilide hydroxamic acid (SAHA)

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Introduction

While cancer has traditionally been associated with genetic damage, pharmacologic interventions for some forms of malignancies have recently focused on epigenetic damage. Epigenetic damage (i.e., the deactivation of genes after multiple cell divisions), which occurs due to factors such as aging and chronic inflammatory processes, has led to many treatment-resistant cancers such as myelodysplastic syndrome. DNA methylation is an important epigenetic marker; malignancy has been associated with hypomethylation of human tumor DNA as well as hypermethylation of tumor suppressor genes. Additionally, the acetylation of

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core nucleosome histone proteins remodels chromatin, increases access to DNA of transcription factors and other co-activator proteins, and promotes gene transcription. Histone acetylation is accomplished by histone acetyl transferases (HATs), whereas the deacetylation of histones is accomplished by histone deacetylases (HDACs) [1]. In normal cells, HAT and HDAC activities are balanced and tightly regulated by homeostasis. However, excess HDAC activity is common in cancer cells and contributes to oncogenic transformation by mediating the function of oncogenic translocation products [2–4]. In patients with gastrointestinal (GI) malignancies, epigenetic deactivation of genes through DNA hypermethylation and histone deacetylation has been implicated, particularly in gastric cancer, in which patients are often affected by chronic gastritis due to *H. pylori* infection [5–7].

The activity of HDACs has been further elucidated recently to include modification of non-histone proteins such as transcription factors, tumor suppressor genes, cell cycle regulators, mediators of signal transduction, a cytoskeletal modifier, the molecular chaperone Hsp90, and SRY [8]. As a result, inhibition of HDACs was identified as a possible target for pharmacologic antineoplastic agents; clinical research with HDAC inhibitors has since validated these agents in a variety of solid tumor and hematologic malignancy settings [9–12].

There are 3 major classes of HDACs that include at least 18 isozymes; HDAC classes are separated based on size, cellular localization, number of catalytic active sites, and homology to yeast HDAC proteins. Class I HDACs are generally localized to the nucleus of cells and include HDAC1, HDAC2, HDAC3, and HDAC8 while class II HDACs shuttle between the nucleus and the cytoplasm and include two subclasses (Class IIa includes HDAC4, HDAC5, HDAC7, and HDAC9, each of which contains a single catalytic active site, and Class IIb includes HDAC6 and HDAC10, which both contain two active sites. Class III HDACs operate by a NAD^+-dependent mechanism unrelated to the other HDAC proteins.

Vorinostat (suberoylanilide hydroxamic acid) is a small molecule inhibitor of class I and II HDAC enzymes that has been shown to promote cell cycle arrest and apoptosis of cancer cells through regulation of gene expression [12, 13]. Vorinostat has demonstrated activity against various types of tumors in vitro and also improved survival and/or produced antitumor effects in animal models [9]. Interestingly, HDAC inhibitors, including vorinostat, reactivated RUNX3, a gastric tumor suppressor in gastric cancer-derived cells lines that is epigenetically silenced [14]. In addition, the loss of transforming growth factor-β (TGFβ) response contributes to oncogenesis and has been described in GI cancer [15, 16]. Vorinostat can restore TGFβ activity [17].

Vorinostat had a favorable toxicity profile in phase I and II trials in Japanese and non-Japanese patients [10, 11, 18–20]. Phase I trials to evaluate the safety and activity of vorinostat were conducted in patients with advanced solid and hematologic malignancies and demonstrated that oral vorinostat was well tolerated [18, 20]. Dose-limiting toxicities (DLTs) included anorexia, dehydration, diarrhea, fatigue, and thrombocytopenia. The maximum tolerated doses of oral vorinostat were determined to be 400 mg qd or 200 mg bid as continuous dosing, and 300 mg bid for 3 consecutive days per week, or 200 mg orally bid or tid for 14 days followed by 7 days of rest [18]. In two phase II trials, vorinostat 400 mg qd as continuous dosing was safe and effective, with an overall response rate of 24–30% in refractory advanced patients with cutaneous T-cell lymphoma (CTCL) including large cell transformation and Sézary syndrome [10, 19]. In October 2006, vorinostat was approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous manifestations in patients with CTCL who have progressive, persistent or recurrent disease on or after two systemic therapies [21].

Based on these promising preclinical and clinical findings, a phase I trial of vorinostat in Japanese patients with solid tumors was conducted. In the study, vorinostat was generally well tolerated up to 500 mg daily for 14 days followed by 7 days of rest. The safety profile and pharmacokinetics data from Japanese patients were similar to those from non-Japanese patients [18, 22]. The current study was conducted in order to evaluate the safety, tolerability, and pharmacokinetics of two non-Japanese maximum tolerated doses (MTDs) of vorinostat (400 mg orally every day as continuous dosing, and 300 mg orally bid for 3 consecutive days per week) in Japanese patients; these dosing schedules were selected based on their dose intensities. An exploratory objective in this study was to determine if vorinostat has antitumor activity against GI cancer, especially gastric cancer.

Methods

This phase I study (Protocol 048) was conducted at 3 study centers in Japan and approved by Institutional Review Boards at each study center. All patients provided written informed consent prior to enrollment in accordance with principles of Good Clinical Practice. This study was conducted at the following sites: National Cancer Center Hospital East, Chiba, Japan; National Cancer Center, Tokyo, Japan; Oita University, Oita, Japan; Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Oita, Japan; and Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan.

Eligibility criteria

Patients who were eligible to enroll in this study included those with a histologically or cytologically diagnosed solid tumor with no standard therapy available or those who had failed to respond to standard therapy, with ECOG performance status of 0–2, whose life expectancy was ≥3 months after enrollment, and who were ≥20 years of age.

Patients were not eligible for enrollment if they had adverse events (AEs) from previous anti-cancer treatments that were National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 grade 2 or more severe (with the exception of alopecia); were positive for HIV, HBV, or HCV; had a brain tumor or brain metastasis; had any concurrent malignancy (unless they had tumors localized in mucosa/epithelium or those who had been in remission for ≥5 years); had anemia requiring blood transfusions within 2 weeks before enrollment; had bone marrow, hepatic, or renal dysfunction beyond predefined criteria; had peritoneal or pleural effusion requiring treatment; or had any uncontrolled concomitant illness (arrhythmias, unstable angina, congestive heart failure, uncontrolled hypertension, infections requiring systemic treatment, or continuous use of steroids). Additionally, patients were excluded if they required immunotherapy, radiotherapy, surgery, or chemotherapy or if they underwent these procedures within 4 weeks before enrollment; had hematopoietic cytokine treatment (e.g., G-CSF) within 2 weeks before enrollment; had mitomycin C or nitrosoureas within 6 weeks before enrollment; had a history of radiotherapy directed toward >25% of hematopoietic marrow cells; or had previously participated in a clinical trial of an HDAC inhibitor. The use of prophylactic concomitant use of colony stimulating factors, antibiotics, or antiemetics was prohibited during the 1st cycle.

Treatment plan

The doses studied in this clinical trial were selected based on their dose intensities. A dose regimen of 200 mg bid for 14 days followed by 7 days of rest (a dose intensity of 5600 mg) had already been determined to be well tolerated in Japanese patients with solid tumors. Because this was the first study in Japanese patients with GI cancer treated with multiple prior chemotherapies, an initial dosing regimen of 300 mg bid for 3 consecutive days per week was chosen due to a lower dose intensity (5400 mg). The 400 mg qd dose was chosen for this study because it is the regimen recommended internationally for other cancers.

Treatment was administered at a hospital for the first cycle and at home for each subsequent cycle. Two dosing regimens that had been used in the previous clinical studies conducted outside Japan were investigated in this study: group 1 and group 2.

For group 1, vorinostat 300 mg (3 × 100-mg oral tablets) was administered twice daily for 3 consecutive days (within 30 min after breakfast and dinner) followed by 4 off-drug days; this was repeated 3 times for each cycle of treatment. For group 2, vorinostat 400 mg qd was administered for 21 consecutive days.

At least 3 evaluable patients for a dose-limiting toxicity (DLT) were enrolled in each dosing regimen using a standard "3 + 3" design. In order to assess the safety of each dosing regimen, we followed the procedure detailed in Fig. 1.

Additional patients were enrolled at the same level up to a total of 10 patients for each dosing regimen (a total of 20 patients) to evaluate pharmacokinetics once safety was confirmed. If a patient developed a DLT during a treatment cycle, the patient was to stop treatment for the rest of the days in the cycle, and the dose was reduced to 200 mg bid for 3 consecutive days followed by 4 off-drug days if the

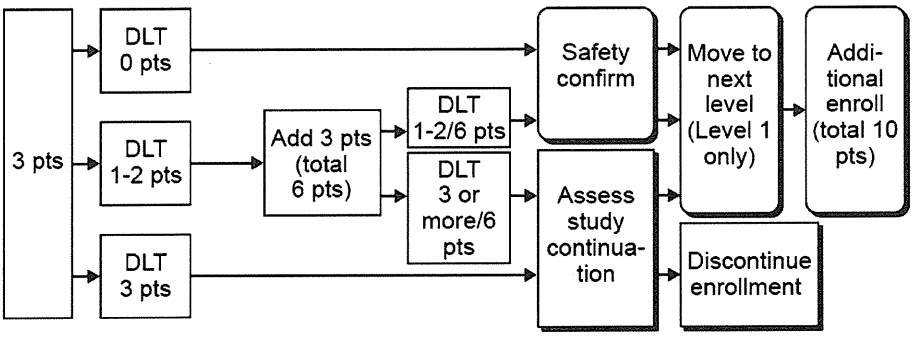


Fig. 1 Procedure for evaluating dose levels for safety based on DLTs: (1) If none of the first 3 patients at a level developed a DLT during the first cycle, the dose was deemed tolerable. (2) If 1 or 2 of the first 3 patients enrolled at a level developed a DLT during the first cycle, 3 additional patients were enrolled at the same level to further assess tolerability with 6 patients; if 2 or fewer of the 6 patients developed a DLT, the dose was deemed tolerable. If 3 or more of the 6 patients developed a DLT, continuation of the level was to be determined by the sponsor after assessment by the efficacy and safety board. (3) If all 3 patients enrolled at a level developed a DLT during the first cycle, continuation of the level was to be determined by the sponsor after assessment by the efficacy and safety board

patient was in group 1, or the dose was reduced to 300 mg qd for 21 consecutive days if the patient was in group 2 in the subsequent cycles.

Safety

Adverse experiences (AEs) were evaluated by investigators who determined their relationship to the study drug and degree of severity. The CTCAE version 3.0 was used to grade AEs. DLTs were defined as the manifestation of one of the following drug-related AEs: (1) grade 4 neutropenia persisting for more than 5 days; (2) grade 3 or higher neutropenia with fever; (3) grade 3 thrombocytopenia requiring platelet transfusions or grade 4 thrombocytopenia; (4) grade 3 or higher non-hematological toxicities; (5) AST/ALT elevation of over 300 IU/L.

Pharmacokinetics

Serum vorinostat concentration was analyzed using a turbulent flow on-line extraction format for analyte isolation followed by reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection. Pharmacokinetic parameters (AUC, C_{max}, T_{max} and $t_{1/2}$) were calculated according to a noncompartmental analysis from the serum concentration of vorinostat based on actual blood sampling time pre-dose and post-dose (at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after administration of vorinostat) on Day 1, Day 3 of the 1st cycle at dose level 1 and Day 1, Day 21 of the 1st cycle at dose level 2. WinNonLin Professional version 5.0.1 (Pharsight Corp., Mountain View, CA, USA) was used for the pharmacokinetic analysis.

Efficacy

Tumor response was assessed according to the RECIST Version 1.0 guidelines [23] in patients with evaluable lesions. Tumor markers were chosen by investigators based on the type of cancer. All assessments required at baseline were performed within 4 weeks of the initiation of the study treatment. After initiation of treatment, tumor response was assessed by imaging at least every 6 weeks (every 2 cycles).

Target lesions were set by choosing up to 5 measurable lesions in one organ and up to 10 measurable lesions in the whole body. Tumor response was assessed by using the following criteria: complete response (CR) was assigned when all target lesions disappeared for 4 weeks; partial response (PR) was assigned when the sum of the longest diameters of the target lesions were reduced by $\geq$30% for 4 weeks; progressive disease (PD) was assigned when the sum of the longest diameters of the target lesions increased by $\geq$20% from the minimum sum recorded during treatment; and stable disease (SD) was assigned when the change in tumor size was not sufficient to assign PR or PD. For non-target lesions, CR was assigned when all non-target lesions disappeared or levels of all tumor markers had been normalized; incomplete response (IR) or SD was assigned when one or more non-target lesion persisted or levels of one or more tumor markers were higher than the upper limit of normal; PD was assigned for non-target tumors when there was an apparent aggravation of pre-existing non-target lesions. New lesions were recorded and treated as non-target lesions. An evaluation of overall response was also conducted by evaluating the response to target lesions, non-target lesions, and presence of new lesions.

Statistical methods

The primary purpose of the present study was to confirm safety. Therefore, the sample size was dependent on the occurrence of DLTs, although 20 patients were to be enrolled in order to obtain pharmacokinetics data. Because data were insufficient for the purpose of estimating the width of confidence intervals, there was no power calculation. A significance level of 5% (two-tailed) was used for all analyses. No adjustments were made for multiplicity since the primary objective of the study was to confirm safety.

For the evaluation of safety, the incidence of patients with AEs, drug-related AEs, and DLTs were summarized by dose levels and grades. For laboratory test parameters, vital sign parameters, and body weight, summary statistics (mean, standard error, minimum, and maximum) were provided. For the 12-lead ECG, a table of the number and percent of patients experiencing abnormalities was summarized by dose levels and time points.

For pharmacokinetic analysis, summary statistics of each pharmacokinetic parameter (AUC, C_{max}, T_{max} and $t_{1/2}$) were calculated. For calculation of AUC and C_{max}, logarithmic transformed values were used. To assess the effect of a repeated administration on pharmacokinetic parameters, the geometric mean ratio of $AUC_{0-12\ h}$ (Day 3/Day 1) and its 90% confidence interval at dose level 1 was calculated. On dose level 2, only the geometric mean ratio of $AUC_{0-24\ h}$ (Day 21/Day 1) was calculated because of limited available data ($n = 2$). For Day 8 at dose level 2, summary statistics were calculated as the trough value of serum concentration of vorinostat. For the other pharmacokinetic parameters, the appropriate transformation was done.

The exploratory analysis of efficacy was performed by summarizing the response of each dosing regimens using RECIST Version 1.0 guidelines.

Results

Patient characteristics

A total of 16 patients were enrolled in this study; 10 at dose level 1 (group 1) and 6 at dose level 2 (group 2). Baseline patient characteristics are shown in Table 1. The specific diagnoses for the patients who enrolled in this study included gastric cancer, colon cancer, and rectal cancer. The median numbers of prior regimens were 3.5 (range 2–6) for patients in group 1 and 4.5 (range 3–6) for those in group 2.

Table 1 Baseline characteristics

	300 mg bid × 3 days/ week ($n = 10$)	400 mg qd 21 consecutive days ($n = 6$)
Median age, years [range]	61 [43–73]	55 [32–66]
Male (n)	8	4
Female (n)	2	2
ECOG performance status (n)		
0	9	2
1	1	4
Disease type (n)		
Gastric cancer	8	2
Colon cancer	1	1
Rectal cancer	1	3
Number of prior chemotherapy regimens [range]	3.5 [2–6]	4.5 [3–6]

Safety and tolerability

Group 1 (300 mg bid for 3 consecutive days followed by 4 rest days)

There were 10 patients who received vorinostat in group 1. The median treatment duration was 52.0 days (range 17–243). In this group of patients, no DLTs were observed. The most common drug-related AEs included anorexia, nausea, and fatigue (Table 2). Of these drug-related AEs, the instances of thrombocytopenia were considered grade 3/4.

Four patients in group 1 experienced serious AEs. Of these, abdominal pain (grade 2) and diarrhea (grade 2), and vomiting (grade 2) and abdominal pain (grade 1) were considered by the investigator to be drug-related. Since these events required hospitalization for a follow-up, they corresponded to serious AEs. Disease progression and hyperbilirubinaemia were considered to be unrelated to the study drug.

One death was reported during this study. The patient, who had a primary disease of gastric cancer, showed disease progression at the end of the first cycle and completed the study. The patient died 26 days after the end of study therapy. The death was considered to be due to underlying disease progression and not related to the study drug.

Group 2 (400 mg qd for 21 consecutive days)

There were 6 patients who received vorinostat in group 2. The median treatment duration was 51.5 days. Of these 6 patients, 2 patients did not complete the first cycle and were not included in the DLT assessment. Of the 4 remaining patients, 2 patients developed DLTs of grade 4 thrombocytopenia. The two patients in group 2 had dose reductions from 400 mg qd to 300 mg qd. The most

Table 2 Most common drug-related hematologic and non-hematologic AEs

	Total ($n = 16$)		300 mg bid × 3 days/ week ($n = 10$)		400 mg qd 21 consecutive days ($n = 6$)	
	Grade 1 or 2	Grade 3 or 4	Grade 1 or 2	Grade 3 or 4	Grade 1 or 2	Grade 3 or 4
Hematologic						
Thrombocytopenia	4	5	2	1	2	4
Lymphopenia	7	0	4	0	3	0
Non-hematologic						
Anorexia	15	0	9	0	6	0
Nausea	14	0	8	0	6	0
Fatigue	11	0	8	0	3	0
Hyperglycemia	11	0	6	0	5	0
Vomiting	9	0	5	0	4	0
Blood creatinine increased	9[a]	0	4	0	4	0

[a] One patient experienced blood creatinine increase after dose reduction from 400 mg qd to 300 mg qd

common drug-related AEs included anorexia, nausea and thrombocytopenia (Table 2). There were 4 cases of thrombocytopenia that were considered to be grade 3/4 in group 2.

One patient in group 2 discontinued due to AEs. The patient experienced acetonemic vomiting and gastric hemorrhage due to primary disease.

Pharmacokinetics

In group 1, the maximum serum concentrations (C_{max}) of vorinostat were observed at 0.50–5.97 h after the first dose on Day 1 and 0.25–6.00 h after the morning dose on Day 3 following 3 days of multiple oral doses of vorinostat 600 mg daily (300 mg × 2) with food. Vorinostat was then rapidly eliminated with apparent $t_{1/2}$ of 0.94–1.05 h on average. The $AUC_{0-12\ h}$ was 3.92 ± 1.52 μM h after the first dose on Day 1 and 4.19 ± 1.84 μM h after the morning dose on Day 3. C_{max} was 1.17 ± 0.43 μM h after the first dose on Day 1 and 1.32 ± 0.75 μM h after the morning dose on Day 3. These results suggest that there was no significant change in absorption or elimination of vorinostat. The accumulation ratio of vorinostat following 3 days of multiple oral dose was 1.07 (90% confidence interval; 097, 1.18), suggesting no accumulation after administration of vorinostat with this dose regimen (Table 3).

In group 2, the C_{max} were observed by 3.80–6.00 h after the first dose and 2.98–3.67 h after the final dose. Vorinostat was eliminated rapidly with an apparent $t_{1/2}$ of 1.17–1.49 h on average. The $AUC_{0-24\ h}$ was 7.97 ± 3.05 μM h after the first dose and 8.45 μM h after the final dose. C_{max} was 1.62 ± 0.52 μM h after the first dose and 2.04 μM h after the final dose. At this dosing level, we were unable to evaluate the effect of multiple dosing on the pharmacokinetic parameters because the parameters following the final dose were calculated for only 2 patients. In these 2 patients, the accumulation ratio was 1.50, but because of limited data, these results should be viewed with caution (Table 4).

Patients with higher AUC values had more AEs compared with those who had lower AUC values. The other studies show the similar result of the correlation between AUC and AEs.

Efficacy

In group 1, of the 10 patients who received vorinostat, 5 patients achieved stable disease ≥8 weeks as best response: 4 patients with gastric cancer, 1 patient with colon cancer. Of these, one patient with gastric cancer showed sustained stable disease for up to 245 days. The median duration of time to progression (TTP) was 70 days (range 21–245 days).

In group 2, of the 6 patients who received vorinostat, 3 patients achieved stable disease as best response. Of these, 2 patients achieved stable disease ≥8 weeks: 1 patient with colon cancer and 1 patient with rectosigmoid cancer; no patients in group 2 with the specific diagnosis of gastric cancer had SD or better (Fig. 2).

Table 3 Summary of serum pharmacokinetic parameters at 300 mg bid × 3 days/week

Parameter	Day 1 (n = 10, fed state)	Day 3 (n = 10, fed state)
$AUC_{0-\infty}$ (μM h)	3.94 ± 1.56	4.15 ± 2.15[a]
$AUC_{0-12\ h}$ (μM h)	3.92 ± 1.52	4.19 ± 1.84
C_{max} (μM)	1.17 ± 0.43	1.32 ± 0.75
T_{max} (h)	1.99 (0.50–5.97)	0.99 (0.25–6.00)
$t_{1/2}$ (h)	1.05 ± 0.32	0.94 ± 0.54[a]
Accumulation ratio[b]	–	1.07 (0.97, 1.18)

$AUC_{0-\infty}$, area under the concentration time curve from zero to infinity; $AUC_{0-12\ h}$, AUC from time to zero to 12 h; C_{max}, maximum concentration; $t_{1/2}$, terminal half life; $AUC_{0-\infty}$, $AUC_{0-12\ h}$ and C_{max}, geometric mean ± geometric SD; T_{max}, median (range); $t_{1/2}$, harmonic mean ± Jackknife SD

[a] n = 9 (Since the terminal elimination phase was not able to be evaluated in one patient, the $t_{1/2}$ and $AUC_{0-\infty}$ could not be determined.)

[b] $AUC_{0-12\ h,\ Day\ 3}/AUC_{0-12\ h,\ Day\ 1}$ (geometric mean)

Table 4 Summary of serum pharmacokinetic parameters at 400 mg qd for 21 consecutive days

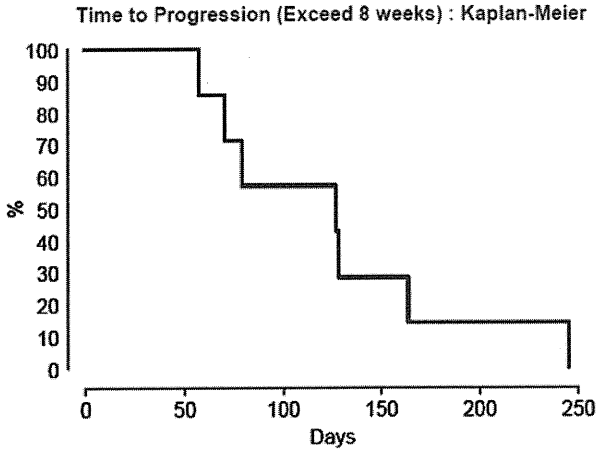
Parameter	Day 1 (n = 5[a], fed state)	Day 21 (n = 2[b], fed state)
$AUC_{0-\infty}$ (μM h)	7.75 ± 2.79	8.30
$AUC_{0-24\ h}$ (μM h)	7.97 ± 3.05	8.45
C_{max} (μM)	1.62 ± 0.52	2.04
T_{max} (h)	3.93 (3.80–6.00)	3.33 (2.98–3.67)
$t_{1/2}$ (h)	1.49 ± 0.82	1.17
Accumulation ratio[c]	–	1.50

$AUC_{0-\infty}$, area under the concentration time curve from zero to infinity; $AUC_{0-24\ h}$, AUC from time to zero to 24 h; C_{max}, maximum concentration; $t_{1/2}$, terminal half life; $AUC_{0-\infty}$, $AUC_{0-24\ h}$ and C_{max}, geometric mean ± geometric SD; T_{max} median (range); $t_{1/2}$, harmonic mean ± Jackknife SD, accumulation ratio:geometric mean

[a] Serum pharmacokinetic parameters on Day 1 in one patient were unavailable for calculation of mean and SD because the subject vomited after administration on Day 1

[b] Mean serum pharmacokinetic parameters on Day 21 were calculated from 2 patients

[c] $AUC_{0-24\ h,\ Day\ 21}/AUC_{0-24\ h,\ Day\ 1}$ (geometric mean)



Type of Cancer	Number of Prior Chemotherapy Regimens	Best Response	Time to Progression (days)
300 b.i.d × 3 days/week			
Rectal cancer	3	SD	127
Gastric cancer	2	SD	164
Gastric cancer	6	SD	70
Gastric cancer	2	SD	245
Gastric cancer	4	SD	128
400 q.d.			
Rectosigmoid cancer	6	SD	79
Colon cancer	3	SD	57

Fig. 2 Patients who achieved stable disease lasting ≥8 weeks (56 days)

Discussion

Previous studies, conducted in Japanese and non-Japanese populations, have evaluated vorinostat in a variety of conditions, including hematologic and solid malignancies, with various dosing regimens [10, 11, 18–20]. The results of the present study demonstrated that vorinostat was generally well tolerated. The most common drug-related AEs were anorexia, nausea, fatigue, and hyperglycemia; these AEs occurred in both dosing regimens and have been observed in previous vorinostat studies [10, 11, 18–20]. These drug-related AEs were grade 1/2. In patients who experienced DLTs, pharmacokinetic exposure was relatively higher; patients with higher AUC values also had more AEs compared with those who had lower AUC values in other studies.

In the treatment of CTCL, the dosing regimen approved by the United States FDA is 400 mg qd as continuous dosing [10, 19, 21]. It should be noted that CTCL differs from solid cancers such as the gastric cancer treated in the present study. In general, prior therapies in patients with CTCL include topical treatments such as interferon-γ and bexarotene or systemic treatments such as monoclonal antibodies, immune response modifiers (IFNs and retinoids), and well-tolerated antiproliferative drugs such as methotrexate [24], whereas combination chemotherapy is the standard therapy for patients with gastric and colorectal cancer [25].

Given the greater number and different types of prior therapies in patients with gastric and colorectal cancer, it is likely that the tolerability results observed in this study would reflect the heavily pretreated nature of this patient population. Indeed, with regard to DLTs and grade 3/4 AEs, it was apparent that the 300 mg bid dose for 3 consecutive days followed by 4 days of rest [a dose associated with a lower dose intensity (5400 mg per cycle) compared with 400 mg qd (6300 mg per cycle)] resulted in greater tolerability compared with the 400 mg qd dose. Additionally of note, because of the platelet-suppressing effects of vorinostat, hematologic effects, such as thrombocytopenia, are expected with vorinostat use. With regard to hematologic toxicities, there was an apparent advantage to the 300 mg bid dosing regimen; grade 3/4 thrombocytopenia AEs were observed in 4 out of 6 patients who received 400 mg qd for 21 consecutive days compared with only 1 out of 10 patients who received 300 mg bid for 3 consecutive days followed by 4 days of rest.

Another Phase I study recently assessed the safety and pharmacokinetics of vorinostat 100 mg bid, 200 mg bid, 400 mg qd, and 500 mg qd in 18 Japanese patients with solid tumors (roughly half of the patients had non-small cell lung cancer; the rest had bile duct cancer, invasive thymoma, esophageal cancer, and malignant mesothelioma) [20]. The results of that study were similar to those observed in the present study in terms of the types of AEs that patients experienced (thrombocytopenia, anorexia, and fatigue). However, the 400 and 500 mg qd doses in that study were better tolerated compared with the 400 mg qd dose in the present study. The mean drug exposure observed in that study was comparable to that observed in the current study for the 300 mg bid dose level, but lower than the 400 mg qd dose level in the present study [20]. Of note, the number of prior chemotherapy regimens among patients in the present study was higher compared with the number of prior chemotherapy regimens among patients in the Fujiwara et al. [20] study, again highlighting the possibility that tolerability may be affected by the nature of prior therapies. On the other hand, the serum exposure of the 400 mg qd dose level in the present study was higher than those of the 400 and 500 mg doses in that study. Identification of the reason is difficult due to the small number of enrolled patients. Also, the relationship between change in pharmacokinetics with vorinostat and the following factors cannot be demonstrated because of variation in concomitant therapy as well as the small number of enrolled patients. However, potential factors could include differences in health status of patients enrolled in the study and/or concomitant therapy during dosing with vorinostat.

These may affect physiology (for example, migration rate in GI tract, epithelial cells in GI tract, blood flow rate, etc.), and produce large inter-individual variability in vorinostat pharmacokinetics. Therefore, there is a possibility that the high serum exposures observed in some of the enrolled patients were due to such multiple factors. Patients with metastatic disease were not examined in this trial and the evidence for the effect of vorinostat in patients with metastatic disease is scant. In small studies in patients with metastatic breast cancer, head and neck cancer, and thyroid carcinoma, stand-alone vorinostat was generally well tolerated but led to neither complete nor partial response in any patient, although the stable disease achieved by some patients warrants further research in combination therapy [26–28].

Although efficacy in the treatment of gastric cancer was not a primary objective for this study, 5 patients in group 1 (300 mg bid) achieved stable disease $\geq$8 weeks, with 1 patient in particular having duration of TTP of 245 days. In contrast, there were two patients in group 2 (400 mg qd) who achieved stable disease $\geq$8 weeks, possibly due to the lower tolerability observed with this dosing regimen. We observed these results despite the fact that 300 mg bid resulted in lower mean drug exposure compared with 400 mg qd, indicating that the lower drug exposure associated with the 300 mg bid dose level led to greater tolerability with no deleterious effects on efficacy compared with the higher observed drug exposure at the 400 mg qd dose level. Objective responses were not observed in this study. However, considering the cytostatic effect of vorinostat in preclinical models, these data appear to be encouraging [9, 29]. In a previous phase I study in non-Japanese patients, the administration of vorinostat with 300 mg or 400 mg bid for 3 consecutive days followed by 4 days rest regimen showed PR in 2 patients, and stable disease $\geq$16 weeks in 3 patients out of 13 patients with malignant pleural mesothelioma [30]. Therefore, from a safety and efficacy perspective, this dosing regimen is promising for Japanese patients with GI cancer. Currently, a phase III study is on-going to evaluate 300 mg bid for 3 consecutive days followed by 4 days rest in non-Japanese and Japanese patients with mesothelioma.

When viewing these data, the limitations of the current study should be considered. Specifically, the results from this study are limited due to the small number of patients studied, and further investigation is needed to assess tolerability in a larger patient population. More research is also needed to further characterize the efficacy of vorinostat with regard to whether or not efficacy is dose-dependent and whether differentiated gastric cancer is more responsive to treatment than undifferentiated gastric cancer.

In conclusion, vorinostat given to patients with GI cancer was well tolerated when given 300 mg bid for 3 consecutive days followed by 4 days of rest when compared with 400 mg qd dosing regimen for 21 consecutive days per cycle in Japanese patients. Additionally, 5 patients receiving 300 mg bid and 2 patients receiving 400 mg qd maintained stable disease for >8 weeks, with the maximum duration being 245 days. The current study supports further investigation of vorinostat alone or in combination with other anti-cancer agents in patients with gastric cancer who may be sensitive to epigenetic treatment with an HDAC inhibitor, such as those who exhibit aberrant DNA methylation of p16. Of particular interest will be the evaluation of overlapping hematologic toxicities for the study of a combination approach with other agents with low rates of toxicities. For further study of vorinostat alone, the rate of efficacy will need to be evaluated in a larger population of patients to ensure adequate treatment of gastric cancer.

Conflict of interest Noguchi and Otsuki are employees of MSD K.K., a subsidiary of Merck & Co., Inc., and may own stock or stock options in the company. Mehta is an employee of Merck Sharp & Dohme, Corp., and may own stock or stock options in the company. The other authors report no conflicts of interest.

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ORIGINAL ARTICLE

Efficacy of trastuzumab in Japanese patients with HER2-positive advanced gastric or gastroesophageal junction cancer: a subgroup analysis of the Trastuzumab for Gastric Cancer (ToGA) study

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Abstract
Background The Trastuzumab for Gastric Cancer (ToGA) study is the first international trial to include Japanese patients with human epidermal growth factor 2 (HER2) positive advanced/metastatic gastric or gastroesophageal junction cancer. ToGA showed that trastuzumab plus chemotherapy (capecitabine/cisplatin or 5-fluorouracil/cisplatin) improved overall survival in the overall population (hazard ratio 0.74). Regional differences in outcome in favor of Japanese populations were observed in other studies; therefore, subgroup analyses of ToGA may contribute to the evaluation of the potential benefits of this regimen in Japanese patients.
Methods We performed subgroup analyses on 101 Japanese patients enrolled into ToGA (trastuzumab plus chemotherapy, $n = 51$; chemotherapy, $n = 50$).
Results Median overall survival in the Japanese subgroup was 15.9 months (95% confidence interval 12–25) for trastuzumab plus chemotherapy and 17.7 months (95% confidence interval 12–24) for chemotherapy (hazard ratio 1.00; 95% confidence interval 0.59–1.69). After adjusting

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for prespecified covariates, the estimated hazard ratio for overall survival was 0.68 (95% confidence interval 0.36–1.27). Further post hoc and exploratory examinations supported the robustness of the adjusted hazard ratios.

Conclusions After adjusting for imbalanced patient backgrounds between arms, overall survival of Japanese patients with human epidermal growth factor 2 positive advanced/metastatic gastric or gastroesophageal junction cancer who received trastuzumab plus chemotherapy was improved compared with patients who received chemotherapy alone.

Keywords Trastuzumab · Drug therapy · Stomach neoplasms · Randomized controlled trial

Background

Approximately 110,000 people in Japan develop gastric cancer each year [1], with 65,000 estimated deaths (which is second only to lung cancer among cancer-related deaths [1]). For advanced disease, the oral fluoropyrimidine S-1, in combination with cisplatin, has become the standard treatment for gastric cancer in Japan, based on the results of the SPIRITS trial [2]. However, the prognosis still remains poor, and therefore new therapies such as molecular-targeted drugs are needed. Trastuzumab is a recombinant monoclonal antibody that targets human epidermal growth factor receptor 2 (HER2). Trastuzumab derives its anticancer effects from inducing antibody-dependent cytotoxicity, inhibiting HER2-mediated signaling, and preventing cleavage of the extracellular domain of HER2 [3].

Trastuzumab has been approved for use in HER2-positive metastatic breast cancer and as a postoperative adjuvant therapy for HER2-positive breast cancer, and is now the standard of care worldwide for these indications, including in Japan. The Trastuzumab for Gastric Cancer (ToGA) study was the first international randomized controlled phase III trial to include Japanese patients with HER2-positive advanced/metastatic gastric or gastroesophageal junction (GEJ) cancer. The percentage of patients with HER2-positive gastric cancer, as assessed by immunohistochemistry (IHC; 3+ on a scale of 0 to 3+) or fluorescence in situ hybridization (FISH; *HER2*:CEP17 ratio $\geq$2.0) was 22.1% in the overall ToGA population. The proportion of patients with HER2-positive disease was similar for Europe (23.6%), Asia (23.5%), and Japan (27.6%) [4], and similar to that seen in patients with breast cancer in other trial populations (25–30%) [5]. ToGA showed that patients who received combination treatment with trastuzumab and chemotherapy [capecitabine plus cisplatin (XP) or fluorouracil plus cisplatin (FP)] had significantly improved survival compared with those who received chemotherapy alone: the median overall survival (OS) in the intent-to-treat (ITT) population was 13.8 months in the trastuzumab plus chemotherapy arm and 11.1 months in the chemotherapy-only arm [hazard ratio (HR) 0.74, 95% confidence interval (CI) 0.60–0.91; $P = 0.0046$] [6].

There were substantial differences in OS reported from recent phase III trials of chemotherapy for gastric cancer, and these are especially evident between Japan and other countries. Recent trials in Japan have demonstrated that combination therapy resulted in longer survival than was seen in studies outside of Japan, with a median survival exceeding 1 year [7, 8], as compared with around 10 months in Western trials [9, 10]. There are considered to be two reasons for the longer survival observed in Japanese trials. Firstly, up to 70% of Japanese patients receive subsequent chemotherapy following failure of first-line therapy [11–13]. Secondary, there may be differences in the eligibility criteria and baseline patient characteristics between the Japanese and non-Japanese trials; the studies in Japan included patients with and without measurable metastatic disease, whereas non-Japanese trials usually included patients with measurable metastatic disease only [11]. Since the primary endpoint of the ToGA study was OS, there is a possibility that the impact of trastuzumab on survival might be reduced in Japanese patients due to inherently longer survival in this population. To evaluate the efficacy of trastuzumab in combination with chemotherapy specifically in the Japanese population of ToGA, we conducted preplanned and post hoc subgroup analyses.

Patients and methods

The details of the ToGA trial design and methods have been reported elsewhere [6].

Japanese patient subgroup

To evaluate the efficacy and safety of the combination treatment (trastuzumab plus XP) in the Japanese population

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