Phase I and pharmacokinetic study of trastuzumab emtansine in Japanese patients with HER2-positive metastatic breast cancer

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Abstract

Objective: Trastuzumab emtansine (T-DM1), an antibody–drug conjugate composed of the cytotoxic agent DM1 conjugated to trastuzumab via a stable thioether linker, has shown clinical activity in human epidermal growth factor receptor 2-positive metastatic breast cancer patients. This study evaluated the maximum tolerated dose, toxicity and pharmacokinetics of trastuzumab emtansine in Japanese breast cancer patients.

Methods: Inoperable advanced or recurrent human epidermal growth factor receptor 2-positive breast cancer patients were administered trastuzumab emtansine intravenously at a dose of 1.8, 2.4 or 3.6 mg/kg every 3 weeks. The maximum tolerated dose was estimated using the continual reassessment method.

Results: This study enrolled 10 patients who were administered trastuzumab emtansine for a median of seven cycles. The dose-limiting toxicity was Grade 3 elevation of aspartate aminotransferase/alanine aminotransferase at the 2.4 mg/kg dose level. The maximum tolerated dose was estimated to be 3.6 mg/kg because at the point when dose-limiting toxicity was evaluable in 10 patients, the probability of dose-limiting toxicity estimated using the continual reassessment method was closest to 25% at a dose of 3.6 mg/kg and this was unchanged by the results for patients enrolled after that. The most frequent adverse events were nausea, arthralgia, fever, fatigue and decreased appetite. Adverse events were generally tolerable. The maximum concentration and area under the concentration–time curve increased linearly with the dose.

Conclusions: Trastuzumab emtansine up to 3.6 mg/kg was well tolerated by Japanese breast cancer patients. Although thrombocytopenia and hepatotoxicity tended to be more severe than was seen in
Western patients in previous trastuzumab emtansine trials, those adverse events recovered without special supportive treatment.

Key words: trastuzumab emtansine, HER2-positive, breast cancer, pharmacokinetics, safety

Introduction

Approximately 15–20% of human breast cancers have gene amplification or overexpression of human epidermal growth factor receptor 2 (HER2) (1). HER2 is a member of the epidermal growth factor receptor family of tyrosine kinase transmembrane receptors, and overexpression of HER2 is associated with aggressive tumor growth and poor clinical outcomes (1–3). Trastuzumab is a recombinant humanized monoclonal antibody targeted to the extracellular domain of HER2, and has significantly improved survival in patients with HER2-overexpressing metastatic breast cancer (MBG) (4, 5).

Trastuzumab emtansine (T-DM1) is a novel antibody–drug conjugate consisting of trastuzumab covalently bound via a thioether linker to DM1, a maytansinoid drug that binds to microtubules. Maytansine competes with vinca alkaloids for binding on the beta subunit of tubulin, with activity that is 20–100 times as potent as that of vincristine (6–8). In addition to its cytotoxic capabilities, T-DM1 retains the properties of trastuzumab, namely it inhibits HER2 cell proliferation signaling, and has antibody-dependent cellular cytotoxicity (9, 10).

In a Phase I study, the safety and tolerability of T-DM1 administered every 3 weeks was evaluated in HER2-positive MBC with prior trastuzumab treatment, and the maximum-tolerated dose (MTD) was determined to be 3.6 mg/kg with Grade 4 thrombocytopenia identified as the dose-limiting toxicity (DLT) (8). Based on results from that Phase I study, Phase II study was conducted with T-DM1 administered at 3.6 mg/kg every 3 weeks to patients with HER2-positive MBC who had received prior HER2-directed therapies; that Phase II study demonstrated an objective response rate of 25.9% (95% CI, 18.4–34.4%) (11). In another Phase II study, the ORR was 34.5% (95% CI, 26.1–43.9%) in patients with HER2-positive MBC who have previously received at least two lines of therapy including trastuzumab and lapatinib (12). A subsequent randomized Phase III study (EMILIA) compared T-DM1 with the combination of lapatinib plus capecitabine in patients with HER2-positive MBC who had previously been treated with trastuzumab and a taxane. Median progression-free survival was 9.6 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine; \( P \leq 0.001 \), and overall survival at the second interim analysis crossed the stopping boundary for efficacy (30.9 months versus 25.1 months, \( P \leq 0.001 \)) (13).

The primary objectives of this study were to determine the MTD and the recommend dose (RD) of T-DM1 in Japanese patients in whom the disease had progressed despite chemotherapy containing trastuzumab, the standard therapy for HER2-positive MBC.

Patients and methods

Patients

Eligible patients had histologically documented, inoperable advanced or recurrent HER2-positive breast cancer (centrally confirmed by immunohistochemistry or fluorescence in situ hybridization). All patients had a history of progression during, or up to 60 days after, treatment with a trastuzumab-containing regimen.

Additional eligibility criteria included the following: women aged 20 years or older; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; adequate organ function [absolute neutrophil count, \( \geq 1500/\text{mm}^3 \); platelet count, \( \geq 100,000/\text{mm}^3 \); hemoglobin, \( >9.0 \text{ g/dl} \); total bilirubin, \( \leq 1.5 \text{ mg/dl} \); hepatic transaminases, \( \leq 2.5 \) times the upper limit of normal (ULN) (\( \leq 5 \) times the ULN if hepatic or bone metastases were present); alkaline phosphatase (ALP), \( \leq 2.5 \) times the ULN (\( \leq 5 \) times the ULN if bone metastases were present); serum creatinine, \( \leq 1.5 \text{ mg/dl} \); and creatinine clearance, \( \geq 60 \text{ ml/min} \); no history of significant cardiac or hepatic disease; left ventricular ejection fraction, \( \geq 50\% \); cumulative anthracycline dose, \( \leq 360 \text{ mg/m}^2 \) doxorubicin or equivalent (e.g. 720 mg/m\(^2\) in the case of epirubicin); peripheral neuropathy, Grade \( \leq 1 \); no treatment containing trastuzumab or lapatinib within 2 weeks of enrollment; no experimental therapy within 4 weeks of enrollment; no requirement for supplemental oxygen; and no history of Grade \( \geq 3 \) hypersensitivity to trastuzumab. All patients provided written informed consent. This study was approved by the Institutional Review Board at each site according to local clinical guidelines and was performed as a registration-directed trial in accordance with the Good Clinical Practice guidelines laid down by the declaration of Helsinki, the study protocol and the revised Pharmaceutical Affairs Act in Japan.

Study design and treatment

T-DM1 was administered by intravenous infusion every 3 weeks. Pre-medications were not required prior to T-DM1 infusion. If an infusion-related reaction occurred, pre-medications (acetaminophen and diphenhydramine hydrochloride) could be given with subsequent cycles per investigator discretion. The drug was infused over 90 min. If prior infusions were well tolerated, subsequent infusions were shortened to 30 min.

When this Phase I study was planned, two of three patients enrolled in the 4.8 mg/kg cohort experienced dose-limiting Grade 4 thrombocytopenia and MTD of 3.6 mg/kg every 3 weeks had been confirmed in the previous Phase I study. Therefore, a starting dose of 1.8 mg/kg, which was half of the previous Phase I RD (3.6 mg/kg), and three dose levels (Level 1, 1.8 mg/kg; Level 2, 2.4 mg/kg; Level 3, 3.6 mg/kg) were selected for this study. The continual reassessment method (CRM) with non-informative prior was used to determine the dose for each patient in this study (14). The planned maximum number of patients was 12. If the MTD was determined before the planned number of subjects was reached, enrollment was to be stopped. The MTD was defined as the dose at which the estimated probability of DLT was closest to 25%, which is the target probability of DLT. The probability of DLT at each dose was estimated using the one-parameter logistic model. The probability of DLT at each dose was estimated using the modified CRM.

DLTs were defined as any of the following adverse events (AEs) that occurred during the DLT observation period (21 days from the first infusion of the investigational product): (i) Grade \( \geq 3 \) non-hematologic toxicity (excluding therapy diarrhea, nausea and vomiting manageable with standard support therapy); (ii) Grade \( \geq 4 \) decreased platelet count; (iii) Grade \( \geq 4 \) decreased hemoglobin; (iv) Grade \( \geq 4 \) decreased absolute neutrophil count lasting for \( \geq 4 \) days or accompanied by a fever of \( \geq 38\degree \text{C} \); (v) Grade \( \geq 3 \) increased total
hilar or increased aspartate aminotransferase (AST), alanine aminotransferase (ALT) or ALP; or (vi) Grade ≥3 cardiotoxicity. Hematologic parameters and blood chemistry were tested on Days 1, 2, 3, 5, 8, 11, 15 and 19 in Cycle 1. The severity of the AE was assessed in accordance with the National Cancer Institute’s Common Terminology Criteria for Adverse Events version 3.0 (15). Tumor responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (16).

Pharmacokinetic studies

Whole-blood samples (3.5 ml tube for T-DM1 and total trastuzumab in serum, and 2 ml heparinized tube for free DM1 in plasma) were drawn before infusion (pre) and at 30 min, 4, 24, 48, 96 h, and 7, 10, 14, 17/18 days (±6 h) after the end of infusion at Cycle 1; pre and 30 min after the end of infusion in subsequent cycles; and 4 h after the end of infusion at Cycle 3 and at termination of the study. Samples were inverted 5–10 times immediately, left 30 min at room temperature, then centrifuged at 1500–2000 g for 10–15 min, and aliquots of the resultant serum and plasma were stored at −70°C until analysis. Concentrations of T-DM1 and total trastuzumab in serum were quantitated using validated enzyme-linked immunosorbent assay methods by Genentech, Inc. (South San Francisco, CA, USA). Concentration of free DM1 in plasma was quantitated using validated liquid chromatography and tandem mass spectrometry by Quest Pharmaceutical Services (Groningen, the Netherlands). The lower limits of quantification were 40 ng/ml for T-DM1 in serum, 40 ng/ml for total trastuzumab in serum and 0.737 ng/ml for free DM1 in plasma.

Each patient’s plasma concentration profiles of T-DM1, total trastuzumab and free DM1 were used to estimate the terminal half-life (t1/2), area under the serum (plasma) concentration–time curve (AUC), total body clearance and volume of distribution (Vss) by a non-compartment analysis using the Phoenix WinNonlin software (version 6.1, Pharsight Corp., CA, USA). The maximum concentration (Cmax) of T-DM1, total trastuzumab and free DM1 was determined from the actual measured values. The AUC was calculated by the trapezoidal rule. The AUCinf was obtained by summation of the AUC0 – t and the extrapolated area estimated by taking the ratio between the last measurable concentration and the apparent elimination rate constant. Regression analyses of each of individual Cmax, AUC0 – t, and AUCinf values versus the dose were performed to evaluate the pharmacokinetic linearity. SAS software (version 9.2, SAS Institute, Inc., NC, USA) was used for statistical analysis.

Whole-blood samples (5 ml tube containing EDTA-2Na) were collected prior to the first dose for analysis of genetic polymorphisms (FcyR, β1 tubulin). These were analyzed at Quest Diagnostics, Inc. (CA, USA).

Whole-blood samples (3.5 ml) for detection of antitherapeutic antibodies (ATA) were drawn at pre-dose in every cycle and at termination of the study. ATA was quantitated using validated enzyme-linked immunosorbent assay at Genentech, Inc.

Results

Patient characteristics

Ten patients were enrolled from three centers in Japan between September 2009 and July 2010, and all patients received at least two cycles of T-DM1 (median, 7 cycles; range, 2–26 cycles). Patient characteristics are shown in Table 1.

Of the 10 patients, 9 withdrew from this study but 1 patient was still continuing as of the data cutoff date (29 July 2011). Of the 9 patients withdrawn, 6 patients were withdrawn because of disease progression and 3 patients were withdrawn because of AEs (Grade 3 thrombocytopenia at 2.4 mg/kg, Grade 3 cholelithiasis at 2.4 mg/kg, and Grade 2 ALP increased at 3.6 mg/kg).

Pharmacokinetic and immunogenicity analyses

Blood samples for pharmacokinetic analysis were available from all 10 enrolled patients in the first cycle. Serum concentration–time curves are shown in Figs. 1 and 2, and pharmacokinetic parameters for T-DM1 in serum are shown in Table 2. The serum T-DM1 in Cycle 1 showed a multiphasic elimination pattern, with peaks reached at 30 min and 4 h after the end of infusion, and rapid elimination from Day 1 to Day 2 after infusion with gradual elimination thereafter. Systemic clearance of T-DM1 was more rapid than clearance of total trastuzumab: at the MTD (3.6 mg/kg), clearance of T-DM1 was 10.6 ± 1.26 ml/day/kg and clearance of total trastuzumab was 5.45 ± 1.98 ml/day/kg. At the MTD, the terminal half-life of T-DM1 was 3.74 ± 1.15 days and terminal half-life of total trastuzumab was 6.47 ± 2.40 days. The AUC of free DM1 in plasma was <1/10 000 that of serum T-DM1 by mass and 10 of serum T-DM1 by molar equivalent. The pharmacokinetics of T-DM1 was linear in the range of 1.8–3.6 mg/kg.

Anti-T-DM1 antibodies were assessed for all enrolled patients. In the 3.6 mg/kg cohort, one patient was positive for anti-T-DM1 antibodies after the completion of Cycle 8. This patient, whose best response was partial response, experienced no Grade ≥3 adverse events.

Toxicities

At least one drug-related all grade AE was reported in each of the 10 patients. Types and incidences of AEs are listed in Table 3. AEs (all grades) observed in three or more patients were nausea and arthralgia (n = 7), fever (n = 6), fatigue and decreased appetite (n = 5), diarrhea,

<table>
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<th>Table 1. Patient baseline demographics and disease characteristics (N = 10)</th>
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ECOG, Eastern Cooperative Oncology Group; PS, performance status; ER, estrogen receptor; PgR, progesterone receptor.
malaise, headache, and rash (n = 4), and constipation, vomiting, myalgia, chills and cystitis (n = 3). There were no deaths during the study period. Drug-related alopecia and neuropathy were not reported.

Grade 3 or 4 AEs were observed in three of the four patients in the 2.4 mg/kg cohort (elevations of AST/ALT, cholelithiasis and thrombocytopenia, respectively). None of the five patients in the 3.6 mg/kg cohort experienced any Grade 3 or 4 AEs. Serious AEs occurred in two patients: one was cholelithiasis in the 2.4 mg/kg cohort, and the other was gastric ulcer hemorrhage in the 3.6 mg/kg cohort. AEs that resulted in discontinuation of treatment were thrombocytopenia (n = 1, 2.4 mg/kg cohort), cholelithiasis (n = 1, 2.4 mg/kg cohort), blood ALP increased (n = 1, 3.6 mg/kg cohort).

Dose modification due to elevation of AST/ALT was performed in one patient in the 2.4 mg/kg cohort. In five patients, treatment cycles were prolonged because of AEs (in the 2.4 mg/kg cohort, one patient with thrombocytopenia, one patient with neutropenia and elevation of serum amylase; in the 3.6 mg/kg cohort, one patient with thrombocytopenia, one patient with elevation of total bilirubin and gastric ulcer hemorrhage, and one patient with elevation of ALP).

Determination of MTD
The one patient who was treated at the 1.8 mg/kg dose level did not experience any grade ≥3 AE. Of the four patients in the 2.4 mg/kg cohort, one experienced Grade 3 elevation of AST/ALT that met the definition of DLT in the first cycle. This patient continued the therapy without delay by dose reduction. Of the five patients in the 3.6 mg/kg cohort, none experienced any Grade ≥3 AE. The MTD was determined to be 3.6 mg/kg at the point when DLT evaluation period was completed in 10 patients because MTD would be unchanged even if subsequent two patients were enrolled.

Efficacy
The best overall response was determined in nine patients (one patient was still continuing as of the cutoff date, 29 July 2011). No complete response was observed. Partial response was observed in one patient in the 3.6 mg/kg cohort. Stable disease was observed in six patients (three in the 2.4 mg/kg cohort; three in the 3.6 mg/kg cohort), and progressive disease was observed in two patients (one in the 1.8 mg/kg cohort; one in the 3.6 mg/kg cohort).

Discussion
In this Phase I study, we assessed the safety, tolerability, pharmacokinetics and efficacy of T-DM1 when intravenously infused every 3 weeks.
in Japanese patients with HER2-positive MBC. In the previous Western first-in-human study (TDM3569g), the MTD was determined to be 3.6 mg/kg [8]. The MTD in Japanese patients was also estimated to be 3.6 mg/kg, and this dose was generally well tolerated. In generally, dose escalation, 3 + 3 design case, the patient can be enrolled in next cohort after DLT assessment of last patient at each cohort was completed and six patients are required to move to next cohort or determine MTD if a DLT is observed in the cohort. In this study, patients can be enrolled more seamlessly by using CRM, and therefore study period was shortened.

The pharmacokinetic profile of serum T-DM1 revealed moderate interindividual variability. It was concluded that the pharmacokinetics of serum T-DM1 is linear when the dose of T-DM1 is in the range of 1.8–3.6 mg/kg. Pharmacokinetic data do not suggest any apparent difference between Japanese patients and Western patients. The observed pharmacokinetics profile of serum T-DM1 in the 3.6 mg/kg cohort (Table 2) was similar to that seen in TDM3569g (C_{max}: 82.0 versus 74.3 μg/ml; AUC_{inf}: 346 versus 295.2 μg·day/ml; t_{1/2}: 3.74 versus 3.5 days; V_{ss}: 59.1 versus 60 ml/kg; clearance: 10.6 versus 12.9 ml/day/kg) [8]. The Grade ≥3 AEs observed in this study were elevation of hepatic transaminases, thrombocytopenia and cholelithiasis. There were no cases presenting with clinically significant bleeding events. The most frequent AEs were nausea, arthralgia, fever, fatigue and decreased appetite (Table 3). Incidence of these common AEs was moderate (all events of nausea occurring in this study were Grade 1) and tended
to persist for 1–3 days; 5 of 7 events of nausea first occurred in Cycle 1 (two events occurred within 24 h from T-DM1 administration). These toxicities of T-DM1 were tolerable in Japanese patients. Hypokalemia was not observed in Japanese patients, whereas in TDM3569g and the Phase II study (TDM4258g and TDM4370g) in the USA, hypokalemia was reported in 4.2% (1 of 24 patients), 24.1% (27 of 112 patients) and 20.9% (23 of 110 patients), respectively. Hypokalemia was not associated with vomiting, diarrhea or diuretic use, and the mechanism of the hypokalemia was unclear (8, 11, 12).

One of the mechanisms of transient thrombocytopenia and hepatotoxicity is thought to be internalization of T-DM1 into Fc-gamma receptor-bearing megakaryocytes and Kupffer cells with subsequent release of free DM1 or DM1-related hepatic injury due to the low levels of free DM1 (8). DM1 is a thiol-containing maytansinoid derived from the naturally occurring ester ansamitocin P-3. The related plant ester, maytansine, has been studied as a chemotherapeutic agent. Gastrointestinal toxicities (nausea, vomiting and diarrhea) are common and are dose-limiting toxicities. A pattern of transient, but also mild gastrointestinal toxicities and also the reversible thrombocytopenia and hepatotoxicity seen in this study may possibly be attributed to transient and low levels of circulating DM1. Other mechanisms may contribute to the occurrence of the other AEs.

In conclusion, this is the first trial of T-DM1 in Japanese patients with HER2-positive MBC previously treated with chemotherapy containing trastuzumab. The results of this study indicate that T-DM1 may be dosed similarly in Japanese and Western patients, and that AEs are generally manageable. Transient thrombocytopenia and hepatotoxicity were observed in most cases. The different trends in severity and incidence rates of thrombocytopenia, hepatotoxicity and hypokalemia are expected to be verified by the results of the Japanese Phase II study (JO22997) evaluating the efficacy and safety of T-DM1 in Japanese patients with heavily pre-treated HER2-positive MBC.

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Conflict of interest statement

Kenjiro Aogi, Hiroji Iwata and Chikako Shimizu have received speaking fee from Chugai. Yasuhiro Fujiiwara has received speaking fees from AstraZeneca, Eisai, Kyowa Hakko Kirin, GlaxoSmithKline, Sanofi-Aventis, Daichii Sankyo, Taiho, Takeda, Chugai, Eli Lilly, Novartis, Bristol-Myers and NEC Corporation. Yuriko Igawa, Takashi Asakawa and Mari Matsubara are employees of Chugai Pharmaceutical Co., Ltd. All remaining authors have declared no conflict of interest.

References

Appendix

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