


Nimotuzumab combined with concurrent chemoradiotherapy in Japanese patients with esophageal cancer: A phase I study

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Nimotuzumab is a humanized anti-epidermal growth factor receptor IgG1 monoclonal antibody. This phase I study assessed the tolerability, safety, efficacy, and pharmacokinetics of nimotuzumab in combination with chemoradiotherapy in Japanese patients with esophageal cancer. Patients with stage II, III, and IV esophageal cancer were enrolled. Patients were planned to receive nimotuzumab (level 1: 200 mg/wk for 25 weeks; or level 2: 400 mg/wk in the chemoradiation period, 400 mg biweekly in an additional chemotherapy period [8 weeks after the chemoradiation period] and a maintenance therapy period [after chemotherapy to 25 weeks]) combined with cisplatin (75 mg/m² on day 1) and fluorouracil (1000 mg/m² on days 1-4) in the chemoradiation and additional chemotherapy periods. Radiotherapy was given concurrently at 50.4 Gy. A total of 10 patients were enrolled in level 1. Dose-limiting toxicities were observed in 2 patients (grade 3 infection and renal disorder). Maximum-tolerated dose was estimated to be at least 200 mg/wk and the dose was not escalated to level 2. The most common grade ≥ 3 toxicities were lymphopenia (90%), leukopenia (60%), neutropenia (50%), and febrile neutropenia, decreased appetite, hyponatremia, and radiation esophagitis (30% each). Neither treatment-related death nor grade ≥ 3 skin toxicity was observed in any patient. Complete response rate was 50%. Progression-free survival was 13.9 months. One- and 3-year survival rates were 75% and 37.5%, respectively. Immunogenicity was not reported in any patient. Nimotuzumab in combination with concurrent chemoradiotherapy was tolerable and effective for Japanese patients with esophageal cancer.

KEYWORDS

chemoradiotherapy, epidermal growth factor receptor, esophageal cancer, Japanese, nimotuzumab

Abbreviations: AE, adverse event; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CRT, chemoradiotherapy; CTCAE, Common Terminology Criteria for Adverse Events; CT, computed tomography; CTV, clinical target volume; DLT, dose-limiting toxicity; DMC, Data Monitoring Committee; ECOG, Eastern Cooperative Oncology Group; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded; G-CSF, granulocyte colony-stimulating factor; GGT, gamma-glutamyltransferase; GTV, gross tumor volume; HAHA, human anti-humanized antibody; IHC, immunohistochemistry; LAEC, locally advanced esophageal cancer; MTD, maximum-tolerated dose; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; PS, performance status; PTV, planning target volume; RECIST, Response Evaluation Criteria in Solid Tumors; SAE, serious adverse event; TGF- α , transforming growth factor alpha.

Clinical trial registration: JapicCTI-101319, first released on October 25, 2010. The trial protocol number is DE766-A-J102.

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1 | INTRODUCTION

The prevalence of esophageal cancer has been increasing in Japan and is higher than that in the USA or Europe.^{1–3} Molecular-targeted drugs and chemotherapeutic agents for esophageal cancer have not been developed very actively as a result of the limited number of patients in the USA and Europe. Radical treatment, such as surgery and radiotherapy, is carried out in more than 60% of esophageal cancer cases.⁴ However, local recurrence or distant metastasis occurs in approximately 30%–60% of cases of esophageal cancer.^{5–7} Standard therapy for LAEC is neoadjuvant chemotherapy or CRT followed by surgery. Definitive CRT is a treatment option for resectable LAEC and standard therapy for unresectable LAEC patients.⁸ The 5-year survival rate in patients with resectable stage II or III LAEC who underwent neoadjuvant chemotherapy or CRT followed by surgery is reported to be 55%–57%.^{9,10} However, mortality and morbidity of esophagectomy after intensive neoadjuvant therapy have been of concern in patients with esophageal cancer who usually have low nutrition status and poor general condition.

In a multicenter randomized controlled phase III trial (FFCD9901 trial; ClinicalTrials.gov: NCT00047112), preoperative CRT did not improve survival in patients with stage I or II esophageal cancer.¹¹ However, improved survival with CRT has been reported in some studies. For resectable stage II/III LAEC patients who receive definitive CRT, median overall survival and 3-year survival rate are reported to be 30 months and 45%–60%, respectively.^{7,12,13} For unresectable LAEC patients who receive definitive CRT, the median overall survival and 2-year survival rate are reported to be approximately 10 months and 30%, respectively.^{14–16} Despite the survival outcomes and acceptable tolerability of CRT, the incidence of local recurrence is high, and novel strategies are needed for CRT.¹³ Introduction of new anticancer drugs, such as molecular targeted drugs, may improve the outcomes in LAEC patients who receive CRT.

EGFR is known to be overexpressed in a wide variety of human tumors and overexpression of EGFR is considered to be associated with a poor prognosis.¹⁷ High levels of EGFR expression are reported in esophageal cancer, which makes this malignancy one possible target for anti-EGFR antibodies.^{18–20} Suppression of distant metastasis and improvement of the local control rate are the main concerns for the treatment of locally advanced esophageal cancer, and CRT combined with anti-EGFR antibodies is therefore expected to become the new treatment option for locally advanced esophageal cancer. Nimotuzumab is a recombinant humanized IgG1 monoclonal antibody directed against EGFR.²¹ Nimotuzumab binds to EGFR, thereby blocking the binding of EGF and TGF- α to EGFR, and eventually inhibiting the EGFR signaling pathway. Nimotuzumab has also been shown to have antibody-dependent cellular cytotoxicity activity and complement-dependent cytotoxicity activity. Through these activities, nimotuzumab is considered to cause cell cycle arrest, apoptosis induction, and inhibition of angiogenesis in tumor tissues.^{22–24} Available clinical data for nimotuzumab support an

enhanced antitumor effect in patients with head and neck cancer in combination with radiotherapy, and nimotuzumab has been reported not to affect the tolerability of combination therapy.²²

With high expression of EGFR, esophageal cancer is considered a potential target for nimotuzumab therapy, and nimotuzumab in combination with CRT is expected to improve the prognosis of esophageal cancer. The present study was conducted to confirm the tolerability of nimotuzumab in combination with cisplatin and fluorouracil CRT, which is the standard chemotherapeutic regimen used for the management of esophageal cancer, in Japanese patients with esophageal cancer.

2 | MATERIALS AND METHODS

2.1 | Eligibility criteria

All patients had histologically confirmed (using the UICC-TNM classification²⁵) stage II, III (excluding T4), or IV (M1 lymph node involvement, excluding T4) esophageal cancer. Patients were previously untreated for esophageal cancer and received no prior treatment for other malignant tumors (chemotherapy, radiotherapy, or treatment with EGFR-targeted antibodies). Definitive radiotherapy at a dose of 50.4 Gy was indicated based on the judgment of a radiation oncologist. Further inclusion criteria included: age 20–74 years; ECOG PS 0 or 1; adequate renal function (serum creatinine \leq 1.2 mg/dL, creatinine clearance \geq 60 mL/min); adequate hepatic function (total bilirubin \leq 1.5 mg/dL, ALT and AST \leq 100 IU/L); adequate bone marrow function (white blood cell count \geq 4000 cells/mm³, neutrophil count \geq 2000 cells/mm³, platelet count \geq 100 000 cells/mm³, hemoglobin \geq 10.0 g/dL); adequate lung function (partial pressure of arterial oxygen \geq 70 torr, left ventricular ejection fraction \geq 60%), and patient willingness to forgo surgical resection as an initial treatment. Patients with another active malignant tumor (other synchronous malignant tumor or metachronous malignant tumor with a disease-free interval of <5 years), severe stenosis or esophago-mediastinal fistula, apparent pulmonary fibrosis, or interstitial pneumonia on chest CT were excluded from the study.

The protocol was approved by the independent ethical committee at each center and the study was carried out according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent before study entry. Written informed consent for the biomarker analysis was additionally obtained from those who submitted their tumor tissues.

2.2 | Treatment schedule

Overall treatment duration was up to 25 weeks. The treatment period consisted of 3 parts.

1. A chemoradiation period (from day 1 to 4 weeks after the end day of radiotherapy): combination of nimotuzumab, cisplatin and fluorouracil chemotherapy, and radiotherapy.

- An additional chemotherapy period (8 weeks after the chemoradiation period): combination of nimotuzumab and cisplatin and fluorouracil chemotherapy.
- A maintenance therapy period (from after the additional chemotherapy period to 25 weeks after the start of treatment): nimotuzumab only.

Nimotuzumab was planned to be given i.v. over 30 minute in 250 mL normal saline solution (level 1: 200 mg/wk for 25 weeks; or level 2: 400 mg/wk in the chemoradiation period and 400 mg biweekly in the additional chemotherapy period [8 weeks after the chemoradiation period] and the maintenance therapy period [from after the additional chemotherapy period to 25 weeks]). Cisplatin (75 mg/m² on day 1) and fluorouracil (1000 mg/m² on days 1-4) were given after an interval of at least 30 minute following nimotuzumab when nimotuzumab and chemotherapy were given on the same day.

During the chemoradiation period, radiotherapy was given at a dose of 1.8 Gy once daily on 5 days per week up to a total of 50.4 Gy on a total of 28 days, and delivered with megavoltage equipment (≥ 6 MV) using the multiple-field technique. CT-based 3-D treatment planning was required for all enrolled patients. GTV was defined as the volume of the primary tumor shown on CT and/or esophageal barium exam, plus that of metastatic lymph nodes in the mediastinum, which were defined as nodes of ≥ 1 cm on the long axis. CTV of the primary tumor was defined as the GTV for the primary tumor plus its subclinical extension of approximately 2 cm in a craniocaudal plane. PTV were generated by adding an appropriate margin (approximately 0.5-1 cm in the lateral plane and approximately 1-2 cm in the craniocaudal plane) to the CTV, incorporating a margin for error based on displacement as a result of respiration and the patient's fixed reproducibility.

2.3 | Safety

Dose-limiting toxicity was defined as the following toxicities assessed to be related to the study treatment during the chemoradiation period. The grade was determined according to the CTCAE (version 4.0): grade ≥ 3 non-hematological toxicities (excluding toxicities reported with chemoradiotherapy [anorexia, nausea, vomiting, oral mucositis, pharyngeal mucositis, laryngeal mucositis, esophagitis, and dysphagia], and transient abnormal electrolytes); grade 3 diarrhea lasting ≥ 4 days, even after supportive therapy; febrile neutropenia lasting ≥ 4 days, even after G-CSF treatment; grade 4 thrombocytopenia; skipping of 3 consecutive doses of nimotuzumab as a result of toxicity; and "infection associated with grade 3 or 4 neutropenia" lasting ≥ 4 days and requiring i.v. antibiotics or treatment with antifungal or antiviral drugs.

Nimotuzumab was given at a dose of 200 mg (level 1), and the presence or absence of a DLT was confirmed during the chemoradiation period; nimotuzumab was then planned to be given at an increased dose of 400 mg (level 2) to a new treatment group. DLT evaluation was carried out on 6 patients at each level, and up to 10 patients were then enrolled at the same level to evaluate the safety

during the entire study period. When fewer than 2 of 6 patients at a certain dose level had a DLT, dose escalation was planned to start after enrolling up to 10 patients. When 3 of 6 patients had a DLT, the decision on whether to enroll up to 10 patients was made in consultation with the DMC. Termination of dose escalation was planned when 4 or more of the 6 patients had DLT. MTD was estimated based on the status of DLT occurrence in 6 patients during the DLT evaluation period. Safety evaluation was carried out based on the AE that occurred in 10 patients at each level during the study period, and the MTD was finally determined by the DMC.

For patients who discontinued treatment prematurely owing to treatment-related toxicity, follow up was continued on a weekly basis until the associated toxicity was resolved. However, for patients who discontinued treatment for reasons other than treatment-related toxicity, follow up was continued until disease progression or the start of a new anticancer treatment.

2.4 | Evaluation of efficacy

The antitumor effect, complete response rate, overall response rate, PFS, and survival rate were evaluated. The antitumor effect was determined in accordance with RECIST version 1.1 combined with endoscopy of the primary esophageal tumor.²⁶ Complete response of a primary site was defined according to the criteria of Japanese Classification for Esophageal Cancer.²⁷

Patients were followed up at an interval of 3 months for the first 12 months after registration, every 4 months for 12-24 months after registration, and every 6 months for 24-36 months after registration.

2.5 | Pharmacokinetic analysis

Blood samples (3 mL) were collected prior to the first dosing and at 5 minute, 3 hour, 8 hour, 24 hour, 48 hour, and 72 hour after dosing on day 1, and prior to and 5 minute after dosing on days 8, 71, 85, 99, and 127. Serum concentration of nimotuzumab was analyzed using a validated ELISA method. The lower limit of quantification was 2 μ g/mL. PK parameters after the first dose were calculated by a non-compartment analysis using Phoenix WinNonlin (Pharsight, Mountain View, CA, USA). Maximum concentration (C_{max}) and time to reach C_{max} (t_{max}) were obtained as observed values. Elimination half-life ($t_{1/2}$), area under the concentration vs the time curve up to infinity (AUC_{0-inf}), and total body clearance (CL_t) were estimated by linear regression after \log_e transformation of the concentrations.

2.6 | Immunogenicity

Serum samples for the analysis of HAHA were collected prior to the first administration, at the end of the chemoradiation period, at the end of the additional chemotherapy period, and during the follow-up assessment period. HAHA was tested for by using a validated ELISA method.

2.7 | Biomarker research using tumor tissue samples

Immunohistochemical staining for EGFR and HER2, and evaluation of amplification of the EGFR gene were carried out using paraffin-embedded sections of tumor samples. Mutations of the *K-ras* gene, which are associated with resistance against EGFR antibody therapies, were also assessed. EGFR expression on the tumor samples was measured by IHC using an EGFR pharmDx kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. DNA was extracted from FFPE tumor samples using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. EGFR gene amplification levels in tumor tissues were measured by FISH. DNA segments including codons 12 and 13 of *K-ras* were amplified by PCR, and *K-ras* mutations in codons 12 and 13 were assessed by direct sequencing. HER2 expression on tissue samples was measured by IHC using the HercepTest II kit (Dako) according to the manufacturer's instructions. Positive cell rate (%) was calculated based on the number of positive cells per 100 tumor cells under a microscope.

2.8 | Statistical analysis

The planned sample size was 10 subjects at each level to evaluate the safety of nimotuzumab combined with cisplatin, fluorouracil, and radiation. OS was defined as the period between the initiation of treatment and death as a result of any cause. PFS was defined as the period between the registration and progression or death as a result of any cause. OS and PFS were estimated using the Kaplan-Meier method. All *P*-values are 2-sided, and 95% confidence intervals were calculated. Statistical analyses were carried out using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

3 | RESULTS

3.1 | Patient characteristics

In total, 10 patients were enrolled and treated (December 2010 to April 2015). Baseline patient characteristics are summarized in Table 1. The median duration (range) of nimotuzumab treatment (maximum 25 weeks) was 21.1 (0.1-24.6) weeks. One subject who developed anaphylaxis withdrew from the study after initial dose of nimotuzumab and did not receive any dose of CRT. The proportion of patients who received at least 18 doses (maximum 25 doses) of nimotuzumab was 60%. In 9 patients, the median duration (range) of cisplatin/fluorouracil treatment (maximum 15 weeks) was 14.1 (0.1-14.6) weeks for both. The proportion of patients who received the specified dose of 50.4 Gy of radiotherapy was 88.9% (8/9) and median total dose (range) was 50.4 (19.8-50.4) Gy.

3.2 | Safety

Dose-limiting toxicities were assessed in 9 subjects and infection and renal disorder (both grade 3) were reported in 2 patients under

treatment with nimotuzumab at 200 mg (level 1) during the chemoradiation period (Table 2). The frequency of DLT was 22.2%. The frequency of DLT at 200 mg (level 1) satisfied the criteria for dose increase specified in the protocol and the MTD was estimated to be at least 200 mg. The dose was not evaluated at level 2 because there was an ongoing phase II study with a 200-mg dose, and this dose will be evaluated in a future phase III study.

The major AEs related to treatment are presented in Table 3. Grade 3 or higher AEs occurred in all 10 patients, and those with an incidence of $\geq 30\%$ were lymphocytopenia (90%), leucocytopenia (60%), neutropenia (50%), and febrile neutropenia, appetite loss, hyponatremia, and radiation esophagitis (all 30%). Eight SAEs developed in 6 (60%) of the 10 patients, and included radiation esophagitis, gastric cancer, pain, herpes zoster, and bile duct cancer reported in 1 patient each, and anaphylactic reaction, laryngeal edema, and dyspnea reported in the same patient. All except 2 (pain and bile duct cancer) were related to the study drug and were followed until resolution. Late AEs related to radiotherapy occurred in 3 (30%) of the 10 patients and all were grade 1 radiation pneumonitis. In 1 patient, approximately 3 months after the start of the study, bile duct cancer was suspected based on CT images and laboratory data (ALP, AST, ALT, and GGT) and confirmed by high CA19-9 levels. There had been no sign of the cancer in the pre-enrollment imaging. The study treatment was discontinued, and the patient received gemcitabine hydrochloride for treatment of bile duct cancer. The patient died approximately 9 months after registration.

Two patients (20%) withdrew from the study treatment because of AEs during the chemoradiation period, which was the DLT evaluation period. One of them experienced an anaphylactic reaction, dyspnea, and laryngeal edema within 15 minute of receiving the initial dose of nimotuzumab, and therefore withdrew from the study treatment and was excluded from the analysis set. The other experienced renal disorder and radiation pneumonitis, and withdrew from the study treatment to proceed to post-anticancer treatment based on the investigator's judgment. There were no protocol deviations or grade 3 or higher skin and subcutaneous tissue disorders in this study.

3.3 | Efficacy

Efficacy findings are summarized in Table 4. Two subjects did not undergo tumor evaluation after receiving the study drug; therefore, the efficacy analysis was conducted in 8 subjects. At the end of CRT and follow-up treatment, the overall response rate was 62.50%. The CR rate was 50% (95% CI: 15.7%-84.3%; 4/8 patients), and the non-CR rate was 12.5% (95% CI: 0.3%-52.7%; 1/8 patients). PD was seen in 37.5% (3/8 patients; 95% CI: 8.5%-75.5%).

The median PFS was 13.9 (95% CI: 4.2-17.1) months and an event was reported in all 8 patients, of whom 6 patients had PD and 2 patients had a fatal outcome. The 1-year, 2-year, and 3-year survival rates (95% CI) were 75.0% (31.5%, 93.1%), 62.5% (22.9%, 86.1%), and 37.5% (8.7%, 67.4%), respectively.

TABLE 1 Demographics and baseline characteristics of patients

Characteristics	Nimotuzumab level 1 (200 mg) n (%)
Sex	
Male	7 (70)
Female	3 (30)
Age (years)	
Median (range)	63.0 (51–71)
ECOG performance status	
0	4 (40)
1	6 (60)
Location of primary tumor	
Upper thoracic esophagus	1 (10)
Midthoracic esophagus	5 (50)
Lower thoracic esophagus	4 (40)
Histological type of cancer	
Squamous cell cancer	10 (100)
T category	
T1	0
T2	4 (40)
T3	6 (60)
N category	
N0	4 (40)
N1	6 (60)
M category	
M0	6 (60)
M1a (cervical lymph nodes) ^a	1 (10)
M1b (cervical and/or abdominal lymph nodes) ^a	3 (30)
Cancer stage at entry (UICC-TNM 6th edn)	
II	4 (40)
III	2 (20)
IV	4 (40)

ECOG, Eastern Cooperative Oncology Group; UICC, Union for International Cancer Control.

^aAll lesions were included in the radiation field.

TABLE 2 DLT assessment (MTD analysis^a set)

System organ class Preferred term	Level 1 (200 mg) N = 9 N (%)
Non-DLT	7 (77.8)
DLT	2 (22.2)
Infections and infestations	1 (11.1)
Infection	1 (11.1)
Renal and urinary disorders	1 (11.1)
Renal disorder	1 (11.1)

DLT, dose-limiting toxicity; MTD, maximum-tolerated dose.

^aCoded with MedDRA version 18.0 (MedDRA MSSO, McLean, VA, USA).

TABLE 3 Summary of study treatment-related adverse events

Adverse event	Level 1 (200 mg) n (%)	
	All Grades	Grade ≥ 3
Hematotoxicity		
Lymphocyte count decreased	9 (90)	9 (90)
Neutrophil count decreased	9 (90)	5 (50)
White blood cell count decreased	9 (90)	6 (60)
Platelet count decreased	8 (80)	2 (20)
Anemia	8 (80)	0
Non-hematotoxicity		
Decreased appetite	8 (80)	3 (30)
Nausea	8 (80)	0
Hypoalbuminemia	6 (60)	0
Hyponatremia	6 (60)	3 (30)
Fatigue	6 (60)	0
Weight decreased	5 (50)	0
Protein total decreased	4 (40)	0
Febrile neutropenia	3 (30)	3 (30)
Diarrhea	3 (30)	0
Stomatitis	3 (30)	0
Herpes zoster	2 (20)	1 (10)
Infection	1 (10)	1 (10)
Anaphylactic reaction	1 (10)	1 (10)
Dyspnea	1 (10)	1 (10)
Laryngeal edema	1 (10)	1 (10)
Renal disorder	1 (10)	1 (10)
Skin and subcutaneous tissue disorder		
Alopecia	3 (30)	0
Skin disorder	2 (20)	0
Dermatitis acneiform	1 (10)	0
Nail disorder	1 (10)	0
Radiation-related toxicity		
Radiation esophagitis	8 (80)	3 (30)
Radiation pneumonitis	4 (40)	0
Radiation skin injury	1 (10)	0

The table shows only the adverse events observed in ≥ 3 patients, or of grade ≥ 3 in at least 1 case. "Skin and subcutaneous tissue disorders" and "Radiation-related toxicity" are shown regardless of number of events because of their relevance to the treatment.

3.4 | Pharmacokinetics

Pharmacokinetic parameters are summarized in Table 5. Serum nimotuzumab concentration–time profiles following the initial dose and multiple doses are shown in Figures 1 and 2, respectively. PK reached the steady-state within 70 days after the first dose. At the steady-state, PK was comparable to the previously reported values.²⁸ C_{\max} at steady-state was twice the C_{\max} after the first dose.

TABLE 4 Efficacy of treatment

	Level 1 (200 mg) N = 8 n (%) [95% CI]
CR	4 (50.0) [15.7, 84.3]
Non-CR	1 (12.5) [0.3, 52.7]
Progressive disease	3 (37.5) [8.5, 75.5]
Not evaluated	0 (0.0) [0.0, 36.9]
Overall response rate	62.50%
Median PFS	13.9 (months) [4.2, 17.1]
Survival rate	(%) [95% CI]
12 months	75.0 [31.5, 93.1]
24 months	62.5 [22.9, 86.1]
36 months	37.5 [8.7, 67.4]

CI, confidence interval; CR, complete response; PFS, progression-free survival.

TABLE 5 Pharmacokinetic parameters after single dose of nimotuzumab

Pharmacokinetic parameter (mean ± SD)	Level 1 (200 mg) N = 7
C_{max} (µg/mL)	47.43 ± 19.982
t_{max} (h)	1.679 ± 1.5423
AUC_{0-inf} (µg·h/mL)	3299 ± 2452.2
CL_t (mL/h)	77.12 ± 27.772
$t_{1/2}$ (h)	40.74 ± 6.2218

AUC_{0-inf} , area under the concentration vs the time curve up to infinity; C_{max} , maximum concentration; CL_t , total body clearance; t_{max} , time to reach C_{max} ; $t_{1/2}$, elimination half-life; SD, standard deviation.

3.5 | Immunogenicity

Of the 9 evaluable patients, all tested negative for the development of HAHA.

3.6 | Biomarkers

Results relating to biomarkers are shown in Table 6. EGFR protein level in tumor tissues was 3+ in all 10 specimens provided by the patients. EGFR gene amplification levels could be measured in all 10 specimens provided by the patients, and gene amplification was not seen in any of the patients, although high polysomy was found in 3 patients. KRAS mutations in tumor tissues were not found in any of the 10 specimens. HER2 levels could be measured in 8 patients, and levels of 1+ and 0 were found in 2 and 6 patients, respectively.

4 | DISCUSSION

In this phase I study, we assessed the tolerability, safety, efficacy, and PK of nimotuzumab in combination with CRT in Japanese patients with esophageal cancer. At the time when 6 patients

treated with nimotuzumab at 200 mg (level 1) completed the chemoradiation period, 2 patients were assessed to have DLT. Subsequently, up to 10 patients were enrolled and no AE falling under the criteria for dose increase were observed as DLT remained limited to 2 patients. Therefore, the frequency of DLT, the primary endpoint, satisfied the criteria for dose increase specified in the protocol, and the MTD was estimated to be at least 200 mg. The results are highly suggestive for a phase III study to be conducted at 200 mg/wk, the dose used in an ongoing phase II study.²⁹ Nimotuzumab was confirmed to be tolerated at 200 mg in this study, and the dose was not increased to 400 mg, taking into consideration the progress of the phase II study at the time when it had to be decided whether to increase the dose in this study, as well as the development plan including a phase III study.

In our study, a grade 3 anaphylactic reaction developed within 15 minute of giving nimotuzumab in a 63-year-old female patient. Treatment with nimotuzumab and, subsequently, with cisplatin, fluorouracil, and radiation was discontinued. The patient was treated with glucocorticoids, antihistamines, and oxygen therapy and improved within 70 minute, and her recovery was further confirmed. This patient withdrew from the study and was excluded from the MTD, per protocol, and PK analysis sets.

Unlike other anti-HER-1/EGFR monoclonal antibodies, such as cetuximab, weaker skin toxicity such as a lower incidence of skin rash is reported with nimotuzumab, a wholly recombinant humanized monoclonal antibody.^{28,30,31} There were no SAE that occurred in 2 or more patients, and there was no trend towards an increased incidence of specific SAE. However, 1 patient was discovered to have bile duct cancer and subsequently died. This event was considered to be secondary cancer that occurred incidentally, and was assessed to be unrelated to the study treatment. Excluding 1 patient who had a fatal outcome, all patients who experienced SAE were confirmed to have recovered or to be recovering with drug treatment, hospitalization, or prolongation of the existing hospitalization. There was no report of grade 3 or higher AE that were classified as skin and subcutaneous tissue disorders in this study. Regarding skin-related AE, only grade 2 or lower alopecia, skin disorder, dermatitis acneiform, eczema, and nail disorder were reported and no delayed skin disorder was observed, indicating no significant concerns about skin disorders for this regimen.

Efficacy was evaluated based on the PFS and the survival rate during the follow-up period of 3 years in the present study. In 4 of the 10 patients with stage IV cancer, the median PFS was 13.9 months. The 1-year and 3-year survival rates were 62.5% and 37.5%, respectively. These results are comparable to those reported in previous studies, at lower stages of the disease. In previous reports of CRT in patients with stage II/III esophageal cancer, the median PFS and 3-year survival rate were reported to be 6-12 months and 23.9%-63.8%, respectively.¹²⁻¹⁶

In a phase II trial evaluating the additive effect of nimotuzumab to CRT in stage II to stage IV esophageal cancer (n = 42), PFS was 10 months and the 3-year survival rate was 26.2%.³² Similar promising results have been reported in other studies.^{33,34}

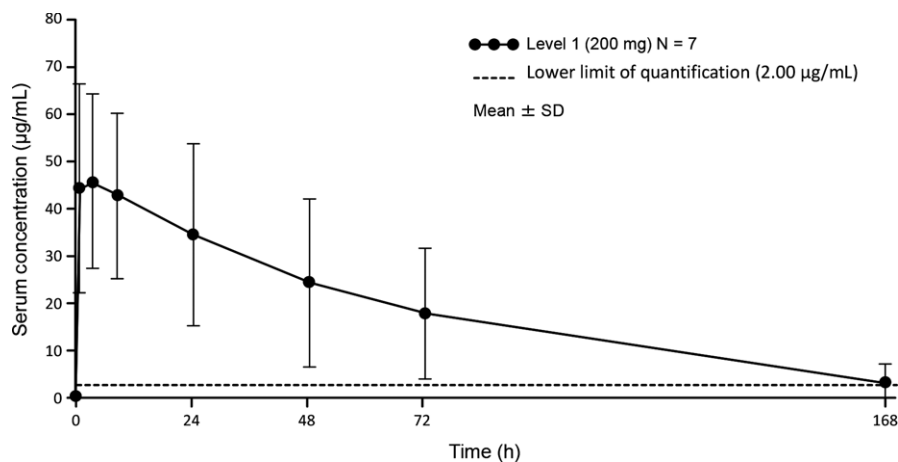


FIGURE 1 Time course of serum nimotuzumab concentrations with single dose (linear scale)

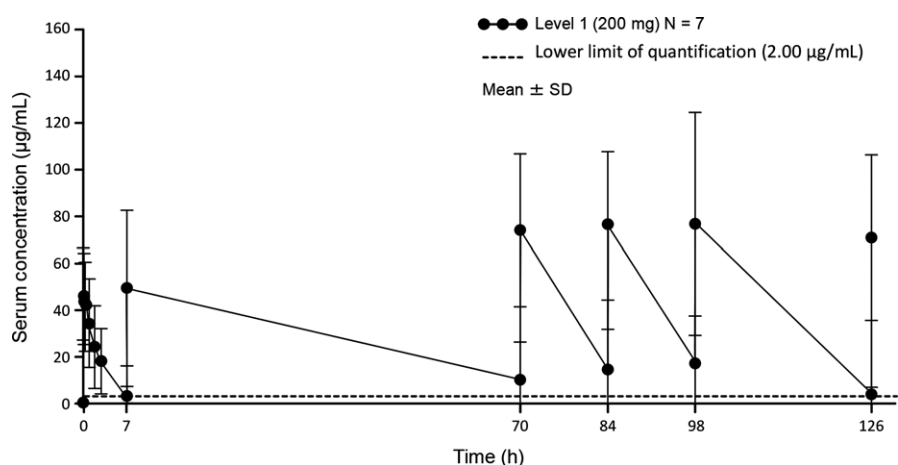


FIGURE 2 Time course of serum nimotuzumab concentrations with multiple dosing (linear scale)

High expression levels of EGFR may predict the long-term efficacy of nimotuzumab when used in combination with chemotherapy for metastatic esophageal squamous cell carcinoma.³⁵ In this study, EGFR was highly expressed in all patients. *Ras* mutation is known to be a marker of poor responses to anti-EGFR antibodies in colorectal cancer, but a low frequency of *K-Ras* mutations has been reported in esophageal cancer.³⁶ *K-Ras* mutations were not observed in this study cohort.

When compared to earlier reports of PK of nimotuzumab in Japanese patients,²⁸ a greater t_{max} was observed in the current study. Blood sampling time points in the current study (end of dosing and 3 and 8 hour after dosing) differed from those in the earlier study (end of dosing and 1, 3, and 8 hour after dosing), resulting in the different findings.

Limitations of the present study include the small sample size and limited numbers ($n = 4$) of patients with stage IV disease. It will be necessary to verify the efficacy of this combination in further studies with an adequate sample size. Because of the small sample size, we cannot draw any conclusions about possible relationships between biomarkers and efficacy. It will be necessary to assess

biomarkers in the next phase of development of nimotuzumab to evaluate which biomarkers can predict efficacy. Another limitation of this study is that the impact of concomitant treatment on MTD has not been assessed. In this study, nimotuzumab was used alone (during the additional chemotherapy and maintenance therapy periods) and in combination with chemotherapy and CRT. Stratification of AEs by concomitant therapy was not done. Further, the impact of target volume of radiation therapy on MTD was not assessed. In summary, 200 mg nimotuzumab weekly, in combination with concurrent CRT, is safe and well tolerated. In Japanese patients with esophageal cancer, the preliminary results of this treatment are encouraging.

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TABLE 6 Biomarkers in the present study

	Level 1 (200 mg), N = 10 n (%)
EGFR protein expression levels by IHC	
0	0
1+	0
2+	0
3+	10 (100)
EGFR gene amplification levels by FISH	
Disomy	0
Low trisomy	5 (50)
High trisomy	0
Low polysomy	2 (20)
High polysomy	3 (30)
Gene amplification	0
<i>K-ras</i> mutations	
Wild	10 (100)
Mutant	0
HER2 ^a	
0	6 (75)
1+	2 (25)
2+	0
3+	0

EGFR, epidermal growth factor receptor; IHC, immunohistochemistry.

^aHER2 expression levels could be measured in 8 subjects.

CONFLICTS OF INTEREST

Ken Kato has received research funds from Daiichi Sankyo Co., Ltd., Ono Pharmaceuticals Co., Ltd., Merck & Co., and Shionogi & Co., Ltd., Wasaburo Koizumi has received research funds and speaker fees from Daiichi Sankyo Co., Ltd., Takashi Ura has received research funds from Daiichi Sankyo Co., Ltd., and Ono Pharmaceuticals Co., Ltd., and speaker fees from Merck Serono, Chuga Pharmaceuticals Co., Ltd., Bayer Yakuhin, Ltd., and Taiho Pharmaceuticals Co., Ltd., Satoru Iwasa has received research funds from Daiichi Sankyo Co., Ltd., and Merck Serono. Chikatashi Katada has received research funds and personal fees from Daiichi Sankyo Co., Ltd., and personal fees from Meiji Seika Kaisha, Astra Zeneca K.K., Eisai Co., Ltd., Astellas Pharma Inc., Yakult Honsha, Takeda Pharmaceuticals Co., Ltd., Olympus Medical Science Sales Co., Ltd., EA Pharmaceuticals Co., Ltd., and Ono Pharmaceuticals Co., Ltd., Mizutomo Azuma has received research funds from Daiichi Sankyo Co., Ltd., Satoshi Ishikura has received personal fees from Daiichi Sankyo Co., Ltd., and Zeria Pharmaceuticals Co., Ltd., Yoshinori Nakao and Hiroshi Onuma are employees of Daiichi Sankyo Co., Ltd., Kei Muro has received research funds from Daiichi Sankyo Co., Ltd., and speaker fees from Takeda Pharmaceuticals Co., Ltd., Chugai Pharmaceuticals Co., Ltd., and Taiho Pharmaceuticals Co., Ltd.

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