Jpn J Clin Oncol 2009;39(4)260–266
doi:10.1093/jjco/hyp006

Phase I and Pharmacokinetic Study of HER2-targeted rhuMAb 2C4 (Pertuzumab, RO4368451) in Japanese Patients with Solid Tumors

Noboru Yamamoto, Yasuhide Yamada, Yutaka Fujiwara, Kazuhiko Yamada, Yasuhito Fujisaka, Toshio Shimizu and Tomohide Tamura

Division of Internal Medicine, National Cancer Center Hospital, Tokyo, Japan

Received November 7, 2008; accepted January 12, 2009; published online March 4, 2009

Objective: rhuMAb 2C4 (pertuzumab, RO4368451), a human epidermal growth factor receptor-2 (HER2) targeted antibody that binds to an epitope distinct from trastuzumab, blocks ligand-associated heterodimerization of HER2 with other HER receptor family members. This study evaluated the toxicity, pharmacokinetics and anti-tumor activities of pertuzumab in Japanese patients with solid tumors.
Methods: Patients with solid tumors refractory to standard therapy were administered pertuzumab 5, 10, 15, 20 and 25 mg/kg intravenously once every 3 weeks. Grade 3 toxicities were considered as dose limiting. The maximum tolerated dose (MTD) was a dose at which two out of six patients had Grade 3 toxicities.
Results: Eighteen patients, aged 38–66 (median 57) years, with solid tumors were enrolled and a total of 32 cycles of pertuzumab were administered. Toxicities were generally acceptable. Grade 3 elevation of gamma-glutamyl transpeptidase was observed in one patient at 25 mg/kg and was considered to be dose limiting. MTD was not reached up to a dose level of 25 mg/kg. The serum concentration of pertuzumab declined slowly (terminal half-life is approximately 3 weeks). The AUC proportionally increased over the dose range tested. There was limited evidence of activity (stable disease 2; progressive disease 13; and not evaluable 3); however, tumor shrinkage and tumor marker decrease were observed in an ovarian cancer and a non-small-cell lung cancer patient, respectively.
Conclusions: Pertuzumab is well tolerated up to 25 mg/kg. Although objective tumor response was not observed, it is worth evaluating as a flat dose and in combination with other cytotoxics and molecular-targeted agents.

Key words: pertuzumab – Phase I – MTD – pharmacokinetics – solid tumors

INTRODUCTION

The human epidermal growth factor receptor (HER) family consists of four tyrosine kinase receptors: HER1/ErbB-1 [epidermal growth factor receptor (EGFR)], HER2/ErbB-2/Neu, HER3/ErbB-3 and HER4/ErbB-4 (1). These receptors are highly expressed in many solid tumor types, including lung (2), breast (3), ovarian (4), colorectal (5) and prostate (6). They also play an important role in the proliferation, differentiation, motility, adhesion, protection from apoptosis and transformation of tumor cells (1,7,8). Receptor dimerization is essential for HER pathway activation, leading to phosphorylation and downstream signal transduction (1). Unlike HER1, HER3 and HER4, HER2 is considered to be an orphan receptor: no direct ligand for HER2 has been discovered. HER2 assumes an open conformation, with its dimerization domain permanently exposed for interaction with other ligand-activated HER receptors (9). HER2 is the preferred partner for dimer formation, and pre-clinical studies have demonstrated that HER2-containing heterodimers are the most mitogenic and have the highest transformation potential of all the HER complexes (1,10,11).

Pertuzumab (rhuMAb 2C4, RO4368451) is a recombinant, humanized monoclonal antibody that targets an epitope within the HER2 dimerization domain (12,13). Pertuzumab

For reprints and all correspondence: Noboru Yamamoto, Division of Internal Medicine, National Cancer Center Hospital, 5-1-1, Tsukiji Chuo-ku, Tokyo 104-0045, Japan. E-mail: nbryamam@ncc.go.jp

inhibits the formation of the HER2 heterodimer, independent of HER2 expression levels, and its binding site does not overlap with the epitope on HER2 that is recognized by trastuzumab (Herceptin) (14,15). Pertuzumab has demonstrated growth inhibition in ovarian, lung, breast and prostate tumor cells without HER2 overexpression (15). In the initial US Phase I study, the dose of pertuzumab was escalated from 0.5 to 15 mg/kg. The majority of adverse events were of Grades 1–2 severity and included asthenia, vomiting, nausea, abdominal pain, rash, diarrhea, pain and anemia. A Grade 3 gastrointestinal hemorrhage was observed at the 15 mg/kg dose level and this was determined as a dose-limiting toxicity (DLT); however, the maximum tolerated dose (MTD) was not reached up to the dose level of 15 mg/kg. Two out of 21 patients, one with ovarian cancer and the other with pancreatic islet cell carcinoma, achieved a partial response (16).

In light of these promising pre-clinical and clinical findings, we conducted a Phase I study of pertuzumab in Japanese patients with solid tumors. The objectives of this study were to determine the toxicity, pharmacokinetics and anti-tumor activities of pertuzumab.

PATIENTS AND METHODS

Patient Eligibility

Patients were eligible if they had histologically or cytologically confirmed malignant solid tumors that were resistant to standard therapies, or for which there was no effective treatment. HER2 status was not used in the selection process, except for those patients with breast cancer. Eligibility criteria were: age 20–74 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1; life expectancy >12 weeks; no previous chemotherapy, radiation therapy or surgery within 4 weeks before treatment with pertuzumab (6 weeks for previous treatment with nitrosoureas or mitomycin); adequate bone marrow (absolute neutrophil count $\geq$1500/mm^3, platelet count $\geq$75 000/mm^3 and hemoglobin level $\geq$9.0 g/dl), hepatic [serum total bilirubin $\leq$1.5 mg/dl, aspartate amino transferase (AST) $\leq$80 IU/l, alanine amine transferase (ALT) $\leq$80 IU/l and alkaline phosphatase (ALP) $\leq$1400 IU/l], renal (serum creatinine $\leq$1.3 mg/dl), coagulation [prothrombin time international normalized ratio (PT-INR) <1.3, activated partial thromboplastin time (APTT) <60 s], pulmonary [arterial oxygen pressure (PaO_2) $\geq$70 torr], and cardiac [left ventricular ejection fraction (LVEF) $\geq$50% by echocardiography] functions. Exclusion criteria included: pregnancy or lactation; symptomatic brain metastasis; HER2-positive breast cancer by fluorescence *in situ* hybridization; previous treatment with antibody agents for HER receptors; a history of hypersensitivity reactions to any drug; pleural effusion and ascites that required drainage; cumulative doxorubicin dose of $\geq$360 mg/m^2 given prior to study; hepatitis B or C or HIV; and serious pre-existing medical conditions such as uncontrolled infections, hypertension, hypercalcemia, diabetes, severe heart disease or psychogenic disorders.

Written informed consent was obtained from all patients. The study was approved by the institutional review board at the National Cancer Center, and conducted in accordance with Japanese Good Clinical Practice (GCP) guidelines.

Drug Administration and Dose Escalation Procedure

Pertuzumab (rhuMAb 2C4, RO4368451) was supplied by Chugai Pharmaceutical Co. Ltd (Tokyo, Japan). Each 10 ml vial contained approximately 175 mg pertuzumab formulated in 10 mmol/l L-histidine (pH 6.0), 240 mmol/l sucrose and 0.02% polysorbate 20. Pertuzumab was diluted in 250 ml saline immediately prior to administration. The calculated dose was administered by a 90 min intravenous infusion at the initial cycle of treatment, and repeated every 3 weeks. No prophylactic pre-medication to reduce hypersensitivity reaction was given. If no hypersensitivity reaction (related to pertuzumab administration) was observed, administration was shortened to a 30 min infusion after the second treatment cycle.

The starting dose was 5 mg/kg, with subsequent dose escalations to 10, 15, 20 and 25 mg/kg. The upper dose of 25 mg/kg was set based on the serum trough level estimation of efficacy in pre-clinical models (25 μg/ml) with the aim of exploring the safety range in Japanese patients considering the differences in body weight between patients from western countries and those from Japan. At least three patients were entered at each dose level. Three additional patients were entered at the same dose, if a DLT was observed in one of the initial three patients. The MTD was defined as the dose level at which two of three to six patients experienced DLT. DLT was defined as: Grade 4 hematological toxicities; Grade 3 or 4 non-hematological toxicities except for AST/ALT and serum creatinine elevations; AST/ALT elevations >150 IU/l; or serum creatinine elevation >2.0 mg/dl.

Pre-treatment Assessment and Follow-up Studies

Complete clinical assessments, including physical examination, ECOG PS, blood pressure, weight, chest X-ray, ECG, echocardiography and routine laboratory tests, were performed for all patients before study entry and prior to each treatment cycle. Routine laboratory tests included complete blood count and differential testing of electrolytes, urea nitrogen, serum creatinine, serum total protein, serum albumin, glucose, total bilirubin, AST, ALT, ALP, lactic dehydrogenase, gamma-glutamyl transferase, PaO_2, adequate tumor markers, PT-INR, APTT and urinalysis. With the exception of PaO_2 and tumor markers, these laboratory tests were repeated on Days 1, 8, 22, 29 and 42, and then every 3 weeks. PaO_2 was assessed on an as needed basis. Tumor markers were assessed on alternate treatment cycles (cycles

1 and 3). Anti-pertuzumab antibody was assessed before each treatment cycle.

Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. Tumor responses were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria (17).

Pharmacokinetics

Pharmacokinetic evaluation was performed in all patients. Venous blood samples (5 ml) were taken and immediately centrifuged at 1500 rpm for 10 min. Serum was aliquoted and stored at -70°C or less in polyethylene tubes until analysis. Pharmacokinetic sampling points in the initial treatment cycle were before infusion, at the end of infusion, after 1.5, 4 and 8 h, and at 8, 15 and 22 days after completion of infusion. In the second treatment cycle, sampling points were before infusion, at the end of infusion, at 4 and 8 h, and at 8 and 22 days. For the third cycle, pharmacokinetic samples were taken before infusion, at the end of infusion, and 8 and 22 days after treatment.

The concentration of pertuzumab in serum was measured by receptor-binding enzyme-linked immunosorbent assay using $p185^{HER2}$ extracellular domain to capture pertuzumab. Bound pertuzumab was detected with mouse anti-human Fc-horseradish peroxidase (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), and tetramethyl benzidine (KPI Inc., Gaithersburg, MD, USA) was used as the substrate for color development to quantify serum pertuzumab against a known standard curve. The minimum quantifiable concentration was 0.025 μg/ml.

Analyzed pharmacokinetic parameters included the maximum plasma drug concentration (C_{max}), area under the plasma drug concentration–time curve (AUC), distribution volume at steady state (V_{dss}), terminal half-life, mean residence time (MRT) and clearance (CL). These data were analyzed by two-compartmental method analysis using the WinNonlin software program version 4.1 (Pharsight Corporation, CA, USA).

RESULTS

Patient Characteristics

Eighteen patients (11 males and 7 females; ECOG PS 0 or 1) were entered in the study. Patient characteristics are shown in Table 1. Median age and body weight were 57 (range 38–66) years and 57.9 (42.9–73.5) kg, respectively. Non-small-cell lung cancer (NSCLC) was the predominant tumor type. Thirteen patients had received surgical resection for primary tumors, 18 had received prior chemotherapy and 8 had $\geq$4 prior treatment regimens. A total of 32 cycles of pertuzumab were administered, and the median number of cycles administered per patient was 2 (range 1–4). All 18 patients were included in the toxicity evaluation and 15 patients met the RECIST criteria. Three patients were not evaluable for efficacy because they did not meet RECIST criteria for adequate measurable lesions.

Table 1. Patient characteristics

Characteristics	No. of patients
Total no. of patients	18
Male/female	11/7
ECOG performance status	
0	5
1	13
Tumor type	
NSCLC	7
Rectum	3
Stomach	2
Ovary	2
Breast	1
Esophagus	1
Germ cell	1
Primary unknown	1
Prior treatment	
Surgery	13
Radiotherapy	6
Chemotherapy	18

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small-cell lung cancer.

Safety and Tolerability

The main toxicities seen in all cycles of treatment are shown in Table 2. The majority of toxicities were mild (Grades 1–2) in severity, and diarrhea, rash, brain natriuretic peptide (BNP) increase and lymphopenia were the most frequent toxicities seen.

Diarrhea was observed in 11 (61.1%) of 18 patients; 10 of these were defined as Grade 1, and 1 was Grade 2. The onset of diarrhea was within 1 week of treatment and resolved on treatment with a Lactobacillus preparation. No watery diarrhea was observed and treatment with loperamide was not required.

Rash was experienced by 9 (50%) of 18 patients, 6 of which were defined as Grade 1 and 3 as Grade 2, and mostly consisted of acne and seborrhea. The onset of these skin toxicities generally occurred within 7–14 days of the initial treatment cycle, persisting for 1–2 weeks and almost asymptomatic with no medication required.

BNP increase and lymphopenia were observed in nine (50%) and seven (38.9%) patients, respectively. The largest elevation of BNP was 119.5 pg/ml in the 20 mg/kg cohort, but this patient was asymptomatic. No cardiac dysfunction (assessed by troponin-T, ECG and echocardiography)

Table 2. Major toxicities in all cycles

Pertuzumab dose (mg/kg)	5		10		15		20		25		Total	%
No. of patients	3		3		3		3		6			
NCI-CTC grade	1, 2	≥3	1, 2	≥3	1, 2	≥3	1, 2	≥3	1, 2	≥3		
Diarrhea	2	0	3	0	3	0	2	0	1	0	11	61.1
Rash	3	0	2	0	1	0	0	0	3	0	9	50.0
BNP increase	2	0	0	0	2	0	1	0	4	0	9	50.0
Lymphopenia	1	0	0	0	1	1	2	0	1	1	7	38.9
Anemia	1	0	1	0	0	0	1	0	2	0	5	27.8
Leukocytosis	2	0	1	0	1	0	1	0	0	0	5	27.8
Hyperglycemia	0	0	2	0	2	0	0	0	1	0	5	27.8
Neutropenia	2	0	1	0	1	0	0	0	0	0	4	22.2
Leukopenia	1	0	0	0	0	0	2	0	1	0	4	22.2
Nausea	2	0	0	0	0	0	0	0	1	0	3	16.7
Stomatitis	0	0	0	0	1	0	0	0	2	0	3	16.7
Asthenia	1	0	0	0	1	0	1	0	0	0	3	16.7
Gamma-GTP increase	0	0	0	0	0	0	0	0	1	1[a]	2	11.1
HSR	0	0	0	0	0	0	0	0	2	0	2	11.1

BNP, brain natriuretic peptide; Gamma-GTP, gamma-glutamyl transpeptidase; HSR, hypersensitivity reaction; NCI-CTC, National Cancer Institute Common Toxicity Criteria.
[a]Dose-limiting toxicity.

was observed among any patients. Lymphopenia was also asymptomatic, and the severity appeared to be dose-related.

Hypersensitivity reactions were observed in two patients immediately following pertuzumab administration: one experienced Grade 1 fever and the other had Grade 2 fever. Both resolved without medication. No other hypersensitivity symptoms, such as dyspnea, bronchospasm, hypotension or tachycardia, were observed.

One patient at the 25 mg/kg dose level had a Grade 3 gamma-glutamyl transpeptidase (gamma-GTP) increase. This patient was a 57-year-old man, with ECOG PS 1, who was diagnosed with advanced gastric cancer with multiple liver metastases. The Grade 3 gamma-GTP increase was observed on the third day of pertuzumab treatment, but it was asymptomatic and resolved without medication. It was considered to be a DLT according to the DLT definition.

Up to the 25 mg/kg dose level, one DLT (Grade 3 gamma-GTP increase at a dose of 25 mg/kg) was observed. Other toxicities were considered acceptable. Therefore, the MTD was not reached, and the acceptable tolerability of pertuzumab doses up to 25 mg/kg was confirmed.

Anti-tumor Activity

Fifteen of 18 patients were evaluable for anti-tumor response. Two patients had stable disease (one rectal cancer and one NSCLC), and 13 had progressive disease. There was limited evidence of anti-tumor activity in this study; therefore, a relationship between pertuzumab dose and anti-tumor response could not be determined.

Pharmacokinetics

Serum sampling for pharmacokinetic analysis was performed in all 18 patients in the initial cycle of treatment. In subsequent cycles, samples from only 9 of 18 patients were available for pharmacokinetic analysis; the other 9 patients were withdrawn due to disease progression. The pharmacokinetic profile of pertuzumab is summarized in Table 3, and trough concentrations of subsequent cycles are summarized in Table 4. Also, the mean serum concentration–time profiles of pertuzumab are illustrated in Fig. 1.

The concentration–time profile of pertuzumab was biphasic, with mean initial half-life and terminal half-life of 11.6 h and 17.3 days at the 25 mg/kg dose level, respectively. V_{dss} and CL showed moderate inter-individual variability and the mean ± SD values (CV%) for V_{dss} and CL were 92.4 ± 15.2 (16.5 CV%) ml/kg and 4.30 ± 1.72 (40.1 CV%) ml/day/kg, respectively. At doses of 5–25 mg/kg, CL, V_{dss}, the distribution volume of the central compartment (V_c) and the terminal half-life did not change with dose. The peak serum concentration (C_{max}) and the AUC increased

Table 3. Pharmacokinetic parameters

Dose (mg/kg)	No. of patients	C_{max} (mg/ml)	AUC (day*mg/ml)	CL (ml/day/kg)	V_c (ml/kg)	V_{dss} (ml/kg)	MRT (days)	$t_{1/2}$ initial (h)	$t_{1/2}$ terminal (days)
5	3	105 ± 14.2	928 ± 162	5.51 ± 1.03	46.0 ± 6.54	88.5 ± 14.5	16.3 ± 2.89	7.17 ± 4.47	11.5 ± 1.95
10	3	178 ± 31.1	2190 ± 813	4.95 ± 1.60	56.0 ± 9.12	95.1 ± 15.3	20.0 ± 3.83	13.6 ± 7.89	14.3 ± 2.66
15	3	314 ± 65.1	4220 ± 2090	4.24 ± 2.17	48.6 ± 11.1	96.3 ± 32.5	24.7 ± 7.32	26.5 ± 10.7	18.2 ± 5.17
20	3	334 ± 62.4	3870 ± 576	5.25 ± 0.848	57.7 ± 8.22	85.6 ± 6.70	16.7 ± 3.59	5.02 ± 5.22	11.7 ± 2.61
25	6	495 ± 91.8	6490 ± 2090	4.30 ± 1.72	50.4 ± 12.7	92.4 ± 15.2	24.2 ± 9.37	11.6 ± 9.81	17.3 ± 6.97

Values are mean ± SD. AUC, area under plasma drug concentration–time curve; CL, clearance; C_{max}, maximum plasma drug concentration; MRT, mean residence time; $t_{1/2}$, half-life; V_c, distribution volume of the central compartment; V_{dss}, distribution volume at steady state.

Table 4. Trough concentration ratio of subsequent cycles

Dose (mg/kg)	No. of cycles	No. of patients	Mean	Standard deviation	CV (%)	Median	Min	Max
5	2	2	1.43	0.610	42.6	1.43	1.00	1.86
	3	2	2.29	0.0427	1.9	2.29	2.26	2.32
10	2	1	1.72	—	—	1.72	1.72	1.72
15	2	1	1.43	—	—	1.43	1.43	1.43
20	2	1	1.76	—	—	1.76	1.76	1.76
25	2	4	1.88	0.0805	4.3	1.86	1.81	2.00
	3	1	2.31	—	—	2.31	2.31	2.31

CV, coefficient of variation.

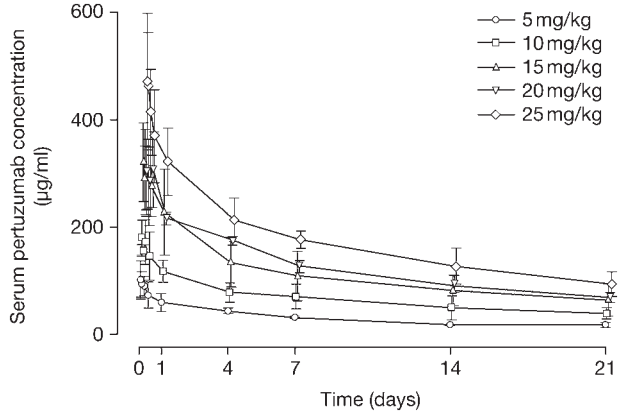

Figure 1. Mean serum concentration–time profiles of pertuzumab.

proportionally with the dose of pertuzumab ($r = 0.914$ and $r = 0.808$, respectively), suggesting linear pharmacokinetics within the dose range in this study.

DISCUSSION

Pertuzumab is a humanized monoclonal antibody that inhibits dimerization of HER2 with other ligand-activated HER kinases. At the start of this study, the pertuzumab study with a flat dose (i.e. unadjusted by body weight) was also in preliminary stages in western countries. Its planned higher dose (1050 mg/person) was based on the highest pertuzumab dose in the US Phase I study (15 mg/kg) and a median body weight of approximately 70 kg. As the body weight of Japanese patients was expected to be lower than US patients, it was thought that this study needed to determine the safety range of pertuzumab doses of more than 15 mg/kg in this population. Although one DLT was observed at the 25 mg/kg dose level, pertuzumab was generally well tolerated and the MTD of pertuzumab was not reached up to a dose level of 25 mg/kg.

The most frequent toxicities observed were diarrhea, rash, BNP increase and lymphopenia. The most frequent adverse event was diarrhea (61.1%), which was generally consistent with findings from the initial Phase I study in the USA (43%) (16), though higher than that observed with other humanized antibodies (trastuzumab, bevacizumab and cetuximab: <10%) (18–23). However, severity was generally mild (10 of 11 cases were Grade 1) and intensive supportive treatment such as loperamide was not required. The precise mechanism of the diarrhea observed has not been determined, but it was considered that pertuzumab was tolerable in Japanese patients.

Rash was experienced by 50% of patients, mostly consisting of acne and seborrhea. The profile of rash was similar to that seen in the US study (16). As pertuzumab inhibits heterodimerization of HER1/HER2, this rash was considered as

possibly related to treatment. However, all these events were mild in severity and it was thought that pertuzumab-related rash was milder than the reported skin disorders due to EGFR inhibition with cetuximab (22–24).

Hypersensitivity reactions were observed in two (11.1%) patients. These reactions were transient fever and headache and resolved immediately without medication. Furthermore, no anti-pertuzumab antibody production was observed. Therefore, the administration of pertuzumab could be considered as safe as other humanized monoclonal antibodies, such as bevacizumab and cetuximab.

Following prior experience with trastuzumab (25), cardiotoxicity was closely monitored throughout this study. A BNP increase was observed in 50% of patients; however, no clinically significant cardiotoxicity or other laboratory abnormalities, such as troponin-T increase and LVEF decrease, were observed.

A DLT (Grade 3 gamma-GTP increase) was observed in one patient at the 25 mg/kg dose level. This resolved without medication. As pertuzumab is not metabolized by the liver, the causality could not be considered as definite. Routine measurement of gamma-GTP levels was not included in the initial study design, so the frequency of such increases is unknown. Routine assessment of gamma-GTP levels should be considered for future studies.

The pharmacokinetic profile of pertuzumab revealed moderate inter-individual variability and linear pharmacokinetics. The mean terminal half-life at the 25 mg/kg dose level was 17.3 days, and the concentration–time profile was similar to that of other monoclonal antibodies such as trastuzumab (25.5 days) and bevacizumab (18.4 days). The observed pharmacokinetic profile was similar to that seen in the US Phase I study (16). The variability of pertuzumab steady-state trough serum concentrations and exposure after fixed, body-weight-based, or body-surface area (BSA)-based dosing in cancer patients had previously been examined (26), and demonstrated the feasibility of administration using flat dosing in specific patients such as ovarian or breast cancer patients.

In this Phase I study, the safe range of pertuzumab dosing in Japanese patients was determined to be up to 25 mg/kg. This dose level was higher than that determined in the US Phase I study (15 mg/kg). However, the median body weight of enrolled patients in this study was 57.9 kg, compared with a median body weight of 69.0 kg for patients in the US study. Considering the results from these studies and the respective patient body weights, it is thought that this pertuzumab dose range may apply for common dose settings such as a flat dose in further studies.

In conclusion, the MTD of pertuzumab was not reached up to a dose of 25 mg/kg and acceptable tolerability was confirmed. Although an objective anti-tumor effect was not observed in this study, the profile of pertuzumab is unique and encouraging, and further investigation with flat doses and in combination with other cytotoxic or molecular-targeted drugs for various tumor types is warranted.

Funding

This study was supported by Chugai Pharmaceutical Co. Ltd. Funding to Pay the Open Access publication charges for this article was provided by Chugai Pharmaceutical Co., Ltd.

Conflict of interest statement

None declared.

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001;2:127–37.
2. Meert AP, Martin B, Delmotte P, Berghmans T, Lafitte JJ, Mascaux C, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J* 2002;20:975–81.
3. Witton CJ, Reeves JR, Going JJ, Cooke TG, Bartlett JM. Expression of the HER1-4 family of receptor tyrosine kinases in breast cancer. *J Pathol* 2003;200:290–7.
4. Campiglio M, Ali S, Knyazev PG, Ullrich A. Characteristics of EGFR family-mediated HRG signals in human ovarian cancer. *J Cell Biochem* 1999;73:522–32.
5. Maurer CA, Friess H, Kretschmann B, Zimmermann A, Stauffer A, Baer HU, et al. Increased expression of erbB3 in colorectal cancer is associated with concomitant increase in the level of erbB2. *Hum Pathol* 1998;29:771–7.
6. Agus DB, Akita RW, Fox WD, Lofgren JA, Higgins B, Maiese K, et al. A potential role for activated HER-2 in prostate cancer. *Semin Oncol* 2000;27:76–83; discussion 92–100.
7. Holbro T, Civenni G, Hynes NE. The ErbB receptors and their role in cancer progression. *Exp Cell Res* 2003;284:99–110.
8. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. *Oncogene* 2000;19:6550–65.
9. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, et al. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol Cell* 2003;11:495–505.
10. Tzahar E, Waterman H, Chen X, Levkowitz G, Karunagaran D, Lavi S, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol Cell Biol* 1996;16:5276–87.
11. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J* 1997;16:1647–55.
12. Adams CW, Allison DE, Flagella K, Presta L, Clarke J, Dybdal N, et al. Humanization of a recombinant monoclonal antibody to produce a therapeutic HER dimerization inhibitor, pertuzumab. *Cancer Immunol Immunother* 2006;55:717–27.
13. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* 2004;5:317–28.
14. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, et al. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* 2002;2:127–37.
15. Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Res* 1990;50:1550–8.
16. Agus DB, Gordon MS, Taylor C, Natale RB, Karlan B, Mendelson DS, et al. Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer. *J Clin Oncol* 2005;23:2534–43.
17. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
18. Fountzilas G, Razis E, Tsavdaridis D, Karina M, Labropoulos S, Christodoulou C, et al. Continuation of trastuzumab beyond disease progression is feasible and safe in patients with metastatic breast cancer: a

retrospective analysis of 80 cases by the hellenic cooperative oncology group. *Clin Breast Cancer* 2003;4:120–5.
19. Sawaki M, Ito Y, Tada K, Mizunuma N, Takahashi S, Horikoshi N, et al. Efficacy and safety of trastuzumab as a single agent in heavily pretreated patients with HER-2/neu-overexpressing metastatic breast cancer. *Tumori* 2004;90:40–3.
20. Burger RA. Experience with bevacizumab in the management of epithelial ovarian cancer. *J Clin Oncol* 2007;25:2902–8.
21. Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther* 2006;28: 1779–802.
22. Saltz LB, Meropol NJ, Loehrer PJ, Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;22:1201–8.
23. Hanna N, Lilenbaum R, Ansari R, Lynch T, Govindan R, Jänne PA, et al. Phase II trial of cetuximab in patients with previously treated non-small-cell lung cancer. *J Clin Oncol* 2006;24: 5253–8.
24. Package insert. Erbitux (Cetuximab). Branchburg, NJ: Imclone Systems and Bristol-Myers Squibb 2008.
25. Keefe DL. Trastuzumab-associated cardiotoxicity. *Cancer* 2002;95: 1592–600.
26. Ng CM, Lum BL, Gimenez V, Kelsey S, Allison D. Rationale for fixed dosing of pertuzumab in cancer patients based on population pharmacokinetic analysis. *Pharm Res* 2006;23:1275–84.