

Original article

Title: An Extended Phase Ib Study of Epertinib, an Orally Active Reversible Dual EGFR/HER2 Tyrosine Kinase Inhibitor, in Patients with Solid Tumours

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Abstract:

Background: Dose-escalation of epertinib (S-222611), a new potent oral EGFR/HER2 inhibitor, has established a recommended daily dose of 800 mg in patients with solid tumours. In this study, we have recruited a larger number of patients to assess further the safety, tolerability, pharmacokinetics (PK) and antitumour activity.

Patients and methods: Patients with solid tumours expressing EGFR or HER2 received a single dose of epertinib at 800 mg on Day 1 to assess PK over 7 days, followed by continuous once-daily dosing from Day 8.

Results: We treated 76 patients with breast (n = 27), upper gastrointestinal (GI; n = 30), head and neck (n = 12) or renal cancers (n = 7). Epertinib was well-tolerated with mostly Grade 1 and 2 adverse events (AE). The most frequent AE was diarrhoea, which was generally manageable with loperamide. The objective response rate (ORR) in patients with heavily pretreated breast and upper GI cancers was 16.0% (4 PRs) and 8.3% (1CR, 1PR), respectively. All 6 responding patients had HER2-positive tumours; the ORR for HER2-positive breast and upper GI cancer populations was 19.0% and 20.0%. Partial response in the brain disease of one breast cancer patient lasted 7.5 months.

Conclusion: Once-daily dosing of epertinib at 800 mg was well-tolerated, and demonstrated promising antitumour activity in patients with heavily pretreated HER2-positive breast and upper GI cancer, including those with brain metastases.

EudraCT Number: 2009-017817-31

Keywords: Epertinib, S-222611, EGFR, HER2, tyrosine kinase inhibitor

1. Introduction

Human epidermal growth factor receptor 2 (HER2) is known to be overexpressed in approximately 25% of breast cancers and around 20% of gastric cancers, and is associated with poor clinical outcome [1, 2]. Trastuzumab, a monoclonal antibody that binds to the extracellular domain of HER2, has been established for HER2-positive breast cancer treatment in the metastatic and early settings, and for HER2-positive metastatic gastric cancer treatment [3-5]. However, in metastatic breast cancer response rates to trastuzumab are only 15% as monotherapy and 49% in combination with paclitaxel [6, 7]. Also, eventually resistance develops in responding patients, and a significant incidence of cardiac toxicity particularly in patients previously exposed to anthracyclines has been reported [8]. Several additional anti-HER2 agents, including lapatinib, neratinib, pertuzumab and ado-trastuzumab emtansine (T-DM1), are approved, but efficacy is still not completely satisfactory despite overall survival improvement with T-DM1 and pertuzumab [3, 9-13]. Therefore, there remains an unmet need for HER2 inhibitors that are effective and well tolerated with chronic administration.

Epertinib (S-222611, Shionogi & Co. Ltd, Osaka, Japan) is an orally active, reversible, selective and potent inhibitor of epidermal growth factor receptor (EGFR), HER2 and HER4 kinases. Compared with lapatinib, which has a similar mechanism of action, in mouse xenograft models epertinib showed more prolonged inhibition of EGFR and HER2 phosphorylation *in vitro*, and 4-6 fold greater antitumour activity [14]. Superior survival was also observed in a brain metastasis model of breast cancer, with good penetration of the blood brain barrier [14].

We previously performed a phase I dose-escalation study in 33 patients with solid tumours expressing EGFR and/or HER2 [15]. Oral epertinib was well tolerated at doses

ranging from 100 mg to 1600 mg, and a maximum tolerated dose was not reached. Drug exposure increased dose-proportionally over a range from 100 mg to 800 mg and tumour responses were observed across a range of doses. Based on these results, a dose of 800 mg was selected for further study. We now report an extended study of epertinib in patients with advanced solid tumours over-expressing EGFR and/or HER2.

2. Methods

2.1. Patients

Eligible patients were ≥ 18 years old with histologically and/or cytologically confirmed EGFR and/or HER2-positive adenocarcinoma of the breast, with or without cerebral metastases; adenocarcinoma of the stomach, gastro-esophageal junction or esophagus; renal cell carcinoma; or squamous cell carcinoma of the head and neck, that had progressed following treatment with approved therapies, or for which no standard therapy was available. The EGFR and HER2 expression were assessed at central or local laboratories using fresh tumour biopsy obtained at screening or archival tissue for confirmation of the eligibility, while the EGFR/HER2 status assessed by the central review was used for evaluation of efficacy. Eligible patients were required to have Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, and adequate hematological, renal, hepatic and left ventricular function ($\geq 50\%$). Patients with brain metastasis from HER2-positive breast cancer were allowed to be enrolled in this study based on the evidence showing that superior efficacy in suppressing tumour growth of brain metastases compared to lapatinib in an intracranial implantation mouse model [14]. The study was approved after review by the relevant regulatory and independent ethics committees (EudraCT Number: 2009-017817-31), and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice. All patients provided written informed consent before enrollment.

2.2. Study design

Epertinib was initially administered as a single dose on Day 1, followed by continuous daily dosing from Day 8 to Day 28. Patients received continuous dosing with epertinib in 28-day

cycles until disease progression, intolerable toxicity or withdrawal of consent. The primary objective was to assess further the safety and tolerability of epertinib. Secondary objectives included pharmacokinetics (PK) and an extended assessment of efficacy.

2.3. Safety assessment

Adverse events (AEs) were monitored and clinical laboratory parameters, weight, vital signs, 12 lead ECGs, physical and ophthalmic examinations, cardiac function including left ventricular ejection fraction, and ECOG performance status were assessed at baseline and throughout the study, and lead II continuous monitoring was carried out for 8 hours post-dosing on Day 1. All AEs were coded using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

2.4. Pharmacokinetic analysis

Blood samples for the measurement of plasma concentrations of epertinib and its active de-alkylated and lactam metabolites were taken at the following times during treatment: Day 1 (pre-dose, 2, 4, 6, and 12 hours post-dose), Day 2 (24 hours post-dose), Day 3 (48 hours post-dose), Day 5 (96 hours post-dose), Days 8, 15, 22 and 29 (pre-dose). Samples were also taken before dosing on Days 15 and 29 of the first two cycles of Part B. Plasma concentration measurement and calculation of the PK parameters of epertinib and its active metabolites were performed in the same manner as described in our previous report [15].

2.5. Tumour evaluation

Tumour response was assessed every 8 weeks according to Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.1 [16]. Patients who had at least one additional scan after baseline, and patients who had no additional scan but discontinued due to clinical disease progression, were considered evaluable for response.

3. Results

3.1. Safety and tolerability

Between July 2010 and January 2014, a total of 76 patients with breast (n = 27), upper GI (n = 30), head and neck (n = 12) and renal cell carcinoma (n = 7) were enrolled at 9 sites in the United Kingdom and France. Patient demographics and baseline characteristics are summarised in Table 1. As shown in patient disposition (Figure S1), 14 patients (7 patients in Part A and 7 patients in Part B) discontinued due to treatment-emergent adverse events (TEAEs). Only 2 patients discontinued due to TEAEs (nausea and vomiting, and bilirubin elevation) that were considered drug-related. Disease progression accounted for all other discontinuations except for one patient who was withdrawn due to deteriorating quality of life.

Table 2 summarises all TEAEs of any grade occurring in at least 10% of patients, and all Grade 3 or higher TEAEs. There were no treatment-related deaths. Eperitinib at 800 mg once daily was well tolerated by most patients, with 21.1% (16/76) of patients requiring dose reduction. The majority of AEs were Grade 1 or 2 in intensity, and no significant hematological or biochemical toxicity was observed. Overall, the most frequently reported AEs were diarrhoea, nausea, rash and anorexia. Diarrhoea was generally self-limiting or effectively controlled with loperamide; in 10 (13.2%) patients it was Grade 3. Nausea was almost always Grade 1 or 2 and was effectively managed with antiemetics and dose interruptions. Rash was reported in 32 (42.1%) patients, of which none was more severe than Grade 2. There were no effects on ECG, and no evidence that eperitinib treatment affected cardiac function.

Bilirubin elevation was observed in 23 patients (30.3%); this elevation was Grade 3 in 5 patients (6.6%), and Grade ≤ 2 in all others. The increase in bilirubin was not associated with any symptoms, elevation of liver enzymes or hematologic abnormalities. When the

unconjugated and conjugated bilirubin was measured in 14 of the 23 patients, the elevation was predominantly attributable to unconjugated bilirubin. Dose reduction was required due to bilirubin elevation in 1 patient (1.3%), and resulted in study discontinuation in one (1.3%) other patient.

3.2. Pharmacokinetic analysis.

The PK parameters after single administration and trough plasma concentrations after repeated doses at 800 mg are summarised in Table S1. Epertinib was absorbed with a median T_{max} of 4.1 hours and geometric mean C_{max} of 454 ng/mL following a single oral dose. The geometric mean $t_{1/2,z}$ was 33.8 hours and steady-state was achieved within 7 days of once-daily dosing. The pharmacologically active dealkylated and lactam metabolites of epertinib were rapidly formed, and the exposures (AUC_{0-inf} and C_{max}) to these metabolites were 5-7% and 6-8% on a molecular basis of the parent epertinib, respectively.

3.3. Clinical response.

Sixty five of 76 patients were evaluable for tumour response. The tumour responses for each cancer are shown in Table S2. Two patients with gastro-esophageal junction tumours had CR and PR, and 4 patients with breast cancer experienced PR. The objective response rate (ORR) for breast, upper GI, head and neck and renal cell cancers were 16.0% (4/25), 8.3% (2/24), 0% (0/9) and 0% (0/7), respectively. As shown in Table 3, all of the 4 responding breast cancer patients were HER2-positive, but 2 of them were EGFR-negative. All 4 responding patients had previously received trastuzumab, and 3 of the 4 patients were previously treated with lapatinib. The 2 responding upper GI cancer patients were also HER2-positive, and one

was EGFR-negative. These two patients had been treated with trastuzumab-based therapy and multiple lines of chemotherapy. The ORRs in patients with HER2-positive breast and upper GI cancers were 19% (4/21) and 20% (2/10), respectively. Figures 1A and 1B demonstrate the changes in the sum of longest diameter of target lesions and treatment period in patients with breast cancer having at least one evaluable scan (n = 21), and Figures 1C and 1D in patients with upper GI cancers (n = 20). Two of the 4 responding patients with breast cancer continued on study beyond 40 weeks and the other 2 patients continued beyond 16 weeks. The patient with gastro-esophageal junction disease experiencing CR completed a total treatment period of 52 weeks and another with PR continued treatment until 39 weeks.

Five patients with brain metastases from breast cancer were evaluable for radiological assessment, and 4 of the 5 patients had brain and/or extra-cranial lesions for assessment by RECIST v1.1 (Figure 1B). One of the 4 patients whose only target lesion was a brain metastasis achieved PR (Figure 1E) with a sustained response of 7.5 months, and 2 patients experienced prolonged SD (> 6 months).

4. Discussion

This was an extended phase I study aiming to assess the safety, tolerability and PK of epertinib, as well as to provide a preliminary indication of antitumour activity. As suggested by the earlier dose-escalation study of this drug [15], once-daily oral dosing of epertinib at 800 mg was well-tolerated with predominantly low-grade toxicities including diarrhoea, nausea, rash and anorexia; no Grade 3 rash, but Grade 3 diarrhoea observed in 10 patients (13.2%) and Grade 2 in 14 out of 76 patients (18.4%). Diarrhoea probably caused by EGFR inhibition [17, 18] could be managed by anti-diarrhoeal medication; prophylactic use was not allowed in this study. No patient required discontinuation of epertinib treatment due to diarrhoea. There was no evidence of a drug-related decline in ejection fraction or effects on ECG QT interval, despite the known expression of HER2 on cardiac myocytes [19]. As reported in the dose-escalation study [15], elevation of blood bilirubin of any grade was observed in 30% of patients in the expansion phase but this was not associated with evidence of other hepatic or hematologic abnormalities. Epertinib is known to inhibit the conjugating enzyme UGT1A1 *in vitro* with an IC₅₀ value of 3.7 µmol/L, but not the multidrug resistance protein 2 (MRP2) involved in conjugated bilirubin transport. The elevated bilirubin was predominantly associated with unconjugated bilirubin in patients measured. Therefore, it is considered that the frequent elevation of total blood bilirubin in epertinib-treated patients arises from inhibition of UGT1A1-mediated bilirubin conjugation.

PK parameters of epertinib at 800 mg once-daily were consistent with those reported previously [15]. The trough concentrations of epertinib at steady-state markedly exceeded those that were seen to inhibit EGFR and HER2 *in vitro* [14], and the t_{1/2} was consistent with the currently recommended daily dosing schedule. The active dealkylated and lactam metabolites which were identified in human but not in mouse have similar activity to that of the parent compound against HER2 and EGFR kinase; the IC₅₀ values of the dealkylated and

lactam metabolites are known to be 1.24 nmol/L and 2.32 nmol/L for EGFR and 6.31 nmol/L and 18.89 nmol/L for HER2, respectively, which are almost equivalent to or lower than those for epertinib. However, the plasma concentration of these metabolites accounted for only 5-7% and 6-8% of the parent epertinib on a molecular basis, respectively, suggesting that the contribution of these metabolites to efficacy is modest.

In this study, PRs were reported in five patients with breast or upper GI cancers who had been previously treated with HER2-targeting drugs, and one CR in another gastroesophageal junction cancer patient previously treated with trastuzumab combination. The response rate in patients with HER2-positive breast cancer was 19.0%, which compares favorably with 6.9% for lapatinib monotherapy and 10.3% for lapatinib plus trastuzumab for HER2-positive breast cancer [20]. Interestingly, we observed one PR and 2 long SDs among 5 patients with intracranial disease, which is of particular relevance for this challenging clinical scenario. Of the 24 upper GI cancer patients evaluable for response, 10 patients were HER2-positive and the other 14 patients were HER2-negative. Two of the 10 patients (20.0%) with HER2-positive upper GI cancer responded. For HER2-positive upper GI cancer, trastuzumab is combined with chemotherapy as a standard second-line therapy, with response rates reported to be 37.0% (17/46) in trastuzumab-naïve patients [21] and 9.3% (3/32) in trastuzumab-refractory patients [22]. These preliminary data on the activity of epertinib in HER2-positive upper GI cancer are promising and warrant further evaluation.

All the patients with head and neck and renal cancers included in this trial were EGFR-positive but HER2-negative, and no response was observed in these cancers. Overall, none of the 31 patients with HER2-negative cancers enrolled in the expansion phase study responded. In the earlier dose-escalation, 18.2% (4/22) of patients whose tumours were HER2-negative and EGFR-positive responded, including 3 PRs in upper GI cancer and 1 PR in renal cancer, but the response was lower than the 30.0% (3/10) observed in patients with HER2-positive

cancers. Combining the data from the dose-escalation and expansion phases suggests that HER2-positive status is a better predictive marker for the activity of epertinib than EGFR over-expression, although EGFR inhibition may also contribute in part to the efficacy of epertinib. Additional pan-erbB inhibitors have been studied in breast cancer patients selected for HER2-positivity [23, 24].

In conclusion, continuous once-daily dosing with oral epertinib is well tolerated, and shows encouraging signs of antitumour activity particularly in patients with HER2-positive breast cancer with or without brain metastasis, even after previous treatment with HER2-targeted therapies, and also in patients with HER2-positive upper GI cancer. Based on these findings, a phase 1/2 study of epertinib in combination with trastuzumab, or with trastuzumab plus chemotherapy, was initiated in patients with HER2-positive breast cancer, including those with brain metastases.

Funding:

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Conflict of interest statement

J. Posner and K. Donaldson undertake consultancy for and J. Spicer has received honoraria from Shionogi & Co., Ltd. Japan, R. Baird and J. Garcia-Corbacho undertake consultancy for and have received travel expense reimbursement from Shionogi Limited UK. R. H. Wilson has received honoraria plus travel expenses for advisory boards and educational meetings from Merck Serono, Servier, Sirtex, Amgen and BMS. D. Tosi has received industrial research grants for his preclinical lab activity from Novartis, Astellas and Janssen. I. Kawabata and A. Arimura are employees of Shionogi Co., Ltd. All remaining authors have declared no conflicts of interest.

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Figure legend

Figure 1

Antitumour activity. Response in target lesions (A and C), and duration of response and treatment exposure (B and D), in the patients with breast (A and B, n = 21) and upper GI cancers (C and D, n = 20), according to HER2 and EGFR status. Partial response in a 43-year-old patient with brain metastases from HER2-positive breast cancer who received epertinib monotherapy at 800 mg (E). This patient, who had previously been treated with HER2-based therapies including trastuzumab, lapatinib and capecitabine, had a target lesion in the brain for evaluation of tumour response according to RECIST version 1.1. The response was observed at 16 weeks and continued until 40 weeks.

BM: Patients with brain metastases. ^a Not evaluated by central review but HER2-positive by local assessment.

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2. Methods

2.1. Patients

Eligible patients were ≥ 18 years old with histologically and/or cytologically confirmed EGFR and/or HER2-positive adenocarcinoma of the breast, with or without cerebral metastases; adenocarcinoma of the stomach, gastro-esophageal junction or esophagus; renal cell carcinoma; or squamous cell carcinoma of the head and neck, that had progressed following treatment with approved therapies, or for which no standard therapy was available. The EGFR and HER2 expression were assessed at central or local laboratories using fresh tumour biopsy obtained at screening or archival tissue for confirmation of the eligibility, while the EGFR/HER2 status assessed by the central review was used for evaluation of efficacy. Eligible patients were required to have Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1, and adequate hematological, renal, hepatic and left ventricular function ($\geq 50\%$). Patients with brain metastasis from HER2-positive breast cancer were allowed to be enrolled in this study based on the evidence showing that superior efficacy in suppressing tumour growth of brain metastases compared to lapatinib in an intracranial implantation mouse model [14]. The study was approved after review by the relevant regulatory and independent ethics committees (EudraCT Number: 2009-017817-31), and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice. All patients provided written informed consent before enrollment.

2.2. Study design

Epertinib was initially administered as a single dose on Day 1, followed by continuous daily dosing from Day 8 to Day 28. Patients received continuous dosing with epertinib in 28-day

cycles until disease progression, intolerable toxicity or withdrawal of consent. The primary objective was to assess further the safety and tolerability of epertinib. Secondary objectives included pharmacokinetics (PK) and an extended assessment of efficacy.

2.3. Safety assessment

Adverse events (AEs) were monitored and clinical laboratory parameters, weight, vital signs, 12 lead ECGs, physical and ophthalmic examinations, cardiac function including left ventricular ejection fraction, and ECOG performance status were assessed at baseline and throughout the study, and lead II continuous monitoring was carried out for 8 hours post-dosing on Day 1. All AEs were coded using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

2.4. Pharmacokinetic analysis

Blood samples for the measurement of plasma concentrations of epertinib and its active de-alkylated and lactam metabolites were taken at the following times during treatment: Day 1 (pre-dose, 2, 4, 6, and 12 hours post-dose), Day 2 (24 hours post-dose), Day 3 (48 hours post-dose), Day 5 (96 hours post-dose), Days 8, 15, 22 and 29 (pre-dose). Samples were also taken before dosing on Days 15 and 29 of the first two cycles of Part B. Plasma concentration measurement and calculation of the PK parameters of epertinib and its active metabolites were performed in the same manner as described in our previous report [15].

2.5. Tumour evaluation

Tumour response was assessed every 8 weeks according to Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.1 [16]. Patients who had at least one additional scan after baseline, and patients who had no additional scan but discontinued due to clinical disease progression, were considered evaluable for response.

3. Results

3.1. Safety and tolerability

Between July 2010 and January 2014, a total of 76 patients with breast (n = 27), upper GI (n = 30), head and neck (n = 12) and renal cell carcinoma (n = 7) were enrolled at 9 sites in the United Kingdom and France. Patient demographics and baseline characteristics are summarised in Table 1. As shown in patient disposition (Figure S1), 14 patients (7 patients in Part A and 7 patients in Part B) discontinued due to treatment-emergent adverse events (TEAEs). Only 2 patients discontinued due to TEAEs (nausea and vomiting, and bilirubin elevation) that were considered drug-related. Disease progression accounted for all other discontinuations except for one patient who was withdrawn due to deteriorating quality of life.

Table 2 summarises all TEAEs of any grade occurring in at least 10% of patients, and all Grade 3 or higher TEAEs. There were no treatment-related deaths. Eperitinib at 800 mg once daily was well tolerated by most patients, with 21.1% (16/76) of patients requiring dose reduction. The majority of AEs were Grade 1 or 2 in intensity, and no significant hematological or biochemical toxicity was observed. Overall, the most frequently reported AEs were diarrhoea, nausea, rash and anorexia. Diarrhoea was generally self-limiting or effectively controlled with loperamide; in 10 (13.2%) patients it was Grade 3. Nausea was almost always Grade 1 or 2 and was effectively managed with antiemetics and dose interruptions. Rash was reported in 32 (42.1%) patients, of which none was more severe than Grade 2. There were no effects on ECG, and no evidence that eperitinib treatment affected cardiac function.

Bilirubin elevation was observed in 23 patients (30.3%); this elevation was Grade 3 in 5 patients (6.6%), and Grade ≤ 2 in all others. The increase in bilirubin was not associated with any symptoms, elevation of liver enzymes or hematologic abnormalities. When the

unconjugated and conjugated bilirubin was measured in 14 of the 23 patients, the elevation was predominantly attributable to unconjugated bilirubin. Dose reduction was required due to bilirubin elevation in 1 patient (1.3%), and resulted in study discontinuation in one (1.3%) other patient.

3.2. Pharmacokinetic analysis.

The PK parameters after single administration and trough plasma concentrations after repeated doses at 800 mg are summarised in Table S1. Epertinib was absorbed with a median T_{max} of 4.1 hours and geometric mean C_{max} of 454 ng/mL following a single oral dose. The geometric mean $t_{1/2,z}$ was 33.8 hours and steady-state was achieved within 7 days of once-daily dosing. The pharmacologically active dealkylated and lactam metabolites of epertinib were rapidly formed, and the exposures (AUC_{0-inf} and C_{max}) to these metabolites were 5-7% and 6-8% on a molecular basis of the parent epertinib, respectively.

3.3. Clinical response.

Sixty five of 76 patients were evaluable for tumour response. The tumour responses for each cancer are shown in Table S2. Two patients with gastro-esophageal junction tumours had CR and PR, and 4 patients with breast cancer experienced PR. The objective response rate (ORR) for breast, upper GI, head and neck and renal cell cancers were 16.0% (4/25), 8.3% (2/24), 0% (0/9) and 0% (0/7), respectively. As shown in Table 3, all of the 4 responding breast cancer patients were HER2-positive, but 2 of them were EGFR-negative. All 4 responding patients had previously received trastuzumab, and 3 of the 4 patients were previously treated with lapatinib. The 2 responding upper GI cancer patients were also HER2-positive, and one

was EGFR-negative. These two patients had been treated with trastuzumab-based therapy and multiple lines of chemotherapy. The ORRs in patients with HER2-positive breast and upper GI cancers were 19% (4/21) and 20% (2/10), respectively. Figures 1A and 1B demonstrate the changes in the sum of longest diameter of target lesions and treatment period in patients with breast cancer having at least one evaluable scan (n = 21), and Figures 1C and 1D in patients with upper GI cancers (n = 20). Two of the 4 responding patients with breast cancer continued on study beyond 40 weeks and the other 2 patients continued beyond 16 weeks. The patient with gastro-esophageal junction disease experiencing CR completed a total treatment period of 52 weeks and another with PR continued treatment until 39 weeks.

Five patients with brain metastases from breast cancer were evaluable for radiological assessment, and 4 of the 5 patients had brain and/or extra-cranial lesions for assessment by RECIST v1.1 (Figure 1B). One of the 4 patients whose only target lesion was a brain metastasis achieved PR (Figure 1E) with a sustained response of 7.5 months, and 2 patients experienced prolonged SD (> 6 months).

4. Discussion

This was an extended phase I study aiming to assess the safety, tolerability and PK of epertinib, as well as to provide a preliminary indication of antitumour activity. As suggested by the earlier dose-escalation study of this drug [15], once-daily oral dosing of epertinib at 800 mg was well-tolerated with predominantly low-grade toxicities including diarrhoea, nausea, rash and anorexia; no Grade 3 rash, but Grade 3 diarrhoea observed in 10 patients (13.2%) and Grade 2 in 14 out of 76 patients (18.4%). Diarrhoea probably caused by EGFR inhibition [17, 18] could be managed by anti-diarrhoeal medication; prophylactic use was not allowed in this study. No patient required discontinuation of epertinib treatment due to diarrhoea. There was no evidence of a drug-related decline in ejection fraction or effects on ECG QT interval, despite the known expression of HER2 on cardiac myocytes [19]. As reported in the dose-escalation study [15], elevation of blood bilirubin of any grade was observed in 30% of patients in the expansion phase but this was not associated with evidence of other hepatic or hematologic abnormalities. Epertinib is known to inhibit the conjugating enzyme UGT1A1 *in vitro* with an IC₅₀ value of 3.7 µmol/L, but not the multidrug resistance protein 2 (MRP2) involved in conjugated bilirubin transport. The elevated bilirubin was predominantly associated with unconjugated bilirubin in patients measured. Therefore, it is considered that the frequent elevation of total blood bilirubin in epertinib-treated patients arises from inhibition of UGT1A1-mediated bilirubin conjugation.

PK parameters of epertinib at 800 mg once-daily were consistent with those reported previously [15]. The trough concentrations of epertinib at steady-state markedly exceeded those that were seen to inhibit EGFR and HER2 *in vitro* [14], and the t_{1/2} was consistent with the currently recommended daily dosing schedule. The active dealkylated and lactam metabolites which were identified in human but not in mouse have similar activity to that of the parent compound against HER2 and EGFR kinase; the IC₅₀ values of the dealkylated and

lactam metabolites are known to be 1.24 nmol/L and 2.32 nmol/L for EGFR and 6.31 nmol/L and 18.89 nmol/L for HER2, respectively, which are almost equivalent to or lower than those for epertinib. However, the plasma concentration of these metabolites accounted for only 5-7% and 6-8% of the parent epertinib on a molecular basis, respectively, suggesting that the contribution of these metabolites to efficacy is modest.

In this study, PRs were reported in five patients with breast or upper GI cancers who had been previously treated with HER2-targeting drugs, and one CR in another gastroesophageal junction cancer patient previously treated with trastuzumab combination. The response rate in patients with HER2-positive breast cancer was 19.0%, which compares favorably with 6.9% for lapatinib monotherapy and 10.3% for lapatinib plus trastuzumab for HER2-positive breast cancer [20]. Interestingly, we observed one PR and 2 long SDs among 5 patients with intracranial disease, which is of particular relevance for this challenging clinical scenario. Of the 24 upper GI cancer patients evaluable for response, 10 patients were HER2-positive and the other 14 patients were HER2-negative. Two of the 10 patients (20.0%) with HER2-positive upper GI cancer responded. For HER2-positive upper GI cancer, trastuzumab is combined with chemotherapy as a standard second-line therapy, with response rates reported to be 37.0% (17/46) in trastuzumab-naïve patients [21] and 9.3% (3/32) in trastuzumab-refractory patients [22]. These preliminary data on the activity of epertinib in HER2-positive upper GI cancer are promising and warrant further evaluation.

All the patients with head and neck and renal cancers included in this trial were EGFR-positive but HER2-negative, and no response was observed in these cancers. Overall, none of the 31 patients with HER2-negative cancers enrolled in the expansion phase study responded. In the earlier dose-escalation, 18.2% (4/22) of patients whose tumours were HER2-negative and EGFR-positive responded, including 3 PRs in upper GI cancer and 1 PR in renal cancer, but the response was lower than the 30.0% (3/10) observed in patients with HER2-positive

cancers. Combining the data from the dose-escalation and expansion phases suggests that HER2-positive status is a better predictive marker for the activity of epertinib than EGFR over-expression, although EGFR inhibition may also contribute in part to the efficacy of epertinib. Additional pan-erbB inhibitors have been studied in breast cancer patients selected for HER2-positivity [23, 24].

In conclusion, continuous once-daily dosing with oral epertinib is well tolerated, and shows encouraging signs of antitumour activity particularly in patients with HER2-positive breast cancer with or without brain metastasis, even after previous treatment with HER2-targeted therapies, and also in patients with HER2-positive upper GI cancer. Based on these findings, a phase 1/2 study of epertinib in combination with trastuzumab, or with trastuzumab plus chemotherapy, was initiated in patients with HER2-positive breast cancer, including those with brain metastases.

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Conflict of interest statement

J. Posner and K. Donaldson undertake consultancy for and J. Spicer has received honoraria from Shionogi & Co., Ltd. Japan, R. Baird and J. Garcia-Corbacho undertake consultancy for and have received travel expense reimbursement from Shionogi Limited UK. R. H. Wilson has received honoraria plus travel expenses for advisory boards and educational meetings from Merck Serono, Servier, Sirtex, Amgen and BMS. D. Tosi has received industrial research grants for his preclinical lab activity from Novartis, Astellas and Janssen. I. Kawabata and A. Arimura are employees of Shionogi Co., Ltd. All remaining authors have declared no conflicts of interest.

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Figure legend

Figure 1

Antitumour activity. Response in target lesions (A and C), and duration of response and treatment exposure (B and D), in the patients with breast (A and B, n = 21) and upper GI cancers (C and D, n = 20), according to HER2 and EGFR status. Partial response in a 43-year-old patient with brain metastases from HER2-positive breast cancer who received epertinib monotherapy at 800 mg (E). This patient, who had previously been treated with HER2-based therapies including trastuzumab, lapatinib and capecitabine, had a target lesion in the brain for evaluation of tumour response according to RECIST version 1.1. The response was observed at 16 weeks and continued until 40 weeks.

BM: Patients with brain metastases. ^a Not evaluated by central review but HER2-positive by local assessment.

Table 1. Patient demographics and baseline characteristics (*N* = 76)

Characteristics	
Age (years)	
Mean	59.8
Range	31-81
Sex	
Female	33 (43.4%)
Male	43 (56.6%)
Primary cancer	
Upper gastrointestinal adenocarcinoma	30 (39.5%)
Breast adenocarcinoma	27 (35.5%)
Head & neck squamous cell carcinoma	12 (15.8%)
Renal cell carcinoma	7 (9.2%)
ECOG PS at screening	
0	24 (31.6%)
1	51 (67.1%)
2	1 (1.3%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Table 3. Tumour response by HER2 and EGFR status in patients with breast, upper GI, head and neck and renal cancers

HER2	EGFR	Number of patients (%) with tumour response				
		Breast N = 25	Upper GI ^a N = 24	H&N N = 9	Renal N = 7	Total N = 65
Positive	Positive	2/9 (22.2)	1/8 (12.5)	0/1 (0)	0/0 (0)	3/18 (16.7)
Positive	Negative	2/12 (16.6)	1/2 (50.0)	0/0 (0)	0/0 (0)	3/14 (21.4)
Negative	Positive	0/1 (0)	0/13 (0)	0/8 (0)	0/7 (0)	0/29 (0)
Negative ^b	Negative	0/1 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/2 (0)
Not evaluable ^c		0/2 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/2 (0)

^a Upper GI includes adenocarcinoma of stomach, gastro-esophageal junction and esophagus.

^b Results shown are those obtained at central review. 2 patients were enrolled based on HER2-positivity assessed by the local laboratory at the treating site, but in each case both HER2 and EGFR were negative on central review.

^c 2 patients' tumours which had HER2-positive status from the local assessment were not evaluable at the central laboratory.

Supplementary Table S1. Geometric mean (Geometric CV %) pharmacokinetic parameters of epertinib and its pharmacologically active dealkylated and lactam metabolites after single oral administration of 800 mg on Day 1 and mean trough plasma concentrations on Days 15, 22 and 29 after repeated administration of 800 mg started on Day 8

		Pharmacokinetic parameters after single dose administration (Day 1)					Trough plasma concentrations after repeated dose administration			
	<i>n</i>	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-inf} (ng·h/mL)	CL/F (L/h)	V_z/F (L)	t_{1/2,z} (h)	Conc. (ng/mL) Day 15	Conc. (ng/mL) Day 22	Conc. (ng/mL) Day 29
Epertinib		454 (53.3)	4.06 (1.98-12.13)	11100 (45.0) ^a	72.2 (44.9) ^a	3510 (47.6) ^a	33.8 (17.4) ^c	331 (124) ^d	320 (125) ^f	330 (137) ^g
Dealkylated	76	23.3 (50.8)	6.00 (2.12-24.17)	685 (47.1) ^a	-	-	48.6 (21.8) ^a	35.4 (16.8) ^d	36.2 (16.5) ^f	37.3 (15.1) ^g
Lactam		35.8 (51.5)	5.98 (2.00-24.17)	770 (41.6) ^b	-	-	30.6 (24.0) ^b	16.7 (8.42) ^e	15.8 (6.62) ^f	15.3 (7.52) ^g

Geometric Mean (Geometric Coefficient of Variation [CV] %) values except T_{max} for which median and range are shown for pharmacokinetic parameters. Mean (SD) values are shown for trough plasma concentrations.

Abbreviations: C_{max}, maximum plasma concentration; T_{max}, time to C_{max}; AUC_{0-inf}, area under plasma concentration versus time curve from time zero extrapolated to infinity; CL/F, apparent total body clearance; V_z/F, apparent volume of distribution and t_{1/2,z}, terminal half-life; Conc. concentration.

^a N = 73. ^b N = 72. ^c N = 74. ^d N = 58. ^e N = 57. ^f N = 56. ^g N = 51.

Supplementary Table S2. Tumour response in patients with breast, upper GI, head and neck and renal cancers

	Breast	Upper GI^a	H&N	Renal	Total
Overall response rate	4/25	2/24	0/9	0/7	6/65
<i>N</i> (%)	(16.0)	(8.3)	(0)	(0)	(9.2)
CR	0	1	0	0	1
PR	4	1	0	0	5
SD	12	11	6	3	32
PD	9	11	3	4	27

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; GI, gastrointestinal; H&N, head and neck

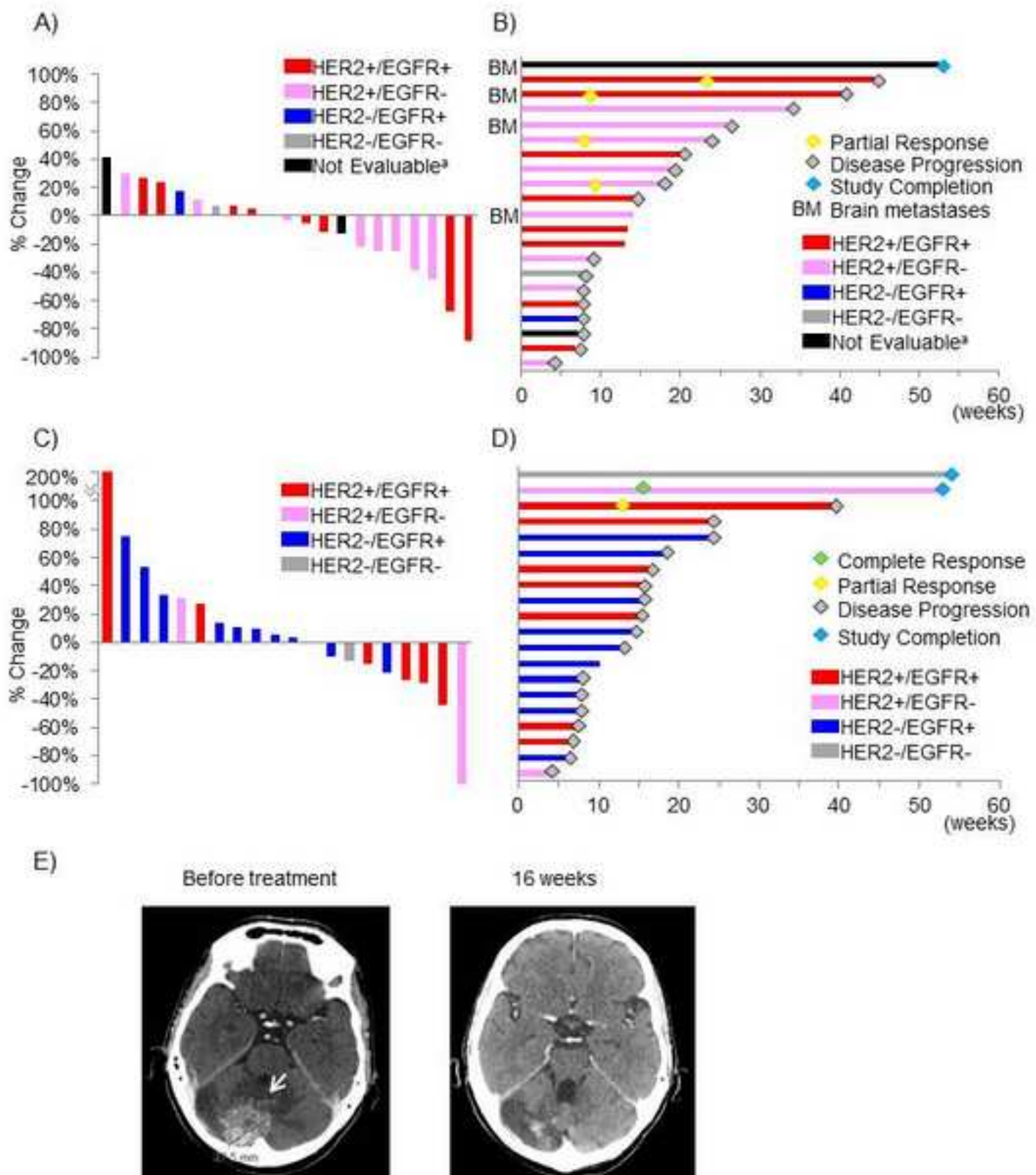
^aUpper GI includes adenocarcinoma of stomach, gastro-esophageal junction and esophagus

Table 2. Treatment-emergent adverse events occurring in $\geq 10\%$ of patients

	Epertinib 800 mg (N = 76)	
	<i>N (%)</i>	
	All grades	Grade $\geq 3^a$
Diarrhoea	59 (77.6)	10 (13.2)
Nausea	37 (48.7)	3 (3.9)
Rash	32 (42.1)	-
Decreased appetite	31 (40.8)	4 (5.3)
Fatigue	30 (39.5)	7 (9.2)
Vomiting	29 (38.2)	3 (3.9)
Bilirubin elevation	23 (30.3)	5 (6.6)
Constipation	20 (26.3)	-
Headache	19 (25.0)	-
Anaemia	15 (19.7)	6 (7.8)
Abdominal pain	13 (17.1)	-
Dry skin	13 (17.1)	-
Cough	13 (17.1)	-
Asthenia	11 (14.5)	3 (3.9)
Nasopharyngitis	10 (13.2)	-
Pruritus	10 (13.2)	-
Pyrexia	10 (13.2)	-
Weight loss	9 (11.8)	-
Oedema peripheral	9 (11.8)	-
Dyspnoea	9 (11.8)	4 (5.3)
Abdominal pain upper	8 (10.5)	-

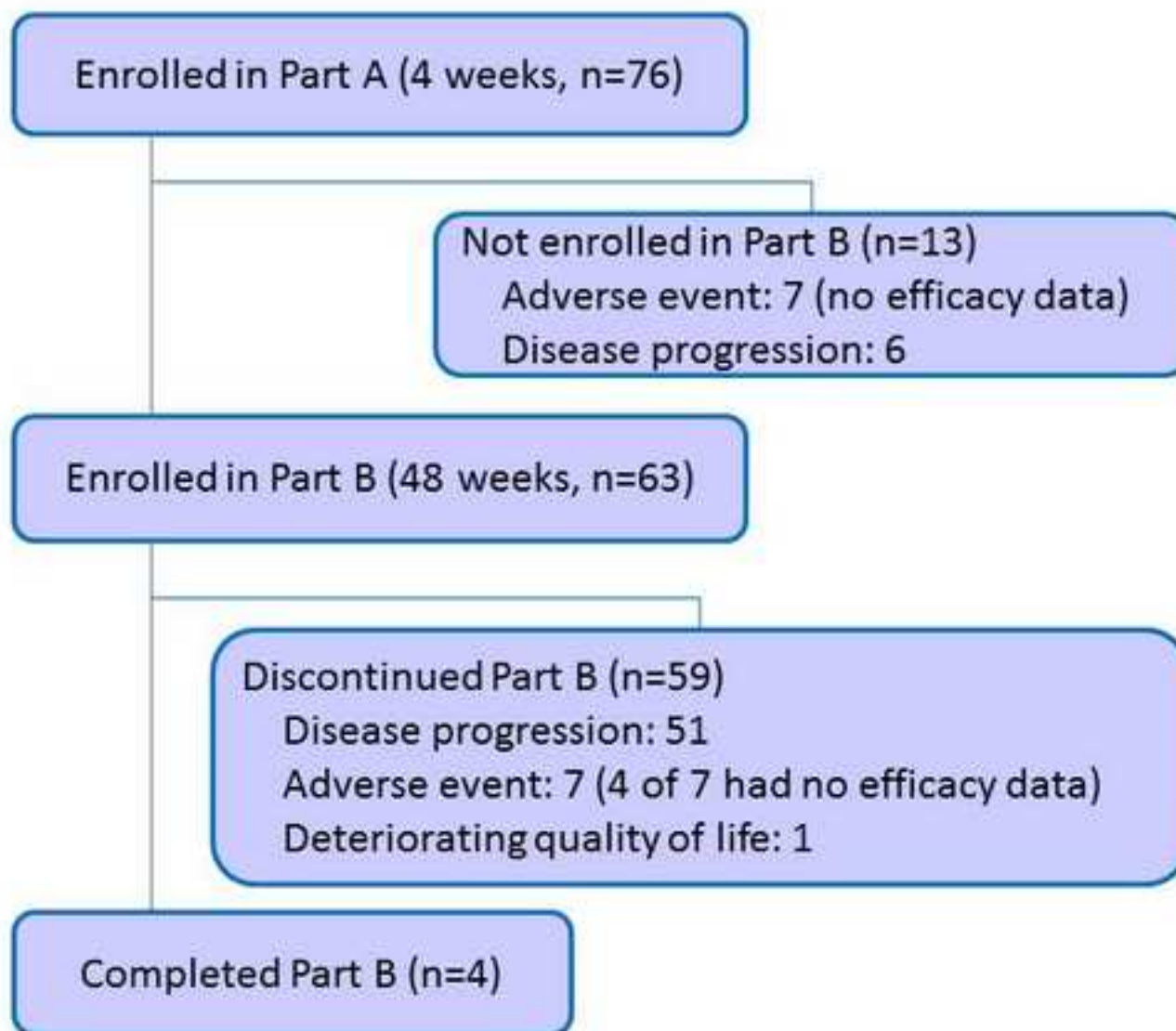
^aThere were no Grade 4 or 5 treatment-related adverse events.

Figure 1
[Click here to download high resolution image](#)



Supplemental Figure S1.

Patient disposition



Conflict of interest statement

J. Posner and K. Donaldson undertake consultancy for and J. Spicer has received honoraria from Shionogi & Co., Ltd. Japan, R. Baird and J. Garcia-Corbacho undertake consultancy for and have received travel expense reimbursement from Shionogi Limited UK. R. H. Wilson has received honoraria plus travel expenses for advisory boards and educational meetings from Merck Serono, Servier, Sirtex, Amgen and BMS. D. Tosi has received industrial research grants for his preclinical lab activity from Novartis, Astellas and Janssen. I. Kawabata and A. Arimura are employees of Shionogi Co., Ltd. All remaining authors have declared no conflicts of interest.