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A Phase I, Dose-Escalation Study of the Multi-Targeted Receptor Tyrosine Kinase Inhibitor, Golvatinib, in Patients with Advanced Solid Tumors

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Statement of Translational Relevance

Golvatinib is a highly potent, small-molecule ATP-competitive inhibitor of c-Met and multiple members of the Eph receptor family, as well as c-Kit and Ron, based on isolated kinase assays. Golvatinib showed significant antitumor effects in mouse xenograft models of cancer cell lines with MET gene amplification. This first-in-human, phase I, dose-escalation clinical trial of oral golvatinib established the recommended phase II dose for a once-daily continuous dosing schedule at 400 mg and showed evidence of biological activity. These observations have provided a rationale to support the continued evaluation of golvatinib in phase II combination studies in different tumor types: gastric cancer, squamous cell carcinoma of the head and neck, and hepatocellular carcinoma where c-Met signalling plays a role in pathogenesis.
Golvatinib in Patients with Advanced Solid Tumors

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Abstract

Purpose: Receptor tyrosine kinases c-Met and Ron transduce signals regulating cell migration and matrix invasion. This phase I dose-escalation trial tested golvatinib, a highly potent, small-molecule, ATP-competitive inhibitor of c-Met and multiple members of the Eph receptor family plus c-Kit and Ron.

Experimental Design: Patients with advanced solid tumors received golvatinib orally, once daily, continuously. Using a “3+3” design, dosing started at 100 mg once daily, escalating to the maximum tolerated dose (MTD) defined by dose-limiting toxicities. Pharmacokinetic, pharmacodynamic, and preliminary antitumor activity was assessed during dose escalation and in a MTD expansion cohort.

Results: Thirty-four patients were treated at 6 dose levels. The MTD was determined as 400 mg once daily. Three dose-limiting toxicities were observed: grade 3 increased gamma-glutamyltransferase and alkaline phosphatase (200 mg), repeated grade 2 fatigue, and grade 3 fatigue (450 mg). Frequent treatment-related adverse events (with incidence >10%) included diarrhea (58.8%), nausea (50%), vomiting (44.1%), fatigue (41.2%), decreased appetite (32.4%), elevated alanine aminotransferase (32.4%), elevated aspartate aminotransferase (20.6%), dry skin (11.8%), and dysgeusia (11.8%). Best overall response was stable disease (median duration 85 days, range 85–237). Pharmacokinetics demonstrated high variability, although maximum plasma concentration and area under the plasma concentration–time curve increased with dose. Soluble urokinase-type
plasminogen activator receptor, VEGFR2, c-Met, and angiopoietin-2 levels increased post-dose. Post-treatment decrease in either p-c-Met or p-ERK was observed in 3 of 4 paired biopsies at MTD.

Conclusions: Golvatinib at the MTD of 400 mg once daily was well tolerated with pharmacodynamic evidence of c-Met target modulation.
Introduction

The protein product of the MET proto-oncopogene, c-Met, a receptor tyrosine kinase (RTK), is a prototype for the c-Met RTK subfamily (1). A second member of the family is Ron (2). Both RTKs share similar structural and biochemical properties, existing as heterodimers of an α and ß chain. c-Met is activated by the ligand hepatocyte growth factor (HGF; scatter factor) while Ron is activated by macrophage-stimulating protein (HGF-like protein; [2, 3]). The c-Met signaling pathway engages with other pathways, including that of the epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor/MAPK/ERK pathway; this pathway is critical in driving the pathogenesis of several cancers (4, 5). In non-small-cell lung cancer (NSCLC), c-Met signaling is also thought to contribute to the development of resistance to EGFR inhibitors (6, 7). c-Met signaling can also promote angiogenesis through interaction with the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) pathway (8, 9). In particular, c-Met signaling can downregulate the antiangiogenic thrombospondin-1, and upregulate VEGF (10), and there is evidence that upregulation of HGF and c-Met occurs after VEGF inhibition and represents a potential mechanism of resistance to antiangiogenic therapy (11).

Ligand-dependent or -independent activation (MET amplification and mutation) of the receptors leads to increased cell proliferation, migration, matrix invasion, and invasive growth (1, 2). The most frequent cause of constitutive activation of c-Met is protein overexpression and this has been demonstrated in NSCLC, renal cell cancer (RCC), mesothelioma, breast
cancer, ovarian cancer, colorectal cancer (CRC), and squamous cell carcinoma of the head and neck (SCCHN; [12, 13]). MET amplification has been demonstrated in upper gastrointestinal cancers, CRC, NSCLC, medulloblastomas, and glioblastomas (14–17). Activating mutations are less frequently reported than amplification, and have been described in papillary RCC (18). More frequent sporadic mutations have been demonstrated in RCC, SCCHN, gastric cancer, small-cell lung cancer, NSCLC, and mesothelioma (19–24).

The Eph receptors are the largest family of RTKs existing as transmembrane receptors stimulated by plasma membrane bound ligands, the ephrins (25). They are divided into 2 sub-classes: sub-class A receptors which are membrane bound and preferentially bind all A-type ephrins and sub-class B receptors that preferentially bind all B-type ligands (26). Signaling by Ephs has been shown to lead to vascular development, vessel stability mediated through pericyte stabilization, and angiogenesis, altered cell mobility, migration, and adhesion; while lowly expressed in normal adult tissues, they are expressed and re-expressed at high levels during organogenesis and tumorigenesis (27, 28, 29). Overexpression of Eph receptors has been demonstrated in several tumor types, including NSCLC, ovarian, breast, endometrial, and gastric cancers, as well as glioblastomas, where it correlates with a poor survival (30–34); as such, Eph receptors represent potential novel therapeutic targets.

Golvatinib is a highly potent, small-molecule, ATP-competitive inhibitor of c-Met and multiple members of the Eph receptor family, as well as c-Kit and

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Ron (35), with an IC$_{50}$ for c-Met of 0.001 µM, for Eph members (A6, B2, A8, A7, B4, B1, A5) between 0.007 and 0.018 µM, for Kit of 0.010 µM, and for Ron of 0.017 µM (Eisai, data on file). It has demonstrated significant preclinical antitumor effects, including tumor regressions in MET-amplified mouse xenograft cancer cell lines (36). Additional in vitro pre-clinical data (measuring the network length of HUVECs on a monolayer of human brain vascular pericytes) has shown that golvatinib disrupts pericyte function and thus vascular integrity, via the inhibition of EphB4 (and Tie-2) (37). Preclinical toxicology (Eisai data on file) indicated primarily gastrointestinal toxicity. Here we report on a phase I first-in-human study of golvatinib in patients with advanced solid tumors. The primary objective of this study was to determine the maximum tolerated dose (MTD) based on the dose-limiting toxicities (DLTs) of golvatinib. Secondary objectives were to assess safety and tolerability, determine the pharmacokinetic (PK) profile, explore the pharmacodynamic (PD) effects, and assess antitumor activity.

Materials and Methods

This was an open-label, phase I, dose-escalation study with an expansion cohort at the MTD (ClinicalTrials.gov trial registration ID: NCT00869895). The study was conducted in 2 UK centers: The Royal Marsden NHS Foundation Trust, Sutton and The Christie NHS Foundation Trust, Manchester. The study was approved by the relevant regulatory and independent ethics committees and conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice.
Guidelines. All patients provided written informed consent before any study procedures were performed.

Patient selection

Eligibility criteria

Patients aged ≥18 years with histologically or cytologically confirmed advanced or metastatic solid tumors unresponsive to standard treatment, or for which no standard treatment was available, were eligible provided they met the following criteria: adequate bone marrow function (hemoglobin >9.0 g/dL; absolute neutrophil count >1.5 x 10^9/L; platelet count >100 x 10^9/L), renal function (serum creatinine <1.5 mg/dL [133 μM/L] or calculated creatinine clearance >50 mL/minute per the Cockcroft and Gault formula), and liver function (bilirubin <1.5 × upper limit of normal [ULN]; alkaline phosphatase [ALP], alanine transaminase [ALT], and aspartate transaminase [AST] <3 × ULN or <5 × ULN in the presence of liver metastases); Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; and life expectancy of >3 months. At the MTD expansion, up to 6 patients recruited were required to have a lesion amenable for paired (pre- and post-treatment) tumor biopsies. Patients were excluded in the case of any condition that precluded oral intake or oral absorption; untreated or unstable known primary or metastatic central nervous system tumors; uncontrolled hypertension, clinically significant cardiac impairment, or unstable ischemic heart disease within the previous 6 months; pregnancy or lactation or any other significant comorbidity; or if therapeutic anticoagulation was required.
Study design

This study utilized a “3+3” dose-escalation design, followed by recruitment to a MTD expansion cohort. Golvatinib was administered orally, once daily (QD), continuously, in 28-day cycles, starting at 100 mg. Dosing was on an empty stomach, with a 2-h fast post dose. Dose escalation in subsequent cohorts was in 100% increments until the emergence of grade ≥2 drug-related toxicities; from this point, dose increments were by 50% in the event of grade 2 toxicity, or 25–33% in the event of grade 3 toxicity. The MTD was defined as the highest dose at which no more than 1 out of 6 patients experienced a DLT during cycle 1. DLT was defined during cycle 1 as any clinically significant treatment-related adverse event (AE) that met any of the following criteria: any grade 3 or higher hematologic or nonhematologic toxicity, any repeated grade 2 hematologic or nonhematologic toxicity requiring dose reduction, or failure to administer >75% of the planned dosage (i.e., failure to take at least 21 days of treatment over the first 28 days). Patients who failed to take at least 75% of the daily dose during cycle 1 for reasons other than toxicity were not evaluable for DLT and were replaced in the cohort. Once the MTD was determined, up to 6 additional patients with at least 1 tumor lesion suitable for paired biopsies were enrolled for further evaluation of safety, PK, and PD profiles, and preliminary antitumor activity. Golvatinib was administered until disease progression, unacceptable toxicity, or withdrawal of consent.

Safety and efficacy assessments

Safety assessments included medical review, physical examination, vital signs, clinical laboratory tests (complete blood count, clinical chemistry, and urinalysis), and electrocardiogram. All assessments were conducted at
baseline, weekly during cycle 1, and biweekly thereafter. All AEs were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Radiological assessments were performed at baseline and at the end of every 2 cycles (every 8 weeks) according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 (38).

**Study drug**

Golvatinib was supplied as 10-mg, 50-mg, and 100-mg pale yellowish-red, film-coated, round, bi-convex tablets for oral administration, manufactured at the Eisai Formulation Research Laboratories, Japan.

**Pharmacokinetics**

Blood samples were collected during cycles 1 and 2, day 1 (predose, 0.5, 1, 2, 4, 6, and 8 hours post-dose). Further predose samples were collected in cycle 1 (days 2, 8, 15, and 22), cycle 2 (days 2 and 15), and days 1 and 15 of subsequent cycles. Plasma samples were stored at -20°C. Plasma concentrations of golvatinib and its metabolite M2 were determined using a validated liquid chromatography/tandem mass spectrometry method (6). Noncompartmental analysis, using Phoenix® WinNonlin® 6.2.1 (Pharsight Corporation, CA), was used to calculate area under the curve from 0 to time t (AUC_{(0-t)}); area under the curve from 0 to time of last quantifiable concentration (AUC_{(0-t)}); maximum plasma concentration (C_{max}); minimum plasma concentration (C_{min}); fraction of the dose excreted in urine (Fe [%]); and time to C_{max} (T_{max}).
Twenty-four hour urine collection for PK analysis was conducted during cycles 1 and 2, on days 1 and 2. Aliquots from each collection time point were stored at -20 °C until analysis.

**Pharmacodynamics**

**Plasma circulating biomarkers** Blood samples for PD analysis were collected predose in cycle 1 (day 1, 2, 8, 15, and 22) and cycle 2 (day 1 and 15), and on day 1 of subsequent cycles. Plasma samples were analyzed using commercially available ELISAs or multiplex assays for concentrations of soluble protein markers, including soluble c-Met, urokinase-type plasminogen activator and its receptor (uPAR), HGF, VEGF, interleukin 8, angiopoietin-2 (Ang-2), follistatin, granulocyte colony-stimulating factor, leptin, platelet-derived growth factor BB, platelet/endothelial cell adhesion molecule 1 (PECAM-1), and soluble KDR. Serum samples were analyzed to measure markers of tumor cell death activated caspases 3/7, cytochrome c, and cytokeratin 18 derived antigens recognized by the M30 and M65 monoclonal antibodies.

**Tumor tissue biomarkers** Tumor biopsies for PD analysis (MET gene copy number and protein expression) were taken predose on cycle 1 day 1 and post-dose between cycle 1 day 22 and cycle 2 day 22. Biopsies were optional during dose escalation, and mandatory at the MTD expansion, where up to 6 patients with at least 1 tumor lesion suitable for biopsy were required. For analysis of MET gene copy number, 5 µm formalin-fixed, paraffin-embedded (FFPE) tissue sections were subjected to dual-color FISH assays using the
MET/CEP7 probe set from Abbott Vysis Molecular (Abbott Park, IL) labeled with spectrum red and spectrum green, respectively. FISH assays were performed according to a modified manufacturer’s protocol; signals were visualized using a Leica DMI4000 epifluorescence microscope equipped with band-pass filters for spectrum green, spectrum red, and 4,6-diamidino-2-phenylindole (DAPI). For analysis of c-Met protein expression, ERK and c-Met phosphorylation, 5 µm FFPE tissue sections were analyzed by immunohistochemistry (IHC) using antibodies against total c-Met, p-c-Met, or p-ERK1/2. Slides were digitized using the Aperio ScanScope™ and viewed using the Aperio ImageScope™ software (Aperio Technologies, Vista, CA). IHC staining was quantified by visual scoring and by using Aperio image analysis algorithms.

**Statistical methods**

Descriptive statistics was applied to summarise extent of exposure, AEs including serious AEs (SAE) and DLTs. Plasma and urine PK concentrations of E7050 and M2 were summarized descriptively by dose level/schedule and treatment cycle. Individual and mean plasma concentration vs. time profile plots were produced on linear and semi-log scales. PK parameters were obtained from plasma and urine concentration data after a single dose on Day 1 of Cycle 1 for E7050 and M2 and after repeat dosing on Day 1 of Cycle 2 and summarized descriptively by day and cycle. Dose linearity of E7050 following a single dose of E7050 and following repeat dosing of E7050 was assessed using graphical and tabular methods only. Dose-normalized PK parameters versus administered dose were plotted.
Levels of soluble protein markers were summarized descriptively by cancer type, dose level/schedule, treatment cycle, and overall. Individual and mean plasma concentration of the above markers vs. time profile plots were produced for changes from baseline by dose. Changes in the median levels of the plasma biomarkers at each time point were analyzed using a Mann-Whitney test ($P < 0.05$ considered statistically significant).

In tumor samples, c-Met phosphorylation, c-Met expression, and ERK phosphorylation were assessed using IHC and summarized descriptively by dose level/schedule, treatment cycle, and overall, using the product of the percentage of cells staining positive and the corresponding intensity score. This information was also summarized descriptively by cellular location (membrane, nuclear, and cytoplasm), if available, by dose level/schedule, treatment cycle, and overall. The percentage of subjects with amplified vs. nonamplified c-Met in their tumor samples, as measured by FISH, were summarized by cancer type (if necessary), dose level/schedule, treatment cycle, and overall. Individual and mean products of the percentage of cells staining positive and the corresponding intensity score of the above tissue markers were displayed in a scatter plot for each assessment for changes from baseline by dose.

Preliminary antitumor activity (best overall tumor response, duration of response and duration of stable disease) was summarized by cancer type, dose level/schedule, and overall.
Results

Patient characteristics

Between February 2009 and August 2011, 45 patients were enrolled; of these, 5 patients did not meet the inclusion/exclusion criteria and an additional 6 subjects discontinued before being dosed (5 patients due to disease related AEs [pain, n=2; cord compression, anemia and haemoptysis, bowel obstruction, n=1 each; 1 patient was withdrawn due to rapid progressive disease). Thirty-four patients were dosed with golvatinib. The most common tumor type was CRC (n = 15) and 65% had received 2 or 3 prior systemic therapies (Table 1).

Exposure, dose escalation, and MTD

All patients who received at least one dose of study drug were evaluable for safety (n=34). During dose escalation, two subjects failed to complete at least 75% of dosing during cycle 1 for reasons other than toxicity and were not evaluable for DLT (one subject in each of the 200mg and 400mg dosing cohorts). Golvatinib was escalated through 5 dose levels: 100 (n=3), 200 (n=6), 270 (n=4; 1 not evaluable), 360 (n=3) and 450 mg (n=2). The first DLT was observed at the 200-mg dose level: a grade 3 increased gamma-glutamyltransferase (GGT) and ALP. Two further DLTs were observed at the 450-mg dose level: 1 patient experienced repeated grade 2 fatigue after dose interruption for the same, and a second patient experienced grade 3 fatigue. The dose was then de-escalated to an intermediate dose of 400mg where 7 patients (1 not evaluable) were treated to define this as the MTD (no DLTs were observed). This dose was also selected based on the
convenience of administration of 4 x 100-mg tablets, as opposed to 3x300-, 1x50- and 1x10-mg tablets. A further 9 subjects were treated at this dose level of 400 mg QD in the MTD expansion cohort (for further safety evaluation, and assessment of PK, PD biomarkers and preliminary anti-tumor activity of E7050). Overall, patients completed a median of 2 cycles of golvatinib (range, 1–8). Two patients completed >6 cycles of study drug.

Safety and tolerability

The majority of patients (94%) experienced at least 1 treatment-related AE. As Table 2 shows, the most common treatment-related AEs with an incidence >10% (n, %) were: diarrhea (20, 58.8), nausea (17, 50), vomiting (15, 44.1), fatigue (14, 41.2), decreased appetite (11, 32.4), elevated ALT (11, 32.4), elevated AST (7, 20.6), dry skin (4, 11.8), and dysgeusia (4, 11.8). Grade 3 treatment-related events observed (n, %) included: fatigue (5, 14.7), decreased appetite (3, 8.8), renal impairment (1, 2.9), elevated GGT, and elevated ALP (1 each, 2.9). There were no grade 4 events or treatment-related deaths. Six patients treated at 200 mg and at the MTD (400 mg) required study drug withdrawal for treatment-related gastrointestinal AEs including nausea, vomiting, diarrhea, and acute pancreatitis. Dose reductions were required in 2 patients at 400 mg (fatigue with anorexia) and 450 mg (vomiting).

Pharmacokinetics

All patients were evaluable for PK analysis, and PK parameters are summarized in Table 3. Peak exposures were achieved within 4 to 6 hours.
after a single dose of golvatinib. Golvatinib exposures, based on AUC\(_{(0-t)}\) and steady-state C\(_{\text{max}}\), generally increased with dose. Due to the small numbers of patients at each dose level and the high variability in PK parameters, no formal statistical analysis was performed to assess dose proportionality. The half-life was 45 hours. Median steady-state T\(_{\text{max}}\) occurred approximately 2 to 4 hours after dosing. Trough concentrations increased between days 2 and 8 but remained relatively constant across days 8, 15, and 22. Steady-state plasma concentrations were achieved between days 8 and 22. Peak-to-trough fluctuation values ranged from 48.6% to 82.9%. Mean accumulation ratios based on peak exposure (C\(_{\text{max}}\)) ranged from 1.74 to 2.84 and those based on total exposure (AUC\(_{(0-t)}\)) ranged from 3.01 to 4.65, indicating moderate accumulation at steady state (Fig. 1). The apparent volume of distribution (Vz/F) ranged between 325 and 707 L.

Urine excretion after a single dose (0.93–2.4%) and at steady state (3.59–4.91%) was low, consistent with minimal renal excretion.

**Pharmacodynamic studies**

**Circulating biomarker studies** Twenty-five patients had evaluable measurements on circulating biomarkers for analysis. Increases in the median levels of the plasma biomarkers, c-Met, VEGFR2, uPAR, and Ang-2 were observed after treatment with golvatinib: a Mann-Whitney test demonstrated statistically significant increases (P < 0.05) for c-Met on days 2, 8, 15, and 22, for VEGFR2 on days 8, 15, and 22, for uPAR on days 8 and 22, and for Ang-2 on day 22. However, there was no correlation with dose (Fig. 2).
Tumor pharmacodynamic analysis Eight patients underwent tumor biopsies at baseline (patients 1019, 1020, 1021, 1022, 1024, 1025, 1026, and 1028). All biopsies were evaluated by FISH (for MET gene copy number, representative images are shown in Fig. 3A–C) and by IHC (for expression levels of c-Met protein, representative images are shown in Fig. 3D–F). In addition, paired (pre- and post-dose) biopsies were evaluable in 4 patients at the MTD (patients 1019, 1020, 1024, and 1026). These four paired biopsies were evaluated by IHC for levels of phosphorylation of c-Met (Tyr1234/1235) and for levels of phosphorylation of ERK (Thr202/Tyr204).

An increase in MET gene copy number was seen in 2 patients with papillary RCC (patient 1020) and CRC (patient 1028) (Fig. 3B and 3C); in both patients this was associated with high levels of c-Met protein expression by IHC (Fig. 3E and 3F). The increase of MET gene copy number in the patient with papillary RCC (patient 1020) was associated with an increase in the copy number of chromosome 7 (possibly a polysomy of chromosome 7; Fig. 3B). The trisomy of chromosome 7, where both the HGF and MET genes reside, has been reported in the literature to occur frequently in papillary RCC (39). Patient 1028 with CRC also demonstrated clustered amplification of the MET gene. Six patients (1019, 1021, 1022, 1024, 1025, and 1026) did not show an increase in MET gene copy number (a representative image of a tumor without MET gene amplification, patient 1022, is shown in Fig. 3A). Consistent with normal MET gene copy number, the level of c-Met protein expression in patient 1022 was low (Fig. 3D). IHC analysis in paired pre- and post-dose biopsies showed a post-treatment decrease in p-c-Met in 2 patients (Fig. 3G and 3I; patients 1020 and 1024). Patient 1020 was the patient with papillary...
RCC with an increased copy number of chromosome 7 and MET gene who demonstrated a reduction in the number of cells staining positive for p-c-Met from 46% to 3.7%. Patient 1024, with CRC, demonstrated a decrease in cells expressing p-c-Met, from 40% to 17%. In patients 1019 (diagnosed with melanoma) and 1026 (diagnosed with carcinoma of unknown primary), levels of c-Met phosphorylation were barely detectable pre-treatment and did not change with treatment. However, in patient 1026 (carcinoma of unknown primary) there was a decrease in the level of ERK phosphorylation after dosing with golvatinib, from 52% to 2% (Fig. 3H and 3J).

Response evaluation

Thirty-one patients were included in the efficacy population. There were no complete responses. One patient with transitional cell carcinoma of the bladder (patient 1022) demonstrated an unconfirmed partial response with a 35% decrease in the target lesions by RECIST after cycle 2. Six patients demonstrated stable disease (SD) lasting ≥85 days (range, 85–237 days). The patient with SD lasting 237 days had an esophageal carcinoma treated at 200 mg.

Two patients with SD lasting 50 (patient 1020, RCC) and 54 days (patient 1024, CRC) demonstrated downregulation of p- cMet (Fig 3G, I). Patient 1026 (CUP) who demonstrated downregulation of p-ERK) was not evaluable for response.
Discussion

This phase I study defined the MTD of golvatinib, an RTK inhibitor of c-Met, Ron, c-Kit, and Eph receptors, as 400 mg administered orally, QD, in 28-day cycles. The DLTs were fatigue and raised ALP and GGT. Fatigue was common and not insignificant, occurring in 50% of patients at the MTD, and resulted in a dose reduction in 2 patients treated at this dose level. Other frequently occurring AEs were diarrhea, nausea, and vomiting, which were manageable with supportive therapy. A concurrent phase I study using a twice-daily (BID) dosing schedule defined a MTD of 400 mg per day (200 mg BID; [40]). The toxicity profile was also similar, with DLTs of elevated ALT ($n = 1$) and nausea, vomiting, and anorexia ($n = 1$). In our study, although PK linearity was not shown in view of the small numbers and variability, peak $C_{\text{max}}$ and $AUC_{(0-t)}$ increased with dose. Although not tested in this study, but based on the results of a previous healthy volunteer study, food effect differences in golvatinib exposure were modest and considered clinically insignificant ([41]). The results of this latter study became available with 2 patients remaining on our study; and so for PK assessments, all patients were dosed on an empty stomach.

Paired pre- and post-treatment biopsies were evaluable in 4 patients at the MTD and provided preliminary evidence of target inhibition and downstream pathway modulation. Two out of 4 patients, with papillary RCC and CRC, demonstrated a detectable decrease in the number of cells staining positive for p-c-Met. One patient with carcinoma of unknown primary demonstrated down-regulation of p-ERK post-treatment. Pharmacodynamic analysis of
plasma biomarkers showed an increase in the median levels of plasma biomarkers, in particular soluble c-Met and a marker of angiogenesis, Ang-2. Limited conclusions can be drawn regarding these results of circulating PD analyses, given the small number of patients; however, elevated soluble c-Met suggested inhibition of the c-Met receptor while Ang-2 is a marker of angiogenesis. We have no PD data from this, or other clinical studies of golvatinib that demonstrate an inhibitory effect on Eph signalling. Golvatinib was originally developed as a TKI of c-Met, but was subsequently found to be a potent inhibitor of EphB4 and Tie2 in preclinical studies (37). However, preclinical data using unique in vitro assay systems of 2D and 3D endothelial cells/pericyte co-culture sprouting assays, demonstrated that the EphB4 inhibitory activity of golvatinib disrupted anti-VEGF therapy-resistant tumor vasculature in a combination with lenvatinib (a tyrosine kinase inhibitor [TKI] of VEGFR1-3, FGFR1-4, PDGFRα, RET and KIT [42]). We can however confirm that the unbound concentration of golvatinib at $C_{min,ss}$ (400mg) of 55.35-67.65 ng/mL, equivalent to 0.0707-0.086 μM, is above the IC50 of 0.015μM for EphB4 (data on file, Eisai).

This study provides some evidence that golvatinib is associated with clinical and PD activity in patients with advanced cancers. Although there were no complete or partial responses, 2 out of 3 patients with PD evidence of target modulation (patient 1020 with papillary renal carcinoma and patient 1024 with CRC) demonstrated SD lasting >50 days. However, what is not clear from our work is the duration of PD modulation of cancer cells. It is possible that if this
PD effect were sustained, more clinically meaningful outcomes may have been demonstrated.

Despite the evidence of target modulation, the efficacy of golvatinib as a single agent was limited. Further dose escalation was prevented by fatigue which, although not uncommon with RTK inhibitors, is difficult to manage. An intermittent dosing schedule may be conceivable, but the impact on PD effects would have to be closely assessed. The observation of elevated Ang-2 levels raises the hypothesis that angiogenesis may represent a mechanism of resistance to targeting c-Met, and it is possible that simultaneous targeting of both c-Met and angiogenic signalling may yield a greater therapeutic benefit. Preclinical data cited previously certainly supports the combination therapy of golvatinib and lenvatinib, which may be promising to overcome anti-VEGF therapy resistance (43). A phase I/II study of golvatinib in combination with lenvatinib, an inhibitor of VEGFR2 and -3, is presently underway.

Other clinical studies support this hypothesis. Phase I and II studies of foretinib, an inhibitor of both c-Met and VEGFR2, not only demonstrated IHC evidence of target modulation but also meaningful clinical responses and meaningful SD (44,45). In addition, HGF-c-MET signalling is also implicated in resistance to EGFR TKI therapy, and rational combinations with anti-EGFR therapies have also been tested. Other c-Met inhibitors such as tivantinib, cabozantinib, and onartuzumab have been combined with erlotinib in order to try to overcome this HGF-c-MET-mediated resistance to EGFR TKI therapy (46–48).
Golvatinib in Patients with Advanced Solid Tumors

In developing future treatment paradigms of personalized medicine, it is likely that golvatinib’s efficacy as monotherapy or in combination will be best demonstrated when applied to a molecularly defined patient population that is more likely to show clinical benefit. Foretinib demonstrated activity in a phase I setting in patients with papillary RCC, which is associated with activating mutations of MET. Activating mutations of MET have been shown in the germline of patients with hereditary papillary RCC and up to 13% of patients with sporadic papillary RCC (18). Furthermore, a majority of sporadic RCCs have a duplication of chromosome 7, where MET is located (39). An initial phase I study of foretinib indicated an efficacy signal in papillary RCC with a tolerable side-effect profile (44). A phase II study subsequently demonstrated significant activity in patients with germline cMET mutations (45). A second phase II study in 74 patients with advanced gastric cancer showed SD as the best response in 15 patients (49). However, just 3 patients demonstrated MET amplification at baseline, and one of these demonstrated SD. The authors concluded that foretinib was of limited value in unselected gastric cancer patients, and furthermore, as a single agent. Phase II and III studies of tivantinib with erlotinib in NSCLC showed evidence of clinical benefit for subsets of patients with MET high tumors. Although the phase III study was negative for the defined primary endpoint of progression-free survival (PFS) and overall survival (OS), an analysis of 40% of trial participants showed that PFS and OS were longer in patients with 2+ MET-positive immunostaining in more than 50% of cells (12, 46, 50). Likewise, a randomized phase II study of onartuzumab with erlotinib (versus placebo and erlotinib) in EGFR TKI-naïve patients with NSCLC demonstrated a significant PFS and OS benefit in a
subset of patients with MET diagnostic-positive tumors (48). Based on the results of these studies, current phase III trials of tivantinib in hepatocellular carcinoma and onartuzumab with erlotinib in NSCLC are focused on MET diagnostic-positive patients.

Our study was initiated prior to the concept of prospective patient selection having established itself in the clinic. However, the encouraging PD data supported by the developmental route taken by other c-Met inhibitors suggest that this approach of patient selection can be applied to future trials of golvatinib.

In summary, this phase I study of golvatinib met its primary and secondary objectives of establishing a recommended phase II dose to take forward for further development in combination with other targeted therapies. These studies may require modification of the schedule to counteract fatigue and, more importantly, focus on enrolling MET-positive patients to maximize the PD effect and efficacy.
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41. Mistry B, Nishioka Y, Fan J, Verbel D. Assessment of effect of low- and high-fat diet on E7050 pharmacokinetics and characterization of E7050 pharmacokinetics after 100 mg, 200 mg, and 400 mg single oral doses in


50. Scagliotti G. A phase III study of tivantinib plus erlotinib did not meet a primary endpoint in patients with locally-advanced or metastatic, non-squamous NSCLC [congress paper]. Presented at The European Cancer Congress of the European Society for Medical Oncology (ESMO), Amsterdam, Netherlands. 2013, abstract [E17-1821].
### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range) age, years</td>
<td>63.5 (32–78)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (61.8)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (38.2)</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13 (38.2)</td>
</tr>
<tr>
<td>1</td>
<td>21 (61.8)</td>
</tr>
<tr>
<td>Prior systemic therapies</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>2</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>3</td>
<td>11 (32.4)</td>
</tr>
<tr>
<td>4</td>
<td>5 (14.7)</td>
</tr>
<tr>
<td>5–8</td>
<td>5 (14.6)</td>
</tr>
<tr>
<td>Tumor types</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>15 (44)</td>
</tr>
<tr>
<td>Lung</td>
<td>4 (11.8)</td>
</tr>
<tr>
<td>Renal</td>
<td>4 (11.8)</td>
</tr>
<tr>
<td>Esophageal</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2 (5.9)</td>
</tr>
<tr>
<td>Others(^a)</td>
<td>7 (20.6)</td>
</tr>
</tbody>
</table>
Others included peritoneal, peripheral nerve, mesothelioma, bile duct, parotid gland, bladder, unknown origin.
Table 2. Grade 1/2 treatment-related AEs with an overall rate of >10%

<table>
<thead>
<tr>
<th>Treatment-related AEs</th>
<th>100 mg&lt;br&gt;N = 3</th>
<th>200 mg&lt;br&gt;N = 6</th>
<th>270 mg&lt;br&gt;N = 4</th>
<th>360 mg&lt;br&gt;N = 3</th>
<th>400 mg&lt;br&gt;N = 16</th>
<th>450 mg&lt;br&gt;N = 2</th>
<th>Total&lt;br&gt;N = 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>0 (0%)</td>
<td>3 (50.0%)</td>
<td>3 (75.0%)</td>
<td>2 (66.7%)</td>
<td>10 (62.5%)</td>
<td>2 (100.0%)</td>
<td>20 (58.8%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1 (33.3%)</td>
<td>2 (33.3%)</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>13 (81.3%)</td>
<td>0</td>
<td>17 (50.0%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>1 (16.7%)</td>
<td>1 (25.0%)</td>
<td>0</td>
<td>11 (68.8%)</td>
<td>2 (100.0%)</td>
<td>15 (44.1%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>3 (50.0%)</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>8 (50.0%)</td>
<td>2 (100.0%)</td>
<td>14 (41.2%)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>9 (56.3%)</td>
<td>1 (50.0%)</td>
<td>11 (32.4%)</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>0</td>
<td>1 (16.7%)</td>
<td>2 (50.0%)</td>
<td>2 (66.7%)</td>
<td>4 (25.0%)</td>
<td>2 (100.0%)</td>
<td>11 (32.4%)</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>0</td>
<td>1 (16.7%)</td>
<td>1 (25.0%)</td>
<td>1 (33.3%)</td>
<td>2 (12.5%)</td>
<td>2 (100.0%)</td>
<td>7 (20.6%)</td>
</tr>
<tr>
<td>Dry skin</td>
<td>2 (66.7%)</td>
<td>1 (16.7%)</td>
<td>0</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>0</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (18.8%)</td>
<td>1 (50.0%)</td>
<td>4 (11.8%)</td>
</tr>
</tbody>
</table>
Table 3. Key pharmacokinetic parameters of golvatinib at steady state (ss)

<table>
<thead>
<tr>
<th>Parameter/dose level</th>
<th>100 mg N = 3</th>
<th>200 mg N = 6</th>
<th>270 mg N = 4</th>
<th>360 mg N = 3</th>
<th>400 mg N = 16</th>
<th>450 mg N = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) C&lt;sub&gt;ss,max&lt;/sub&gt;, ng/mL</td>
<td>2340 (1230) (n = 3)</td>
<td>6290 (2240) (n = 5)</td>
<td>9180 (940) (n = 2)</td>
<td>9160 (7270) (n = 2)</td>
<td>9730 (3810) (n = 6)</td>
<td>NA</td>
</tr>
<tr>
<td>Median (range) t&lt;sub&gt;ss,max&lt;/sub&gt;, h</td>
<td>2.00 (2.00–2.00) (n = 3)</td>
<td>4.02 (1.98–4.08) (n = 5)</td>
<td>4.06 (2.02–6.10) (n = 2)</td>
<td>3.42 (2.83–4.00) (n = 2)</td>
<td>3.01 (1.80–6.00) (n = 6)</td>
<td>NA</td>
</tr>
<tr>
<td>Mean (SD) AUC&lt;sub&gt;(0,t)&lt;/sub&gt;, ng/mL h</td>
<td>39,100 (20,900) (n = 3)</td>
<td>107,000 (50,000) (n = 5)</td>
<td>145,000 (116,000) (n = 1)</td>
<td>150,000 (116,000) (n = 2)</td>
<td>171,000 (77,400) (n = 6)</td>
<td>NA</td>
</tr>
<tr>
<td>Mean (SD) C&lt;sub&gt;ss,min&lt;/sub&gt;, ng/mL</td>
<td>960 (400) (n = 3)</td>
<td>3720 (1980) (n = 5)</td>
<td>4830 (382) (n = 2)</td>
<td>3800 (2250) (n = 2)</td>
<td>6150 (2660) (n = 6)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Mean concentrations of golvatinib at steady state. Pharmacokinetic blood collection was not taken for the 450-mg dose group due to DLTs.

Figure 2. Median levels of plasma biomarkers c-MET (A), uPAR (B), Ang-2 (C) and soluble (s) VEGFR2 (D) during cycle 1.

Figure 3. Immuno-histochemical assessment of c-Met protein expression and FISH analysis of MET gene copy number (expanded MTD cohort 400 mg). Changes in levels of phospho-cMet and phospho-ERK in pre and post-treatment tumor biopsies.
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L. Rhoda Molife, Emma Dean, Montserrat Blanco Codesido, et al.

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