HGF as a circulating biomarker of onartuzumab treatment in patients with advanced solid tumors.

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Abstract:

The objective of this study was to evaluate circulating hepatocyte growth factor (cHGF) as a pharmacodynamic biomarker of Met inhibition for onartuzumab (MetMAb, OA5D5v2) in a Phase I trial in patients with advanced cancers and a Phase II trial in non-small cell lung cancer (NSCLC).

The Phase I study was a dose escalation trial with onartuzumab administered intravenously once every three weeks. The Phase II study was a randomized two-arm trial in which onartuzumab or placebo was administered in combination with erlotinib in 137 patients with second and third line (2/3L) NSCLC. Circulating HGF (cHGF) levels were evaluated by enzyme-linked immunosorbent assay (ELISA) at multiple time-points over the treatment period.

Onartuzumab administration resulted in an acute and sustained rise in cHGF in both the Phase I and II studies. Elevation in cHGF was independent of dose or drug exposure and was restricted to onartuzumab treatment. Neither higher baseline nor elevated change in cHGF levels upon treatment could simply be attributed to tumor burden or number of liver metastasis.

We have shown that elevated cHGF can consistently and reproducibly be measured as a pharmacodynamic biomarker of onartuzumab activity. The elevation in cHGF is independent of tumor type, dose administered or dose duration. While these studies were not powered to directly address the contribution of cHGF as a predictive, on-treatment, circulating biomarker, these data suggest that measurement of cHGF in future expanded studies is warranted.
Introduction:

Activation of the Met pathway in both epithelial and endothelial cells by its only known ligand, HGF (also known as scatter factor, SF) results in a wide array of biological responses ranging from cell proliferation and cell invasiveness to more complex morphological changes, such as branching morphogenesis (1-4). These diverse roles in response to Met signaling contribute to tumor growth and metastasis. As a consequence, aberrant Met activation due to overexpression of HGF and/or Met is poorly prognostic in multiple cancer types, including NSCLC, breast and, colorectal cancers (5-9). Recent phase I and phase II clinical studies have substantiated the clinical potential for Met inhibitors with preliminary evidence of anti-tumor activity using the anti-Met antibody, onartuzumab (MetMAb, OA5D5v2: Genentech, Inc), or Met tyrosine kinase inhibitors (TKIs) (9-11). In addition, dual inhibition of Met and EGFR signaling by co-administration of onartuzumab with erlotinib in a phase II trial showed both PFS and OS benefit compared to treatment with erlotinib alone in second line NSCLC patients with overexpression of tumor Met (11).

Onartuzumab is a recombinant, humanized, monovalent monoclonal antibody that binds to the SEMA domain of Met thereby blocking HGF binding and subsequent Met activation (12, 13). In preclinical studies, inhibition of Met signaling with onartuzumab results in pathway suppression and blockade of ligand-induced cell proliferation, migration, invasion, and cell survival (13, 14). Onartuzumab has preclinical anti-tumor activity in multiple tumor models when used as a single agent as well as in combination with various targeted and standard of care therapeutics (13, 14). Onartuzumab is currently being evaluated in phase II and phase III clinical studies for the treatment of multiple cancer types in combination with erlotinib, bevacizumab and chemotherapy.

Biomarkers are increasingly being evaluated in early Phase I trials to demonstrate proof of target engagement, pathway inhibition and for confirmation of mechanism of action, thereby increasing the probability of success of novel molecular entities in Phase II
studies (15, 16). The circulation (serum, plasma, whole blood) provides a non-invasive medium to evaluate dose response and biological activity of therapeutic agents. Circulating HGF is associated with poor outcome in multiple disease indications including NSCLC, CRC and head and neck cancers (17-19). Moreover, high levels of circulating HGF in plasma are also associated with resistance to receptor tyrosine kinase and BRAF inhibition (20-23). Given that onartuzumab blocks HGF binding to Met (13), we hypothesized that target engagement might result in elevated levels of cHGF as a consequence of ligand displacement from the receptor or by compensatory up-regulation. Thus we evaluated circulating levels of HGF as a potential pharmacodynamic biomarker in Phase I and Phase II clinical trials of onartuzumab administered as a single agent or in combination with the EGFR inhibitor erlotinib (10, 24, 25). In addition to assessing pharmacodynamic effects, we looked for correlations between HGF levels and liver metastasis, tumor burden and, clinical benefit from onartuzumab.

**Materials and Methods**

**Reagents**

Affinity-purified goat polyclonal antibodies to human HGF were generated at Genentech Inc. (South San Francisco, CA). Recombinant human HGF was also generated at Genentech Inc. (South San Francisco, CA).

**Study Design**

The Phase Ia, OAM4224g study, was an open-label, dose escalation study of the safety and pharmacology of onartuzumab in 34 patients with locally advanced or metastatic solid tumors. The trial consisted of a dose escalation of single agent onartuzumab (Stage 1) administered intravenously at 1, 4, 10, 15, 20 and 30 mg/kg once every three weeks and a single agent expansion at the recommended Phase II dose (Stage II) The Phase II study (ClinicalTrials.gov identifier NCT00854308, OAM4558g) was a
randomized, double-blind, placebo-controlled study in 137 patients with locally advanced or metastatic (stage IIIb/IV) NSCLC comparing erlotinib (150 mg oral, daily dose) plus placebo or onartuzumab (15 mg/kg IV q3wks).

**Serum and Plasma Samples**

Procured plasma (K2-EDTA anti-coagulant) from mBC, CRC, and NSCLC patients, as well as healthy individuals, was obtained from Conversant Healthcare Systems, Inc. Patient serum samples were obtained from 32 patients in the Phase I OAM4224g study (ClinicalTrials.gov Identifier NCT01068977) at pre-dose cycle 1 (C1D1), cycle 1 day 2 (C1D2), pre-dose cycle 2 (C2D1) and pre-dose cycle 3 (C3D1). Optional, exploratory serum and plasma (K2-EDTA anti-coagulant) samples were also obtained from 82 patients in the Phase II OAM4558g study, at screening (-14 to Day1), pre-dose cycle 1 (C1D1), 2.5 hours post-dose cycle 1, cycle 1 day 10-14 (C1D10-14), pre-dose cycles 2-4 (C2D1, C3D1, C4D1) and then at the study drug discontinuation visit (SDDV) which occurred ~30 days after the last dose. All patients on the trials and vendor collections provided written informed consent for use of bio-fluids for exploratory analysis of biomarkers.

**HGF quantification by Enzyme-Linked Immunosorbent Assay (ELISA)**

Plasma HGF levels were measured by ELISA. Briefly, wells of NUNC MaxiSorp microtiter plates were coated with 0.5 μg/mL of affinity purified goat anti-human (hu)HGF polyclonal antibody overnight at 4°C and then blocked with assay diluent (phosphate buffered saline (PBS) pH 7.4 with 0.5% bovine albumin, 0.05% Tween 20, 0.25% CHAPS, 0.35M NaCl, 5mM EDTA and, 10 ppm Proclin 300). Standards, controls, and plasma samples were diluted with assay diluent, loaded in duplicate, and incubated for 2 hours at room temperature. Affinity -purified goat anti-huHGF-biotin was diluted to 150 ng/mL in assay diluent and added to plates and incubated for 1 hour at room temperature. Following incubation of avidin-conjugated horseradish peroxidase (diluted to 40 ng/mL in PBS, pH 7.4, containing 0.5% BS, 0.05% Tween 20, and 10 ppm Proclin 300) for 1 hour, the reaction was visualized by the addition of a chromogenic
substrate (3', 3, 5, 5'-tetramethylbenzidine) for 15 minutes. The reaction was stopped with 1 M phosphoric acid, and absorbance was measured at 450 nm using an ELISA plate reader: the 630 nm reference wavelength was subtracted from the recorded spectra in accordance with the manufacturer’s instructions. Wash buffer with PBS containing 0.05% Tween 20 was used, plates were washed three times for the first two wash steps and four times for the remainder of the procedure. As a reference for quantification, a standard curve was established by the serial dilution of recombinant huHGF (2000–15.625 pg/mL). Lower Limit of Quantification (LLOQ) for the assay was 210 pg/ml.

**Statistical Analysis**

Pearson and Spearman correlations and Mann-Whitney tests were performed on cHGF from plasma verses serum, plasma from healthy donors verses cancer patients, and plasma from patients pre-treatment verses post-treatment using GraphPad Prism version 5, GraphPad Software, La Jolla California.

**Results:**

**Circulating HGF is elevated in cancer patients**

The prevalence of cHGF at baseline (pre-dose) was evaluated in procurred, non-study associated plasma samples from healthy donors and metastatic breast (mBC), CRC and NSCLC indications. cHGF in plasma from healthy donors was detected above the LLOQ in only 10/45 samples. The levels in these samples ranged from 215-692 pg/ml with a median of 261 pg/ml. cHGF was significantly elevated in cancer plasma from all three indications relative to healthy donors. Moreover, the levels measured in CRC and NSCLC (median values of 750 and 663 pg/ml respectively) were approximately 2-fold higher than those in mBC (median value 326 pg/ml) (Fig. 1A). In order to examine this dynamic in the clinic, cHGF was measured in serum from patients on the dose-escalating Phase I study of onartuzumab that enrolled patients with locally advanced or metastatic tumors. In this study, a wide range of baseline cHGF in serum across all
indications from 250-8058 pg/ml (median value of 1104 pg/ml) was observed (Fig. 1B). As compared to age-matched serum from healthy donors (median value 460 pg/ml) we observed a median 2.5 fold elevation in cHGF in these patients. Similarly, baseline cHGF was also measured in plasma from the Phase II study of onartuzumab conducted in second/third line NSCLC. Baseline cHGF levels in the phase II study were in the range of 210-11375 pg/ml (median value of 466 pg/ml). There was no significant difference between baseline cHGF in the placebo verses the onartuzumab arm (Fig. 1C) and similar to the Phase I study, median baseline levels in the Phase II population were on average ~2 fold higher than plasma from healthy donors, and were similar to levels in the acquired NSCLC plasma samples. Previous observations by Sakon et. al. (26) have demonstrated increased levels of HGF in serum when compared to plasma. In order to compare results from the phase I and phase II studies, cHGF was measured in a subset of patients in both serum and plasma from the Phase II onartuzumab trial. While baseline plasma and serum measurements from 48 patients correlated (Fig. 1D), the baseline values in serum were generally elevated relative to the levels measured in plasma corroborating previous observations. In a larger subset of patients with parallel measurements in serum and plasma across all time points a very strong correlation was observed ($r=0.974$) (Fig 1E) thus allowing for comparison between the two trials.

**Serum HGF is a biomarker of Met target engagement**

We hypothesized that onartuzumab binding to the Met receptor may lead to ligand displacement resulting in elevated levels of ligand and hence evaluated cHGF as a biomarker of target engagement in the single agent Phase Ia trial (10). Serum samples at various pre- and post-treatment time points from 29 patients (of 34 patients enrolled in the Phase Ia study) were evaluated for HGF levels using ELISA assays. Administration of onartuzumab was associated with an acute rise in serum HGF at day 2 in 40% of the patients, showing at least a 1.5 fold increase in cHGF (Fig. 2A). As a measure of the PK/PD relationship cHGF levels were monitored over the dose escalation. The rise in cHGF was sustained through the dosing cycle and appeared to be independent of dose administered (Fig. 2B) or disease indication. Three of the 29
patients evaluated showed an acute 3 to 8 fold decrease in serum HGF at day 2. Coincidentally, these three patients had the highest levels of HGF at baseline in the Phase I study ranging from 4000-8000pg/mL (Fig. 2C). Of these, a single gastric cancer patient showed an immediate and sustained decrease in cHGF from a baseline of 3441±301.8 pg/mL to near physiologic levels of 526.8±91.98 pg/mL for the duration of the treatment cycle. This was the only patient to achieve a RECIST complete response to single agent onartuzumab (Fig. 2B). Catenacci characterizes this patient as having evidence for an autocrine production of HGF (24).

A shed protein that constitutes the extracellular domain (ECD) of Met can be found in circulation and shows a dose-dependent elevation upon onartuzumab treatment in the Phase Ia study (27). This rise in shed Met is likely due to drug binding to the shed protein resulting in altered clearance of the receptor (27). We did not observe a correlation between levels of shed Met and cHGF upon onartuzumab administration in the Phase I study (supplemental Fig. 1).

cHGF elevation upon onartuzumab treatment in a Phase II study in NSCLC

Observations from the Phase I clinical trial prompted further evaluation of cHGF as a biomarker of onartuzumab in the OAM4558g Phase II study in advanced NSCLC. OAM4558g is a randomized, double-blind, placebo-controlled study with 137 patients treated with onartuzumab or placebo, in combination with erlotinib. In this study, serial analysis of cHGF was conducted in 44 patients in the placebo plus erlotinib arm and 38 patients in the onartuzumab plus erlotinib arm. Baseline cHGF levels remained relatively stable as assessed by parallel measurements at screening and at the pre-dose time-point (supplemental Fig. 2). In the placebo group, cHGF levels generally remained unchanged for the duration of the first cycle of drug administration (Fig. 3A). In contrast, cHGF levels in the onartuzumab treated patients changed from a median
baseline value of 515 pg/ml to 1167 pg/ml prior to the second cycle. While baseline values in the placebo and onartuzumab-containing arms were comparable, a consistent increase and broader distribution of cHGF with an average increase of 2.6 fold was observed in the onartuzumab/erlotinib arm (Fig. 3B). cHGF levels increased significantly at days 10-14 post-dose and plateaued through multiple cycles of therapy (Fig.3C). Additional evidence for cHGF as a pharmacodynamic biomarker of onartuzumab target engagement was found in patients in the placebo arm who crossed over to the onartuzumab/erlotinib arm at disease progression. Baseline, C2D1, cross-over pre-dose and, C2D1 post cross-over measurements of cHGF were available in 10/24 patients. In all but two cases, cHGF increased upon cross-over (Fig. 3D) with an average 3.7 fold increase over pre-dose values.

**Relationship between cHGF and tumor expression of Met receptor by IHC**

Evaluation of efficacy in patients with high tumor Met receptor expression defined by an IHC score of 2+ or 3+ (Met positive) was a co-primary endpoint of the OAM4558g Phase II trial (25). The addition of onartuzumab to erlotinib improved both PFS and OS in Met positive patients compared to erlotinib alone, resulting in a near 3-fold reduction in the risk of death (25). We evaluated the relationship between cHGF and Met expression as defined by IHC scores of 0, 1+, 2+ or 3+. Baseline cHGF levels were comparable in all four subgroups of Met expressing patients (Fig. 4A). Consistent with this observation, no statistically significant association between OS and HGF levels was observed (Yauch et al., in review) demonstrating that Met IHC is still the best predictive diagnostic assay for onartuzumab.

Next, we examined if magnitude of pharmacodynamic modulation of cHGF correlated with tumor Met expression. Consistent with data shown in Fig. 3 there was little change in cHGF levels in the placebo treated patients regardless of tumor Met status. In the onartuzumab-containing arm differences in cHGF modulation were detected in the
tumor Met expressing groups but no statistically significant trend was observed (Fig. 4B).

cHGF modulation and response to onartuzumab

The diverse roles of HGF as a cellular growth, motility and morphogenic factor led us to evaluate potential associations between cHGF levels and liver metastasis, tumor stage, tumor burden or tumor response. Neither the presence of liver metastasis (Fig. 5A) or stage of the tumor (Fig. 5B), at diagnosis, correlated with levels of cHGF. Comparisons of baseline cHGF did not correlate with either initial tumor burden (Fig.5C) or tumor response (Fig. 5B). Similarly, there was no relationship between changes in cHGF levels and tumor response. More statistically rigorous analysis was performed to evaluate a relationship between changes in cHGF levels and PFS and/or OS in the Met-positive group but these were inconclusive due to the small sample size.

Discussion

A number of agents targeting Met signaling are currently in clinical trials including small molecule inhibitors to Met such as tivantinib (ARQ 197) and antibodies targeting HGF such as rilotumumab (AMG 102) (28). Clinical studies that have evaluated HGF as a biomarker of Met pathway inhibition for tivantinib and rilotumumab suggest that different modes of Met inhibition can yield different results on levels of cHGF. For example, rilotumumab administration led to a dose dependent elevation in cHGF in a Phase I study, likely due to stabilization of drug-bound protein resulting in increased half-life of cHGF (29). In Phase I clinical studies evaluating the small molecule inhibitor tivantinib, no obvious changes in cHGF were observed upon drug treatment (30).
In this study, we identified cHGF as a robust, pharmacodynamic biomarker of onartuzumab treatment in cancer patients receiving this agent. The effect is specific to onartuzumab treatment as unequivocally demonstrated in the Phase II study where the placebo/erlotinib-containing regimen had little effect on cHGF as compared to the onartuzumab-containing arm. It is unlikely that feedback mechanisms, such as differential expression of HGF in tumor cells, explain this phenomenon as evidence for autocrine activation in NSCLC tumors from the Phase II study was infrequent (unpublished observation). However, up-regulation of HGF derived from the stroma cannot be excluded. No correlation was observed between drug exposure and cHGF levels upon onartuzumab administration in both the Phase I and II studies, suggesting that PK alone does not drive the magnitude of biomarker modulation (data not shown). One possible explanation of the elevation in cHGF may be that onartuzumab displaces HGF binding to Met, resulting in increased circulating levels.

HGF was discovered as a complex mitogen produced by liver kupfer cells and sinusoidal endothelial cells, capable of promoting liver regeneration via stimulation of hepatocytes (3, 31-33). cHGF is frequently associated with liver dysfunction or damage (32) and is elevated in liver diseases including cirrhosis and hepatitis independent of Met receptor expression (34). cHGF is also elevated in many cancers and is associated with increased incidence of tumor cell invasion, distant metastases and poor prognosis (18, 35, 36). Within tumors, HGF expression is generally restricted to the stromal compartment with fibroblasts being the predominant source of HGF production (37). In addition, tumor resident vascular endothelial cells, smooth muscle cells, macrophages and neutrophils are also cellular sources of HGF (38). In tumors that are driven by autocrine production of HGF, the contribution of tumor-cell derived HGF to total plasma levels may be significant. Consistent with this hypothesis, the one patient with HGF driven autocrine disease (24) with a complete response in the Phase I study presented with supraphysiologic levels of baseline cHGF that decreased to near physiologic levels.
upon onartuzumab administration. Evaluation of cHGF as an on-treatment predictive marker of response (sustained decrease in cHGF upon onartuzumab administration) in autocrine-driven disease warrants further investigation. However, it is unlikely that supraphysiologic levels of baseline cHGF alone reflect autocrine driven disease. Although we observed patients in the NSCLC Phase II study with similar high levels of baseline cHGF, tumor HGF mRNA expression in archival tumors from patients in this study was low. Furthermore, tumor HGF mRNA expression did not correlate with levels of cHGF (unpublished observation).

Recent studies have suggested an important role for stromal derived HGF as a mechanism for drug resistance, highlighting the importance of tumor microenvironment derived growth factors on tumor growth and response to therapy (22, 23). Wilson et. al., showed that high baseline cHGF in patients with melanoma was associated with worse outcome (PFS and OS) in a trial testing the BRAF inhibitor vemurafenib (22). These studies primarily assessed associations of pre-treatment levels of HGF with response to therapies. However changes in HGF levels, potentially as an on-treatment circulating biomarker of response to treatment, was not evaluated. The relationship between cHGF levels, on-treatment, and PFS and OS in the Met-positive group in the Phase II onartuzumab study was inconclusive due to small sample size but will be evaluated in future studies with onartuzumab. The relative ease of collecting samples for blood based-biomarker analysis would undeniably allow for easier implementation of the biomarker strategy in global clinical trials.

We have shown that elevated circulating HGF can consistently and reproducibly be measured as an on-target pharmacodynamic biomarker of onartuzumab activity. While the Phase II was not powered to directly address the contribution of cHGF as a predictive on-treatment biomarker, these data suggest that measurement of cHGF in additional Phase II or expanded Phase III studies is warranted.
11. Spigel DR ET, Ramlau R, Daniel DB, Goldschmidt JH, Blumenschein GR, Krzakowski MJ, et.al. Final efficacy results from OAM4558g, a randomized phase II
study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC.


Figure Legends

Figure 1.

Baseline levels of cHGF are elevated in plasma from different cancer indications as well as from, Phase I and Phase II Onartuzumab clinical studies relative to plasma from healthy donors.

(A) Plasma samples from healthy donors, metastatic breast cancer (mBC), colorectal (CRC) and non-small cell lung cancer (NSCLC) are plotted as scatter plots. Medians are indicated as bars and the LLOQ of the assay is plotted as a dotted line. Mann Whitney comparisons were performed on plasma from healthy donors relative to each of the cancer indications.

(B) Pre-dose (baseline) serum was collected from patients in the Phase 1 study. cHGF serum levels are plotted relative to serum samples from healthy donors. Mann Whitney analysis was performed to evaluate the difference between the two populations.
(C) Pre-dose (baseline) plasma was collected from patients in the Phase II study. cHGF levels from the placebo/erlotinib arm are plotted compared to the onartuzumab/erlotinib arm.

(D) Correlation of serum and plasma cHGF measurements. A correlation analysis was performed between cHGF values from 48 paired, baseline serum and plasma patient samples.

(E) Correlation analysis performed for cHGF levels from 124 serum and plasma parallel measurements across multiple time points.

Figure 2.

cHGF is elevated upon onartuzumab administration in Phase I patients.

(A) cHGF was measured at pre-dose, 24h after onartuzumab infusion (C1D2) and at the end of the first cycle, C2D1 (day 21), for patients in the Phase Ia study. Data represent ratios of cHGF to pre-dose levels for each individual patient.

(B) Phase I dose escalation cHGF measurements. The ratio of pre-dose cHGF and the levels upon dose escalation of single agent onartuzumab administered intravenously at 1, 4, 10, 15, 20 and 30 mg/kg once every three weeks.

(C) cHGF levels for all patients measured at pre-dose, C1D2 and C2D1 were plotted in ascending order of baseline levels and compared to % change in tumor response as assessed by measurement of sum of the longest unidimensional diameter (SLD) for lesions of interest using computed tomography (CT) scans.

Figure 3.

cHGF from Phase II plasma samples is elevated upon onartuzumab administration.

(A) cHGF was measured at pre-dose and at the end of the first cycle, C2D1 (day 21) after onartuzumab infusion for patients in the Phase II study. cHGF values (pg/ml) are plotted as pre- and post-dose for both the placebo/erlotinib and onartuzumab/erlotinib arms. Mann Whitney analysis was performed comparing pre-dose verses post-dose populations.

(B) A frequency distribution analysis was performed for cHGF from both arms of the Phase II study. Measurements are ratios of cHGF to pre-dose levels.

(C) cHGF was measured in plasma samples at pre-dose, 2.5 hours after the first infusion (C1D2.5h), at day 10 to 14 after the first infusion (C1D10-14), at the end of the first infusion cycle (C2D1) and, again at the end of the second infusion cycle (C3D1) in both placebo/erlotinib and onartuzumab/erlotinib treatment arms. Data represent ratios of cHGF measurements relative to pre-dose levels for each individual patient.
(D) Plasma was collected from 10 placebo/erlotinib treated patients that crossed over to the onartuzumab/erlotinib regimen. Plasma was obtained at pre-dose and at 21 days after infusion with placebo/erlotinib (C2D1), then at pre-dose prior to cross-over onto the onartuzumab/erlotinib regimen (XO-pre-dose) and, again at 21 days after infusion on the onartuzumab/erlotinib regimen (XO-C2D1). Data represent a time course of cHGF levels for each patient.

Figure 4.

There is no relationship of cHGF and Met expression at either baseline or change from baseline in response to onartuzumab administration.

(A) Pre-dose cHGF measurements were evaluated in each of the IHC defined Met expression level groups (0, 1+, 2+ or, 3+). Numbers of samples in each group are set in parenthesis. Whisker plots are displayed with 5-95 percentile ranges. Bars represent the median and (+) represent the average. There is no statistical significant difference of cHGF at baseline between the Met expression groups.

(B) Pharmacodynamic measurements of cHGF as a change from pre-dose levels, represented as a ratio, from both the placebo/erlotinib and onartuzumab/erlotinib treatment arms are plotted relative to Met IHC measurements. Neither comparison met statistical significance.

Figure 5.

There is no correlation between the pharmacodynamic assessment of cHGF and liver metastasis, tumor stage, or tumor response.

(A) The ratio of cHGF relative to pre-dose for each patient was evaluated with respect to the presence or absence of liver metastasis. There were 24 patients with liver metastasis and 59 patients without liver metastasis.

(B) The ratio of cHGF relative to pre-dose for each patient was evaluated with respect to stage of the tumor as defined by tumor stage 1 (n=11),2 (n=41),3 (n=14) or 4 (n=15).

(C) Baseline cHGF from the Phase II clinical study are plotted against tumor size as assessed by measurement of sum of the longest unidimensional diameter (SLD) for lesions of interest using computed tomography (CT) scans (tumor burden).

(D) Baseline cHGF from the Phase II clinical study are plotted against the change in size of the tumor from assessment day 42 relative to the initial assessment at day 1 as assessed by measurement of sum of the longest unidimensional diameter (SLD) for lesions of interest using computed tomography (CT) scans (tumor response).

(E) The ratio of cHGF levels from pre-dose (Day1) and post-dose (Day21) were plotted relative to the change in size of the tumor from assessment day 42 relative to the initial
assessment at day 1 (tumor response). The dotted line indicates a reduction of $\geq 30\%$ of tumor size.
Figure 1

(A) Serum and plasma HGF levels in Healthy donors, mBC, CRC, and NSCLC patients.

(B) Serum HGF levels in Healthy donors and Phase I patients.

(C) Plasma HGF levels in Phase II placebo and Phase II onartuzumab patients.

(D) Linear regression for plasma HGF in Healthy donors: R² 0.6795, Pearson r 0.8243, n=48.

(E) Linear regression for plasma HGF in Phase II onartuzumab patients: R² 0.9499, Pearson r 0.9746, n=124.
Figure 2

(A) HGF (Ratio to pre-dose) vs. C1D2 and C2D1.

(B) HGF (Ratio to pre-dose) vs. Onartuzumab (mg/kg).

(C) HGF (pg/ml) vs. % Tumor response for Patients C1D1, C1D2, C2D1, and C3D1.
Figure 3

(A) Pre-dose vs. Post-dose (C2D1) HGF (pg/ml)

Placebo + erlotinib
Mann Whitney
p = 0.4911

Onartuzumab + erlotinib
Mann Whitney
p < 0.0001

(B) Frequency distribution (%)

HGF (Ratio to pre-dose)

(C) Placebo + erlotinib

Onartuzumab + erlotinib

(D) HGF (pg/ml)

Pre-dose C2D1 Pre-dose XO C2D1 XO

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(A) Pre-dose HGF (pg/ml) vs MET IHC Score

(B) HGF (ratio to pre-dose) vs MET IHC Score

- Placebo + erlotinib
- Onartuzumab + erlotinib

n= 8 7 12 9 15 14 3 7
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