Pharmacokinetic and Pharmacodynamic analysis of circulating biomarkers of anti-NRP1, a novel anti-angiogenesis agent, in two Phase I trials in patients with advanced solid tumors.

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Running title: Circulating biomarkers and PK/PD relationship for anti-NRP1
Statement of translational relevance

Blockade of VEGF signaling with monoclonal antibodies or small molecule inhibitors to the receptors (VEGFR1, VEGFR2) results in an increase in systemic PlGF. We have shown for the first time, that blocking neuropilin-1 (NRP1), a co-receptor for VEGFR2, results in an increase in plasma PlGF, total circulating soluble NRP1, and VEGF in humans. Furthermore, MNRP1685A, a function blocking antibody to NRP1, enhances pharmacodynamic response to bevacizumab-mediated VEGF pathway blockade, as demonstrated by an increase in the magnitude of PlGF elevation when combined with bevacizumab. Pharmacokinetic (PK) and pharmacodynamic (PD) analysis of biomarkers in the Phase I population allowed for identification of doses at which maximal sustained biomarker modulation was observed. These results are pertinent as they demonstrate an integrated PK/PD approach to guiding dose and schedule selection in humans.
Abstract

**Purpose:** MNRP1685A is a monoclonal antibody to neuropilin-1 (NRP1). We evaluated blood-based pharmacodynamic (PD) biomarkers of MNRP1685A in two Phase I studies to assess exposure/response relationships to inform target dose and regimen selection.

**Experimental Design:** The Phase I studies evaluated escalating doses of MNRP1685A as a single agent or in combination with bevacizumab. Plasma Placental Growth Factor (PlGF), VEGF and circulating NRP1 (cNRP1) were evaluated at multiple time-points using meso-scale discovery (MSD) assays and ELISA, respectively. Plasma PlGF was also measured in a Phase I/II trial of bevacizumab in metastatic breast cancer (AVF0776). The association between PlGF and MNRP1685A dose was described by a sigmoid Emax model. cNRP1 and MNRP1685A PK profiles were described using a two-target quasi-steady state (QSS) model.

**Results:** A dose and time dependent increase in plasma PlGF and cNRP1 was observed in all patients treated with MNRP1685A. PK/PD analysis showed that bevacizumab and MNRP1685A had an additive effect in elevating PlGF. Predictions based on the two-target QSS model showed that the free drug concentration to maintain greater than 90% saturation of membrane NRP1 (mNRP1) and cNRP1 is about 8 μg/mL.

**Conclusion:** These data show that MNRP1685A inhibits the VEGF pathway in humans as assessed by an increase in plasma PlGF. MNRP1685A appears to enhance bevacizumab-mediated VEGF pathway blockade, as demonstrated by an increase in the magnitude of PlGF elevation when combined with bevacizumab. PK/PD analysis of biomarkers in the Phase I population allowed identification of doses at which apparent maximal pathway modulation was observed.
Introduction

The neuropilins, NRP1 and NRP2, are non-tyrosine kinase receptors for class 3 semaphorins and VEGF. NRP1 was first characterized as a semaphorin receptor mediating axonal guidance (1, 2). Shortly after this discovery, it was shown that NRP1 is also a receptor for VEGF<sub>165</sub> (3). It was then demonstrated in genetic studies, that NRP1 is required for vascular endothelial sprouting and remodeling during embryonic development (4). The mechanism by which NRP1 mediates its effects on endothelial function is complex. Since the cytoplasmic domain of NRP1 is short, it is generally believed that coupling with other receptors is required for signal transduction, therefore, NRP1 is proposed to act as a co-receptor for VEGF<sub>165</sub> (3). VEGFR2-NRP1 complexes are formed due to bridging when VEGF<sub>165</sub> binds to both receptors simultaneously, resulting in increased VEGFR2 signaling (5). VEGF<sub>121</sub> also binds to the b1,b2 domain of NRP1 and promotes endothelial cell migration and sprouting (6).

MNRP1685A is a fully human monoclonal antibody directed to the b1b2 domain of NRP1(7). This antibody blocks the binding of VEGF<sub>165</sub> and VEGF<sub>121</sub> to NRP1 (6, 7). In preclinical xenograft models, administration of a function blocking antibody anti-NRP1<sup>b</sup>, a precursor of MNRP1685A, designed specifically to block VEGF binding to NRP1, demonstrates marginal tumor growth delay as a single agent. However, an additive effect in reducing tumor growth and tumor vascular density is observed when anti-NRP1<sup>b</sup> is combined with murine anti-VEGF (8). It is proposed that anti-NRP1<sup>b</sup> enhances anti-VEGF activity by inhibiting vascular remodeling, as evident by a reduction of pericyte association with vessels, thereby rendering vessels more susceptible to anti-VEGF therapy (8). Given the promising preclinical anti-angiogenesis profile, MNRP1685A has been evaluated in first in human Phase I studies as a single agent (Ia) and in combination with bevacizumab (Ib) to investigate safety, tolerability and PK with the goal to further improve therapeutic benefit of bevacizumab by combining with MNRP1685A in humans with advanced solid malignancies (9, 10).
Biomarkers were included in the Phase I studies of MNRP1685A to evaluate doses at which target modulation was observed. These included circulating biomarkers for anti-angiogenesis agents such as VEGF, PlGF and fibroblast growth factor-2 (bFGF) among others (11-13). We also assessed the levels of total cNRP1 as a surrogate marker for receptor occupancy (14). The objectives of these analyses were to explore the relationship between drug exposure and biomarker response to inform target dose and regimen selection for MNRP1685A.

Materials and Methods

Exploratory biomarker analyses in cynomolgus monkeys

Cynomolgus monkeys received a single intravenous (IV) dose of MNRP1685A at 0, 0.5, 3, 15, or 50 mg/kg and were monitored for a period of 50 days. Plasma samples were collected at predose (baseline) and various time points post dose to evaluate the effect of NRP1 pathway inhibition on circulating levels of VEGF pathway markers including PlGF, VEGF, bFGF and soluble VEGFrl1, using a four-plex assay (Meso Scale Discovery, Gaithersburg, MD).

Biomarker evaluation in MNRP1685A Phase I studies

The Phase Ia study (NCI study# ANP4509g) consisted of a standard 3+3 dose escalating trial design with IV MNRP1685A administered once every three weeks (q3w) at 2, 5, 10, 15, 20, 30, or 40 mg/kg. All patients enrolled provided written informed consents for exploratory biomarker analysis. Serum for MNRP1685A PK and plasma (EDTA-plasma) for exploratory biomarkers were collected at predose (baseline), and 1, 3, 7, 14, and 21 days after the first dose of MNRP1685A from 32 patients.

Arm A of the Phase Ib trial (NCI study # ANP4667g) consisted of escalating doses of MNRP1685A at 7.5, 15, or 24 mg/kg administered along with a standard dose of 15 mg/kg bevacizumab (both IV, q3w). All patients enrolled provided written informed consents for biomarker analysis. Serum for PK and plasma (EDTA-plasma) for
exploratory biomarkers were collected at predose (baseline), and 1, 7, and 14 days after the first dose of MNRP1685A from 10 patients. Premedications such as dexamethasone were allowed in both studies to manage infusion related symptoms following drug administration. To avoid confounding the biomarker assessment by the premedications, the earliest time point chosen to evaluate plasma biomarkers was 8 days after drug administration to allow for washout of the premedications from patients (dexamethasone has a half-life of 36-54 hours).

PlGF, VEGF, bFGF, and soluble VEGFr1 were measured using a four-plex assay (Meso Scale Discovery, Gaithersburg, MD). EDTA plasma from 28 healthy donors (ages 21-60) was collected to measure the circulating levels of these markers in healthy population. While the VEGF assay does not cross-react with VEGF-B, C or D, the assay preferentially recognizes the VEGF 121 and 165 isoforms (manufacturer communication). The PlGF assay recognizes both the PlGF 1 and 2 isoforms with varying sensitivities (detection antibody).

MNRP1685A concentrations were determined using a validated enzyme-linked immunosorbent assay (ELISA). MNRP1685A was detected using a mAb specific to the IgG framework (MAb 10C4), labeled with biotin. This step was followed by addition of avidin-digoxin-HRP conjugate. The assay’s lower limit of quantitation (LLOQ) was 75 ng/ml.

Total cNRP1 (including free and complex) concentration in plasma was measured from patients in Phase Ia study using an ELISA as previously reported (14). In addition, cNRP1 was measured in plasma from healthy donors and vendor procured plasma from mBC, CRC, and NSCLC patients (Conversant Healthcare Systems, Inc). A rabbit anti-human sNRP1 polyclonal antibody was used as both coat and detection reagents. The assay LLOQ was 0.02 ng/mL in plasma.

PlGF modulation by bevacizumab

In a Phase I/II trial (AVF0776), seventy-five patients with metastatic breast cancer were treated with escalating doses of bevacizumab ranging from 3 to 20 mg/kg administered
intravenously every other week (15). Plasma samples were available at predose (baseline) and 14 and 28 days after the first dose from 66 patients. Plasma PI GF was measured from 15 patients in the 20mg/kg cohort using a four-plex assay (Meso Scale Discovery, Gaithersburg, MD).

**MNRP1685A/PIGF PK/PD analysis**

The PK/PD relationship between plasma PI GF level (normalized to baseline) and MNRP1685A dose, with (Arm A of Phase Ib) or without (Phase Ia) co-administration of bevacizumab was evaluated. Various analyses were explored to correlate the MNRP1685A and PI GF exposure parameters, including dose of MNRP1685A, concentration at specified time-point, or area under concentration-time curve (AUC). The dose of MNRP1685A and PI GF AUC (AUC$_{PIGF}$) showed more pronounced relationship, which is well described by a sigmoid Emax model (Equation 1).

\[
E = E_0 + (E_{max} - E_0) \times \frac{Dose}{Dose + ED_{50}}
\]

(1)

where E is the drug (MNRP1685A) effect on PI GF, $E_0$ is the baseline PI GF level, $E_{max}$ is the maximum drug effect on PI GF level, $E_{max} - E_0$ is the change in PI GF level, Dose is the MNRP1685A dose level (mg/kg), and ED$_{50}$ represents the dose of MNRP1685A that leads to 50% maximum drug effect. Note that instead of using the PI GF level at a specific time point, the area under PI GF concentration (baseline-normalized)-time curve (AUC$_{PIGF}$) from 0 to 14 days post MNRP1685A administration was used to reflect PI GF level change over the time. The analysis was done in S-PLUS (version 8.2, TIBCO Software Inc., Palo Alto, CA).

**MNRP1685A/cNRP1 PK/PD analysis**

Because MNRP1685A binds to both mNRP1 and cNRP1, the two-target quasi-steady-state (QSS) model (16) has been shown to adequately describe the free drug MNRP1685A and total cNRP1 concentration-time profiles in cynomolgus monkeys (17).
In this model, MNRP1685A distributes to both central and peripheral compartments, with parallel linear and nonlinear clearance of free drug, and binds to free cNRPI in the central compartment. In order to understand the target occupancy after MNRP1685A administration, free cNRPI and mNRPI profiles were simulated using the parameters obtained from the two-target QSS model to predict free receptor levels, at the studied dose regimens.
Results:

**PIGF: A biomarker of systemic pathway inhibition for anti-NRP1**

Plasma biomarkers related to angiogenesis were evaluated in cynomolgus monkeys administered with a single dose of MNRP1685A with the aim of identifying a systemic marker of pathway inhibition. Of the four markers tested, namely VEGF, PlGF, bFGF and FLT1, plasma PlGF was the only biomarker that showed an immediate 1.5-2 fold elevation at 24 hours, the earliest time point evaluated (Fig. 1). Doses of 15mg/kg and higher showed sustained elevation in PlGF for the duration of the study (28 days). At day 28, quantifiable circulating drug concentrations (110 ± 50.1 µg/ml) were present only at the 50 mg/kg dose which may explain the sustained increase in PlGF at this dose level.

cNRP1 was also evaluated in the cynomolgus monkeys as a biomarker reflecting drug binding to target (14). A dose and time dependent elevation in total cNRP1 was observed, again more pronounced at the 3mg/kg and higher dose levels (14).

Given the effect of MNRP1685A on systemic PlGF in non-clinical models, plasma PlGF evaluation was included in the Phase I study as a marker of target modulation (Table 1). As shown in Fig. 2A, patients on the Phase I study had six times higher baseline levels of plasma PlGF compared to healthy donors. As expected from preclinical studies, single agent MNRP1685A administration resulted in a 2 fold elevation in systemic plasma PlGF at doses of 10mg/kg and higher. Sustained elevation in PlGF through the duration of the dosing cycle, was observed at doses of 20 mg/kg and higher (Fig. 2B). In Arm A of the Phase Ib study, when combined with bevacizumab, PlGF levels increased to 2.4 fold at day 8 and reached 1.8 fold by the end of the dosing cycle at the first dose (7.5 mg/kg) administered (Fig. 2C). Drug concentration for this dose cohort at day 7 was 13.2±3.75µg/ml and undetectable at day 21. This observed effect on PlGF is greater than equivalent doses with single agent MNRP1685A based on Phase Ia study. Consistently, increasing the dose of MNRP1685A to 15 and 24 mg/kg showed up to 3.7 fold increase in PlGF levels at day 21 (end of cycle 1). The day 21 drug concentrations for the 15 and 24 mg/kg dose cohorts were 1.63±1.32 µg/ml and 7.69±9.64 µg/ml respectively.
Since Arm A of the Phase Ib trial included co-administration of MNRP1685A with bevacizumab, PIGF modulation by bevacizumab alone was also evaluated to better understand the combination effect of bevacizumab and MNRP1685A. Plasma samples from baseline and days 14 and 28 from the 20 mg/kg dose cohort of AVF0776, a single agent trial of bevacizumab in breast cancer were used to evaluate the degree of PIGF elevation by single agent bevacizumab(15). Patients enrolled on this trial had equivalent levels of PIGF at baseline as those evaluated in the Phase I study (supplemental Fig. 1). Treatment with bevacizumab alone resulted in a consistent ~1.8-2 fold elevation in plasma PIGF that was sustained through the duration of treatment cycle in all patients (Fig. 2D). These observations are similar to those previously reported for bevacizumab (18, 19).

Since bevacizumab does not show greater than 2 fold increase in PIGF, and MNRP1685A as a single agent did not increase PIGF levels at 7.5 mg/kg (based on single agent results from Phase Ia), the sustained and enhanced (15 and 24 mg/kg) PIGF elevation observed in Arm A of the Phase Ib study suggests a potential readout of enhanced pathway inhibition for the two anti-angiogenesis agents.

PK/PD modeling further indicates the additive effect between bevacizumab and MNRP1685A on PIGF elevation. Either as a single agent or in combination with bevacizumab, the profile of $\text{AUC}_{\text{PIGF}}$ versus MNRP1685A dose was well described by a sigmoid Emax model (Equation 1), as shown in Fig. 3. For single agent MNRP1685A (Phase Ia), the estimated parameter value is 9.2 (90% CI: 1.1-12) fold-day for $E_0$, 43 (90% CI: 29-60) fold-day for $E_{\text{max}}$, and 23.5 (90% CI: 4.5-40) mg/kg for $ED_{50}$. These results suggest that the potential pharmacologically active dose is about 20 mg/kg or higher. For the combination of MNRP1685A and bevacizumab, the $E_0$ value is consistent with that in the bevacizumab alone study (AVF0766). After subtracting the 1.8-2 fold elevation of PIGF by bevacizumab alone, the estimated model parameters for $\text{AUC}_{\text{PIGF}}$ versus MNRP1685A dose were similar to those in Phase Ia, with $E_0$ at 9.1 (fold-day), $E_{\text{max}}$ at 42.4 (fold-day), and $ED_{50}$ at 21.7 mg/kg. The results suggest an additive effect of combination of MNRP1685A and bevacizumab on PIGF elevation.
In addition to PlGF, a dose independent elevation in plasma VEGF was observed in a subset of patients in the Phase Ia study (Fig. 4A). This elevation was dependent on baseline values at start of treatment, where patients with baseline levels of 250 pg/ml or less showed a rise in plasma VEGF with drug administration (Fig. 4B). Notably, subjects who showed an increase in plasma VEGF also appeared to exhibit sustained elevation through the dosing period, independent of the dose administered, which is contrary to the observed effects with plasma PlGF. No correlation was observed between elevation of plasma VEGF and duration of therapy.

**cNRP1: A surrogate marker for receptor occupancy**

cNRP1 is abundantly found in plasma from healthy donors with a mean level of 200 ng/ml (Fig. 5A). The Phase Ia patients showed a broader dynamic range in baseline expression with a mean level of 400 ng/ml. We next evaluated vendor procured cancer blood samples to determine the levels of cNRP1 in three major disease indications. Despite being abundantly expressed in healthy donors, circulating levels of cNRP1 appear to be further elevated in cancer blood samples, with higher levels observed in colorectal and non-small cell lung cancer (median level 400 ng/ml) compared to breast cancer (median level 330 pg/ml) (Fig. 5A).

MNRP1685A administration resulted in elevation of total cNRP1 at doses of 10 mg/kg and higher (Fig. 5B). By day 14, doses of 15 mg/kg and higher showed a ~2 fold elevation in total cNRP1. The increase of total cNRP1 was sustained through the end of the cycle at doses of 30 and 40 mg/kg, suggesting a plateau for total cNRP1 at these dose levels.

Although total cNRP1 correlates well with the MNRP1685A dose (Fig. 5B), in cynomolgus monkey studies, the increase of total cNRP1 appears to be driven by the accumulation of drug-cNRP1 complex (17). To better utilize total cNRP1 as a surrogate marker for receptor occupancy in humans, a mechanistic PK/PD model (two-target QSS
model) was applied to predict the profiles of free receptors both the membrane mNRP1 and cNRP1. Totally 414 data points for MNRP1685A (from 44 patients in both Phase Ia and Phase Ib studies) and 275 data points for total cNRP1 (from 30 patients in Phase Ia study) were included in the analysis using NONMEM version VI, level 1.0 (LLC, Globomax, Ellicott City, MD). As shown in Fig. 6, the receptor occupancy profiles of both mNRP1 and cNRP1 changed in a concentration-dependent manner following MNRP1685A administration. Both free mNRP1 and free cNRP1 levels were maintained at greater than 90% target saturation at concentrations above 8 \( \mu \)g/mL. Therefore, the higher the dose, or the more frequent the dose, the longer the duration of full target occupancy. At q3wk doses of 25 mg/kg, both free targets can be maintained at lower than 10% baseline (i.e., greater than 90% saturation) during the dosing intervals at steady state.

**Discussion**

Phase I trials for anti-cancer agents are traditionally designed to primarily evaluate safety and pharmacokinetics of novel drugs, with exploratory efforts focused on pharmacodynamic activity. Phase II dose estimates are typically identified using integrated preclinical and clinical PK/PD and safety in humans. As monoclonal antibodies inhibiting angiogenesis pathways generally have favorable safety profiles and the maximum tolerated dose (MTD) may not be possible to determine in the Phase I dose escalation studies, identification of a recommended Phase II dose relies on the strength of preclinical PK/PD and clinical PK data. In conditions where preclinical models are not representative of disease or molecule biology, the use of preclinical data to identify a recommended Phase II dose is limited. This results in dose finding being extended to a largely underpowered Phase II study, or costly Phase III studies, which in the past have not been definitively informative in selecting dose. Examples include dose seeking Phase III trials conducted for bevacizumab in NSCLC (AVAiL) and breast cancer (AVADO), where both the lower dose of 7.5mg/kg q3wk and higher doses of 15 mg/kg q3wk were evaluated (20, 21). While tumor based biomarkers of efficacy are most informative in confirming target modulation at the site of action, circulating biomarkers have the
potential to inform the lowest doses at which target inhibition is observed. Given the ease of frequent sampling, these biomarkers are also informative in determining the duration of target inhibition through the cycle of therapy. We employed exploratory circulating biomarkers in the Phase I study of MNRP1685A to confirm target modulation and conduct PK/PD analysis with the goal of using the results to contribute to decisions on recommended Phase II dose and schedule.

PlGF is a systemic biomarker of VEGFR2 pathway blockade and has been clinically evaluated as a biomarker of anti-angiogenesis activity for both large molecules, including bevacizumab, and small molecule TKIs, including Sunitinib (19, 22, 23). Treatment of HUVECs in 2D cultures in vitro with bevacizumab or MNRP1685A does not lead to increase in PlGF in the media (data not shown). Yet, administration of these agents in vivo results in a time dependent, and in the case of MNRP1685A, a dose dependent rise in plasma PlGF, potentially as a physiologic response not reflected by healthy endothelial cells in vitro. PlGF is a ligand for NRP1. Using BIAcore assays to assess ligand-receptor interactions, we observed that MNRP1685A is only partially able block the binding of PlGF to NRP1 (supplemental Fig. 2). Thus, ligand displacement from the receptor may not entirely explain the rise in PlGF. Since anti-NRP1 treatment also results in a rise in cNRP1, stabilization of PlGF bound to cNRP1 may explain yet another mode by which plasma PlGF is elevated. We did not however, observe a high degree of concordance in cNRP1 compared to PlGF post drug administration (supplemental Fig. 3). Thus it is likely that plasma PlGF elevation may indeed be reflective of pathway inhibition. Apart from expression on endothelial cells, NRP1 is expressed on smooth muscle cells, pericytes and plasmacytoid dendritic cells (24, 25). These cell types may contribute as likely sources of PlGF upon receptor blockade. In the single agent trial, PlGF elevation was useful to estimate the dose and duration of pathway inhibition, albeit in a surrogate tissue compartment. Assessing the biomarker in arm A in combination with bevacizumab needed additional PK/PD modeling to confirm the additive nature of the combination therapy and identify the dose at which surrogate biomarker modulation occurs.
In addition to PLGF, we observed a sustained increase in plasma VEGF with single agent MNRP1685A in patients with low baseline levels. Similar observations have been reported with small molecule TKIs like Sunitinib where all patients treated with these agents show an increase in systemic plasma VEGF, most likely due to increased release of VEGF from intracellular stores (i.e. platelets) (26). The fact that the effect with MNRP1685A is seen only in patients with low baseline levels may simply be due to loss of dynamic range in patients with higher levels where the ability to detect a 2-3 fold change may be difficult to ascertain. Interestingly, the kinetics of plasma VEGF elevation appear distinct from plasma PLGF or drug exposure where sustained elevations through the duration of the treatment cycle are observed even at doses where drug clearance is non-linear. This suggests that displacement of ligand from the receptor may not be the driver for elevated VEGF levels. It is unlikely a systemic marker given that plasma from non-tumor bearing cynomolgus monkeys dosed with MNRP1685A did not show an effect on circulating VEGF (supplemental Fig. 4). As to the role of these ligands as potential resistance mechanisms of angiogenic escape for the combination therapy, the rise in VEGF is unlikely to provide a potential mechanism for escape as bevacizuamb is administered far in excess of the released VEGF, thus neutralizing the bioactivity of the ligand. Without clear evidence, it is premature to comment on the rise of PLGF and it’s contribution to angiogenic escape as larger, adequately sized studies would be needed to further explore this hypothesis.

NRP1 is abundantly expressed in endothelial cells and can be detected in circulation (cNRP1) in mice, rats, monkeys, and humans in two forms: the naturally occurring human soluble NRP1 isoforms (sNRP1) that contain a1a2 and b1b2 domains of the ECD (27-29) and the complete NRP1 ECD shed from the membrane bound NRP1 (14). Total cNRP1 comprising both the soluble and membrane bound isoforms is expressed at moderately high levels in healthy donors, reflective of the abundant expression of the receptor on endothelial cells. Despite high baseline expression, cancer patients have 2-3 fold higher levels of cNRP1 (fig. 5A). It would be interesting to determine if baseline cNRP1 may serve as a prognostic marker of disease or predictive marker of anti-NRP1
efficacy. As an on-treatment measure of receptor occupancy, what is truly pharmacologically important is the free receptor level, including both the membrane associated mNRP1 and cNRP1. As measurement of mNRP1 level is difficult and quantitation of free cNRP1 is technically challenging, the current study demonstrated an example where circulating receptors can be used as a surrogate marker to assess receptor occupancy for molecules with membrane-bound targets, with the assistance of PK/PD modeling. Optimal dose/regimen can then be selected to achieve maximal drug effect by maintaining the free target as low and as long as possible. This offers great clinical utility, as soluble target is easily accessible. The MNRP1685A/cNRP1 PK/PD relationship is driven by the drug-cNRP1 interaction, thus the results from current analysis are not expected to be impacted by the lack of cNRP1 data from the arm containing the combination regimen with bevacizumab.

In conclusion, in this study, IV infusion of MNRP1685A resulted in a dose and time dependent elevation in plasma PlGF. PK/PD modeling support the use of this biomarker as a means to assess combination activity with bevacizumab. Using PK/PD modeling via monitoring cNRP1 as a surrogate marker for receptor occupancy measurement in the periphery, we were also able to identify a target drug concentration (i.e. 8 µg/mL) and a target dose (i.e., 25 mg/kg q3w) at which maximum target occupancy is achieved. This target dose is also potentially pharmacologically active by elevating the systemic PlGF level greater than 50% of the maximum effect by MNRP1685A. This study demonstrates the incorporation of exploratory systemic biomarkers of pathway inhibition in a Phase I study to guide Phase II dose selection.
Figure Legends

Figure 1. Systemic PlGF is induced upon MNRP1685A administration. Plasma from cynomolgus monkeys was evaluated for PlGF expression at baseline, days 2 and 28 after a single dose of MNRP1685A. Ratios to baseline levels for each animal were generated at each time point. Each dose level included n=3 animals. Data represent mean ratio ± SEM (p<0.05; paired t-test).

Figure 2. PlGF is induced upon MNRP1685A administration in humans. (A) Baseline levels of plasma PlGF were measured in Phase I patients and healthy donors. Data represent mean ±SEM (p<0.0001; student’s t-test) (B) Plasma PlGF measured through the duration of the first cycle (21 days) of single agent MNRP1685A administration is represented as a ratio to predose levels for each subject in the dose escalating arm of the Phase Ia study (*P<0.05; paired t-test) (C) Elevation in plasma PlGF in patients administered increasing doses of MNRP1685A with fixed dose of bevacizumab (*P<0.05; paired t-test). (D) Elevation in plasma PlGF by bevacizumab (20mg/kg) in a metastatic breast cancer study (n=15 patients). Y-axes represents ratio to predose levels for each subject. Data represented as mean ratio± SEM

Figure 3. AUC_{PlGF} versus MNRP1685A Dose (mg/kg): raw data and fitted curve. Black open circles: data from Phase Ia study. Solid black line: fitted curve using the sigmoid E_{max} model and Phase Ia data. Blue open triangles (up): data from Phase Ib study. Solid blue line: fitted curve using the sigmoid E_{max} model and Phase Ib data. Blue open triangle (down): bevacizumab alone (data from study AVF0766)

Figure 4. Plasma VEGF is induced upon MNRP1685A administration. (A) Plasma VEGF measured through the duration of the first cycle (21 days) is represented as a ratio to Predose levels for each subject in the dose escalating arm of the Phase Ia study. Data represent mean ± SEM. (B) Elevation in plasma VEGF at Day 14 is compared to baseline
VEGF levels. Y-axis represents the ratio of VEGF levels at Day 14 to predose levels for each subject.

Figure 5. Circulating NRP1 induced upon MNRP1685A administration. (A) Baseline cNRP1 levels measured in healthy donors (n=28), Phase I (n=32) population and plasma from vendor procured mBC (n=97), NSCLC (n=52), CRC (n=100). Data represented as mean ±SEM (Mann-Whitney test P<0.00001) (B) cNRP1 measured at baseline (predose) and days 7, 14, 21 after the first dose of MNRP1685A and plotted as a ratio to predose for each subject at each time point. Data represent mean Ratio± SEM. (student’s t-test*p<0.05)

Figure 6. Simulated concentration-time profiles of free MNRP1685A (solid black line), free cNRP1 (%baseline, solid blue line), and free mNRP1 (%baseline, dashed blue line) after every-three-week doses of MNRP1685A at 25 mg/kg in patients.
References

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Table 1

Description of trials for biomarker evaluation

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<th>Dose (mg/kg)</th>
<th>Dose Schedule</th>
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<td>Solid tumors</td>
<td>MNRP1685A</td>
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q3wk: once every three week dosing; q2wk: once every two week dosing.
Figure 1
Figure 5

A

B

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Figure 6
Pharmacokinetic and Pharmacodynamic analysis of circulating biomarkers of anti-NRP1, a novel anti-angiogenesis agent, in two Phase I trials in patients with advanced solid tumors.

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