

Research Paper

Pharmacokinetics and Safety of a Fully Human Hepatocyte Growth Factor Antibody, AMG 102, in Cynomolgus Monkeys

Tarundeep Kakkar,^{1,4,5} Mark Ma,¹ Yao Zhuang,² Aaron Patton,² Zheng Hu,² and Barbara Mounho³

Received January 17, 2007; accepted April 16, 2007; published online May 23, 2007

Purpose. AMG 102, a fully human monoclonal antibody that binds to hepatocyte growth factor (HGF), is a potential cancer therapeutic agent because of its ability to disrupt HGF/c-Met signaling pathways which have been implicated in most tumor types. To support a phase 1 study, the pharmacokinetic and safety profile of AMG 102 was assessed in cynomolgus monkeys.

Materials and Methods. Serum concentration-time data from single-(IV and SC) and repeated-dose (IV) studies of up to 13 weeks were used for pharmacokinetic analysis. Safety was assessed in a single-dose safety pharmacology study with IV doses of 0 (vehicle), 25, 100, or 300 mg/kg and a 4-week toxicity study with once weekly IV doses of 0 (vehicle), 5, 25, or 100 mg/kg.

Results. AMG 102 exhibited linear pharmacokinetics over a 600-fold dose range (0.5 to 300 mg/kg) with a mean terminal half-life of 5.6 days after IV dosing. Clearance and volume of distribution at steady state were 1.22 ml/h and 198.3 ml, respectively. Estimated bioavailability was 72% for SC administration. Antibody response to AMG 102 was observed in a small percentage of monkeys. No treatment-related cardiovascular, respiratory, or CNS changes were observed. Administration of AMG 102 for 4 weeks was well tolerated at doses up to 100 mg/kg. Potential treatment-related effects were limited to minimal/moderate gastric mucosa hemorrhage in the mid- and high-dose groups.

Conclusions. The nonclinical pharmacokinetic and safety profile of AMG 102 effectively supports clinical investigation.

KEY WORDS: anti-HGF antibody; cancer; c-Met; hepatocyte growth factor; tyrosine kinase.

INTRODUCTION

The HGF/c-Met axis is a well-characterized receptor tyrosine kinase pathway involved in multiple cellular func-

tions including proliferation, survival, motility, and morphogenesis. Accumulating data suggest that dysregulation of HGF/c-Met signaling plays an important role in many human malignancies (1,2). Expression of both HGF and c-Met in tumor cells leads to constitutive activation through an autocrine loop, a mechanism that has been described in gliomas, osteosarcomas, and breast and prostate cancers (3,4). Activating point mutations in the c-Met receptor have been reported in a variety of human malignancies, including a minority of nonsmall cell and small cell lung carcinomas (5,6). Recently, there are increasing data supporting the inhibition of HGF/c-Met pathways as a new paradigm of therapy for various malignancies (7,8). In addition, preclinical data suggest that HGF/c-Met pathway can be inhibited with peptides/antagonists of HGF, small-molecule tyrosine kinase inhibitors, and antibodies directed against c-Met or HGF (9).

AMG 102 is a fully human monoclonal antibody (IgG₂) that binds and neutralizes human HGF and is currently being developed as a cancer therapeutic agent (16). The safety and pharmacokinetic (PK) profile of AMG 102 is currently being evaluated in a phase 1 first-in-human, open-label, multiple-dose, dose-escalation study in patients with solid tumors (clinical study 20040167). To support the administration of AMG 102 in clinical trials, nonclinical studies evaluating the pharmacokinetics and safety of AMG 102 were conducted in cynomolgus monkeys.

¹Department of Pharmacokinetics and Metabolism, Amgen Inc., Thousand Oaks, California 91320, USA.

²Clinical Immunology, Amgen Inc., Thousand Oaks, California 91320, USA.

³Comparative Biology and Safety Sciences, Amgen Inc., Thousand Oaks, California 91320, USA.

⁴Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, USA.

⁵To whom correspondence should be addressed. (e-mail: tkakkar@amgen.com)

ABBREVIATIONS: AUC, Area under the concentration-time curve; CL, Systemic clearance; CL₁₂, Intercompartmental clearance; C_{max}, Observed maximum concentration; cMet, Tyrosine kinase receptor for hepatocyte growth factor; CNS, Central nervous system; ECG, Electrocardiogram; ELISA, Enzyme-linked immunosorbent assay; F, Apparent bioavailability; FOCE, First order conditional estimation; IgG₂, Immunoglobulin of subclass 2; IV, Intravenous; HGF, Hepatocyte growth factor; Ka, First-order absorption rate; OD, Optical density; PK, Pharmacokinetics; rhHGF, Recombinant human hepatocyte growth factor; SC, Subcutaneous; T_{max}, Time of observed maximum concentration; t_{1/2}, Half-life; V_{ss}, Volume at steady-state; V₁, Volume of central compartment; V₂, Volume of peripheral compartment.

The cynomolgus monkey was chosen for the safety evaluation because human and cynomolgus monkey HGF are 98% homologous, and AMG 102 binds and neutralizes cynomolgus monkey HGF. The binding affinity of AMG 102 to d5-cynomolgus monkey and d5-human HGF were 19 ± 15 and 41 ± 20 pM, respectively. The measured IC_{50} s of AMG 102 to neutralize d5-cynomolgus monkey HGF and d5-human HGF were 1.94 nM and 0.74 nM, respectively. In contrast, the sequence homology for mouse and rabbit to human HGF is 90 and 93%, respectively (the sequence for rat HGF has not been reported). In addition, AMG 102 does not bind either murine or rabbit HGF, nor does it neutralize mouse or rat HGF-mediated cellular responses (Burgess *et al.*, Abstract in AACR 2006). The route of administration and doses used in the nonclinical safety studies were selected to match the route of administration in humans and encompass intended clinical trial doses and provide adequate multiples above the expected human exposure for the determination of safety.

MATERIALS AND METHODS

Test Article

AMG 102 is a Chinese hamster ovarian cell-derived fully human monoclonal antibody against human HGF (IgG₂). AMG 102 was supplied as a frozen liquid formulation containing 30 mg/ml AMG 102 in a 5% sorbitol solution, buffered with 10 mM sodium acetate, adjusted to pH 5.2, and stabilized with 0.004% polysorbate 20. The concentrations of AMG 102 dosing solutions (original or diluted) were confirmed by UV absorption spectrometry.

Animal Husbandry

Cynomolgus monkeys (*Macaca fascicularis*) weighing approximately 2–6 kg were used for all studies. Animals were housed in stainless steel cages in a controlled environment (18–29°C; 30–70% relative humidity) on a 12-h light/dark cycle at the various sites where the in-life portion of the studies were conducted. Purina Certified Primate Diet or Harlan Teklad Primate Diet #2055C was provided daily in amounts appropriate for the size and age of the animals. Tap water was provided ad libitum. At one of the study sites, a non-certified primate diet was provided ad libitum, and the relative humidity was not controlled. Additionally, primate treats, fruits, or vegetables were provided to each monkey. All procedures employed were in compliance with the *Animal Welfare Act Regulations*, 9 CFR 1–4 and were approved by the test facilities' Institutional Animal Care and Use Committees.

In Vivo Study Design

Dose Selection

The doses used in the single-dose PK study were chosen to provide coverage over the first-in-human study dose range and to provide preliminary exposure data in the cynomolgus monkey to guide dose selection for the toxicology studies.

The low dose (5 mg/kg) used in the toxicology studies was chosen to provide more clinically relevant exposure levels whereas the high doses (100 and 150 mg/kg) were chosen to provide reasonable exposure margins to the clinical exposure. The highest dose (300 mg/kg) used in the safety pharmacology study was the maximum feasible IV dose in cynomolgus monkeys.

Single-dose Pharmacokinetic Study

In a single-dose study, 24 male monkeys (Covance Research Products Inc., Alice, TX) were assigned to six groups. Animals were administered a single dose of 0.5, 1, 5, 10, or 50 mg/kg AMG 102 by intravenous (IV) bolus administration and 75 mg/kg AMG 102 by subcutaneous (SC) administration on day 1. Blood samples for PK analysis were collected from each animal predose and at 0.083 (IV groups only), 0.5 (IV groups only), 2, 4, and 8 h postdose. Additional samples were collected on days 2, 3, 4, 5, 8, 9, 11, 15, 22, 29, 36, and 43.

Single-dose Safety Pharmacology Study

Young adult male cynomolgus monkeys (imported from China by Scientific Resources International, Ltd.; Charles River Laboratories, Inc., [Sierra Division], Sparks, NV) were administered a single bolus IV injection of 0 (control), 25, 100, or 300 mg/kg AMG 102 (4/group) on day 1. All animals were evaluated for changes in cardiovascular, respiratory, and CNS function over a 7-day observation period and returned to the in-house colony at study completion. All evaluations were performed in conscious monkeys with surgically implanted telemetry transmitters.

Body temperature, cardiovascular data (heart rate, systolic and diastolic pressure, blood pressure, electrocardiogram, [ECG]) and respiratory data (rate and waveform) were recorded by telemetry. External ECGs were also recorded, and quantitative (manual) measurements (RR, QT, and heart rate-corrected QT [QTc] intervals) were performed. In addition to respiratory data recorded by telemetry, arterial blood samples were collected for determination of blood gases (partial pressure of CO₂ and O₂, pH, and hemoglobin oxygen saturation measurements).

Evaluations of CNS function included examination of behavior (e.g., awareness, alertness, responses to movement outside of the cage), motor function (e.g., strength, coordination, patellar reflex), sensory functions (e.g., eyelid responses, eye movements, auditory response), and proprioception (e.g., postural/gait reactions).

Beginning approximately 60 min prior to dose administration and continuing through 168 h postdose, heart rate, systolic and diastolic pressure, mean arterial blood pressure, respiratory rate, and body temperature were recorded from each animal for 30 s at 3-min intervals. Approximately 60 min prior to dose administration through 2 h postdose, the ECG, arterial blood pressure and respiratory waveforms were recorded for 30 s at 9-min intervals. From 2 through 168 h postdose, ECG, arterial blood pressure and respiratory waveforms were recorded for 30 s at up to 8-h intervals. In addition, quantitative measurements of the QT- and RR-interval from a QRS complex in each 30-s recording were

performed for three timepoints prior to dosing and at approximately 9, 18, 27, 36, 45, 54, 63, 72, 81, and 90 min postdosing and 2, 4, 8, 12, and 24 h postdosing. Arterial blood samples for blood gas analysis were collected prior to dosing, and at 2 and 24 h postdose. CNS evaluations were conducted prior to dosing, and 24 and 168 h postdose.

Blood samples for PK analysis were collected predose and at 2 and 8 h postdose. Additional samples were collected on days 2 and 8.

Repeated Dose: 4-week Toxicity Study

For the 4-week repeated-dose toxicity study, young adult, experimentally naïve male and female cynomolgus monkeys (imported from China by Scientific Resources International, Ltd.; Charles River Laboratories, Inc., [Sierra Division], Sparks, NV) were administered AMG 102 once weekly at doses of 0 (control), 5, 25, or 100 mg/kg by IV bolus injection for four consecutive weeks. Main study animals (four monkeys/sex/group) were terminated on study day 29. Recovery animals (two monkeys/sex in vehicle-control and high-dose groups) were terminated at the completion of a 6-week recovery period after cessation of dosing (day 64) to assess the reversibility of any potential treatment-related effects.

Throughout the study, all animals were observed twice daily (morning and evening) for clinical signs. Food consumption observations occurred once daily as part of the routine cageside observations except when the animals were fasted for study procedures. Individual body weights were recorded for all animals before the first dose (week-2, week-1, and day-1), on study days 7, 14, 21, 28, and for recovery animals on days 35, 42, 49, 56, and 63. ECG and ophthalmic examinations were performed prestudy, on study days 15 (ECG only) and 28 (all animals), and near the end of week 9 (recovery animals).

All animals were evaluated for clinical pathology indices. Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from monkeys (fasted overnight) twice before initiation of dosing and on study days 15 (predose), 29, and 64 (recovery animals). Urine samples for urinalysis were collected before initiation of dosing, on study day 15 (predose), and at necropsy.

Blood samples for PK analysis were collected at predose, 0.25, 0.5, 2, and 8 h postdose on day 1. Additional samples were collected on days 2, 4, 8 (predose), 15 (predose), 22 (predose and 0.25, 0.5, 2, and 8 h postdose), and 29. Main study animals were euthanized after the sample collection on day 29. Blood samples were collected on days 43, 50, 57, and 64 from recovery animals (2/sex each from 0 and 100 mg/kg dose groups) after treatment was stopped. Recovery animals were terminated 6 weeks after the last dose (study day 64).

A complete necropsy was performed on all animals; routine organ weights were measured and organ-to-body weight ratios (using the final body weight obtained prior to necropsy) and organ-to-brain weight ratios were calculated. Tissues were collected, preserved in neutral-buffered 10% formalin (except the eyes [Davidson's fixative] and testes [modified Davidson's fixative]), embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination.

Repeated Dose: 13-week Pharmacokinetic Study

In a 13-week repeated-dose study, 48 (24/sex) monkeys (Covance Research Products Inc., Alice, TX; Covance Laboratories, Madison, WI) were assigned to four groups. Animals were administered 13 once-weekly doses of 0 (control), 5, 25, or 150 mg/kg AMG 102 by IV bolus injection. Blood samples for PK analysis were collected predose, 0.25, 0.5, 2, and 8 h postdose on the first and last day of dosing, days 1 and 85. Additional samples were collected during the treatment period on days 2, 4, and predose on days 8, 22, 36, 50, 64, and 78. After treatment was stopped, blood was collected on day 92, and 32 animals (4/sex/group) were euthanized. Blood samples were collected on days 106, 120, 134, 148, 162 and 169 from the remaining 16 animals in the recovery groups (2/sex/group); these animals were terminated 12 weeks after the last dose (study day 169).

Assay Methods

Recombinant human HGF (rhHGF), and biotin-labeled anti-AMG 102 polyclonal (rabbit) antibody were made at Amgen Inc. Streptavidin-conjugated horseradish peroxidase was purchased from R&D Systems (Minneapolis, MN).

Serum concentrations of AMG 102 were determined with an enzyme-linked immunosorbent assay (ELISA). Briefly, serum samples were diluted within the calibration range and added to microplate wells coated with rhHGF. After unbound AMG 102 was removed by washing the wells, a biotin-labeled anti-AMG 102 polyclonal (rabbit) antibody was added to the wells for detection of the captured AMG 102. This step was followed by addition of streptavidin-conjugated horseradish peroxidase. After another washing step, a substrate was added to the wells and then reacted with the peroxidase to create a colorimetric signal that was proportional to the amount of AMG 102 bound to the rhHGF in the initial step. The color development was stopped after 20 min of incubation, and the intensity of the color (optical density) was measured at 450 nm with reference to 650 nm. The optical density values were compared with a concurrently analyzed standard curve that was regressed according to a 4-parameter model using Watson™ LIMS 7.0 (Thermo, PA).

Reproducibility and accuracy of the assay were determined by spiking control monkey serum with AMG 102 and analyzing replicates ($n=1064$, stored frozen as authentic serum samples at -60°C or below). These samples were stable for at least the time period spanning the collection and analysis of authentic serum samples. The inter-run coefficients of variation ranged from 7 to 10%, respectively, in the concentration range of 31.25 to 2,000 ng/mL. Average assay accuracy ranged from -3 to 7%.

Antibody Analysis

Two validated assays were used to test for anti-AMG 102 antibodies. The first was an acid-dissociation bridging ELISA to detect and confirm the presence of anti-AMG 102 antibodies. Assay criteria for positive or negative results were established and validated with 44 individual donor serum samples.

Once anti-AMG 102 antibodies were found by immunoassay, a second cell-based bioassay was used to detect neutralizing or inhibitory activity toward AMG 102. The bioassay is based on AMG 102 inhibition of HGF-induced c-Met phosphorylation. The presence of neutralizing anti-AMG 102 antibodies would inhibit AMG 102 activity and therefore restore HGF-induced c-Met phosphorylation. Assay criteria for positive or negative results were established and validated with 50 individual donor serum samples. In the single-dose PK study, samples were collected at predose and day 43. In the 4-week multiple-dose study, samples were collected at predose, day 15, day 29, and day 64 (recovery animals). In the 13-week repeated-dose study, samples were collected at predose, day 41, day 85, and day 169 (recovery animals).

Pharmacokinetic Evaluation

Serum concentration-time data from the four studies (IV and SC) described above were fitted simultaneously to a 2-compartment model, with dosing either into the central compartment (for IV dosing) or dosing into the depot compartment followed by first-order absorption into the central compartment (for SC dosing), using NONMEM software (version V) and a population-based approach (10). Actual doses, mg/animal, were used to fit the data. Data from animals that developed anti-AMG 102 antibodies and showed an obvious decrease in exposure were excluded from the analysis. The first-order conditional estimation (FOCE) method was implemented to estimate PK parameters. The primary PK parameters estimated were systemic clearance (CL), intercompartmental clearance (CL12), volume of central compartment (V1), volume of peripheral compartment (V2), first-order absorption rate (Ka), and apparent bioavailability (*F*); PREDPP subroutines ADVAN4 and TRANS4 were used. An exponential error model was used to describe interindividual variability in the PK parameters. For example, interanimal variability in CL was modeled as:

$$CL_i = \theta_1 \times e^{\eta_1}$$

where CL_i denotes individual animal value, θ_1 the true value for clearance and η_1 the interanimal differences in θ_1 . The η s were assumed to be normally distributed with mean equal to 0 and interanimal variance equal to ω^2 . The variance-covariance matrix Ω was unconstrained, ie, the BLOCK option was used.

Residual variability was also assumed to follow an exponential error model:

$$y_{ij} = f \times e^{\varepsilon_{ij}}$$

where y_{ij} is the concentration value for the i th animal at the j th time point; f represents the model predicted concentration value; residual error (ε_{ij}) was assumed to follow a normal distribution with mean of 0 and variance equal to σ^2 . Since NONMEM treats an exponential error model as a proportional error (constant variance) model when applied to residual variability, the log-transformed data were fitted so that an exponential error model was applied:

$$\ln(y_{ij}) = \ln(f) + \varepsilon_{ij}$$

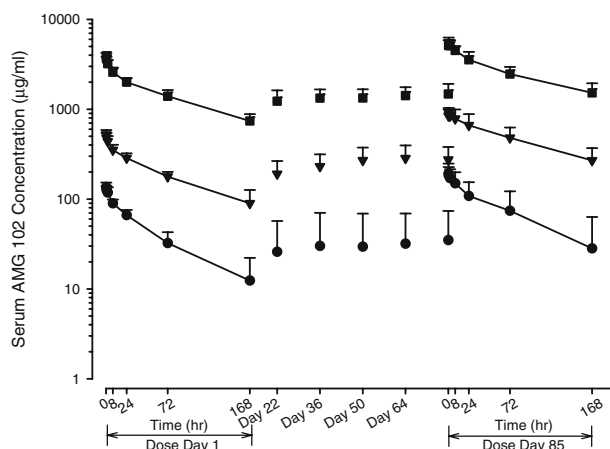


Fig. 1. Mean (+SD) Serum AMG 102 concentration-time profile by dose after once weekly IV administration of 5 (filled circle), 25 (filled triangle), or 150 (filled square) mg/kg AMG 102 for 13 weeks to cynomolgus monkeys ($n=12$ /group).

where \ln denotes the natural log transformation of the variable.

The effects of sex and body-weight as covariates on CL, CL12, V1 and V2 were explored. Model selection criteria included reduction of objective value function, evaluation of precision of parameter estimates, visual inspection of predicted versus observed concentrations, and plots of weighted residuals versus time or predicted concentration (11).

Tissue Cross-reactivity

The tissue cross-reactivity of AMG 102 was evaluated in cryosections from a full panel of normal human and cynomolgus monkey tissues. AMG 102 was fluorescein-conjugated in order to optimize the detection of AMG 102 tissue binding. The cross-reactivity of AMG 102 was also evaluated in a select panel of mouse, rat, and rabbit tissues known to express HGF (e.g., heart, kidney, liver, lung, spleen) to determine if any of these species could be potential animal models for toxicology studies. Fluorescein-conjugated human IgG₂ served as an isotype control antibody. All tissues were evaluated with two different concentrations

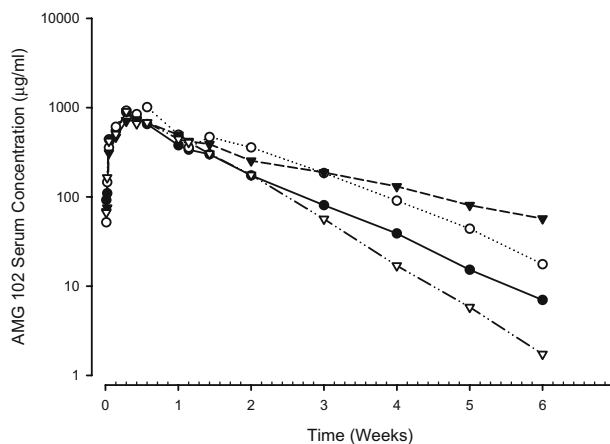


Fig. 2. Individual animal serum AMG 102 concentration-time profiles after a single subcutaneous injection of 75 mg/kg AMG 102 to cynomolgus monkeys ($n=4$).

of fluorescein-AMG 102 or fluorescein-IgG₂, 0.5 µg/ml (the optimal binding concentration) and 2.5 µg/ml (5× the optimal binding concentration).

RESULTS

Pharmacokinetic Evaluation

Serum AMG 102 concentrations after IV administration showed a biexponential elimination profile (Fig. 1). After a

single SC administration, the mean observed maximum concentration (C_{max}) was 884 µg/ml (13% CV), and the median observed time to C_{max} (T_{max}) was 2.5 days (range: 2–4 days; Fig. 2).

Since the PK profiles suggest that a 2-compartment model is appropriate, a 1-compartment model was not evaluated. During the model building process, initially inter-individual variability terms were added to all the PK parameters. Since only 4 of 100 animals were dosed SC, and the T_{max} and C_{max} values were not very different between

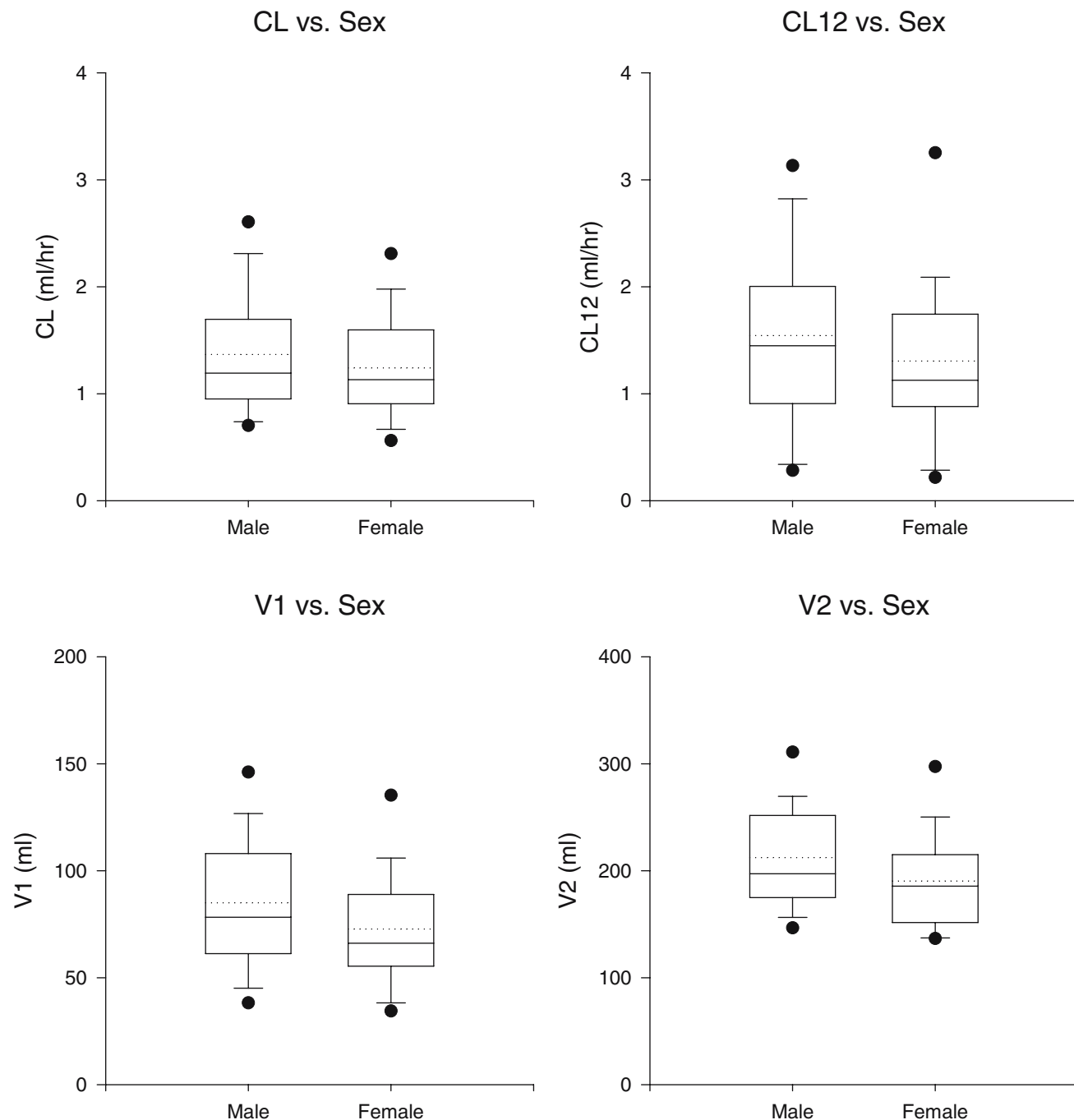


Fig. 3. Box and whiskers plot of CL, CL12, V1 and V2 vs. sex. The boundary of the box closest to zero indicates the 25th percentile, the solid and dotted lines within the box marks the median and mean, respectively, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. The symbol closest to zero indicates the 5th percentile and the symbol farthest from zero indicates the 95th percentile.

Table I. Pharmacokinetic Parameters of AMG 102 Following Intravenous Bolus and Subcutaneous Administration to Cynomolgus Monkeys

Parameter	Estimate	RSE ^a (%)	CV ^b (%)	RSE ^c (%)
CL (ml/h)	1.22	4.2	41	14
CL12 (ml/h)	1.19	13	82	21
V1 (ml)	123	2.2	18	18
V2 (ml)	75.3	7.8	45	35
Ka (h ⁻¹)	0.0216	7.9	–	–
F (%)	72	6.2	–	–
Intra-individual error (%)	21	15	–	–
No. of observations	1,559	–	–	–
No. of subjects	100	–	–	–

^a Relative standard error of estimate for PK parameters.

^b Interanimal variability (coefficient of variation).

^c Relative standard error of estimate for variance parameters.

animals, interindividual variance terms had negligible values for Ka and F, and removal of those terms from the model did not affect the objective function. Each of the variance terms included in the final model caused a significant increase in the objective function if they were removed. Therefore, the final model had interindividual variability terms for CL, CL12, V1 and V2, but not for Ka or F.

The effect of sex as a covariate was explored as shown in Fig. 3 in box plots generated from individual bayesian estimates of PK parameters. Based on a 2-tailed unpaired t-test, statistically ($\alpha=0.05$) only V1 was found to be significantly ($p=0.0354$) different between males and females. However, the difference between the means was only 9.467 ± 4.437 ml (95% confidence interval was 0.6494 to

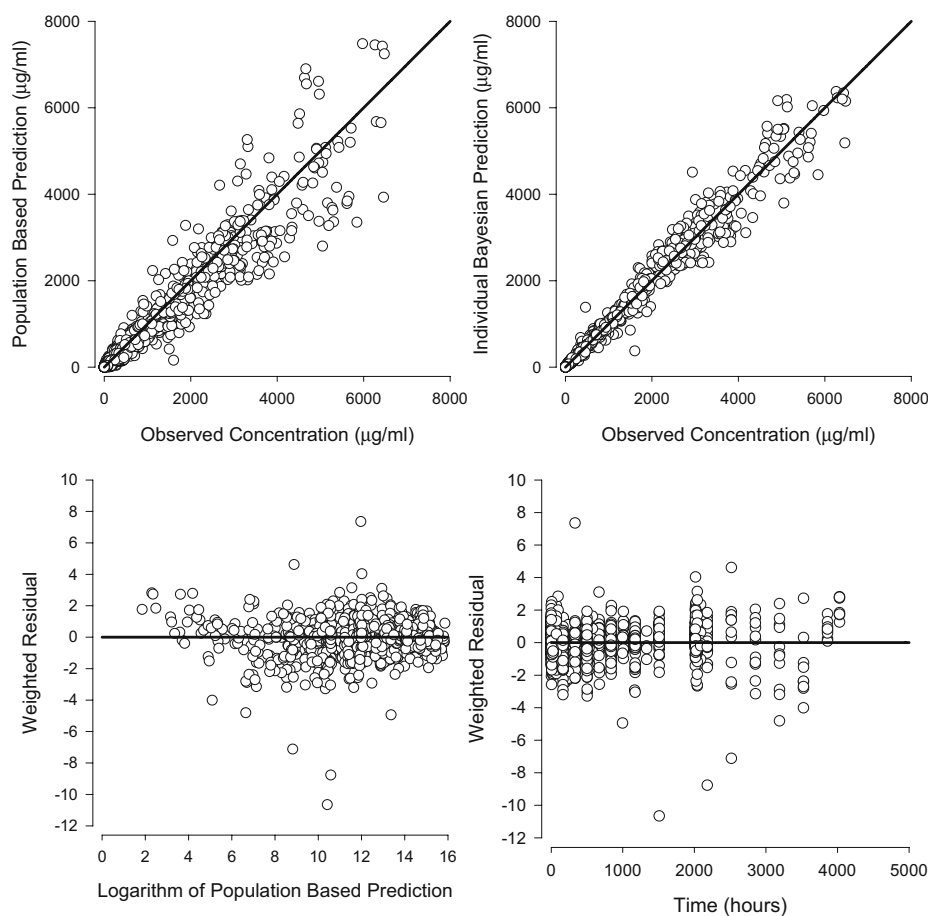


Fig. 4. Diagnostic plots for the final PK model for AMG 102 in monkeys. *Clockwise from the upper left panel* are population predicted values vs. observed values, individual bayesian predicted values vs. observed values, weighted residuals vs. time, and weighted residuals vs. log-transformed population predicted concentration values.

Table II. Four-week Toxicity Study: Incidence and Severity of Gastric Hemorrhage Observed in Cynomolgus Monkeys

Dose (mg/kg) ^a	No. of monkeys (main study)	No. of monkeys (recovery)
0 (vehicle control)	0	2 ^c
5	0	–
25	2 ^a	–
100	4 ^b	1 ^d

^a Gastric hemorrhage was graded as minimal in both animals.

^b Gastric hemorrhage was graded as minimal (two animals), mild (one animal), and moderate (one animal).

^c Gastric hemorrhage was graded as minimal in one animal and mild in the other animal.

^d Gastric hemorrhage was graded as mild.

18.28). Also, when sex was included as a covariate in the final model, the objective function did not decrease appreciably (the maximum difference was 2.767 which was obtained when sex was included as a covariate on V1). These results suggest that PK parameters were not different between males and females. When weight was included as a covariate, the objective function decreased appreciably (the difference was 20.047) only when it was included as a covariate on V1. However, the minimization algorithm in NONMEM did not converge successfully and the population parameter estimate for V1 ranged only from 91% (for the lowest weight of 2 kg) to 121% (for the highest weight of 5.59 kg) of the median (2.8 kg). Therefore, weight was not an important covariate either.

Based on visual examination, the plots of individual Bayesian estimates of PK parameters across the four studies did not show any trend, suggesting that PK did not change across these studies. The parameters were well estimated as shown in Table I. The diagnostic plots did not show any bias for the data fit, suggesting that the 2-compartment PK model fits the data adequately (Fig. 4). These results also suggest that the PK for AMG 102 is linear in monkeys, and the accumulation (accumulation ratio ranged from 1.4 to 2.5) upon multiple dosing was as expected.

The interanimal variability for CL, V1, and V2 ranged from low to moderate, whereas the interanimal variability for CL12 was high, suggesting that distribution among animals was varied. The imprecise estimate of interanimal variability for V2 suggests that the moderately high variability for V2 could also be due to limited PK sampling schedule in the initial distribution phase. The V_{ss} (V1 + V2) estimate of 66 ml/kg (average animal weight was 3 kg) is approximately 1.5 times the serum/plasma volume (12). This estimate of V_{ss} is based on the assumption that AMG 102 is being eliminated from only the central compartment (13). The terminal half-life ($t_{1/2}$) was estimated to be 5.6 days. After SC dosing, the absorption half-life (calculated as $\ln(2)/K_a$) of 1.3 days was much shorter than the $t_{1/2}$ of 5.6 days confirming that drug elimination rather than the SC absorption was the rate-limiting step and also confirming that terminal half-lives after SC and IV dosing were similar.

In the single-dose PK study, two out of 24 monkeys developed anti-AMG 102 antibodies; one of these animals developed neutralizing antibodies, and the exposure to AMG 102 significantly decreased in the terminal phase for this animal. In the 13-week repeated-dose PK study, three out of 36 monkeys developed anti-AMG 102 antibodies; one of these animals developed neutralizing antibodies, and the exposure to AMG 102 was affected in the terminal phase for this animal. None of the animals developed anti-AMG 102 antibodies in the 4-week repeated-dose toxicity study.

Safety Evaluation

Monkey Toxicity Studies

AMG 102 was well tolerated in cynomolgus monkeys at doses up to 300 mg/kg in the safety pharmacology study. No evidence of treatment-related changes were noted for clinical signs, food consumption, body temperature, or evaluated cardiovascular, respiratory, or CNS parameters.

In the 4-week toxicity study, administration of AMG 102 over a 4-week period was well tolerated by cynomolgus monkeys, and all animals survived to their respective scheduled necropsy. No treatment-related effects on clinical observations, food consumption, body weight, ECGs, ophthalmic examinations, clinical pathology parameters (serum chemistry, hematology, coagulation, urine chemistry, urinalysis), or organ weights were observed in any of the animals. Necropsy observations in main study animals (terminated on study day 29) were limited to isolated red foci or red discoloration in the stomach mucosa in two of eight animals in the mid-dose (25 mg/kg) group and two of eight animals in the high-dose (100 mg/kg) group. Correlative microscopic findings of minimal to moderate gastric hemorrhage were noted in two of eight mid-dose animals (the same animals that showed macroscopic changes) and four of eight high-dose animals (two animals showed macroscopic changes; Table II). The gastric hemorrhage was focal to multifocal in the mucosa of affected animals and extended into the submucosa in a single high-dose animal with moderate mucosal hemorrhage. Minimal to mild gastric hemorrhage was also noted in recovery animals (terminated after a 6-week no-treatment period) and was similar in character, frequency, and severity to the gastric hemorrhage observed in the main study animals. The gastric hemorrhage observed in recovery animals was considered incidental because this finding was observed in both vehicle control (two of four monkeys) and high-dose (one of four monkeys) groups. Thus, the gastric hemorrhage observed in treated main study animals may also be incidental with coincidental distribution in treated animals. No other macroscopic or microscopic changes were noted in main study or recovery animals.

Tissue Cross-reactivity

Binding of fluorescein-conjugated AMG 102 (fluorescein-AMG 102) in human and cynomolgus monkey tissues was observed primarily in epithelial cells in various tissues (e.g., breast, liver, lung, skin). Overall, the pattern of tissue binding of fluorescein-AMG 102 in cynomolgus monkey tissues was

similar to human tissues, which further supports the utility of the cynomolgus monkey as a relevant model for nonclinical safety studies. In mouse, rat, and rabbit tissues, binding of fluorescein-AMG 102 was restricted mainly to the endothelium (e.g., heart, liver), and minimal binding to lymphocytes was detected in mouse spleen. Unlike the epithelial cell binding pattern observed in human and cynomolgus monkey tissues, no specific epithelial cell binding was observed in tissues from mouse, rat, or rabbit.

DISCUSSION

HGF was first identified as a molecule that stimulated hepatocyte proliferation. It has been reported also that HGF promotes not only cell proliferation, but also motility, invasiveness, morphogenesis, and angiogenesis (14). HGF is mainly produced by mesenchymal cells, and its biological signal is transmitted from mesenchymal cells to epithelial cells through HGF receptor, a *c-met* proto-oncogene product. Over-expression of *c-Met* cDNA in NIH-3T3 cells has been reported to make the cells tumorigenic and show enhanced invasion and metastasis in nude mice. (15) Thus, blocking the activation of the HGF/*c-Met* pathway may provide an effective therapeutic strategy in the treatment of cancer. The studies presented in this paper report the pharmacokinetics and safety of AMG 102 in cynomolgus monkeys. The cynomolgus monkey was used as the test species because human and cynomolgus monkey HGF are 98% homologous, and AMG 102 binds and neutralizes cynomolgus monkey HGF.

The 2-compartment PK model described the AMG 102 serum concentration-time data from the two single-dose and two repeated-dose studies adequately. Typically, noncompartmental analysis is performed for each study separately (especially data from toxicology studies). If compartmental analysis is conducted, it is performed for PK studies using the 2-stage approach, fitting data from each animal and calculating descriptive statistics for each parameter. The 2-stage approach allows for formal, statistical assessment of dose-proportionality (e.g., comparison of single-dose and steady-state AUC and C_{\max} values) and the 2-stage approach can be useful in estimating initial parameter estimates for typical values in the population-based PK modeling. However, the 2-stage approach is only appropriate if the data set from each animal is adequate to describe the full PK profile. If the data are sparse, as in the case of 1 of the single-dose studies, the data may not be amenable to fitting using the 2-stage approach. The population-based PK modeling was used so that both the complete and sparse datasets could be evaluated simultaneously. This approach allowed a comprehensive evaluation of the PK from all available data and allowed evaluation of any study-related differences in PK. In addition, once the data were compiled, the run times were much faster as compared with the 2-stage modeling approach that was performed initially.

By estimating the intercept (a) and exponent (b) for CL and V_{ss} from linear fit of the equation $\log(\text{CL or } V_{ss}) = \log(a) + b \cdot \log(\text{Weight})$, AMG 102 exposure observed in one species may be used to predict exposure in another. Since AMG 102 binds only to primate HGF, PK analyses from

nonprimate species were not used to perform interspecies scaling. To estimate PK parameters for humans the intercepts for CL and V_{ss} were estimated while the exponents for CL and V_{ss} were fixed to 0.75 and 1, respectively. CL, V_{ss} and $t_{1/2}$ in humans were estimated to be 13.0 ml/h, 4,627 ml, and 10.3 days, respectively. These values are similar to the NONMEM estimated preliminary "population" mean values for CL, V_{ss} , and $t_{1/2}$ from data from 13 subjects in the first-in-human study, which were 13.4 ml, 4,770 ml, and 10.4 days, respectively.

Since AMG 102 is a fully human antibody, it was expected that administration of the drug to monkeys might result in the development of anti-drug antibodies in some animals. Indeed, anti-AMG 102 antibodies were detected in a small number of animals: two of 24 (8%) of animals in the single-dose PK and three of 36 (8%) of the animals in the 13-week repeated-dose study. Among these animals that developed anti-AMG 102 antibodies, approximately half also developed neutralizing antibodies. The exposure to AMG 102 was affected in the terminal phase in these animals. Since the production of anti-drug antibodies affected drug exposure, the affected data from the anti-AMG 102 antibody positive animals were excluded from the PK analysis.

The tissue cross-reactivity study demonstrated that the binding pattern of AMG 102 was similar in human and cynomolgus monkey tissues and was observed primarily in epithelial cells. Single-dose administration of AMG 102 to cynomolgus monkeys was well tolerated at doses up to 300 mg/kg in the safety pharmacology study, and no treatment-related effects on cardiovascular, respiratory, or CNS function were observed. The 4-week toxicity study indicated that once-weekly IV administration of AMG 102 for 4 consecutive weeks was well tolerated by cynomolgus monkeys at doses up to 100 mg/kg/week. Changes noted in this study were limited to microscopic findings of gastric hemorrhage in some main study animals (study day 29) in the mid- and high-dose groups. Gastric hemorrhage was also observed in recovery animals (study day 64); the finding was considered incidental at this time point since it was observed in both vehicle control and AMG 102-treated animals. The gastric hemorrhage observed in treated animals at day 29 was similar to that observed in recovery animals and, therefore, might also be incidental with a coincidental distribution in AMG 102-treated animals. Thus, it is highly unlikely that the gastric hemorrhage was related to AMG 102, although the potential of this change being treatment-related cannot be completely ruled out at this time. Regardless, this finding was not a chronic process and did not adversely affect the health status of the animals.

CONCLUSION

The cynomolgus monkey proved to be an appropriate species to evaluate toxicity in terms of providing a PK profile that allowed close estimation of AMG 102 exposure in humans and demonstrating similar tissue cross-reactivity. AMG 102 administered IV once weekly was well tolerated by cynomolgus monkeys at doses up to 100 mg/kg/week in the 4-week toxicity study conducted to support a phase 1 study. These findings indicate that the nonclinical toxicology program defined a favorable safety profile for AMG 102.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Parnian Zia-Amirhosseini for reviewing this manuscript and providing helpful comments and Dr. Laura Healy for performing a peer review of the microscopic findings in the 4-week toxicity study. Ms. Tsui-Chern Cheah contributed to the technical preparation of this manuscript.

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