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ORIGINAL ARTICLE

# Pharmacokinetics and safety of bevacizumab administered in combination with cisplatin and paclitaxel in cynomolgus monkeys

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**Abstract**
*Purpose* Bevacizumab is the first anti-angiogenic monoclonal antibody approved for use in combination with chemotherapy for treatment of a variety of solid tumors. The objective of this study was to evaluate the safety of bevacizumab when administered concomitantly with paclitaxel and cisplatin to cynomolgus monkeys, and to assess the pharmacokinetic and safety interactions between bevacizumab and the two chemotherapeutic agents.
*Methods* Twenty male cynomolgus monkeys (*Macaca fasicularis*) were randomized to one of four treatment groups: vehicle, bevacizumab alone, cisplatin alone, and the combination of cisplatin and bevacizumab. Blood collection over serial time points allowed determination of the pharmacokinetic parameters of paclitaxel and bevacizumab and the maximum concentration ($C_{max}$) for cisplatin. Drug concentrations were determined by graphite-furnace atomic absorption, high performance liquid chromatography, and enzyme-linked immunosorbent assay methods, for cisplatin, paclitaxel, and bevacizumab, respectively.
*Results* AUC0-t values for bevacizumab when administered alone or in combination with chemotherapy were $6{,}747 \pm 1{,}872$ and $7{,}366 \pm 1{,}599$ μg/ml × day, respectively. AUC0-t values for paclitaxel with or without concomitantly administered bevacizumab were $10.9 \pm 2.9$ and $10.3 \pm 3.7$ μg/ml × day, respectively. No alterations in the $C_{max}$ of bevacizumab, paclitaxel, or cisplatin were observed between any of the treatment groups. As expected, based on their known safety profile, the administration of cisplatin and paclitaxel were associated with vomiting, decreased body weight, and transient decreases in white blood cell and absolute neutrophil counts; concomitant bevacizumab administration did not alter the incidence or severity of these toxicological effects.
*Conclusion* Pharmacokinetic estimates for bevacizumab, paclitaxel and cisplatin indicate that combination of bevacizumab with the two chemotherapeutic agents does not result in a pharmacokinetic interaction. Moreover, the addition of bevacizumab to the chemotherapy regimen did not appear to alter the safety profiles of cisplatin/paclitaxel in cynomolgus monkeys. Results from the present study supported the clinical development of bevacizumab treatment regimens in combination with the chemotherapeutic agents paclitaxel and cisplatin.

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## Introduction

The angiogenic signal cascade, stimulated by proangiogenic factors such as vascular endothelial growth factor (VEGF; also referred to as vascular permeability factor),

leads to phenotypic changes in endothelial cells that line tumor blood vessels, allows the tumor to expand rapidly, invade surrounding tissues, and metastasize [1, 2]. Blockade of the angiogenic signal cascade by antibodies to VEGF has been reported to suppress tumor growth in animal models bearing human tumor xenograft [3]. The synergistic antitumor activity of antibodies to VEGF and cytotoxic chemotherapy has also been demonstrated in animal models [4–7]. The mechanism by which VEGF blockade potentiates the cytotoxic effects of chemotherapeutic agents is not completely understood but may result from changes in vascular functions (i.e. decreased vessel density, diameter and permeability), reduction in interstitial fluid pressure and improvements in intratumoral uptake of chemotherapeutic agents [8, 9].

Bevacizumab (rhuMAb VEGF, Avastin®, Genentech, Inc., South San Francisco, CA, USA) is a humanized monoclonal antibody targeting human VEGF. It contains the human immunoglobulin G1 framework (93%) and murine VEGF-binding complementary-determining regions (7%). Clinical development plans included, among others, the combination of bevacizumab with chemotherapies as treatment for first- or second-line colorectal cancer (in combination with 5-FU/irinotecan/leucovorin) [10, 11], first-line non-small cell lung cancer (in combination with carboplatin/paclitaxel) [12, 13], metastatic breast cancer (in combination with paclitaxel) [14], and cisplatin/paclitaxel in ovarian cancer [15]. Plans for the combination of bevacizumab with a broad variety of small molecule chemotherapeutic agents prompted the investigation of whether bevacizumab may alter the pharmacokinetic disposition and/or safety profile of the chemotherapeutic agents through potential changes in vessel biology or other unknown mechanisms. The cynomolgus monkey served as the nonclinical species for pharmacokinetic and safety evaluation in this study, as human and cynomolgus monkey VEGF were predicted to have identical amino acid sequences [16] and bevacizumab has been shown to bind to VEGF in cynomolgus monkeys. In support of the clinical development plan in ovarian cancer, the purpose of this study were to investigate: (1) potential pharmacokinetic interactions between bevacizumab and the chemotherapeutic agents cisplatin and paclitaxel, and (2) the safety of bevacizumab when administered in combination with cisplatin and paclitaxel.

## Materials and methods

### Test material

Bevacizumab (Lot No. M3-RD595, 10 mg/ml) and bevacizumab vehicle, (Lot No. M3-RD588, 0 mg/ml) were supplied as clear liquids. Paclitaxel and cisplatin (Taxol® and Platinol-AQ®, respectively, Bristol Myers Squibb Oncology, Princeton, NJ) were purchased commercially.

### Species and husbandry

Twenty-six male cynomolgus monkeys (*Macaca fascicularis*) were obtained from HRP, Inc. (Denver, PA) and the Virginia facility of Corning Hazleton Inc. The animals were quarantined for 38 days before initiation of treatment. Animals were approximately 4–10 years old and weighed 3.8–5.9 kg at initiation of treatment. Environmental controls were set to maintain a temperature of 19–26°C (66–79°F) with a relative humidity of 30–70%, and an approximate 12-h light/dark cycle. Animals were housed individually in stainless steel cages. Certified primate diet (PMI® Feeds, Inc.) was provided, except during protocol-defined intervals of fasting. Water was provided ad libitum throughout the study. Fruit, vegetable or additional supplements were provided. There were no known contaminants in the food or water which were expected to interfere with the objectives of the study. The study was approved by the Institutional Animal Care and Use Committee and all procedures in this protocol were performed in compliance with the Animal Welfare Act Regulations, 9 CFR 3.

### Randomization

Twenty animals were stratified by weight and randomly assigned to one of four treatment groups ($n = 5$ animals per group) as shown in Table 1.

### Dosing solution

Bevacizumab (10 mg/ml), bevacizumab vehicle (0 mg/ml) and cisplatin (1.0 mg/ml) were used as supplied. Paclitaxel was prepared in glass vials at a concentration of 1.2 mg/ml using 0.9% sodium chloride as diluent. The doses of cisplatin, paclitaxel and bevacizumab were chosen based on previous experience with these agents in toxicology studies

**Table 1** Dose groups

| Group[a] | No. of animals | Bevacizumab (10 mg/kg) | Cisplatin (1 mg/kg) | Paclitaxel (4 mg/kg) |
|---|---|---|---|---|
| 1 | 5 | 0 | 0 | 0 |
| 2 | 5 | 10 | 0 | 0 |
| 3 | 5 | 0 | 1 | 4 |
| 4 | 5 | 10 | 1 | 4 |

[a] Order of administration on day 18: bevacizumab (or bevacizumab vehicle), cisplatin (or saline), paclitaxel (or saline)

which when dosed on a mg/kg basis would provide an appropriate toxicologic response.

### Dosing procedures

Table 1 outlines the dosing groups in this study. Bevacizumab (10 mg/kg) or bevacizumab vehicle (0 mg/kg) were administered on days 1, 4, 8, 11, 15, and 18 via intravenous (IV) bolus injection. At the time of this study, the pharmacokinetics of bevacizumab in humans was unknown, the dosing regimen used in this study was similar to that used in previous toxicology studies, which were shown to be safe in these animals, attain relevant concentrations quickly, and provide exposures relevant to murine tumor xenograft models and provide concentrations for predicted human exposure based on allometric scaling to allow robust testing of the interaction. Cross-over occurred on day 18, when bevacizumab exposure in the animals was believed to be adequate for investigating drug interaction, although bevacizumab steady state had not been achieved. Cisplatin and paclitaxel were administered on day 18 following the bevacizumab or bevacizumab vehicle dose. Cisplatin (1 mg/kg) or saline were given via IV bolus injection which was followed by a 1-h IV infusion of paclitaxel (4 mg/kg) or saline using IVEX®-HP filter sets with a 0.22-micron in-line filter. Doses were administered into the saphenous vein and were adjusted based on the most recent body weight measured before dosing.

### Antemortem observations

Routine physical examinations were performed twice before the initiation of treatment (days 2 and 1) and during weeks 2, 3, and 6. Animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. Animals were observed twice daily (a.m. and p.m.) on nondosing days; predose and approximately 1 h postdose on days 1, 4, 8, 11, and 15; and predose and approximately 0.5, 1, 2, 4, and 6 h after the start of the infusion on day 18 for signs of poor health or abnormal behavior. Obvious signs were recorded as they were observed. Individual body weight data were recorded weekly before initiation of treatment and at approximately 3-day intervals until day 46 post treatment. Food consumption was confirmed qualitatively by daily visual inspection. Physical examinations and electrocardiographic measurements were performed twice before initiation of treatment and during weeks 2, 3, and 6. Blood and urine were collected from each animal twice before initiation of treatment and during weeks 2, 4, 5, and 7. Animals were fasted overnight, and urine was collected before blood sampling. Routine clinical chemistry, hematology, urinalysis, and urine chemistry endpoints were measured.

### Blood collection procedures and processing

Blood (~1 ml) was collected for bevacizumab serum concentration determination at the following time points: pre-paclitaxel/cisplatin on days 1, 4, 11, and 18; on day 18 at approximately 5, 15, and 30 min, 1, 2, 3, 4, 6, 8, 12 and 24 h after the bevacizumab IV bolus injection, as well as on days 20, 21, 22, 24, 27, 30, 34, 38, 43, and 48. Additional blood samples (~1 ml) were taken for serum anti-bevacizumab antibody determination prior to the initiation of the treatment and on Days 18 (predose), 30 and 48. On day 18, blood (~2 ml) was collected into tubes containing EDTA anticoagulant for paclitaxel and platinum plasma concentration determination at the following times: predose and at approximately 5, 15 and 30 min, 1 h (before the end of infusion), and 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after the start of the 1-h paclitaxel infusion. Plasma was harvested and aliquoted (200 µl for platinum analysis and the remaining plasma for paclitaxel analysis). All serum and plasma samples were stored in a freezer set to maintain −60 to −80°C until analyzed. For paclitaxel and cisplatin PK on day 18, time = 0 corresponded to the start of the paclitaxel (or saline) infusion.

### Assays

Serum concentrations of bevacizumab were determined by a validated enzyme-linked immunosorbent assay (ELISA) method (a binding assay using the truncated form of VEGF for capture and a goat anti-human IgG antibody conjugated with horseradish peroxidase for detection). The standard curve was diluted in 10% cyno serum ranged from 0.39 to 25 ng/ml. The minimum dilution for neat cyno serum samples, 1/10, resulted in a lower limit of 0.0039 µg/ml with no known upper limit. Detectable bevacizumab concentrations were reported for six monkeys (range 0.0063–0.020 µg/ml) receiving bevacizumab vehicle. During validation, non-specific monkey IgG and other humanized antibodies were tested at 10.0 µg/ml and found not to cross-react. These concentrations were at least 2,500-fold less than the lowest concentration detected in bevacizumab-treated monkeys. These data points were most likely a result of non-specific assay interference and were not included in the pharmacokinetic analysis.

Samples collected during week 4 and on days 18, 30 and 48 were analyzed for anti-bevacizumab antibodies using two methods for detecting antibodies to the Fab or the Fc portion of bevacizumab.

Plasma assays for platinum and paclitaxel were performed at Corning Hazleton Inc. (Madison, Wisconsin). An HPLC method (lower limit of quantification: 0.050 µg/ml) was used for determination of plasma paclitaxel concentrations. Total plasma elemental platinum concentrations were

measured using a graphite-furnace atomic absorption method (lower limit of quantification: 0.150 μg/ml). Since total plasma elemental platinum was assayed (free and bound) but free platinum is considered the relevant pharmacologic species, and which shows time-dependent increases in protein binding, only the $C_{max}$ of platinum was reported, since at the completion of the administration, nearly all measurable platinum would be free (unbound), drug [17].

### Pharmacokinetic analysis

Bevacizumab and paclitaxel concentration-time profiles for individual animals were analyzed with compartmental and noncompartmental approaches using a commercially available software package (WinNonlin, version 3.2, Pharsignt Corporation, Mountain View, CA). Nominal times were used in the analysis. Data reported as BLQ (below limit of quantitation) were not used in the analysis.

The maximum drug concentration ($C_{max}$) and time to maximum drug concentration ($T_{max}$) were determined from observed values. The area under the curve (AUC0-t) was calculated for paclitaxel plasma concentration-time profiles using the trapezoidal method derived from noncompartmental analysis from time zero (i.e. start of paclitaxel infusion) to the last detectable concentration. AUC0-t for bevacizumab was defined as the area under the serum concentration-time profile of the last dose (i.e. from the time of bevacizumab IV bolus administration on day 18 to the last detectable concentration on day 48). A two-compartment model was fit to the serum bevacizumab concentration-time profiles following multiple IV bolus doses for each bevacizumab-treated animal with elimination from the central compartment, using an iteratively re-weighted least squares scheme, with weights inversely proportional to the predicted concentration values squared. Similarly, a two-compartment model was fit to plasma paclitaxel concentration-time profiles following IV infusion for each animal. Estimated parameters included weight-normalized initial and steady-state volumes of distribution ($V_c/W$ and $V_{ss}/W$, respectively), weight-normalized clearance (CL/$W$), and terminal half-life.

### Statistical analysis

The statistical analysis of the effect of bevacizumab on cisplatin and paclitaxel PK was conducted on PK parameters $C_{max}$ and AUC0-t. Differences in systemic exposure (i.e. $C_{max}$ and AUC0-t) were tested via Student's $t$ tests, while similarities in systemic exposure were tested by calculating the geometric mean ratio (GMR) and its 90% confidence interval (CI). Significant alteration of PK (i.e. interaction) was concluded at a $P$ value of 0.05 or the 90% CI of GMR does not include unity. Student's $t$ test and GMR calculations were conducted using statistical software JMP version 6 (SAS Institute, Cary, NC). Values reported are mean ± SD unless otherwise noted. No statistical analysis was conducted on PK parameters CL, $V_c$ or $V_{ss}$.

One-way analysis of variance (ANOVA [18]) was used to analyze hematology values. Levene's test [19] was done to test for variance homogeneity. In the case of heterogeneity of variance at $P \leq 0.05$, transformations were used to stabilize the variance. ANOVA was performed on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's multiple comparison $t$ test [20] was used for pair-wise comparisons. One-way analysis of covariance (ANCOVA [20, 21]) was used to analyze body weights, with initial body weights as the covariate. Although Levene's test for variance homogeneity was performed, no transformations were used because covariance adjustment removed extraneous heterogeneity. If the ANCOVA was significant, least squares means $t$ test [22] was used for pairwise comparisons. Covariate-adjusted mean (CAM) body weights were calculated to allow for pairwise comparisons between individual groups (SAS/STAT, SAS Institute, Cary, NC) [22].

## Results

### Bevacizumab pharmacokinetics

Figure 1 illustrates the similarity of the mean (±SD) serum bevacizumab concentration-time profiles following administration of bevacizumab alone or in combination with cisplatin and paclitaxel. Concomitant administration of the

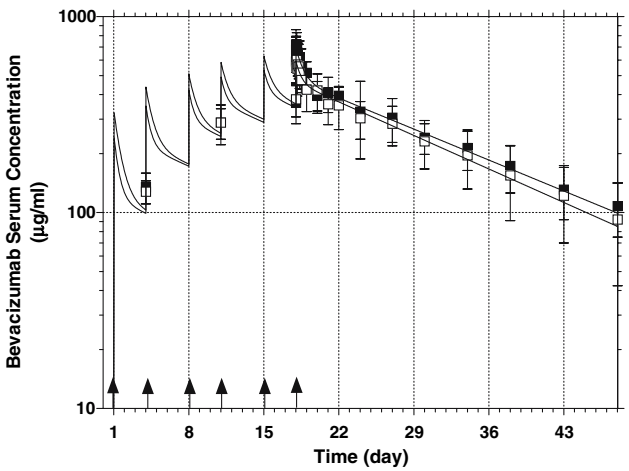


**Fig. 1** Mean (±SD) bevacizumab serum concentrations (μg/ml) after multiple doses of bevacizumab with the model fit to mean values superimposed on the data ($n = 5$ per group). *Open squares* represent treatment with cisplatin/paclitaxel; *filled squares* represent treatment with bevacizumab + cisplatin/paclitaxel; *vertical arrows* represent dosing of bevacizumab

chemotherapeutic agents did not alter the pharmacokinetics of bevacizumab, as evidenced by similar bevacizumab mean (±SD) AUC0-t and $C_{max}$ values with $P$ values larger than 0.05 (Table 2). The 90% CI of the GMR values calculated for these two parameters contained unity, further indicating lack of a difference between the two groups (Table 2). Pharmacokinetic parameters estimated from compartmental analysis are reported in Table 3 Co-administration of cisplatin and paclitaxel with bevacizumab showed no effect on bevacizumab initial distribution volumes ($V_c/W$), which were estimated to be 46.1 ± 10 and 37.1 ± 15 ml/kg (mean ± SD), when administered alone or in combination with the chemotherapeutic agents, respectively ($P = 0.30$). These values are consistent with bevacizumab distribution within the serum volume. There were no other statistically significant PK parameter changes, including estimates of steady state volume of distribution, clearance and half-life. The observed bevacizumab pharmacokinetics in this study is comparable to those determined in previous studies of bevacizumab in cynomolgus monkeys [23].

**Table 3** Bevacizumab and paclitaxel pharmacokinetic parameter estimates

| Parameter | Group 2 Bevacizumab Alone | Group 3 Cisplatin/ paclitaxel | Group 4 Cisplatin/ paclitaxel + bevacizumab | $P$ value |
|---|---|---|---|---|
| Bevacizumab | | | | |
| CL (ml/day/kg) | 4.61 ± 1.3 | – | 3.99 ± 0.76 | 0.38 |
| Terminal $t_{1/2}$ (day) | 13.5 ± 5.1 | – | 14.5 ± 2.7 | 0.70 |
| $V_c$ (ml/kg) | 46.1 ± 10 | – | 37.1 ± 15 | 0.30 |
| $V_{ss}$ (ml/kg) | 78.0 ± 8.4 | – | 72.5 ± 7.0 | 0.55 |
| Paclitaxel | | | | |
| CL (ml/h/kg) | – | 387 ± 130 | 357 ± 94 | 0.70 |
| Terminal $T_{1/2}$ (h) | – | 7.55 ± 1.7 | 7.27 ± 1.4 | 0.78 |
| $V_c$ (ml/kg) | – | 343 ± 95 | 315 ± 56 | 0.60 |
| $V_{ss}$ (ml/kg) | – | 1,490 ± 590 | 1,200 ± 220 | 0.86 |

## Paclitaxel and cisplatin pharmacokinetics

Paclitaxel was administered on day 18 as an IV infusion immediately after a cisplatin IV bolus injection following bevacizumab vehicle (Group 3) or bevacizumab (Group 4) (Table 1). Figure 2 shows the similarity in mean (±SD) paclitaxel plasma concentration versus time profiles following administration with or without concomitant bevacizumab. The presence of bevacizumab did not appear to alter the pharmacokinetics of paclitaxel as evidenced by similar mean (±SD) values of AUC0-t and $C_{max}$ (Table 2) and by the calculated 90% CIs of the GMR for both parameters including unity. In addition, there was no statistically significant change in any other paclitaxel pharmacokinetic parameter including volume of distribution, clearance and half-life (Table 3).

Cisplatin was administered on day 18 as an IV bolus immediately prior to an IV infusion of paclitaxel with (Group 4) or without concomitant bevacizumab administration (Group 3). Since previous studies demonstrated time dependent increases in plasma protein binding of elemental platinum [16, 24], only the $C_{max}$ of the total plasma elemental platinum was measured and reported in this study (Table 2). The mean (±SD) free elemental platinum $C_{max}$ values following cisplatin bolus administration were similar in the presence or absence of bevacizumab (5.28 ± 0.50 and 5.01 ± 0.55 μg/ml, respectively).

## Toxicological observations

All animals survived the study. Administration of bevacizumab alone had no effect on antemortem clinical

**Table 2** Summary of pharmacokinetic parameters

| Parameter (mean ± SD) | Not in combination | In combination[a] | GMR (90%CI) | Difference of mean (%)[b] | $P$ value |
|---|---|---|---|---|---|
| Bevacizumab | | | | | |
| AUC (μg/ml × day) | 6,747 ± 1,872 | 7,366 ± 1,599 | 0.91 (0.73, 1.21) | 9.17 | 0.59 |
| $C_{max}$ (μg/ml) | 676 ± 100 | 744 ± 120 | 0.95 (0.84, 1.07) | 10.06 | 0.36 |
| Paclitaxel | | | | | |
| AUC (μg/ml × day) | 10.3 ± 3.7 | 10.9 ± 2.9 | 0.92 (0.67, 1.22) | 5.83 | 0.76 |
| $C_{max}$ (μg/ml) | 8.33 ± 2.9 | 8.73 ± 1.8 | 0.93 (0.66, 1.3) | 4.80 | 0.80 |
| Cisplatin | | | | | |
| $C_{max}$ (μg/ml) | 5.01 ± 0.55 | 5.28 ± 0.50 | 0.91 (0.91, 1.10) | 5.39 | 0.45 |

[a] "Not in combination"—cisplatin and paclitaxel PK parameters from Group 3 and bevacizumab PK parameters from Group 2; "In combination"—cisplatin, paclitaxel and bevacizumab PK parameters from Group 4

[b] Difference of means = (mean in combination − mean not in combination)/mean not in combination

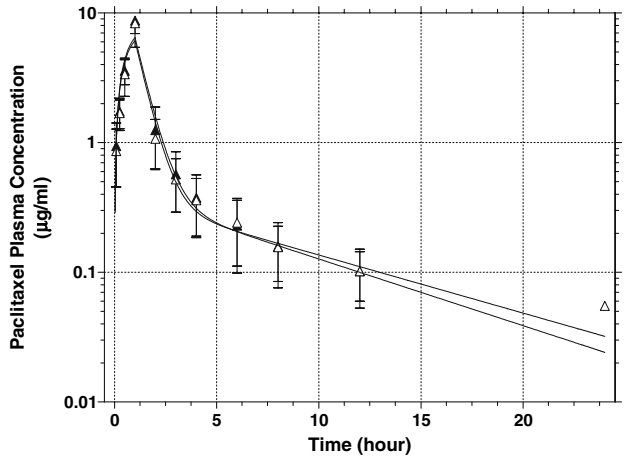


**Fig. 2** Mean (±SD) paclitaxel plasma concentrations (µg/ml) following IV infusion of paclitaxel on day 18 with the model fit to mean values superimposed on the data ($n = 5$ per group). *Open triangles* represent treatment with cisplatin/paclitaxel; *filled triangles* represent treatment with bevacizumab + cisplatin/paclitaxel

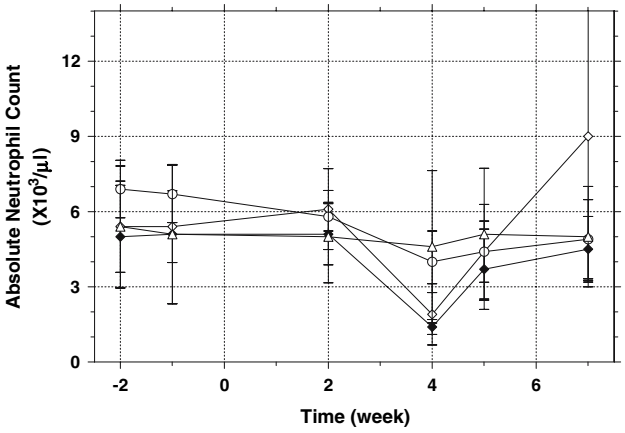


**Fig. 3** Mean (±SD) absolute neutrophil counts. *Open triangles* represent vehicle control; *open circles* represent treatment with bevacizumab alone; *open diamonds* represent treatment with cisplatin/paclitaxel; *solid diamonds* represent treatment with bevacizumab + cisplatin/paclitaxel

observations, body weight, or food consumption. There were no drug-related findings observed upon physical examination or electrocardiographic assessment during weeks 2, 3, or 6 in any of the treatment groups. Administration of cisplatin and paclitaxel was associated with vomiting in four Group 3 and two Group 4 animals on day 18, with no discernible differences between groups. Animals in Groups 3 and 4 had lower CAM body weights on day 22 as compared to control (Group 1) animals. This finding is consistent with observations of low food consumption and vomiting following dosing on day 18. The CAM body weights of the Group 4 animals (given cisplatin and paclitaxel with bevacizumab) were not statistically significantly different from animals in Group 3 (given cisplatin and paclitaxel) from day 18 to day 46, except for a transient 0.1 kg lower body weight observed on day 22. This finding was considered incidental and not related to bevacizumab treatment.

Administration of bevacizumab alone had no effect on clinical pathology parameters. As expected, administration of cisplatin and paclitaxel resulted in transiently decreased absolute neutrophil counts (Fig. 3). During week 4, 8 days following administration of cisplatin and paclitaxel, mean absolute neutrophil counts for the groups given cisplatin and paclitaxel (Groups 3 and 4) were more than 50% lower than the mean counts for groups not given cisplatin and paclitaxel (Groups 1 and 2). Concomitant administration of bevacizumab with cisplatin and paclitaxel had no apparent effect on the magnitude of the leukocyte effect with nearly identical mean nadir counts and similar recovery values at weeks 5 and 7. A similar pattern was observed in the white blood cell count (data not shown).

### Bevacizumab antibodies

Anti-bevacizumab antibodies were not detected in any of the serum samples in bevacizumab-treated animals.

## Discussion and conclusion

This study was performed to investigate the safety and potential pharmacokinetic interactions of concomitant administration of bevacizumab and the chemotherapeutic agents cisplatin and paclitaxel. This investigation was performed in cynomolgus monkeys because (1) bevacizumab has been shown to bind to VEGF in cynomolgus monkeys, thus make cynomolgus monkey a relevant animal model for bevacizumab PK; and (2) cynomolgus monkey closely resembles human in the toxicological responses to paclitaxel and cisplatin. The dose and dosing frequency of bevacizumab for cynomolgus monkeys in this study were chosen taking into consideration of the shorter half-life and to match the clinical relevant exposure in patients (beyond 100 µg/ml).

The rationale for initiating this nonclinical study was that a toxicodynamic interaction could theoretically occur whereby bevacizumab would potentiate chemotherapy toxicities at the vascular and cellular level, rather than from a pharmacokinetic interaction resulting in high drug plasma exposure to the chemotherapeutic agents. The mechanism by which VEGF blockade may potentiate chemotherapeutic cytotoxicity in tumors is not completely understood but may result from changes in vascular functions (i.e. decreased vessel density, diameter and permeability), reduction in interstitial fluid pressure and improvements in

intratumoral uptake of chemotherapeutic agents [8, 9]. The effect of anti-angiogenic agents like bevacizumab on normal vascular system was expected to be minimal based upon nonclinical safety studies (unpublished data on file); however, potential existed for vascular drug effects which could modulate the distribution of chemotherapeutic agents in normal tissues.

The results of this study suggest that concomitant administration of a chemotherapy regimen containing cisplatin and/or paclitaxel does not alter the systemic disposition of bevacizumab in cynomolgus monkeys. Moreover, co-administration of bevacizumab did not affect the plasma pharmacokinetics of either chemotherapeutic agent. Free elemental platinum $C_{max}$ values following cisplatin administration, with or without concomitantly administered bevacizumab, were similar. The absence of a pharmacokinetic drug interaction supports the toxicological observations. Administration of cisplatin/paclitaxel was associated with the expected toxicities of vomiting, body weight loss, and neutropenia [25, 26]. Concomitant bevacizumab administration did not appear to alter the incidence or severity of these effects. The design of this study only allowed for the assessment of acute effects of chemotherapy and therefore any conclusions regarding the effect of bevacizumab treatment on chronic or cumulative dose toxicities of cisplatin or paclitaxel, such as peripheral neuropathy, would require further study. However, in phase III clinical trials in which bevacizumab has been combined with other platinum-based compounds, such as carboplatin or oxaliplatin, no augmentation of platinum-induced neuropathy has been observed [27, 28].

This study provided critical observations for the clinical development of bevacizumab. The lack of pharmacokinetic and toxicodynamic interactions suggested that these platinum-based agents and taxanes could be safely administered in the clinic without compromising dose or tumor exposure. Subsequent controlled clinical trials were performed combining bevacizumab with carboplatin/paclitaxel for the treatment of first-line NSCLC and in combination with paclitaxel in metastatic breast cancer [13, 14]. Positive primary outcomes, as measured by a prolongation of survival, were observed in these trials, demonstrating that bevacizumab could be successfully and safely administered in combination with a variety of small molecule cytotoxic chemotherapies.

The lack of PK interactions between bevacizumab and paclitaxel/cisplatin can be supported by the current understanding of each agent's elimination and/or metabolic pathway. Bevacizumab is a recombinant humanized monoclonal antibody and, like other IgG monoclonal antibodies, is believed to be primarily cleared from systemic circulation via a FcRn receptor-mediated route [29–32]. Distribution and metabolism studies of bevacizumab in rabbits with [$^{125}$I]bevacizumab showed no specific uptake in any organ and limited metabolism [23]. In contrast, paclitaxel undergoes hepatic oxidative metabolism via cytochrome P450 2C8 and 3A4 to form the major metabolites, 6-α-hydroxypaclitaxel and 3′-para-hydroxyphenyl-paclitaxel, respectively [33]. These metabolites subsequently undergo biliary excretion [34] and this pathway of metabolism and excretion would not be expected to be altered by an IgG1 monoclonal antibody. Clearance of cisplatin has also been well characterized with renal elimination of free (non-protein bound) platinum as the major route of clearance; thus co-administration of bevacizumab and cisplatin are unlikely to result in a drug–drug interaction resulting in an alteration of platinum clearance. It is also unlikely that interactions due to protein binding displacement would occur, as, although unknown, it is unlikely that bevacizumab binds to plasma albumin. The distinct metabolic pathways of the three agents investigated in our study support our findings that the pharmacokinetics of each agent are not altered by the others. A previous study also reported the lack of pharmacokinetic or toxicodynamic interaction between bevacizumab and irinotecan in cynomologus monkeys [35].

In summary, we used the cynomologus monkey as a nonclinical model to study the potential pharmacokinetic and toxicological interactions of bevacizumab with a chemotherapeutic regimen containing cisplatin and paclitaxel. No pharmacokinetic interaction was observed between bevacizumab and paclitaxel/cisplatin. Administration of the two chemotherapeutic agents was associated with the expected toxicities of myeloid suppression, anemia, vomiting and body weight loss. Concomitant bevacizumab did not alter the incidence or severity of these effects. Results from the present study and the previous study of irinotecan [35] support the clinical development of bevacizumab in combination with three commonly used small molecule cancer chemotherapeutic agents with an acceptable margin of safety and no compromise of the optimal exposure of these agents.

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