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ORIGINAL ARTICLE

Evaluation of combined bevacizumab and intraperitoneal carboplatin or paclitaxel therapy in a mouse model of ovarian cancer

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Abstract
Purpose To evaluate the pharmacokinetics of bevacizumab following IP and IV administration, and to investigate combined bevacizumab therapy (IP or IV) with IP paclitaxel or carboplatin in a mouse model of ovarian cancer.
Methods Bevacizumab pharmacokinetics were investigated following IV or IP dosing, and mice bearing peritoneal A2780 xenografts were treated with vehicle, IV or IP bevacizumab, IP paclitaxel, IP paclitaxel with co-administration of IV or IP bevacizumab, IP carboplatin, and IP carboplatin with co-administration of IV or IP bevacizumab. Survival time was defined as the time to death or the time to reach 120% of baseline body weight.
Results Following IP administration, bevacizumab was rapidly absorbed and bioavailability was 92.8%. Median survival time, which was 33 days for control mice, was increased by 24% with IP paclitaxel. IP carboplatin failed to increase survival time when administered alone. IV and IP bevacizumab increased survival time by 42 and 33%. Combined bevacizumab and IP paclitaxel was superior to paclitaxel alone ($P = 0.01$ for IV and $P = 0.04$ for IP bevacizumab), and combined bevacizumab and IP carboplatin was superior to carboplatin alone ($P = 0.002$ for IV and $P = 0.02$ for IP bevacizumab). There were no significant differences in survival between groups receiving bevacizumab IV or IP, either alone ($P = 0.586$), in combination with paclitaxel ($P = 0.467$), or in combination with carboplatin ($P = 0.149$).
Conclusions Following IP administration to mice, bevacizumab demonstrates rapid and near complete absorption. Bevacizumab therapy, initiated prior to IP carboplatin or paclitaxel administration, increased survival time significantly in mice, and results were not dependent on the route of bevacizumab administration (IV vs. IP).

Keywords Intraperitoneal chemotherapy · Ovarian cancer · Anti-angiogenic therapy · Pharmacokinetic · Carboplatin · Paclitaxel

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Introduction

Ovarian cancer is a common disease in the United States, with approximately 20,000 new cases diagnosed each year, and it is a leading cause of cancer death. In the majority of diagnosed individuals, the disease remains localized to the peritoneal cavity [1]. Standard treatment for ovarian cancer involves debulking surgery followed by systemic or intraperitoneal (IP) chemotherapy [2]. Clinical trials have demonstrated that IP chemotherapy, with platinum and taxane-based regimens, is superior to intravenous (IV) chemotherapy in women who initiate drug treatment with small-volume residual ovarian cancer [3]. However, IP chemotherapy often fails to produce complete and lasting responses in the treatment of experimental and human peritoneal tumors [4, 5], and there is need to further improve the efficacy of this approach.

There is strong evidence indicating that the efficacy of IP chemotherapy is primarily limited by poor penetration of drug into peritoneal tumors [6, 7]. Tumor blood flow, capillary permeability, and capillary surface area all play an

important role in determining depth of drug penetration into peritoneal tumors following IP chemotherapy [8, 9]. Based on mechanistic, mathematical models presented by Dedrick et al. [10] and Au et al. [11], we recently proposed that co-administration of anti-angiogenic agents would enhance the efficacy of IP chemotherapy by improving drug penetration into peritoneal tumors [12]. By inhibiting neovascularization in tumors, anti-angiogenic agents have been shown to decrease tumor blood flow, tumor capillary surface area, and tumor capillary permeability. Each of these effects may be expected to decrease the rate of capillary removal of drug following IP chemotherapy, thereby allowing greater tumor exposure to drug, and increasing the efficacy of IP chemotherapy [12].

Using computer simulations and animal models of ovarian cancer, we have demonstrated that anti-angiogenic therapy leads to a substantial and selective increase in drug exposure in peritoneal tumors following IP chemotherapy [12]. Additionally, therapeutic studies conducted with two different chemotherapeutic drugs, topotecan and cisplatin, showed that animals receiving combined IP chemotherapy and anti-angiogenic therapy displayed superior survival relative to animals treated with chemotherapy alone (i.e., cisplatin or topotecan), anti-angiogenic therapy alone, or IV chemotherapy with concomitant anti-angiogenic therapy [12].

Our prior work demonstrated that the administration of an anti-angiogenic agent, bevacizumab, improved drug penetration in tumors following IP chemotherapy, but not following systemic chemotherapy. We did not, however, evaluate whether the benefit of this combination therapy was dependent on the route of administration of the anti-angiogenic agent. This is of interest, as there are literature reports advocating the use of IP bevacizumab for the symptomatic treatment of ovarian cancer [13, 14] and local exposure of the anti-angiogenic agent may allow for improved efficacy in the treatment of peritoneal tumors. In this report, we have investigated the pharmacokinetics of bevacizumab following IP and IV dosing in mice. Additionally, using a mouse model of human ovarian cancer, we investigated the influence of the route of bevacizumab administration on the efficacy of bevacizumab alone, and on the efficacy of bevacizumab in combination with IP carboplatin therapy and IP paclitaxel therapy.

Materials and methods

Materials

Paclitaxel, carboplatin, and bevacizumab (Genentech, South San Francisco, CA) were purchased from a local pharmacy. All other reagents were of analytical grade. A2780 cells were obtained from the DCTD Tumor Repository (maintained by Charles River Laboratories, Inc., under contract to the Biological Testing Branch for the National Cancer Institute, Frederick, MD). Cells were cultured in an incubator with 5% CO_2 at 37°C using RPMI 1640 medium (20 mM HEPES, 2 mM L-glutamine) mixed with 10% heat-inactivated fetal bovine serum and 5 mg/l gentamicin. Protocols for animal use were approved by the Institutional Animal Care and Use Committee of the University at Buffalo. Mice were housed in a sterile room on a standard light/dark cycle, with continuous access to food and water.

ELISA for bevacizumab

A species-specific ELISA was developed to determine bevacizumab concentrations in mouse plasma. Bevacizumab working standards were prepared by spiking blank mouse plasma with a standard stock solution. Working standards were then diluted 100-fold with phosphate-buffered saline (pH 7.4) to achieve appropriate standard concentrations (2.5, 10 25, 50, 75 and 100 ng/ml) with 1% mouse plasma (v/v) in the final solution. The precision and accuracy of the ELISA were evaluated by determining the recovery of quality control samples (QCs) of bevacizumab, prepared as described for the standard samples. Quality control samples were prepared at low, mid, and high concentrations (5, 40 and 90 ng/ml) with respect to the standard curve. Standards were prepared immediately prior to use in the assay, and QCs were prepared in bulk, aliquoted, and stored at 4°C. Standards and QCs were run on each microplate, with samples, and a unique standard curve was generated for each microplate.

Briefly, anti-human IgG (Fc-specific) (Sigma, Cat#I2136) was diluted 1:333 in 20 mM Na2HPO4 (no pH adjustment, Sigma). Nunc Maxisorp 96-well plates (Nunc model #62409-002, VWR, Bridgeport, NJ) were then incubated with anti-human IgG (250 µl/well) overnight at 4°C. Subsequently, the plates were washed thrice with PB-Tween (0.05% Tween in 0.02 M Na2HPO4, no pH adjustment), followed by three washes with double distilled water. Plates were then incubated with standards and samples in triplicate (250 µl) for 2 h at room temperature. At the end of incubation, the plates were washed as described above. Next, the plates were incubated with 250 µl of anti-human IgG (Fab Specific- alkaline phosphatase conjugated) (Sigma, Cat #A8542, St. Louis, MO) for 1.5 h at room temperature (1:5,000 dilution of the conjugate with 1% bovine serum albumin in PBS). The plates were then washed and rinsed, and p-nitro phenyl phosphate solution (Pierce, Rockford, IL, 4 mg/ml in diethanolamine buffer, pH 9.8) was added to each well (250 µl/well). The change in absorbance at 405 nm with time (dA/dt) was monitored with a plate reader (Spectra Max 250, Molecular Devices, Sunnyvale,

Table 1 Summary of groups, treatments, and dosage regimens used for the therapeutic study

Group ID	Treatment	Total drug (mg/kg)	Total bevacizumab (mg/kg)	Animals per group
Group-1	Control; No treatment	0	0	10
Group-2	Control; Saline IP	0	0	7
Group-3	Carboplatin; IP q4d × 4	64	0	7
Group-4	Paclitaxel; IP q4d × 4	80	0	7
Group-5	Bevacizumab; IV 2x/week × 3	0	30	7
Group-6	Bevacizumab; IP 2x/week × 3	0	30	7
Group-7	Bevacizumab; IV 2x/week × 3 + Carboplatin; IP q4d × 4	64	30	7
Group-8	Bevacizumab; IP 2x/week × 3 + Carboplatin; IP q4d × 4	64	30	7
Group-9	Bevacizumab; IV 2x/week × 3 + Paclitaxel; IP q4d × 4	80	30	7
Group-10	Bevacizumab; IP 2x/week × 3 + Paclitaxel; IP q4d × 4	80	30	7

CA), and standard curves were obtained by fitting the dA/dt versus bevacizumab concentration using the equation for a straight line ($y = mx + c$).

Pharmacokinetics of bevacizumab

The pharmacokinetics of bevacizumab after IV and IP administration were evaluated in athymic nude mice. Ten animals were randomly divided into two groups, IV and IP, with 5 animals in each group. In each animal, bevacizumab was administered at the dose of 5 mg/kg, the same dose selected for use in subsequent therapeutic studies. Animals in the intravenous group received the drug via penile vein injection and the IP group received the drug via IP bolus injection. The pharmacokinetic study employed a staggered design, where 20–25 µl blood samples were collected from three of the five animals from each group, where sampled animals were selected randomly. For the IV group, samples were collected at 5 min, 2, 4, 8, 12, 24, 36, 48, 72, 96, and 144 h and for the IP group, samples were collected at 5 min, 2, 4, 8, 12, 24, 36, 48, 72, and 120 h. Blood samples were centrifuged at 13,000 rpm for 3 min, and plasma was separated and stored at −20°C until analyzed by ELISA.

Compartmental analysis of the pharmacokinetic data was performed using WinNonlin, version 5.0 (Pharsight Corporation, Palo Alto, CA). Modeling employed a two-compartmental pharmacokinetic model with linear elimination from the systemic compartment and first-order absorption from the peritoneal compartment.

Therapeutic study

To establish a suitable peritoneal tumor model, approximately 2 million A2780 cells were injected IP into athymic nude mice (20–25 g and 4–5 weeks old). Seventy-three animals were randomly divided into 10 groups (Group-1 to Group-10). The number of animals assigned to each group and the treatments assigned to each group are summarized in Table 1. The dosing regimen for carboplatin was 16 mg/kg every 4 days for 4 cycles, administered on day 8, 12, 16, and 20. Paclitaxel was administered on a schedule of 20 mg/kg delivered every 4 days for 4 cycles, administered on day 8, 12, 16, and 20. Bevacizumab was administered at the dose of 5 mg/kg on days 6, 9, 13, 16, 19, and 23 (i.e., twice weekly for 3 weeks).

Paclitaxel and carboplatin were administered IP. For groups 5, 7, and 9, bevacizumab was administered IV, and for group 6, 8, and 10, bevacizumab was administered IP. Saline was administered to control animals in place of bevacizumab, paclitaxel or carboplatin. As required by the University at Buffalo Institutional Animal Use and Care Committee, mice were removed from the study and euthanized when animal weight increased above 120% of baseline body weight (measured the day after tumor implantation). For purposes of assessment of the duration of animal survival, survival time was determined based on the date of animal death or removal from the study. Systemic toxicity was assessed by observing changes into the animal body weight. The percent increase in life span, calculated as $[(\text{median survival time in treated animals}/\text{median survival time in controls}) - 1] \times 100$, was used to compare therapeutic effectiveness of different treatment groups.

Statistical analysis

The Kaplan–Meier product limited method was used to calculate survival percentage. The Log-Rank test was used for statistical comparison of survival curves between selected groups using Prism software (GraphPad software, San Diego, CA). Groups receiving combination therapy (i.e., IP chemotherapy + bevacizumab) were compared with groups receiving IP chemotherapy alone. Groups receiving IP bevacizumab (alone or in combination) were compared with

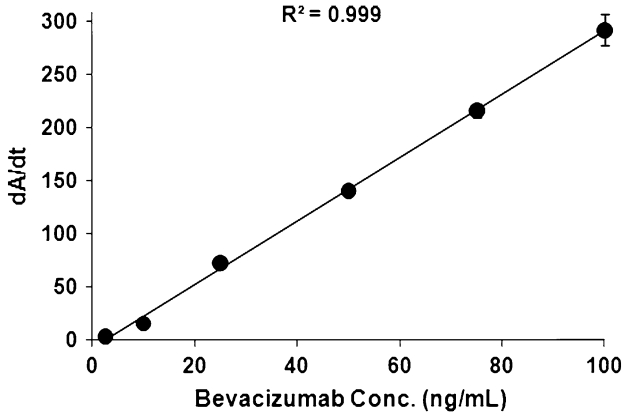


Fig. 1 Representative standard curve for bevacizumab ELISA over the range of 2.5–100 ng/ml. The *curve* is fitted with the equation for a *straight line*, and $r^2 = 0.999$. *Error bars* represent the standard deviation across the mean of 3 replicates

groups receiving IV bevacizumab (alone or in combination). All statistical tests were 2 sided.

Results

Development and validation of bevacizumab ELISA

The calibration curve was linear over the standard curve range and correlation coefficients greater than 0.99 were typically obtained on fitting to the equation: $y = mx + c$ (Fig. 1). Inter-assay recoveries were in the range of 91–115% and the percent coefficient of variation ranged from 0.86 to 8.27%. The working range of the assay was 2.5–100 ng/ml, which corresponds to a limit of quantitation of 250 ng/ml (i.e., since the samples are diluted 1:100 prior to analysis).

Pharmacokinetics of bevacizumab

Drug concentration vs. time profiles for bevacizumab after IP and IV administration of 5 mg/kg doses in athymic nude mice are shown in Fig. 2a. There was no significant lag time observed after the IP administration, and the drug showed a biphasic disposition profile irrespective of the route of administration. Data from both routes of administration were simultaneously fit to a two-compartment pharmacokinetic model with linear elimination, and model fittings are presented in Fig. 2b.

The terminal half-life for the drug was found to be approximately 5.5 days. The volumes of central compartment and peripheral compartment were found to be 49.4 (CV%, 8.06) and 53.8 (CV%, 16.2) ml/kg. The clearance from the central compartment was found to be 6.27 (CV%, 29.8) ml/day/kg, and the distribution clearance was found to be 87 (CV%, 23.2)

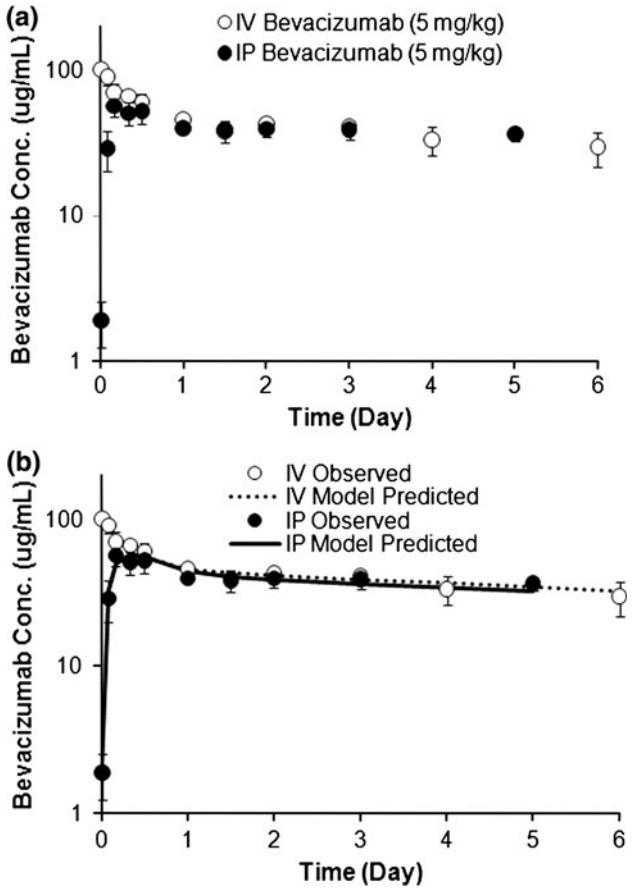
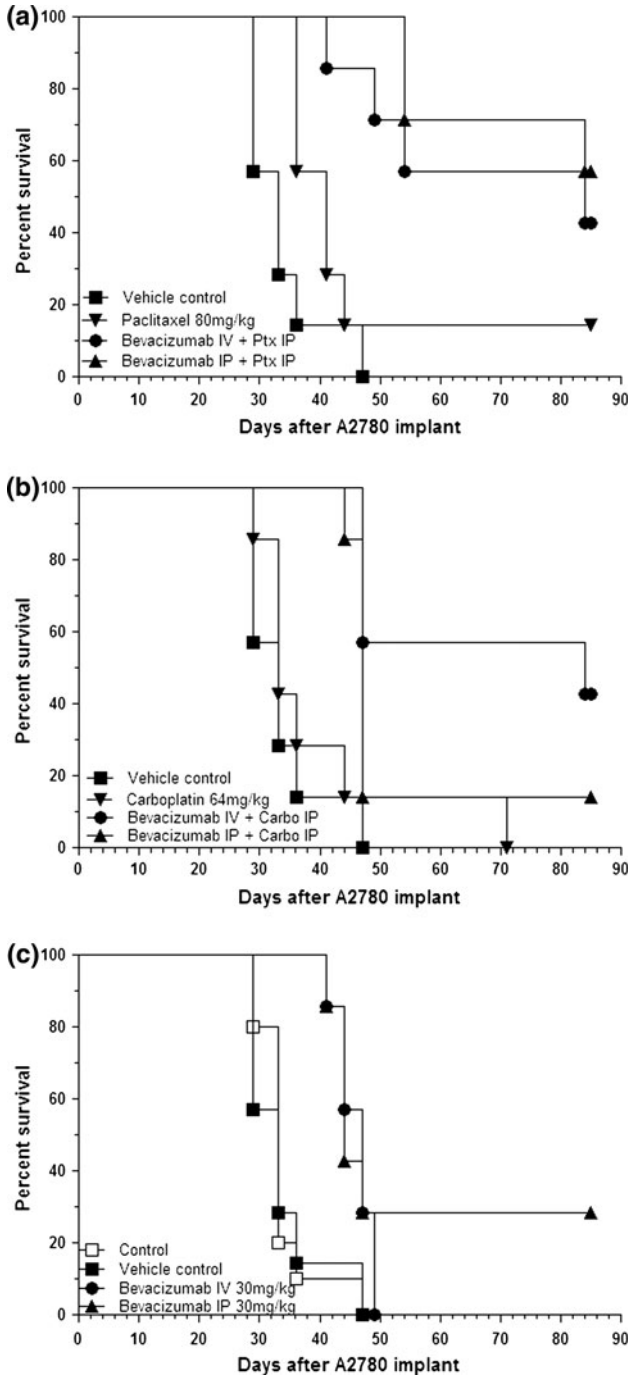


Fig. 2 Bevacizumab pharmacokinetics after IV or IP administration to athymic nude mice. **a** Observed log concentration vs. time profile for bevacizumab after IV or IP administration (5 mg/kg). *Error bars* represent standard deviations. **b** Characterization of systemic pharmacokinetics of bevacizumab after IV or IP administration, using two-compartmental pharmacokinetic model with linear elimination. *Circles* represent the raw data and lines represent model fitting. *Error bars* represents standard deviation. Key: (*open circle*) IV administration; (*filled circle*) IP administration

ml/day/kg. After IP administration of 5 mg/kg bevacizumab, the absorption rate constant from peritoneal to central compartment was found to be 5.79 day^{-1} (CV%, 12.5), and systemic bioavailability was 92.8% (CV%, 5.09).

Therapeutic study

Survival curves for all the groups are shown in Fig. 3, and summary data are provided in Table 2. Median survival time for the control (Group-1), vehicle control (Group-2), carboplatin IP (Group-3), and paclitaxel IP (Group-4) groups was 33 (33–47), 33 (33–47), 33 (33–71), and 41 (41–85) days. Treatment with paclitaxel IP was significantly ($P < 0.05$) better than control and resulted in 24% increase in life span. Median survival time for the bevacizumab IV (Group-5) and bevacizumab IP (Group-6) groups was 47 (44–49) and 44 (44–85) days. Treatment



◀ **Fig. 3** Results from the therapeutic study. After tumor implantation, 73 animals were randomly divided into 10 groups (Group 1–10). The number of animals assigned to each group and the treatment assigned to each group is summarized in Table 1. **a** Survival fractions from combination therapy with IP paclitaxel. The combination of bevacizumab (IP or IV) and IP paclitaxel led to significantly ($P < 0.05$) improved therapeutic outcome compared to IP paclitaxel alone. Differences between bevacizumab IV + IP paclitaxel and bevacizumab IP + IP paclitaxel groups were not statistically significant ($P = 0.467$). **b** Survival fractions from combination therapy with IP carboplatin. The combination of bevacizumab (IP or IV) and IP carboplatin led to significantly ($P < 0.05$) improved animal survival compared to IP carboplatin alone. The differences between bevacizumab IV + IP carboplatin and bevacizumab IP + IP carboplatin groups were not statistically significant ($P = 0.149$). **c** Comparison of IP and IV bevacizumab. The differences between bevacizumab IV and bevacizumab IP groups were not statistically significant ($P = 0.586$)

with bevacizumab therapy alone (IP or IV) was significantly ($P < 0.05$) better than the control group. Median survival time for the bevacizumab IV + carboplatin IP (Group-7) and bevacizumab IP + carboplatin IP (Group-8) groups was 84 (84–85) and 47 (47–85) days. Combined therapy with bevacizumab (IV or IP) and carboplatin IP was significantly ($P < 0.05$) better than the control group. Median survival time for the bevacizumab IP + paclitaxel IP (Group-10) group was 84 (49–85) days. For the bevacizumab IV + paclitaxel IP (Group-9) group, 4 of 7 animals were free of grossly detectable tumors at the end of the experiment, and hence calculation of median survival time was not possible. Combined therapy with bevacizumab (IV or IP) and paclitaxel IP was significantly ($P < 0.05$) better than the control group.

Combined therapy with bevacizumab, IV or IP, and IP paclitaxel was significantly ($P < 0.05$) better than IP paclitaxel alone (Fig. 3a). Bevacizumab IV + IP paclitaxel and bevacizumab IP + IP paclitaxel resulted in long-term cures in 4 of 7 and 3 of 7 animals, respectively. Combined therapy with bevacizumab, IV or IP, and IP carboplatin was also significantly ($P < 0.05$) better than IP carboplatin alone (Fig. 3b). Bevacizumab IV + IP carboplatin and bevacizumab IP + IP carboplatin resulted in long-term cures in 3 of 7 and 1 of 7 animals, respectively. The route of administration for bevacizumab did not significantly impact the survival results. Statistical comparison of IV bevacizumab + IP paclitaxel and IP bevacizumab + IP paclitaxel resulted in significance level of $P = 0.467$, and the comparison of IV bevacizumab + IP carboplatin, and IP bevacizumab + IP carboplatin resulted in significance level of $P = 0.149$ (Fig. 3a, b). As described in Fig. 3c, the difference between IV bevacizumab and IP bevacizumab groups was also statistically insignificant ($P = 0.586$).

There was no substantial weight loss in any of the treatment groups (Table 2), suggesting an absence of significant systemic toxicity in all 10 groups.

Discussion

Despite the landmark clinical trial (GOG-172) showing a significant improvement in survival of ovarian cancer patients treated with IP vs. IV chemotherapy [15], IP therapy has not gained widespread acceptance in the oncology community [16]. Appropriate drugs, doses, schedules, and

Table 2 Summary of survival and weight loss results from the therapeutic study

Treatment	MDD (range)	% Increase in life span[a]	Survivors/group	% Weight loss[b] (Range)
Control	33 (33–47)	–	0/10	0 (0–1.9)
Vehicle control	33 (33–47)	–	0/7	0 (0–1.3)
Carboplatin 64 mg/kg	33 (33–71)	–	0/7	0 (0–1)
Paclitaxel 80 mg/kg	41 (41–85)	24	1/7	1.4 (0–28.7)
Bevacizumab 30 mg/kg; IV	47 (44–49)	42	0/7	0 (0–6.5)
Bevacizumab 30 mg/kg; IP	44 (44–85)	33	2/7	0 (0–2.9)
Bevacizumab IV + Carbo IP	84 (84–85)	155	3/7	0 (0–8.3)
Bevacizumab IP + Carbo IP	47 (47–85)	42	1/7	1.5 (0–4.8)
Bevacizumab IV + Ptx IP	–	–	4/7	0 (0–2.6)
Bevacizumab IP + Ptx IP	84 (49–85)	155	3/7	0 (0–3.3)

Statistical comparisons were performed between selected groups, as presented within the Sect "Results"

MDD median day of death

[a] % Increase in life span, calculated as [(median survival time in treated animals/median survival time in controls) − 1] × 100%

[b] % Weight loss indicates the median percentage weight loss (at nadir), calculated as [(Weight at nadir−Weight on day 0)/(Weight on day 0) × 100%]

indications for IP chemotherapy still remain as areas of uncertainty and debate [3, 16, 17].

Preclinical and clinical studies have demonstrated that the efficacy of IP chemotherapy is limited by poor penetration of drug into peritoneal tumors [6–9, 18]. Based on pharmacokinetic theory that suggests that penetration depth is primarily determined by the rate of drug removal via tumor capillaries, we have hypothesized that adjunct therapy with anti-angiogenic agents will lead to decreased drug removal from peritoneal tumors, increased drug concentrations in tumors, and increased efficacy of IP chemotherapy [12]. This hypothesis has been evaluated *in silico* with the use of a hybrid physiologically based pharmacokinetic model, and computer simulations predicted a considerable increase in the tumor exposure to topotecan, which was selected as a model drug. Results from computer simulations were supported by a series of *in vivo* studies, which demonstrated that administration of bevacizumab led to no changes in drug disposition in peritoneal fluid, in plasma, or in tissues with mature vasculature (e.g., liver and kidney), but did lead to a greater than sixfold increase in topotecan concentrations in peritoneal tumors [12]. It was also discovered, through pharmacokinetic modeling and preclinical experiments, that this effect was observed after IP administration of topotecan but not after systemic administration of the drug. In further work, combined administration of bevacizumab and IP chemotherapy produced superior anticancer effects in a mouse model of human ovarian cancer, using two chemotherapeutic agents (topotecan and cisplatin) in two separate studies [12]. To further investigate this novel combination therapy, we wished to determine whether the route of bevacizumab administration, IP versus. IV, was an important determinant of efficacy, and we wished to evaluate the combination of bevacizumab with IP administration of the first line chemotherapeutic agents for ovarian cancer, carboplatin, and paclitaxel [17].

Combination therapy with paclitaxel and bevacizumab produced significantly improved animal survival ($P < 0.05$) compared to the administration of IP paclitaxel alone (Fig. 3a). Bevacizumab IV with IP paclitaxel and bevacizumab IP with IP paclitaxel resulted in long-term cures in 4 of 7 and 3 of 7 animals, along with a greater than 150% increase in life span for mice bearing human ovarian cancer xenografts. Combination therapy with IP carboplatin and bevacizumab, IV or IP, was also significantly ($P < 0.05$) superior to IP carboplatin alone (Fig. 3b). Bevacizumab IV with IP carboplatin and bevacizumab IP with IP carboplatin resulted in long-term cures in 3 of 7 and 1 of 7 animals, along with a 155 and 44% increase in life span. As such, similar results have been shown for combinations of bevacizumab and IP chemotherapy with four separate drugs, topotecan, cisplatin, paclitaxel, and carboplatin. The consistent results for all four chemotherapeutic drugs were expected, as the determinants of capillary removal of drug from peritoneal tumors (i.e., tumor blood flow, capillary surface area, and capillary permeability) are relevant for all chemotherapeutic drugs. Thus, anti-angiogenic therapy is expected to lead to increased tumor exposure and antitumor efficacy for any chemotherapeutic drug after regional administration (e.g., IP chemotherapy of peritoneal tumors).

Systemic toxicity for the proposed combination therapy was assessed by monitoring the animal body weight. There was no substantial weight loss observed in any of the treatment groups (Table 2), which suggests an absence of significant systemic toxicity. These results were also

anticipated because anti-angiogenic agents primarily inhibit neovascularization [19]; hence, bevacizumab would not be expected to affect blood perfusion or increase drug exposure in organs or tissues with mature blood vessels (including organs and tissues associated with the toxicities of carboplatin and paclitaxel).

The present work has demonstrated rapid absorption of bevacizumab following IP dosing, with nearly complete bioavailability (92.8%). The observed pharmacokinetic data were well characterized with a two-compartment model with linear elimination. The terminal half-life for the drug and the volumes of central compartment was found to be 5.5 days and 49.4 ml/kg, which is similar to the values of 6.8 days and 53 ml/kg reported in the literature [20]. The clearance from the central compartment was found to be 6.27 ml/day/kg, which was somewhat lower than the value of 15.7 ml/day/kg reported by Lin et al. [20]. Computer simulations predicted that steady state peak and trough bevacizumab concentrations were 261 and 160 μg/ml after IV administration, and 202 and 146 μg/ml, respectively, after IP dosing (data not shown). The slightly lower values predicted for IP dosing reflect the incomplete (92.8%) bioavailability of the antibody and the kinetics of bevacizumab absorption. However, irrespective of the route of administration, bevacizumab concentrations are predicted to be far in excess of the equilibrium dissociation constant for bevacizumab binding to VEGF (Kd = 0.33 μg/ml [21]). We found no significant differences in animal survival for groups of mice that received bevacizumab IV vs. IP, either when bevacizumab was administered as monotherapy or in combination with IP chemotherapy (Fig. 3a–c). In contrast to the demonstrated benefits of IP administration of chemotherapy, our findings suggest that, when compared to IV administration, IP dosing of bevacizumab does not provide a significant improvement in survival. This is notable, as the IV route is more convenient and offers improved control of delivery, which may provide safety advantages (e.g., allowing cessation of bevacizumab administration for patients demonstrating signs of bevacizumab infusion reactions).

At present, there are twelve clinical trials (www.clinicaltrials.gov) underway that investigate various combinations of paclitaxel, carboplatin/cisplatin, and bevacizumab to treat peritoneal tumors, with most employing IV administration of the chemotherapeutic agents. NCT00652119 (finishing February 2021) comes closest to the combination therapy that we have investigated in our animal studies. Patients participating in this trial are scheduled for six three-week cycles of therapy. Cycle 1 consists of 60 mg/m^2 IV paclitaxel weekly over 1 h for 3 weeks and carboplatin IV over 1 h on day 1. Cycle 2 calls for 60 mg/m^2 IP paclitaxel weekly over 1 h for 3 weeks, carboplatin IP over 1 h on day 1, and bevacizumab 15 mg/kg IV over 90 min on day 8. For cycles 3–6, the dosing regimen for paclitaxel and carboplatin is the same as in cycle 2, but bevacizumab is administered on day 1 of the cycle instead of on day 8.

The results of our current and prior work support the general design of NCT00652119. However, we believe that the treatment schedule may be improved by earlier initiation of bevacizumab therapy. In the present study, and in our prior investigations with combined bevacizumab and IP chemotherapy, anti-angiogenic treatment was initiated prior to the start of chemotherapy. The putative mechanistic explanation for the enhanced efficacy observed in our preclinical investigations is that decreased neovascularization of developing tumors, which results from pretreatment with anti-angiogenic therapy, leads to a decreased rate of drug removal by tumor capillaries, thereby leading to increased drug exposure in peritoneal tumors following IP (but not following IV) chemotherapy. As such, it is expected that full benefit of the combined therapy requires administration of the anti-angiogenic therapy in advance of the initiation of IP chemotherapy, and it is expected that benefit will be greatest when bevacizumab therapy is initiated as soon as possible following cytoreductive surgery.

In summary, we have found rapid and near complete absorption of bevacizumab following IP dosing, and we have found significant increases in animal survival following combined therapy with bevacizumab and IP carboplatin or IP paclitaxel in a mouse model of human ovarian cancer. The effects of bevacizumab, either alone or in combination with IP carboplatin or IP paclitaxel, were not found to be significantly dependent on the route of bevacizumab administration (IP vs. IV). As such, the data indicate that the route of bevacizumab administration is not an important determinant of its efficacy, and the data suggest that the IP and IV routes may be used interchangeably.

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Conflict of interest None.

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