Population Pharmacokinetics of Bevacizumab in Children with Osteosarcoma: Implications for Dosing

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Abstract

Purpose: To describe sources of interindividual variability in bevacizumab disposition in pediatric patients and explore associations among bevacizumab pharmacokinetics and clinical wound healing outcomes.

Experimental Design: Before tumor resection, three doses of bevacizumab (15 mg/kg) were administered to patients (median age, 12.2 years) enrolled in a multi-institutional osteosarcoma trial. Serial sampling for bevacizumab pharmacokinetics was obtained from 27 patients. A population pharmacokinetic model was fit to the data, and patient demographics and clinical chemistry values were systematically tested as predictive covariates on model parameters. Associations between bevacizumab exposure and wound healing status were evaluated by logistic regression.

Results: Bevacizumab concentration–time data were adequately described by a two-compartment model. Pharmacokinetic parameter estimates were similar to those previously reported in adults, with a long median (range) terminal half-life of 12.2 days (8.6 to 32.4 days) and a volume of distribution indicating confinement primarily to the vascular space, 49.1 mL/kg (27.1 to 68.3 mL/kg). Body composition was a key determinant of bevacizumab exposure, as body mass index percentile was significantly ($P < 0.05$) correlated to body-weight normalized clearance and volume of distribution. Furthermore, bevacizumab exposure before primary tumor resection was associated with increased risk of major wound healing complications after surgery ($P < 0.05$).

Conclusion: A population pharmacokinetic model for bevacizumab was developed, which demonstrated that variability in bevacizumab exposure using weight-based dosing is related to body composition. Bevacizumab dosage scaling using ideal body weight would provide an improved dosing approach in children by minimizing pharmacokinetic variability and reducing likelihood of major wound healing complications. Clin Cancer Res; 20(10); 2783–92. ©2014 AACR.

Introduction

Bevacizumab is a humanized monoclonal immunoglobulin G1 (IgG1) antibody that inhibits VEGF, blocking its interaction with VEGF cell-surface receptors and the subsequent intracellular signaling cascade that promotes proliferation and mobilization of endothelial cells that comprise the tumor vasculature network (1). Preclinical data from xenograft studies suggest that single-agent therapy with bevacizumab impairs tumor growth in a variety of different tumor types (2), and the combination of bevacizumab with other cytotoxic agents or radiotherapy achieves additive or synergistic tumor growth inhibition (2–4). In clinical trials, bevacizumab has shown activity against colorectal cancer (5–10), non-small cell lung cancer (11), renal cell carcinoma (12), ovarian cancer (13), and glioblastoma multiforme (14–16). On the basis of data linking higher VEGF expression levels with poor prognosis and increased risk of metastases in patients with osteosarcoma (17–19), we prospectively evaluated the feasibility of a novel treatment regimen that combines standard chemotherapy with bevacizumab (clinical trial NCT00667342).

The pharmacokinetic disposition of bevacizumab has been previously described in adult patients receiving dosages of 1 to 20 mg/kg every 1, 2, or 3 weeks (20, 21).
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Translational Relevance
Although not yet approved for use in children, bevacizumab is a widely studied antiangiogenic agent in adults, and several early-phase trials are currently evaluating bevacizumab regimens in children. This report describes the only population pharmacokinetic model of bevacizumab in children and establishes, for the first time, bevacizumab pediatric pharmacokinetic parameters including the interindividual and interoccasion variability. This pharmacokinetic/pharmacodynamic analysis also demonstrates a significant link between treatment outcomes (i.e., postsurgical wound healing) and systemic bevacizumab exposure in children and adolescents treated for osteosarcoma. The impact of this finding is clinically significant because surgical tumor resection is an important component of care for patients suffering from osteosarcoma and many other solid tumor types. Pharmacokinetic simulations suggest that dosing of bevacizumab based on ideal body weight would be more appropriate to mitigate pharmacokinetic variability encountered because of discrepancies in body composition between underweight and overweight children and adolescents.

The estimated terminal half-life in this patient population was 22.8 days (range, 13–45 days), consistent with other IgG-like antibodies. A Children's Oncology Group phase 1 trial was recently conducted in which bevacizumab was administered intravenously (5, 10, or 15 mg/kg) every 2 weeks to children with refractory solid tumors; however, pharmacokinetic evaluation was limited to noncompartmental analysis of data from only 8 patients (22). Hence, the potential sources of interindividual variability of bevacizumab disposition in pediatric patients and the implications of this variability upon bevacizumab safety and efficacy remain poorly understood.

One major concern with adding bevacizumab to a treatment regimen for patients with osteosarcoma is that aggressive surgery is an integral part of curative therapy. Data suggest that bevacizumab administration could impede surgical wound healing, owing to the underlying similarities between pathologic angiogenesis and normal wound repair (23–27). Patients undergoing major surgical procedures who receive bevacizumab have an increased risk of postoperative bleeding and wound complications versus patients receiving the same chemotherapy without bevacizumab (5, 28). Reported healing complications include abnormal bruising/bleeding and wound dehiscence (8, 28). Because these data suggest a link between bevacizumab treatment and wound healing complications, one of the key objectives of our trial was to establish whether a significant relationship exists between bevacizumab pharmacokinetic disposition and clinical wound healing outcomes. To address this, we performed intensive serial sampling for bevacizumab pharmacokinetics to define the pharmacokinetic parameters and interindividual variability of bevacizumab disposition in pediatric patients. We then examined the contribution of various patient covariates toward the interindividual variability of bevacizumab pharmacokinetic parameters and investigated the potential relationship between bevacizumab systemic exposure and postoperative wound complications.

Patients and Methods

Patient population and bevacizumab administration
This study was carried out as part of a multi-institutional clinical trial (NCT00667342) to evaluate the feasibility of combining bevacizumab with chemotherapy for treatment of children with newly diagnosed osteosarcoma. The trial was approved by the Institutional Review Board of each participating institution, and written informed consent was obtained from patients, parents, or legal guardians. Patients with either localized or metastatic osteosarcoma were eligible. Preoperative chemotherapy consisted of cisplatin (60 mg/m², days 1 and 2) and doxorubicin (25 mg/m², days 1–3) at weeks 0 and 5 and high-dose methotrexate (12 g/m², day 1) at weeks 3, 4, 8, and 9 (29). Bevacizumab was administered at 15 mg/kg i.v. 3 days before the first dose of chemotherapy at week 0, and on day 1 of week 3 and 5 of chemotherapy. Surgery for resection of the primary tumor was performed at approximately week 10. The first bevacizumab dose was infused over 90 minutes and if tolerated, subsequent doses were infused over 60 minutes and then 30 minutes. After surgery, bevacizumab was held for at least 5 weeks or until adequate wound healing occurred. Patients with localized disease continued on a regimen of cisplatin, doxorubicin, and methotrexate, whereas patients with metastatic disease also received ifosfamide and etoposide.

Pharmacokinetic sampling strategy
Ten serial blood samples (2-mL each) for pharmacokinetic studies were collected with the first (week 0) and third (week 5) bevacizumab doses each at the following times: predose, end of infusion, and at hours 1, 3, 6, 24 (± 4 hours), 48 (± 6 hours), 96 (± 6 hours), 144 (± 24 hours), and 288 hours (± 24 hours) after the end of the infusion. Two blood samples were also collected with the second (week 3) bevacizumab dose both before and at the end of the infusion. After the third bevacizumab dose on week 5, samples were collected weekly until definitive surgery at week 10.

Twenty-seven patients were assessable for bevacizumab pharmacokinetics. Of these, all had bevacizumab concentration–time data for weeks 0, 3, and 5 except 1 patient whose week 0 and week 3 dose was withheld (only week 5 administered). In total, 26 patients were studied on all three planned presurgery bevacizumab dosing occasions to define the interoccasion variability of bevacizumab.

Blood samples were collected in a clot activator containing vacutainer tube that was placed upright to clot at room temperature (~30 minutes), then centrifuged for 10 minutes at 4°C at 3,000 ×g. Extracted serum was stored at −80°C. Serum bevacizumab concentrations were determined using a proprietary GLP compliant validated ELISA (ref. 20; QPS). All samples were analyzed...
as a single batch. The lower limit of sensitivity of this assay was 78 ng/mL.

**Population pharmacokinetic modeling**

In total, concentration data from 749 serum samples were used for the population pharmacokinetic analysis. No samples other than the day 1 preinfusion samples were below the lower limit of quantitation. The pharmacokinetic parameters of bevacizumab were determined by nonlinear mixed-effects modeling via NONMEM (version 7.2), sequentially using the iterative two-stage (ITS), stochastic approximation expectation-maximization (SAEM), and importance sampling (IMP) estimation methods. Bevacizumab concentration–time profiles followed a biexponential decay (Supplementary Figs. S1 and S2), so the data were fit by a two-compartment structural model with first-order elimination from the central compartment (ADVAN6 subroutine) as also described in previously published pharmacokinetic studies (20). The slope of the terminal elimination phase, \( \beta \), was calculated using the post hoc microrate constants, and \( \beta \) was used to determine the terminal half-life, \( t_{1/2} = \frac{\ln(2)}{\beta} \).

The entire population was used to estimate population means and coefficients of variation of the bevacizumab central compartment volume of distribution (\( V_{\text{central}} \)), total systemic clearance (CL, in mL/day/kg), and intercompartmental rate constants between the central and peripheral compartment (\( k_{12} \) and \( k_{21} \) in days\(^{-1} \)). Interindividual variability (IVV) was assumed to be log-normally distributed for all pharmacokinetic parameters, so IVV was exponentially scaled on each population parameter. Thus, for the ith individual:

\[
\theta_i = \theta_{\text{pop}} \times e^{(\sum_{a=1}^{n} \eta_{a,i} + \eta_a)} \tag{1}
\]

where \( \theta_i \) is the value of parameter, \( k_i \) in the given individual, \( \theta_{\text{pop}} \) is the typical value of the parameter in the population, and \( \eta_{a,i} \) is a normally distributed random variable with a mean of zero and a variance of \( \sigma_{\eta_a}^2 \) (estimated by NONMEM). Because bevacizumab was administered on multiple occasions per individual, \( \eta_{a,j} \) represents the variability of occasion \( j \) from individual \( i \) average value (i.e., between-occasion variability) with mean 0 and variance \( \sigma_{\eta_a}^2 \). An occasion was defined as the time from the start of the corresponding infusion to the start of the next infusion (or surgery). The full covariance matrix was implemented with all between-subject \( \eta \) terms. The random-effect residual error model, resulting from assay errors and other unexplained sources, was described by mixed proportional plus additive terms:

\[
C_{i,m} = C_{\text{predicted},i,m} \times (1 + C_{\text{prop},i,m}) + C_{\text{add},i,m} \tag{2}
\]

in which \( C_{i,m} \) is the mth measured concentration of the ith individual, \( C_{\text{predicted},i,m} \) is the corresponding predicted concentration, and \( C_{\text{prop},i,m} \) and \( C_{\text{add},i,m} \) are the normally distributed proportional and additive random variables with mean zero and variances \( \sigma_{\text{prop}}^2 \) and \( \sigma_{\text{add}}^2 \), respectively.

A bootstrap resampling method (\( n = 1,000 \)) was applied for internal validation of the final population model. Finally, a visual predictive check was used to evaluate the adequacy of the final population model by comparing the distribution of observed concentrations with the distribution of simulated concentrations based on the final estimates of parameter/covariate relationship.

**Relationship between pharmacokinetic parameters and covariates**

The following exploratory covariates were tested to explain the interindividual variability of the population pharmacokinetic model: age, gender, height, total body weight (TBW), ideal body weight (IBW), adjusted body weight (AIBW), body mass index percentilie (BMI%), body surface area (BSA) calculated using the Gehan and George formula (30) using TBW and height, serum albumin, total protein, alkaline phosphatase (ALKP), and aspartate aminotransferase (AST). For modeling purposes, measurements were taken at baseline before the first bevacizumab dosing. The \( \chi^2 \) test was used to compare the objective function values (OFV) of nested models (likelihood ratio test). A covariate was considered significant in this analysis if the addition of the covariate to the model reduced the \( -2 \) log-likelihood at least 3.84 units (\( P < 0.05 \), based on the \( \chi^2 \) test for the difference in the \( -2 \) log-likelihood between two hierarchical models that differ by 1 degree of freedom).

BMI was calculated as TBW (kg) divided by height\(^2 \) (m\(^2 \)). IBW and BMI% were determined using the gender-specific BMI-for-age growth charts established for children 2 to 20 years of age published by the Center for Disease Control and Prevention (31). According to these standard guidelines, BMI values characterized in the upper 15th percentile of BMI for age and gender. AIBW was calculated using IBW and TBW by the conventional formula, AIBW = (TBW - IBW) \( \times \) 0.40 + IBW. Clinical studies suggest that the weighting factor of 0.40 in this formula is appropriate because extracellular water space in adipose tissue is approximately 40% of that of other tissues (32–35).

Three different parameterizations were explored to describe the relation between TBW and bevacizumab pharmacokinetic parameters:

**Model parameterization [A]:**

\[
\theta_{\text{prop}, \text{CL}} = \theta_{\text{base}, \text{CL}} \times \text{TBW}^{0.75} \tag{3}
\]

\[
\theta_{\text{prop}, \text{V}} = \theta_{\text{base}, \text{V}} \times \text{TBW}^{1.2} \tag{4}
\]

**Model parameterization [B]:**

\[
\theta_{\text{prop}, \text{CL}} = \theta_{\text{base}, \text{CL}} \times \text{TBW} \tag{5}
\]

\[
\theta_{\text{prop}, \text{V}} = \theta_{\text{base}, \text{V}} \times \text{TBW} \tag{6}
\]

**Model parameterization [C]:**

\[
\theta_{\text{prop}, \text{CL}} = \theta_{\text{base}, \text{CL}} \tag{7}
\]

In model parameterization [A], body weight was implemented a priori as a covariate for clearance and volume of distribution values using an allometric equation with fixed exponent of 0.75 for clearance and 1.0 for volume of...
distribution. In parameterization [B], a fixed linear relationship between TBW and clearance as well as TBW and volume of distribution was assumed because bevacizumab dosages on this protocol were scaled on the basis of patient weight (this relation to body weight is inherently built into all bevacizumab TBW-based clinical dosing regimens). In the third parameterization [C], no a priori relationship between body weight and bevacizumab pharmacokinetic parameters was presumed.

As a preliminary investigation of associations between other potential covariates (aside from TBW) and model parameters, scatter plots of the covariates and post hoc parameter estimates were visually examined. All covariates in this screening process were tested in a univariate fashion in the population model by inclusion in the model as an additional estimated parameter. The relationship between the pharmacokinetic parameters and categorical or continuous covariates (aside from TBW) were described using either a simple multiplicative or an exponential multiplicative model. The exponential multiplicative model codes for a fractional change in the parameter estimate: 

$$\theta_{\text{pop},k} = \theta_{\text{base},k} \times e^{\text{covariate}_p \times \beta_{p,k}}$$

where $\theta_{\text{base},k}$ represents the baseline population parameter estimate not explained by any of the included covariates, and $\beta_{p,k}$ was the effect of covariate $P$ on the model parameter, $k$. Likewise, the simple multiplicative model was coded according to the relationship in equation 4:

$$\theta_{\text{pop},k} = \theta_{p,k} \times \text{Covariate}_p$$

For evaluation of the predictive performance of the models, CL, and $V_{\text{normalized}}$ were calculated for each patient, given the model fixed-effects relationships. The prediction error ($P_e$) for individual parameter estimate was calculated as the population prediction, $k_{\text{POP},i}$, minus the post hoc parameter estimate, $k_{\text{posthoc},i}$ expressed as a relative percentage of the post hoc estimate:

$$P_e = \frac{k_{\text{POP},i} - k_{\text{posthoc},i}}{k_{\text{posthoc},i}} \times 100\%$$

Predictive performance of each model was then assessed by the relative mean prediction error (%MPE) as a measure of parameter estimation bias and the relative root mean square prediction error (RMSE%) as a measure of precision:

$$\%\text{MPE} = \frac{\sum P_e}{n}$$

$$\text{RMSE\%} = \sqrt{\frac{\sum P_e^2}{n}}$$

**Assessment of pharmacokinetic/pharmacodynamic relationship**

The relation of bevacizumab area under the concentration–time curve (AUC) and wound healing complications was investigated because AUC is the best metric of the overall systemic exposure of bevacizumab. Cumulative bevacizumab AUCs were calculated in NONMEM by integrating model-predicted bevacizumab serum concentrations using a dummy compartment. AUC was calculated from the start of infusion of the first dose to the beginning of the second infusion on week 3 ($\text{AUC}_{0,3}$), from the start of infusion of the first dose to beginning of the third infusion at week 5 ($\text{AUC}_{0,5}$), and from the beginning of the third infusion at week 5 to surgery ($\text{AUC}_{5-\text{surgery}}$).

**Statistical analysis**

Wound healing status was represented as a nominal dependent variable with two possible values (e.g., yes or no), whereas AUC data were treated as a continuous independent variable. A univariate logistic model was applied to evaluate the association between bevacizumab systemic exposure and wound healing status. A $P$ value of 0.05 was chosen as the a priori cutoff significance level.

**Results**

**Patient characteristics**

Bevacizumab pharmacokinetic studies were evaluable in 27 patients all of whom had bevacizumab concentration–time data for weeks 0, 3, and 5 except 1 patient whose week 0 and week 3 dose was withheld (only week 5 administered). The median (range) time from the last bevacizumab dose to surgery was 7.3 weeks (5.9 to 9.3). The patients’ baseline characteristics are summarized in Table 1.

**Population pharmacokinetic modeling**

As described in Materials and Methods, three model parameterizations were explored to describe the relation between TBW and bevacizumab pharmacokinetic parameters. To facilitate comparison with prior published TBW-normalized bevacizumab pharmacokinetic data and also emphasize dependency of bevacizumab exposure on body composition in children in this report, we have elected to summarize our analysis of model parameterization [B] (fixed linear relation between TBW and CL/TBW and $V$). Comparison of all model parameterizations are presented in text below and summary results of this assessment are also provided in Supplementary Table S1.

Initial diagnostic plots for the parameterization [B] base population pharmacokinetic model are shown in Fig. 1A and B. The individual predicted concentrations were symmetrically distributed around the line of identity. Conditional weighted residual values were symmetrical and generally distributed around zero. No bias was apparent in the plot of the predicted concentration versus the conditional weighted residual (not shown), suggesting an adequate structural model. Median (range) individual post hoc CL, $V_{\text{normalized}}$, $k_{12}$, and $k_{21}$ estimates were
from the final model bootstrap for parameterization [B] BMI% (Fig. 1D). The population parameter estimates improved after accounting for interpatient differences in prediction versus observed concentration (Fig. 1C) was the negative bias from the line of unity in the population BMI% as a covariate on CL and containing both parameterizations [A] and [B], i.e., those models identified BMI% as a significant covariate for CL.

8.0) –2.27) and 0.34 per day (0.07 to 0.67), respectively.

Independent covariate searches for each model parameterization identified BMI% as a significant covariate for both parameterizations [A] and [B], i.e., those models containing a priori fixed TBW relationships for CL or \(\frac{C}{D}\) OFV confirmed that the negative bias from the line of unity in the population prediction versus observed concentration (Fig. 1C) was improved after accounting for interpatient differences in BMI% (Fig. 1D). The population parameter estimates from the final model bootstrap for parameterization [B] in Table 2 indicate that all final pharmacokinetic parameters for model parameterization [B] were precisely estimated, with relative SEs (RSEs) of <7%. Monte Carlo simulations performed with the final covariate-containing model for parameterization [B] (Supplementary Fig. S3) indicate that the population model successfully captured the distribution of observed bevacizumab serum concentrations by accounting for body composition in the model.

Inclusion of BMI% to correct bias introduced by a priori fixed TBW relationships underscores the dependency of bevacizumab interindividual variability on body composition when administered on a linear TBW-based clinical regimen and furthermore confirms TBW to be a suboptimal body size descriptor for bevacizumab pharmacokinetics in children. This hypothesis is also supported by subsequent covariate analyses of model parameterization [C] indicating bevacizumab CL and \(V_{\text{normalized}}\) scale linearly with IBW in children, rather than TBW (Figs. 2 and 3). Precision and bias of population parameter estimates for parameterizations [A], [B], and [C] (with IBW), summarized in Supplementary Table S1, suggest that linear IBW relationship accounts for a greater proportion of the interindividual variability in children than either of the TBW fixed effects in [A] or [B]. The net change in OFV of model parameterization [C] after inclusion of IBW (\(\Delta\text{OFV} = -62.4\)) was significantly greater than the \(\Delta\text{OFV}\) comparing base parameterization [C] to [B] (\(\Delta\text{OFV} = -30.0\)) or [C] to [A] (\(\Delta\text{OFV} = -35.7\)). Together, these results reveal linear IBW-based parameter scaling to be superior to either of the fixed TBW-based scaling approaches explored in this bevacizumab pediatric pharmacokinetic analysis.

**Assessment of pharmacokinetic/wound healing relationships**

Twenty-six of the 27 patients with bevacizumab pharmacokinetic data were included in the analysis to determine possible associations between bevacizumab exposure and wound complications. One patient was excluded from this analysis because the family refused surgery. All patients had extremity tumors. Limb sparing surgery was performed in 20 patients and amputation in 6. Wound complications were graded using prospectively defined protocol criteria (see Supplementary Material). A complication was either minor or major based on whether it was superficial (above the fascia) or deep (below the fascia). Only wound complications possibly, probably, or definitely related to bevacizumab were considered in the analysis. Six patients had major wound complications after surgery, of which 5 were determined to be related to bevacizumab therapy. Four of these 5 major wound complications occurred before any additional bevacizumab therapy after surgery, and the median time to occurrence of the major wound complication in these 4 patients was 23 days (range, 20–24). Six patients had only minor wound complications after surgery, of which only one was before receiving any additional bevacizumab after surgery. Because only one

<table>
<thead>
<tr>
<th>Table 1. Summary of patient characteristics and laboratory data</th>
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<tr>
<td>Total number of patients</td>
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<tr>
<td>Sex</td>
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<td>Male:female</td>
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<tr>
<td>Race</td>
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<td>White:Black:other(^a)</td>
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<tr>
<td>Stage</td>
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<td>Localized:metastatic</td>
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<td>Age (y)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>Mean (SD)</td>
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<tr>
<td>Body surface area (m(^2))</td>
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<td>Median (range)</td>
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<td>Mean (SD)</td>
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<tr>
<td>BMI%</td>
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<td>Median (range)</td>
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<td>Mean (SD)</td>
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<td>TP (g/dL)</td>
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<td>Mean (SD)</td>
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<td>ALKP (IU/L)</td>
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<td>Albumin (g/dL)</td>
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<td>Median (range)</td>
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<tr>
<td>Mean (SD)</td>
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Abbreviation: TP, total protein.

\(^a\)Hispanic (2), Asian (1).
patient had a minor wound complication before subsequent bevacizumab therapy, no formal statistical analysis was performed assessing effect of bevacizumab exposure on minor wound healing complications.

Univariate logistic regression analysis showed a significant association between AUC5–surgery and major wound healing complications occurring before any subsequent dose of bevacizumab after surgery (Fig. 4A; \( P = 0.03 \)). The odds of a major wound complication increased to 3.1-fold [95% confidence interval (CI), 1.1–9.1] for every 1,000 \( \mu \text{g/mL} \times \text{day} \) increase in AUC5–surgery. One of the children in this study who had a major wound healing complication, a 12-year-old boy with a BMI of 40.6 kg/m², also had the highest estimated bevacizumab AUC5–surgery. Simulations (Fig. 4B) indicate that his AUC5–surgery would likely have fallen in the middle 50th percentile of those patients that experienced no wound complications had he been administered bevacizumab by an IBW-based dosing regimen.

Discussion

This is the first comprehensive population pharmacokinetic/pharmacodynamic study of bevacizumab in children. Our analysis confirms that the disposition of bevacizumab in children is similar to that found in adults, although the terminal half-life is slightly shorter in children. The data suggest that the incidence of major wound complications is related to bevacizumab exposure in the weeks preceding surgery, and secondly, bevacizumab exposure scales linearly with IBW, rather than TBW.

To date, only one published study has explored bevacizumab pharmacokinetics in children (22). In that study, noncompartmental analysis of the pediatric phase I trial

Figure 1. Goodness-of-fit plots from the bevacizumab population pharmacokinetic model (model parameterization [B]). A, observed versus individual predicted concentrations for the covariate-free (base structural) model. B, conditional weighted residual (CWRES) versus time in days for the covariate-free model with LOWESS (solid line). C, observed versus population predicted concentrations for the covariate-free model. D, observed versus population predicted concentrations for the final population model. Dashed lines in panel A, C, and D represent the line of unity.
data showed a median (range) terminal half-life of bevacizumab in children of approximately 11.8 days (3.9 to 14.6 days), consistent with the median (range) in the present analysis of 12.2 days (8.6 to 32.4 days). The median (range) bevacizumab CL from the same phase I trial was 4.1 mL/day/kg (3.1 to 15.5 mL/day/kg), also very similar to the median (range) reported in the present analysis, 4.8 mL/day/kg (2.4 to 7.5 mL/day/kg). The most complete pharmacokinetic information for bevacizumab to date originates from data pooled from 491 adult patients with solid tumors on two phase I, four phase II, and two phase III trials who received bevacizumab dosages of 1 to 20 mg/kg, every week to every 3 weeks (20, 21). In that population analysis, the average terminal (b) half-life was 22.8 days with post hoc estimates ranging from 13 to 45 days. The adult systemic CL of bevacizumab was 0.207 to 0.264 L/day [4.6% coefficient of variation (CV)]. Assuming a typical adult body weight of 74 kg, this estimate falls somewhere in the range of approximately 2.8 to 3.6 mL/day/kg; so overall, the bevacizumab pharmacokinetic parameters derived from our analysis confirm prior published data in children with elimination estimates slightly more rapid than published adult data.

In adults, the clearance and volume of distribution of the central compartment were higher in males than females. In addition, the final adult population pharmacokinetic model table 2. Final population pharmacokinetic parameter median estimates and CI from 1,000 bootstrap replicates of original dataset

<table>
<thead>
<tr>
<th>Parameter/covariate relationship</th>
<th>Median estimate (95% CI)</th>
<th>IIV, % CV&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>Intercoccasion variability, % CV&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/mL/day/kg &lt;sup&gt;1&lt;/sup&gt; = CL × e&lt;sup&gt;BMI%×BML&lt;/sup&gt;</td>
<td>5.16 (4.36–5.74)</td>
<td>18.4% (8.4%–33.9%)</td>
<td>18.2% (13.3%–22.6%)</td>
</tr>
<tr>
<td>CL/&lt;sup&gt;2&lt;/sup&gt;/BMI%&lt;sub&gt;CL&lt;/sub&gt;</td>
<td>–0.004 (–0.006 to –0.002)</td>
<td></td>
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<tr>
<td>V/&lt;sup&gt;3&lt;/sup&gt;/normalized (L/kg) = V × e&lt;sup&gt;BMI%×BML&lt;/sup&gt;</td>
<td>62.7 (57.5–69.0)</td>
<td>13.2% (9.0%–17.6%)</td>
<td>4.7% (1.6%–7.4%)</td>
</tr>
<tr>
<td>V/&lt;sup&gt;4&lt;/sup&gt;/BMI%&lt;sub&gt;V&lt;/sub&gt;</td>
<td>–0.005 (–0.006 to –0.003)</td>
<td></td>
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<tr>
<td>k&lt;sub&gt;12&lt;/sub&gt; (per day)</td>
<td>0.222 (0.187–0.268)</td>
<td>25.0% (12.7–37.3%)</td>
<td></td>
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<tr>
<td>k&lt;sub&gt;21&lt;/sub&gt; (per day)</td>
<td>0.335 (0.250–0.418)</td>
<td>49.8% (15.1–87.7%)</td>
<td></td>
</tr>
<tr>
<td>Residual variability</td>
<td>Proportional</td>
<td>0.014 (0.004–0.018)</td>
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<tr>
<td>Additive</td>
<td>0.003 (–0.003 to 0.010)</td>
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</table>

NOTE: k<sub>12</sub> and k<sub>21</sub> are intercompartmental rate constants.
Abbreviations: CL, clearance; V/<sup>5</sup>/normalized, body-weight normalized volume of central compartment; CV, coefficient of variation.

<sup>a</sup>√<sup>6</sup> × 100% and <sup>b</sup>√<sup>7</sup> × 100% (both from equation 1).

Figure 2. A, the plot of bevacizumab V/<sup>5</sup>/normalized (weight normalized volume of distribution from parameterization [B]) versus TBW implies an apparent nonlinear relationship between TBW and V/<sup>5</sup>/normalized. In cases of extreme body weight, the V/<sup>5</sup>/normalized of bevacizumab was up to 53% lower (BMI > 97th percentile) or up to 32.1% higher (BMI < 3rd percentile) than the population median value. B, the trend in the scatter plot of CL versus BMI% further confirms that body composition is a key determinant of pharmacokinetic interindividual variability when bevacizumab is dosed on a TBW-based regimen.
included several covariates on clearance: gender, TBW, albumin levels, ALKP, AST, and chemotherapy. Gender, TBW, and albumin levels were also identified as significant covariates on the bevacizumab volume of distribution in adults. In contrast with these adult findings, our analysis, performed on the weight-normalized pharmacokinetic parameters for children (model parameterization [B]), identified BMI% as the most important covariate, with individual post hoc estimates for $V_{\text{normalized}}$ (Fig. 2) and CL significantly higher in obese children compared with normal-weight and underweight children. The apparent explanation for this phenomenon is evident in the nonlinear relationship between TBW and $V_{\text{normalized}}$ (Fig. 3B). This nonlinearity is especially evident in pediatric patients who have age- and gender-adjusted BMI% values in percentiles generally associated with overweight/obesity (Figs. 2 and 3B).

Current bevacizumab dosing regimens in children (and adults) assume a linear relationship between volume of distribution and total body weight (i.e., model parameterization [B]). We find that IBW, in contrast, shows a strong linear relationship with bevacizumab $V_{\text{normalized}}$ (Fig. 3C; model parameterization [C]). These results suggest that IBW-based dosing of bevacizumab would decrease the interindividual variability in exposure by at least 10% in the average patient and up to 45% in obese patients and, in turn, help to maintain more consistent serum concentrations across the spectrum of clinical BMI% values observed in our population. The dosing of several other agents with similar physicochemical properties (e.g., IgGs for immunodeficiency disorders, antimicrobials, and anesthetic drugs) are also based on corrected estimates of body size such as lean body mass, AIBW, and IBW (36–39), and it is well established that extravascular distribution of large hydrophilic drugs such as proteins and other therapeutic macromolecules is limited by their permeability across biologic barriers. As a result, such agents are presumably less likely to diffuse into adipose tissue and, therefore, more likely to scale with measures of extracellular fluid volume (ECV) or total body water (40). In children, lean body mass shows a strong positive linear correlation with measured ECV that is similar to that observed in adults (41).
Minimizing interindividual variability in bevacizumab exposure is critical to ensure that therapeutic exposures are attained while mitigating drug-related adverse events. This is highlighted by the evident correlation between bevacizumab exposure and risk of major wound healing complications observed in our patient population. Whereas previous studies have suggested an association between bevacizumab administration and surgical complications, this is the first study to our knowledge to link bevacizumab exposure directly with wound healing outcomes. Obese patients may be inherently predisposed to wound healing complications (42, 43), but the added burden of elevated concentrations of an antiangiogenic agent likely exacerbates the likelihood of such complications. Thus, on the basis of the relationship between bevacizumab AUC, obesity, and incidence of wound healing complications, bevacizumab dosing scaled linearly to IBW provides an improved dosing model in children. The biologic rationale suggests that a similar dosing strategy would be appropriate in adult populations, but further studies will be required to confirm if this is, in fact, true.

In summary, our data provide the first comprehensive description of the pharmacokinetics of bevacizumab in children, and shows that the terminal half-life of bevacizumab is shorter than in adults. Importantly, in patients with osteosarcoma receiving bevacizumab plus standard chemotherapy, we found that the incidence of major wound healing complications after definitive surgery of the primary tumor is correlated with bevacizumab systemic exposure before surgery, and bevacizumab systemic exposure scales linearly with IBW. Thus, the results of this study support dosing bevacizumab based on IBW in this patient population.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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