Development and Evaluation of a Population Pharmacokinetic-Pharmacodynamic Model of Darbepoetin Alfa in Patients with Nonmyeloid Malignancies Undergoing Multicycle Chemotherapy

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ABSTRACT

Anemia is frequently observed in patients undergoing chemotherapy. Administration of darbepoetin alfa, a recombinant erythropoiesis-stimulating agent that has longer residence time than endogenous erythropoietin, to patients with chemotherapy-induced anemia (CIA) increases mean hemoglobin concentration, reduces risk of red blood cell transfusions, and improves patient-reported outcomes. A pharmacokinetic/pharmacodynamic (PkPd) model was developed using data from patients with nonmyeloid malignancies and CIA who were receiving darbepoetin alfa. A 2-compartment Pk model with linear elimination described the Pk data obtained in 140 CIA patients after intravenous and subcutaneous (SC) doses of 2.25 μg/kg every week and SC doses of 6.75 μg/kg every 3 weeks. The population typical values of key Pk parameters were clearance, 2010 mL/day; steady-state volume of distribution, 3390 mL; and bioavailability, 44.3%. A modified indirect response model, wherein serum concentrations stimulated the production of hemoglobin through an Emax-type equation, described the hemoglobin levels after SC doses of 0.5 μg/kg every week to 15 μg/kg every 3 weeks in 573 CIA patients. The estimated incremental maximum stimulation of hemoglobin production was 43.7% and darbepoetin alfa serum concentration at half-maximal stimulation was 3.68 ng/mL. The impact of covariates (body weight and platinum-containing chemotherapy) on the PkPd response was evaluated based on point and interval estimates of parameters, rather than through stepwise hypothesis testing. The final PkPd model adequately predicted hemoglobin response in a test data set, thereby confirming the predictive capability of the model. Based on simulations, it was not possible to categorize the influence of any covariate as clinically important.

KEYWORDS: population PkPd modeling, darbepoetin alfa, covariate analysis

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INTRODUCTION

Patients with cancer undergoing chemotherapy are frequently anemic (up to 90%) as a result of the underlying chronic illness, combined with the myelosuppressive effects of chemotherapy.[1,2] Recombinant erythropoietic agents epoetin alfa (Procrit; recombinant human erythropoietin [rHuEPO]) and darbepoetin alfa (Aranesp) are commonly used to treat chemotherapy-induced anemia (CIA). The administration of these agents increases hemoglobin concentration, which is associated with a decreased risk of red blood cell (RBC) transfusions and improved patient-reported outcomes.[3,4]

Darbepoetin alfa is a hyperglycosylated analog of rHuEPO with increased sialic acid content, resulting in slower clearance and 3- to 4-fold longer elimination half-life,[5] allowing for less frequent administration. The terminal half-life of darbepoetin alfa is longer after subcutaneous (SC) dosing than after intravenous (IV) dosing, presumably because of absorption rate-limited kinetics.[6] The clearance of darbepoetin alfa has also been reported to be lower in CIA subjects than in renal anemia subjects.[7] Recent studies have shown that darbepoetin alfa is effective in raising hemoglobin levels when administered at intervals of every 2 weeks (Q2W) or every 3 weeks (Q3W).[8,9]

A preliminary pharmacokinetic/pharmacodynamic (PkPd) model of darbepoetin alfa in the CIA setting was developed[10]; predictions based on this model have been supported by recent clinical trial data.[11] A recent population pharmacokinetic (Pk) analysis of darbepoetin alfa in healthy subjects[6] suggested that age and bodyweight might be important determinants of the Pk response.

This report focuses on the further refinement of the PkPd model of darbepoetin alfa in the CIA setting using clinical data collected over a wider dose range. Pharmacokinetic data were obtained from 140 subjects after administration of IV (2.25 μg/kg) and SC (6.75 μg/kg) doses. Hemoglobin data were obtained from 573 subjects after administration of various doses ranging from 0.5 to 15 μg/kg.

We also evaluated the role of baseline covariates on the individual hemoglobin response, which has not been published

previously. Identification of covariates influencing the hemoglobin response after erythropoietic treatment has received considerable interest. Beguin et al[12] found that in anemia of kidney failure, fibrinogen and serum transferrin receptor amounts were the best predictors of hemoglobin response after rHuEPO treatment. Drueke[13] reported that iron deficiency status and unknown inflammatory status contributed to the anemia in dialysis patients. In the CIA setting, Beguin hypothesized that the intensity of chemotherapy and functional iron deficiency may influence hemoglobin response after rHuEPO treatment.[14] Statistical analysis of clinical trial data from 1400 CIA patients treated with darbepoetin alfa showed that baseline hemoglobin (Hb_0), type of chemotherapy (Platinum [Pt] vs non-Pt), albumin content, and lactate dehydrogenase (LDH) levels were important predictors of hemoglobin response.[8]

The primary aim of this analysis is to better understand the widely variable Pk and Pd response of darbepoetin alfa in this disease setting, and to aid in the design of more convenient and efficacious dosing regimens for these patients.

Materials and Methods

Summary of Clinical Studies Used in Modeling

Dosing and Pk data were pooled from 2 studies involving a total of 140 patients (Amgen clinical study numbers 990146 and 20010162) (Table 1). In study 990146, 56 CIA patients received either IV or SC darbepoetin alfa. In study 20010162, 84 patients were randomized 1:1 to receive 6.75 μg/kg darbepoetin alfa SC Q3W either on day 1 or day 15 of a 3-week (21-day) chemotherapy cycle.

Hemoglobin data were gathered from a total of 573 CIA patients given multiple doses of darbepoetin alfa ranging from 0.5 to 4.5 μg/kg every week (QW), 3.0 to 9.0 μg/kg Q2W, 4.5 to 15 μg/kg Q3W, and 9.0 to 18.0 μg/kg Q4W (Amgen clinical studies 20010162, 980290, 980291). These data represent the majority of the range of doses that have been administered in the CIA setting. Hemoglobin data for patients in study 990146 were not used for the Pd analysis because the hemoglobin entry criterion was substantially different from the other studies.

Patient Demographics and Baseline Characteristics

The institutional review boards approved the individual clinical study protocols, and all patients provided written informed consent before any study-specific procedures were done. Eligible patients were ≥18 years of age, were receiving cyclic chemotherapy for nonmyeloid malignancies, and were anemic (hemoglobin concentration ≥9.0 g/dL and ≤11.0 g/dL) as a result of cancer or chemotherapy; eligible patients enrolled in study 990146 were required to have hemoglobin ≤13.0 g/dL and as such may not have been anemic (hemoglobin concentration ≤11.0 g/dL). Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, and adequate renal and hepatic function.

Patients with a history of seizures, significant cardiac or inflammatory disease, or primary hematologic disorder that could cause anemia were excluded. Patients who received any rHuEPO; more than 2 RBC transfusions within 4 weeks before study drug assignment; or any RBC transfusion during the current chemotherapy cycle before randomization, were not eligible. Table 2 describes the baseline characteristics of the population used in the development of the Pk and PkPd models.

Analytical Methods

Serum concentrations of darbepoetin alfa were measured using a validated enzyme-linked immunosorbent assay

Table 1. Summary of Dosing and Pharmacokinetic-Pharmacodynamic Sampling Information in the Analysis Data Set*

Study	Route of Administration	Dose and Schedule	Full Pk Profile†	Sampling Schedule Multiple Trough Samples	Hb Samples‡
990146	IV or SC	2.25 μg/kg QW	Weeks 1 and 9	QW, weeks 1 to 9	-
20010162	SC	6.75 μg/kg Q3W	Day 1	QW, weeks 1 to 16	QW, weeks 1-16
980290	SC	0.5 to 4.5 μg/kg QW	-	-	QW, weeks 1-16
	SC	3.0 to 9.0 μg/kg Q2W	-	-	QW, weeks 1-16
980291	SC	4.5 to 15 μg/kg Q3W	-	-	QW, weeks 1-18
	SC	9.0 to 18 μg/kg Q4W	-	-	QW, weeks 1-17

*Pk indicates pharmacokinetic; Hb, hemoglobin; IV, intravenous; SC, subcutaneous; QW, every week; Q3W, every 3 weeks; Q2W, every 2 weeks; and Q4W, every 4 weeks.

†In Study 990146, Pk samples were collected 5 and 30 minutes postdose and 6, 24, 48, 72, 96, 120, and 168 hours postdose. In study 20010162, Pk samples were collected 6, 24, 48, 72, 96, 120, 168, 174, 192, 216, 312, 336, 360, and 480 hours postdose.

‡Hemoglobin samples were taken weekly and before drug administration (on days of drug administration).

Table 2. Baseline Patient Characteristics Used in Pharmacokinetic and PkPd Model Development*

	Pk Model Development Data Set			PkPd Model Development Data Set		
	990146	20010162	Combined (990146 and 20010162)	980290	980291	Combined (20010162, 980290, 980291)
Number of patients	56	84	140	228	261	573
Gender, n (%)						
Males	23 (41%)	21 (25%)	44 (31%)	71 (31%)	78 (70%)	170 (30%)
Females	33 (59%)	63 (75%)	96 (69%)	157 (69%)	183 (30%)	403 (70%)
Age (years)						
Mean (SD)	62.7 (11.8)	62.0 (13.6)	62.3 (12.9)	61.8 (11.8)	57.9 (11.9)	60.1 (12.3)
Min, Max	22, 86	32, 87	22, 87	20, 91	22, 84	20, 91
Body Weight (kg)						
Mean (SD)	68.8 (16.2)	72.2 (17.8)	70.8 (17.2)	71.5 (16.8)	67.7 (14.8)	69.9 (16.2)
Min, Max	36, 112	41, 123	36, 123	39, 129	40, 136	39, 136
Tumor Type, n (%)						
Breast	5 (9%)	33 (39%)	38 (27%)	69 (30%)	78 (30%)	180 (31%)
Lung	7 (13%)	8 (10%)	15 (11%)	45 (20%)	44 (17%)	97 (17%)
Gastrointestinal	13 (23%)	6 (7%)	19 (14%)	41 (18%)	50 (19%)	97 (17%)
Gynecologic	13 (23%)	13 (15%)	26 (19%)	28 (12%)	56 (21%)	97 (17%)
Genitourinary	4 (7%)	8 (10%)	12 (9%)	14 (6%)	17 (7%)	39 (7%)
Lymphoma	10 (18%)	9 (11%)	19 (14%)	0	0	9 (2%)
Other	4 (7%)	7 (8%)	11 (8%)	31 (14%)	16 (6%)	54 (9%)
Baseline Hb (g/dL)						
Mean (SD)	10.38 (1.41)	10.26 (1.08)	10.30 (1.19)	9.91 (0.936)	9.92 (1.03)	9.96 (1.00)
Min, Max	6.3, 12.7	5.5, 12.1	5.5, 12.7	6.9, 12.2	7, 12.3	5.5, 12.3

*PkPd indicates pharmacokinetic-pharmacodynamic; Pk, pharmacokinetic; SD, standard deviation; min, minimum; max, maximum; and Hb, hemoglobin.

(ELISA),[15] which demonstrated recovery of spike experiments, parallelism, accuracy, interassay precision (coefficient of variation for darbepoetin alfa was 5% to 7%), and stability. This assay, using the Quantikine IVD human erythropoietin ELISA kit (R & D Systems, Minneapolis, MN), was performed by MDS Pharma Services (Montreal, Quebec, Canada). The standard curve range was from 0.125 to 5.0 ng/mL, and the lower limit of quantification was 0.14 ng/mL. Endogenous erythropoietin (eEPO) cross-reacted in this ELISA, therefore the reported darbepoetin alfa serum concentrations include a contribution from endogenous erythropoietin.

Blood samples for hemoglobin measurement were taken every week, before administration of darbepoetin alfa on days darbepoetin alfa was administered. Complete blood count (CBC) including RBCs, hematocrit (Hct), and hemoglobin were measured by standard methods. The assay variability for hemoglobin measurements was between 1% and 5%.

Modeling Methodology

A sequential model fitting approach[16,17] was used to develop the PkPd model using the Pk and hemoglobin data. Darbepoetin alfa serum concentration data from studies 990146 and 20010162 were used to first develop a population Pk model. Then, the Pd parameter estimates were obtained based on the available hemoglobin data, and the fixed-effect Pk parameter estimates from studies 20010162, 980290, and 980291. This method was adopted because Pk and Pd data from the same individual in our data set were available in only a small subset of patients (patients in study 20010162). Fixed effect parameters, representing the population typical estimates, consisted of structural PkPd parameters and covariate coefficients characterizing the hypothesized mean covariate-PkPd parameter relationships. Random interindividual variability (IIV) and random residual (unexplained) variability (RRV) were also estimated. Each subject's eEPO level was assumed to be constant during the duration of Pk assessment and was estimated as another Pk parameter. Thus, eEPO constituted another fixed-effects Pk parameter with random IIV.

For any parametric nonlinear mixed-effects model, it is necessary to assume parametric distributions for the random effects. As a starting point for this analysis, all interindividual error terms were described by a log-transformed exponential error model or log-normal parameter distribution (Equation 1). An attempt was made to define a full-block

covariance matrix for the interindividual random effects (Ω) when possible.

$$P_i = TVP \exp\left(\eta^{Pi}\right), \quad (1)$$

where P_i is the estimated parameter value for individual i; TVP is the typical population value (geometric mean) of the parameter; η^{pi} are individual-specific interindividual random effects for individual i and parameter P and are assumed to be distributed $\eta \sim N(0, \omega^2)$ with interindividual variance-covariance matrix Ω.

For pharmacokinetic observations in this analysis, the residual error model was described by a log-transformed exponential error model (Equation 2).

$$\ln(Cij) = \ln\left(\hat{C}ij + \hat{C}i, eEPO\right) + \varepsilon ij, \quad (2)$$

where C_{ij} is the jth measured observation (serum total drug concentration) in individual i; C^ij is the jth model predicted value (serum darbepoetin alfa concentration) in individual i; C^i, eEPO is the ith model predicted eEPO level in individual i; ε_{ij} is residual random error for individual i and measurement j and is assumed to be independently and identically distributed: $\varepsilon \sim NID(0, \sigma_1^2)$.

Pharmacokinetic models with increasing numbers of disposition compartments were used to describe the data. The best model was chosen based on diagnostics including goodness of fit plots, minimum objective function value (MOFV) after accounting for the number of fitted parameters, and precision and plausibility of parameter estimates. The first-order conditional estimation (FOCE) method was used for Pk parameter estimation. The first-order method was used to obtain Pd parameter estimates because the FOCE method did not converge. Analyses were performed separately on the Pk and PkPd models to identify influential covariates on the Pk and Pd parameters, respectively. A covariate modeling approach emphasizing parameter estimation rather than stepwise hypothesis testing was implemented.[18] First, covariate-parameter relationships were identified based on exploratory graphics, scientific interest, mechanistic plausibility, or prior knowledge, and a full model was constructed with care to avoid correlation or colinearity in predictors. Unlike stepwise methods, inclusion in the full model does not imply clinical importance of the covariate; however, inferences about clinical importance of covariates were then based on the resulting parameter estimates and measures of estimation precision (asymptotic standard errors and bootstrap 95% confidence intervals [CI][19]). No hypothesis testing was conducted. Poor precision of parameter estimates would indicate lack of information of effect rather than lack of effect. This notion is a simplification of the global model approach,[20-23] and has been proposed as an alternative to stepwise regression methods typically used for PkPd covariate analyses.[17,24,25]

The effects of continuous covariates were modeled using a normalized power model, while the effects of categorical covariates were similarly described (Equation 3).

$$TVP = \theta_n \cdot \prod_{i=1}^{m} \left(\frac{cov_i}{ref_i}\right)^{\theta_{(n+i)}} \cdot \prod_{j=1}^{p} \theta_{(j+m+n)}^{\mathrm{COV}_j}, \quad (3)$$

where the typical value of a model parameter (TVP) was described as a function of m individual continuous covariates (cov_i) and p individual categorical (0 or 1) covariates (cov_j), such that θ_n is an estimated parameter describing the typical Pk parameter value for an individual with covariates equal to the reference covariate values ($cov_i = ref_i$, $cov_j = 0$), $\theta_{(i+n)}$ and $\theta_{(j+m+n)}$ were estimated covariate coefficients describing the magnitude of the covariate-parameter relationships.

The asymptotic standard errors of the covariate coefficients estimates were obtained using the $COVARIANCE step in NONMEM (Globomax, Ellicott City, MD). Bootstrap analysis was performed similarly to previously described methods.[16] One thousand replicate bootstrap data sets were obtained by resampling with replacement (sampling unit is individual) from the original data set, and the full model was fitted to each of the data sets. The observed median and 95% CI were obtained for each estimated parameter from the distribution of parameter estimates from 1000 NONMEM estimation runs.

The erythropoiesis PkPd model was developed using the fixed-effect parameters of the full covariate Pk model, and hemoglobin and dosing data from 573 CIA patients. The dose range was from 0.5 μg/kg QW to 15 μg/kg Q3W, and there were a total of 6356 hemoglobin observations. The PkPd model is shown in Figure 1. The overall model governing hemoglobin dynamics is similar to the type III model of Dayneka et al,[26] wherein a zero-order production rate of hemoglobin (which is a "net" of the actual production rate of progenitors and their apoptosis) is stimulated by serum darbepoetin alfa concentrations (Equations 4, 5, and 6).

$$\frac{dHb}{dt} = Rin - Kout \times Hb, \quad (4)$$

where Rin is the hemoglobin production rate (g/dL/time), $Kout$ is the first-order rate constant for overall destruction of hemoglobin (time^{-1}), and Hb is the total hemoglobin at time t. At baseline

$$Hb(t = 0) = Hb_0 \quad (5)$$

$$\frac{dHb}{dt} = 0 \Rightarrow Rin = Kout \times Hb_0 \quad (6)$$

In vivo, stimulation of hemoglobin production occurs through prevention of apoptosis of erythroid progenitors in bone marrow, and there is a delay before these progenitors mature into hemoglobin-carrying RBCs. Also, the hemoglobin-carrying RBCs have a lifespan of ~120 days. A delay compartment chain with first-order transit between the compartments, similar to the one employed by Friberg,[27] mimics

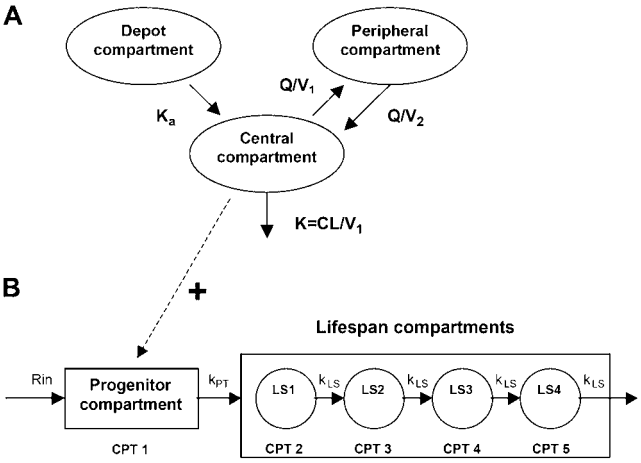

Figure 1. Schematic of the PkPd model showing the link between the 2-compartment Pk model and the indirect effect PD model.

these 2 physiological processes (Figure 1). The progenitor compartment (compartment 1 in Figure 1) with a transit time of RBCPT (first-order transit rate constant k_{PT} = 1/RBCPT) accounts for the delayed hemoglobin response after erythropoietic treatment. Four transit compartments (representing the lifespan of mature RBCs; LS1-LS4 in Figure 1) with equal transit times totaling RBCLS (transit rate constants k_{LS} = 4/RBCLS) approximate the age distribution of mature RBCs. At any time, all mature RBCs in the 4 lifespan compartments (LS1-LS4) are capable of transporting hemoglobin; thus the total hemoglobin is the sum of the hemoglobin amounts in each of the lifespan compartments.

The following differential equations (Equations 7, 8, 9, 10, and 11) govern the rate of change of hemoglobin in the 5 transit compartments; the subscripts denote compartment numbers, CPT1 to CPT5 in Figure 1.

$$\frac{dA_1}{dt} = Rin(1 = Stim) - k_{PT} \times A_1 \quad (7)$$

$$Stim = \left(1 + \frac{S_{\max} C_{DA}}{S_{50} + C_{DA}}\right) \quad (8)$$

$$\frac{dA_2}{dt} = k_{PT} \times A_1 - k_{LS} \times A_2 \quad (9)$$

$$\frac{dA_2}{dt} = k_{LS} \times A_{i-1} - KLS \times A_i i = 3,4,5 \quad (10)$$

$$Hb = \sum_{i=2}^{5} A_i, \quad (11)$$

where A's are amounts in each of the transit compartments, and C_{DA} is serum darbepoetin alfa concentration.

The base model contained 5 fixed-effects parameters: RBCPT (k_{PT}), RBCLS (k_{LS}), S_{max}, S_{50}, and Hb_0. While estimating the Pd parameters, the Pk model consisted of fixed-effects parameters obtained from the full covariate model; random IIV and RRV in the Pk model were ignored. Also, the effect of endogenous EPO level fluctuation on the stimulation of hemoglobin production rate was ignored.

The full PkPd model was evaluated by performing an external predictive check using the fixed-effects and random-effects parameter estimates. Uncertainty in the parameter estimates was ignored. Monte Carlo simulations were conducted against an external data set obtained from a second part of studies 980290 and 980291 that had not been previously used in the modeling process. This data set consisted of 302 patients with population characteristics and administered doses similar to those used in the modeling data set.

The effect of the covariates identified in this analysis on the PkPd response was evaluated by performing predictions with the full model fixed-effect parameters point estimates and 95% CIs, to obtain possible differences in the 16-week hemoglobin response from baseline, at the extremes of the covariate ranges. The ability to achieve and maintain hemoglobin within the range recommended by evidence-based guidelines (11-13 g/dL) is considered a clinically significant response in this setting. For ease of evaluation, however, we considered a hemoglobin response difference of ≥0.5 g/dL as the criteria for evaluating the effect of covariates.

Population PkPd modeling using nonlinear mixed-effects modeling was performed using the NONMEM software version V level 1.2, NMTRAN version III level 1.0, and PREDPP Version IV level 1.0 (Globomax, Ellicott City, MD) running on a Clustered Array of Processors (CAP; CLINapps Inc, Westlake Village, CA) and on a stand-alone laptop (Compaq Evo, running Microsoft Windows 2000, Pentium IV 1.8 MHz processor, Compaq Visual Fortran Version 6.6 compiler). Statistical analysis and graphing were performed using S-Plus (Version 6.2, Insightful Corporation, Seattle, WA). Aggregation of clinical trials data was accomplished using SAS (Version 8.0; SAS Institute Inc, Cary, NC) and S-Plus. Deterministic simulations were performed using SAAM II (SAAM Institute, Seattle, WA).

RESULTS

Pharmacokinetic Model

The final Pk model development data set consisted of 140 patients and 1442 darbepoetin alfa serum concentrations. A 2-compartment disposition model[28] (parameterized with concentration-independent clearance [CL], central volume of distribution [V_1], intercompartmental clearance [Q], peripheral volume of distribution [V_2], first-order absorption rate constant [K_a], bioavailability fraction [F], and endogenous EPO levels [eEPO]) provided the best description of the Pk data obtained after IV and SC doses of 2.25 μg/kg QW and SC doses of 6.75 μg/kg Q3W. The random IIV was obtained for CL, V_1, and K_a. Because Pk data after IV and SC dose administration were obtained from separate sets of patients, IIV of F was not estimated. It was not possible to estimate the IIV of Q and V_2. The interindividual random effect variance-covariance matrix (Ω) contained an

off-diagonal element corresponding to covariance between CL and V_1. A log-transformed exponential error model (Equation 2; variance of residual error: σ_1^2) provided the best fit to the residual variability in the Pk data. This model was used as the base Pk model for subsequent covariate analyses.

Comparison of variability in Pk and hemoglobin response[7,9,29] suggested substantially larger variability in the Pd response compared with that in the Pk response. Therefore, it is possible that covariates that explain Pk variability may not substantially explain the Pd response variability. Nevertheless, covariate-PK parameter relationships were evaluated to confirm this suggestion and to evaluate whether these relationships provide insights into the mechanisms of darbepoetin alfa disposition.

The following covariates were evaluated through a graphical analysis: age, body weight (BWT), sex, race, total chemotherapy count, Pt-containing chemotherapy count (PCNT), and baseline hemoglobin (Hb_0) (plots not shown). It appeared that CL and V_1 increased with increasing BWT, and CL decreased with increasing PCNT. The greatest difference in mean clearance was observed between those with >2 and ≤2 cycles of Pt-chemotherapy during the Pk assessment. The covariate-PK parameter relationships that were explored in the full model are shown below in Equations 12 and 13.

$$TVCL = \bar{\theta}_{CL} \times (\theta_1)^X \times \left(\frac{BWT}{70}\right)^{\theta_2} \quad (12)$$

$$TVV1 = \bar{\theta}_{V1} \times \left(\frac{BWT}{70}\right)^{\theta_3}, \quad (13)$$

where X = 1 if PCNT >2, else X = 0; $\bar{\theta}$'s are the population mean estimates given the reference covariate values; TVs are the typical population values of the parameters, and θ_{1-3} (covariate coefficients) are fitted parameters characterizing the covariate-PK parameter relationships. Estimates of covariate coefficients (θ_{1-3}) were obtained by fitting the model described above to the available Pk data. The final estimates of the covariate coefficients and asymptotic standard errors obtained from a single run of the full model in NONMEM, and the median and 95% CI obtained from the bootstrap analysis are shown in Table 3. Addition of the covariates decreased the IIV on CL (ω^2_{CL}) by 19%, and on V_1 by 15% (data not shown for base model) indicating that none of the covariates significantly accounted for the IIV of Pk parameters in this population. The MOFV decreased by 15 points. The bootstrap 95% CI of estimates of the covariate coefficients (θ_{1-3}) did not overlap their NULL values. The plot of predicted and observed concentrations (Figure 2A) indicated that the model adequately described the observations over the entire range. Most of the weighted residuals were within ±2 SD of the mean (Figure 2B).

PkPd Model

While estimating the Pd parameters, the Pk model consisted of fixed-effects parameters obtained from the full covariate model; random IIV and RRV in the Pk model were ignored. Hemoglobin data for 28 days after an RBC transfusion were censored. We were able to obtain population typical values of all 5 fixed-effects parameters but were not able to estimate random IIV in RBCPT and S_{max}. There was also covariance between S_{50} and Hb_0. A plot of the empirical Bayes estimates of the interindividual random effects parameter (η in Equation 1) of RBCLS and S_{50} obtained from the base PkPd model against baseline covariates indicated that age, BWT, sex, race, and serum levels of lactate dehydrogenase [LDH], ferritin, and albumin did not influence parameter variability (plots not shown). The S_{50} appeared to be higher in Pt-containing chemotherapy-administered subjects. Therefore, this covariate was chosen for evaluation. The covariate-parameter relationship was as follows:

$$TVS50 = \bar{\theta}_{S50} \times (\theta_4)^X, \quad (14)$$

where TVS_{50} is the population typical value of S_{50}; $\bar{\theta}S50$ is the population value under reference covariate conditions; X = 1 if PCNT >0, else X = 0; and θ_4 is the covariate coefficient characterizing the parameter covariate relationship.

The PkPd full covariate model parameters and asymptotic standard errors are shown in Table 4. Bootstrap CIs were not obtained for this model because of the computational intensity of this parameter estimation problem. Approximate symmetric 95% CI was estimated for each parameter as plus or minus 1.96 × standard error (SE) estimated by NONMEM.

We estimated a mean maximum stimulation of production of hemoglobin (S_{max}) of 0.437 (relative standard error [RSE] defined as asymptotic standard error estimated by NONMEM ÷ parameter estimate × 100 = 16%) and an in vivo S_{50} of 3.68 ng/mL (RSE = 38.6%) (Table 4). The IIV in RBCLS and S_{50} were high (CV >100%) illustrating the high variability in hemoglobin response in these patients. Diagnostic plots for the PkPd model (Figure 3) indicate that the model provided an adequate description of the data. Addition of the PCNT covariate reduced the MOFV of the base PkPd model by 11 points. The interindividual variance of S_{50} decreased by ~10%. The typical value of S_{50} was higher in patients receiving Pt-containing chemotherapy. However, the asymptotic 95% CI of the covariate coefficient (θ_4 in Equation 14) overlapped its NULL value indicating lack of information about this effect.

Model Evaluation Using External Predictive Check

The test set contained a total of 302 patients and 1253 hemoglobin observations. The 2.5th to 97.5th quantiles of the predicted data are shown along with all individual observations

Table 3. Full Pharmacokinetic Model Parameter Estimates*

Full Model				
Parameter	**Units**	**Point Estimate†**	**SE (% RSE)**	**Bootstrap Median (95% CI)**
θ_CL	mL/day	2010	128 (6.37)	2010 (1620, 2350)
θ_V1	mL	3390	313 (9.23)	3450 (2900, 4530)
θ_V2	mL	251	92.1 (36.7)	200 (0.337, 393)
θ_Q	mL/day	2900		
θ_Ka	day^{-1}	0.318	0.0275 (8.65)	0.312 (0.269, 0.44)
F	-	0.443	(18.3)	0.458 (0.337, 0.576)
θ_C0	ng/mL	0.415	0.0391 (9.42)	0.421 (0.352, 0.511)
Covariate Coefficients	**NULL Value**	**Point Estimate**	**Asymptotic 95% CI**	**Bootstrap Median (95% CI)**
θ_1 (PCNT on CL)	1	0.737	(0.551, 0.923)	0.756 (0.597, 0.940)
θ_2 (BWT on CL)	0	0.623	(0.172, 1.07)	0.754 (0.221, 1.20)
θ_3 (BWT on V_1)	0	0.639	(−0.01, 1.29)	0.731 (0.169, 1.30)
Variances		**Point Estimate**	**SE (% RSE)**	**Bootstrap Median (95% CI)**
ω^2_{CL}		0.181	0.0397 (21.9)	0.184 (0.112, 0.281)
ω^2_{V1}		0.225	0.0908 (40.4)	0.180 (0.047, 0.373)
$\omega^2_{CL,V1}$		0.134	0.0381 (60.4)	0.119 (0.0478, 0.203)
ω^2_{Ka}		0.0883	0.0272 (30.8)	0.0793 (0, 0.154)
ω^2_{C0}		0.501	0.117 (23.4)	0.476 (0.282, 0.702)
σ_1^2		0.483	0.0348 (7.20)	0.466 (0.409, 0.537)

*SE indicates standard error; RSE, relative standard error calculated as SE/point estimate*100; CI, confidence interval; Hb, hemoglobin; and NULL value, the value of the covariate coefficient that would eliminate the effect of the covariate; PCNT, platinum-containing chemotherapy count; CL, clearance; BWT, body weight; and V_1, central volume of distribution.

†Point estimates and RSE obtained from a single NONMEM run (asymptotic standard errors) and those obtained after a nonparametric bootstrap procedure are shown for the covariate coefficients. Final typical values of the pharmacokinetic parameters are also shown. Comparison of point estimates and CIs of the covariate coefficients obtained from a single NONMEM run (asymptotic standard errors) and those obtained after a nonparametric bootstrap procedure.

in Figure 4. This predictive check indicated that the model could be used in a simulation setting.

Assessment of Effect of Covariates

Differences in the 16-week hemoglobin response for a fixed dose of 150 μg QW (equivalent to the prescribed, weight-based dose of 2.25 μg/kg QW) at the extremes of the covariate range for the covariates identified in this analysis (BWT and Pt-containing chemotherapy) were predicted. The mean (95% CI) for the effect of BWT was 0.939 (0.283-1.45) g/dL and that of chemotherapy was 0.317 (−0.504-0.877) g/dL. While differences were observed, the wide CI around the effect sizes indicated that it was not possible to classify any of the covariates as clinically significant.

DISCUSSION

A population PkPd model was developed using sequential PkPd modeling methods and data from anemic patients with nonmyeloid malignancies receiving multicycle chemotherapy and darbepoetin alfa. This is the first published report of a PkPd model based on transit compartment concepts[30] applied to the Pk and Pd of darbepoetin alfa.

Previous models of erythropoiesis have used physiological components of the erythropoiesis feedback system.[29,31,32] Other, more empirical, models have also been used to describe kinetics of hemoglobin change owing to administered erythropoiesis stimulating proteins.[33,34] Recently, Chapel et al[35] and Ramakrishnan et al,[36,37] applied catenary models based on lifespan concepts, to describe recombinant erythropoietin Pk and Pd, in sheep, monkeys, and healthy subjects, respectively. These PkPd models have attempted to account for different physiological phenomenon such as feedback mechanisms regulating erythropoiesis, and multiple intermediate cell types and their lifespans. The present work, however, is the first attempt to describe the population PkPd response using clinical trial data collected from this patient population. These data are characterized by high intersubject and intrasubject variability because of

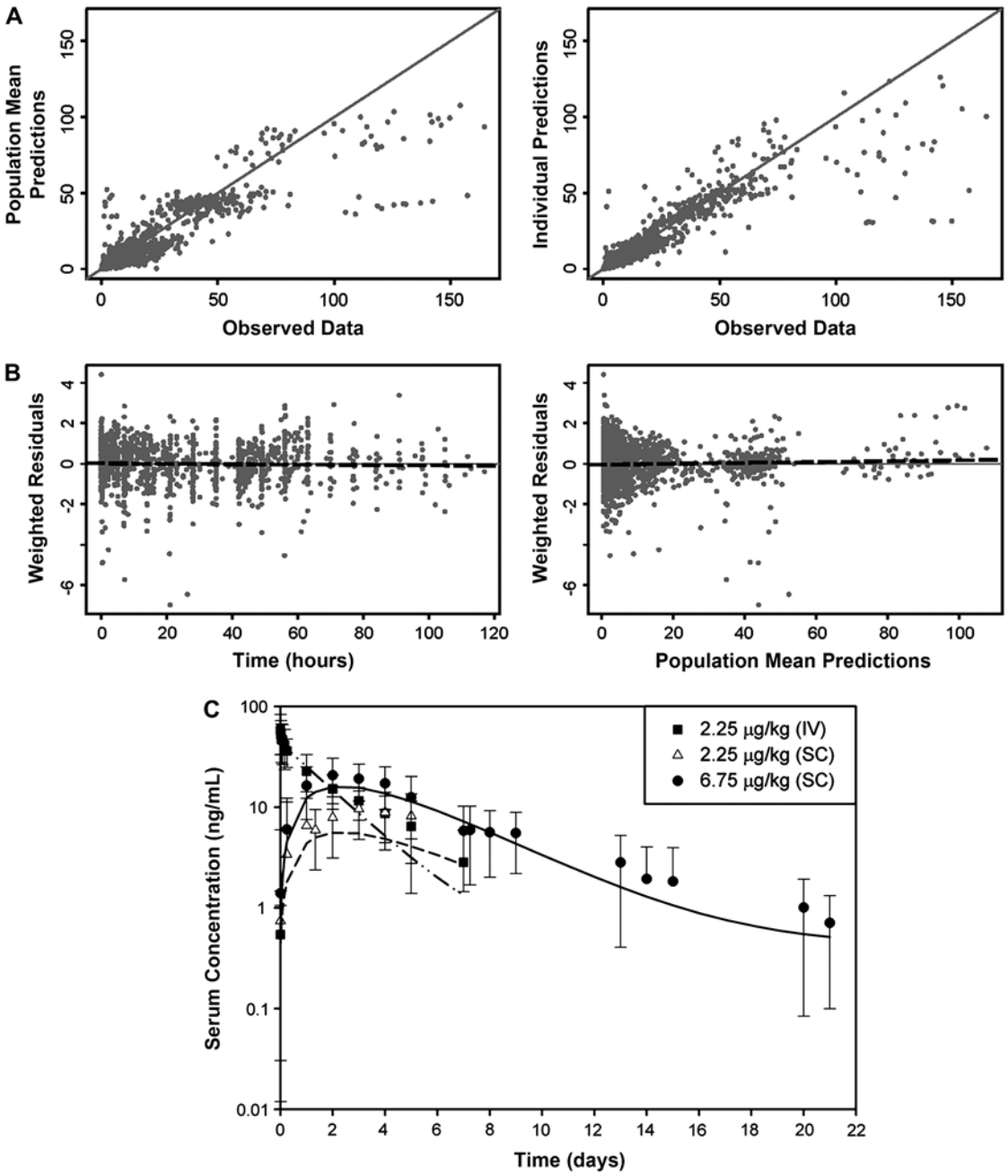

Figure 2. Diagnostic plots of the full covariate Pk model. (A) Mean and individual predicted vs observed serum darbepoetin alfa concentrations are plotted on a log scale. (B) Plot of the weighted residuals vs mean predicted (PRED) and time after dosing (TIME). (C) Plot of mean predicted (solid or dashed line) vs observed serum concentration (open symbols) profile after a single administration of 2.25 µg/kg intravenous (IV), 2.25 µg/kg subcutaneous (SC), or 6.75 µg/kg SC doses. Observed concentrations are shown as mean ± SD.

confounding medical interventions such as transfusions and chemotherapy treatment; detailed data on these interventions have not been collected during clinical studies. The aim of this analysis is to obtain a PkPd model capable of predicting the population hemoglobin response in this setting and to identify covariates that might explain PkPd response variability and help guide future study designs.

The Pk parameters obtained in this analysis (Table 3) generally agreed with previously reported noncompartmental values[7]; minor differences are likely the result of variations in the analysis methods. Comparison of the Pk parameters obtained in the analysis of this patient population with those of other populations (noncompartmental estimates) indicated that the bioavailability of the SC route of dosing was similar, but clearance of darbepoetin alfa in CIA patients is significantly reduced (ca. 40%) compared with that in patients on dialysis.[15,38] This is likely because of the negative effect of chemotherapy on the clearance of erythropoietic proteins. This notion is generally accepted; however, the exact contributory mechanism and the organ/tissue involved are not clearly understood.[39,40]

Table 4. Full PkPd Model Parameter Estimates*

		Full Model		
Parameter	**Units**	**Point Estimate†**	**SE (%RSE)**	**Asymptotic 95% CI**
θ_RBCPT	Days	4.68	1.26 (26.9)	(2.21, 7.15)
θ_RBCLS	Days	82.2	11.3 (13.7)	(60.1, 104)
θ_SMAX	Fraction	0.437	0.0699 (16.0)	(0.300, 0.574)
θ_S50	ng/mL	3.68	1.42 (38.6)	(0.897, 6.46)
θ_Hb0	g/dL	9.92	0.0413 (0.416)	(9.84, 10.0)
Covariate Coefficients	**NULL Value**	**Point estimate**	**SE (%RSE)**	**Asymptotic 95% CI**
θ_4 (PCNT on S_{50})	1	1.86	0.448 (24.1)	(0.982, 2.74)
Variances		**Point estimate**	**SE (% RSE)**	**Asymptotic 95% CI**
ω^2_{RBCLS}		1.63	0.489 (30.0)	(0.672, 2.59)
ω^2_{S50}		8.05	2.73 (33.9)	(2.70, 13.4)
ω^2_{Hb0}		0.00720	0.000560 (7.78)	(0.00610, 0.00830)
$\omega^2_{S50,Hb0}$		0.0539	0.0209 (38.8)	(0.0129, 0.0949)
σ_2^2		0.401	0.0179 (4.46)	(0.366, 0.436)

*SE indicates standard error; RSE, relative standard error calculated as SE/point estimate*100; CI, confidence interval; NULL value, the value of the covariate coefficient that would eliminate the effect of the covariate; and PCNT, platinum-containing chemotherapy count.
†Point estimates and RSE obtained from the NONMEM run (asymptotic standard errors) are shown for the covariate coefficients and the final typical value of the PD parameters.

The high IIV of Pk parameters, especially CL, in this population could be owing to difference in types and intensities of the chemotherapy treatments that were not captured during the clinical trials and, hence, are not accounted for in this model. The Pk of darbepoetin alfa may also be influenced by the timing of chemotherapy administration relative to darbepoetin alfa dosing.[41,42] Another potentially confounding factor is the effect of chemotherapy on endogenous EPO concentrations. It has been shown that endogenous EPO increases 6-fold ~48 hours after chemotherapy but returns to baseline levels by 168 hours.[41,42] However, as the contribution of endogenous EPO to the darbepoetin alfa measurements is minimal (Table 3), the effect on the estimated Pk parameters of darbepoetin alfa is also likely to be minimal.

Pt-containing chemotherapy cycle count and BWT were found to influence the clearance of darbepoetin alfa in these patients. Bootstrap 95% CIs and asymptotic 95% CI indicated that the estimates of these relationships generated from our data were reasonably precise. The influence of Pt-containing chemotherapy should be further examined in light of the hypothesized role of bone marrow as a clearance site of erythropoietic proteins[39] and the myelosuppressive effects of Pt-containing chemotherapy. These covariates reduced the IIV in CL by less than 20%, leaving a significant portion unexplained.

We estimated a mature hemoglobin lifespan of 82.2 days (RSE = 13.7%) in these patients compared with a 120-day lifespan of RBCs seen in healthy adults (Table 4). This is consistent with the myelosuppressive effects of chemotherapy and the shortened RBC lifespan resulting from anemia of chronic disease.[43] Our predicted first-order transit time for the manifestation of a hemoglobin effect, denoted by the RBCPT parameter in our model, was 4.68 days (RSE = 26.9%), consistent with the time taken for erythroid precursor maturation to reticulocytes.[44,45] Direct comparison of the estimated S_{max} and S_{50} values with literature estimates is not possible because of the different models and assay methodologies used. However, Ramakrishnan et al[37] reported a more than 4-fold increase in reticulocyte production in healthy volunteers after rHuEPO administration, although it is unclear how this increase translates to overall steady-state hemoglobin increase. In our analysis, the estimated sensitivity of hemoglobin response, S_{50}, 3.63 ng/mL) was ~10-fold higher than estimated eEPO levels (0.43 ng/mL). In contrast, Ramakrishnan et al[37] and Krzyzanski[46] reported half-maximal stimulation concentrations close to eEPO levels for reticulocyte response in healthy subjects. The high S_{50} for hemoglobin response in the CIA population indicates higher darbepoetin alfa dose requirements for anemia correction. Compared with anemia due to kidney disease, the recommended doses in the CIA setting are known to be higher.[47]

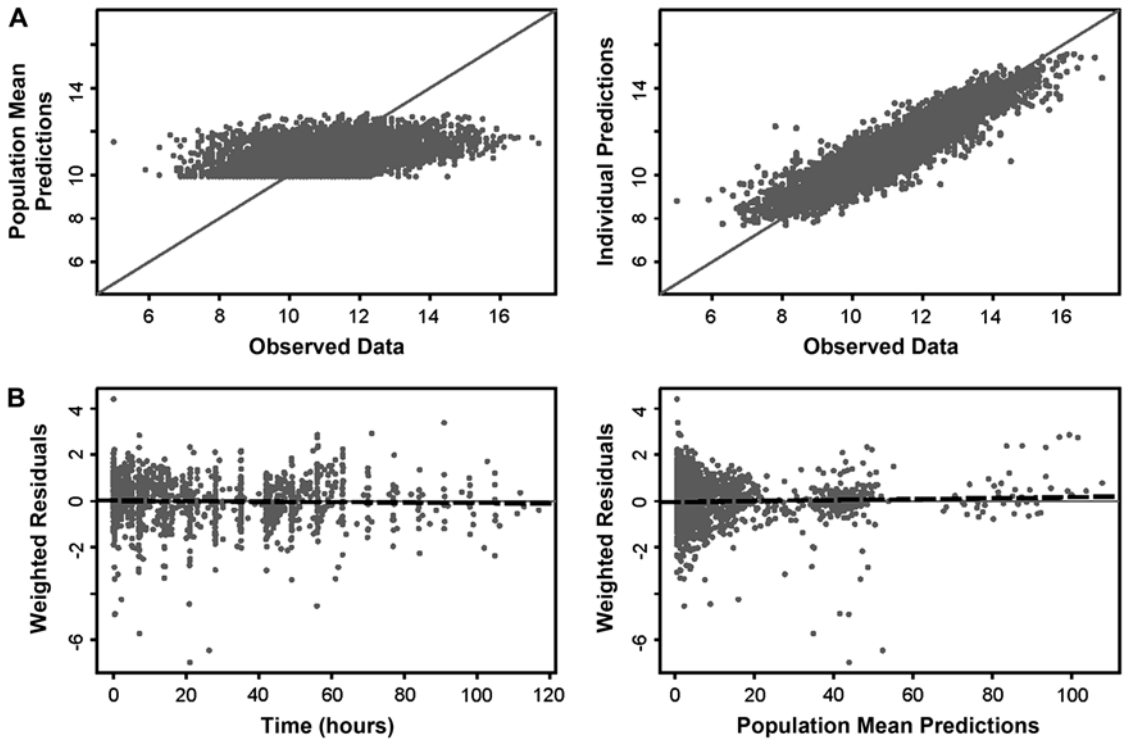

Figure 3. Diagnostic plots for the full covariate PkPd model. (A) Mean and individual predicted vs observed hemoglobin levels. (B) Plot of the weighted residuals vs mean predicted and time after dosing.

The PkPd model revealed high IIV in hemoglobin response as indicated by the estimates of the random effect variance-covariance matrix (Ω) for the Pd parameters (Table 4), consistent with the high degree of heterogeneity within this patient population. Variability in a range of factors such as tumor type, nutritional (eg, iron) status, disease stage, iatrogenic factors (eg, chemotherapy type and dose), and prior concomitant medications all likely contributed to the high variability in hemoglobin response. Some limitations of the analysis may also confound this variability estimate: (1) lack of individual Pk parameters in all subjects; (2) assumption of dose-invariant Pk parameters over the entire dose range; and (3) the first-order likelihood approximation method. As anticipated, the IIV of the Pk parameters were significantly smaller than those of the Pd parameters; therefore, it is likely that fixing the Pk parameters to their typical population values does not inflate the Pd parameter IIV to a substantial extent. The Pk of darbepoetin alfa has been shown to be dose-independent in the range 0.45 to 9 μg/kg[47]; however, the Pk at higher doses has not been fully evaluated.

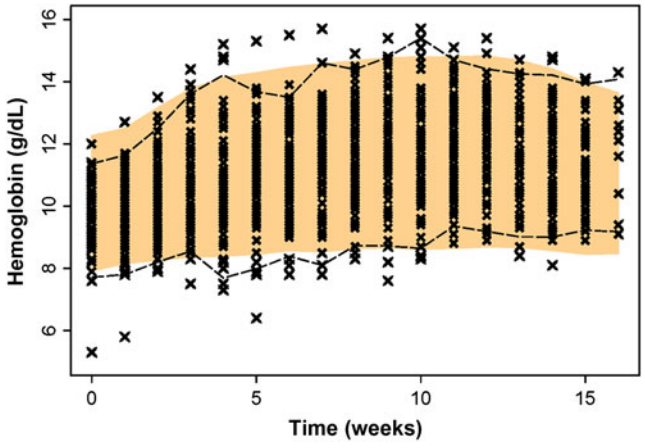

Figure 4. Summary of the external predictive check. The shaded area indicates the 2.5th to 97.5th quantiles of the predicted Hb profiles. The x's indicate the observed hemoglobin in the test data set. The broken line is the 2.5th to 97.5th quantile of the observed data.

We were not able to obtain the estimates of IIV of both S_{max} and S_{50}. This may be owing to the design of the studies included in modeling; for safety reasons, the protocol specified that the dose be withheld when the hemoglobin level reached 14 g/dL for women and 15 g/dL for men and resumed when the hemoglobin level fell to <13 g/dL. It should be noted that because of recent safety concerns,[48,49] lower hemoglobin thresholds (≤12 g/dL) are currently employed clinically. Because interindividual differences in S_{max} were not estimated, care should be exercised in the use of this model to simulate clinical trials with different dose titration algorithms, particularly those with higher hemoglobin targets. An external predictive check, however, did confirm the predictive capability of the full PkPd model under a similar trial design (Figure 4). Because we were not able to estimate S_{max} and S_{50} simultaneously in all patients, our estimate of S_{50} variability may also contain information about S_{max}

variability. Therefore, any conclusions on covariate effects may equally apply to both these parameters. We also used Hb_0 as a model parameter, even though it could be considered as a baseline covariate in the clinical setting. Instead of fixing Hb_0 to the clinical measured value, estimating Hb_0 using the model from all available individual hemoglobin data are likely to provide a more accurate estimate of the baseline hemoglobin and hence the other Pd parameters.

We found that patients not on Pt-containing chemotherapy tended to respond better than those on Pt-containing chemotherapy, similar to that reported by Vadhan-Raj.[8] However, since breast, pancreatic, and prostate cancer patients in our data set typically did not get Pt-containing chemotherapy, it is difficult to differentiate between the effects of tumor type and chemotherapy regimen on hemoglobin response.

Despite these interesting observations, effect size calculations using the final PkPd model indicated that it was not possible to classify any of these relationships as clinically significant. Therefore, dose adjustments based on any of these covariates are not currently recommended. It was recently shown that among CIA patients in the weight range 40 to 130 kg, a fixed dose of 325 μg Q3W was as effective as a weight-based dose of 4.5 μg/kg Q3W in alleviating anemia over the entire weight range and within each weight quartile.[11] Therefore, it appears that BWT has limited clinical effect at the dose levels and weight ranges evaluated. However, the effect of weight beyond the evaluated weight range and at other doses needs to be studied. Exploration of other covariates such as chemotherapy type and intensity is also warranted. Rather than report reduced covariate models, which may imply an absence of covariate effects on PkPd model parameters, the full model approach allowed for an accurate depiction of the effects of the covariates. Given these findings, these trends are being evaluated in the databases of larger clinical trials.

An important limitation of the PkPd model is that it does not account for upregulation of eEPO due to chemotherapy and its contribution to hemoglobin response. As more data are available on individual eEPO response after chemotherapy, the model will be refined to include this effect and also that of rescue treatments such as blood transfusions on measured PkPd response. However, as the predictive check indicates, the model adequately predicts the population hemoglobin response of a different dosing regimen under a similar study design and hence can be used in the design of such clinical trials.

Conclusion

In summary, we have developed and rigorously evaluated a PkPd model of darbepoetin alfa in the CIA setting. The model adequately described the observed variability in PkPd response in a large number of CIA subjects treated with darbepoetin alfa under a wide variety of doses and dosing schedules. The base model performed well in an external predictive check. There was insufficient information in the available data set to conclude whether any of the covariate effects on hemoglobin response in this population were clinically significant. The model is being refined with additional clinical data and is currently being used to help design clinical studies using darbepoetin alfa.

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