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Population-Based Pharmacokinetic and Exposure-Efficacy Analyses of Peginterferon Beta-1a in Patients With Relapsing Multiple Sclerosis

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Abstract

Peginterferon beta-1a reduced annualized relapse rate as compared with placebo and was approved to treat multiple sclerosis patients. A population pharmacokinetic and an exposure-efficacy model were developed to establish the quantitative relationship between pharmacokinetics and annualized relapse rate. The pharmacokinetics was well described by a 1-compartment model with first-order absorption and linear elimination kinetics. Body mass index was the most significant covariate that impacted both clearance and volume of distribution, which in turn impacted area under the curve and maximum serum concentration. Cumulative monthly area under the curve and annualized relapse rate were best described by a Poisson-gamma (negative binomial) model, demonstrating that the improved efficacy of every-2-weeks dosing was driven by greater drug exposure. The results supported the superior efficacy of the every-2-week dosing regimen compared with the every-4-weeks dosing regimen.

Keywords

interferon beta-1a, pegylation, pharmacometric analysis, count data, annualized relapse rate

Peginterferon beta-1a was formed by attaching a 20-kDa methoxy poly(ethyleneglycol) (PEG) polymer to the α-amino group of the N-terminus of interferon (IFN) beta-1a[1] and provides a less frequently injected subcutaneous (SC) therapy for relapsing multiple sclerosis (MS) with efficacy and safety characteristic of the IFN class.

A definitive mechanism of action of peginterferon beta-1a in MS is anticipated to be similar to interferon beta-1a in MS, which binds to the type I IFN receptor on the surface of cells and elicits a cascade of intracellular events leading to the regulation of IFN-responsive gene expression, and modulates immune responses that are believed to play a role in the pathogenesis of MS. However, because the pathogenesis of the disease is complex and multifaceted, the definite mechanism of action of peginterferon beta-1a or interferon beta-1a in MS is unknown.[2,3] Peginterferon beta-1a was developed to reduce the dosing frequency of interferon beta-1a by reducing clearance and prolonging half-life to promote treatment adherence.[4] As shown in a phase 1 study, peginterferon beta-1a had a longer half-life and increased exposure as compared with interferon beta-1a.[2] Following SC administration, peginterferon beta-1a serum concentrations increased rapidly after dosing, reached peak levels after approximately 1 day, plateaued for approximately 3 to 4 days, and then gradually decreased to below the limit of quantification (BLQ) in 7 to 10 days, yielding a peginterferon beta-1a dose-independent terminal half-life of approximately 2 days. Both maximum serum concentration (C_{max}) and area under the curve (AUC) were dose proportional, demonstrating linear pharmacokinetics (PK). Compared to unmodified intramuscular interferon beta-1a 30 μg, peginterferon beta-1a 125 μg SC yielded an approximately 11-fold higher AUC and 2-fold longer terminal half-life.[2] In the registration phase 3 ADVANCE study of 1512 relapsing MS patients, treatment with SC peginterferon beta-1a 125 μg every 2 or 4 weeks, or treatment with placebo, resulted in significantly lower adjusted annualized relapse rates (ARRs; primary endpoint) in both treatment arms vs placebo.[3,5] At week 48, the

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adjusted ARR was 0.397 (95%CI 0.328-0.481), 0.256 (95%CI 0.206-0.318; $P = .0007$), and 0.288 (95%CI 0.234-0.355, $P = .0114$) in the placebo, the every-2-weeks, and the every-4-weeks groups, respectively. Magnetic resonance imaging outcomes also demonstrated treatment benefits. As a secondary endpoint, the adjusted mean number (95%CI) of new or newly enlarging T2-weighted hyperintense lesions at 48 weeks was 10.9 (9.6-12.5), 3.6 (3.1-4.2), and 7.9 (6.9-9.0) for the placebo, the every-2-weeks, and the every-4-weeks groups, respectively. Notably, the primary efficacy endpoint suggested numerically greater, but not statistically significant, better effects in the every-2-weeks dosing group compared with the every-4-weeks dosing group ($P = .40$). Consequently, it has been questioned whether the observed numerical differences in efficacy were a random occurrence and if both dosing regimens should be recommended in the label. To address this question, an exposure-response analysis was carried out to establish the relationship between peginterferon beta-1a exposure and efficacy response quantitatively to provide justifications for recommending every 2 weeks as the only dosing treatment for approval.

We previously described a negative correlation ($r \approx -0.5$) between body size (body mass index [BMI], body surface area [BSA], and weight) and peginterferon beta-1a exposure and a negative correlation between renal function and AUC using a noncompartmental analysis.[6] However, this evaluation was limited to 25 intensive PK subjects and required confirmation by additional analysis. In an effort to confirm and expand on our previous findings, to describe peginterferon beta-1a PK profiles quantitatively, as a basis for the exposure-efficacy analysis, we performed a population PK analysis including all data collected in the ADVANCE trial.

Herein we report the population PK characteristics of peginterferon beta-1a and the exposure-efficacy relationship. The results provided information regarding drug disposition and provided justifications for recommending every 2 weeks as the only approved dosing regimen in the label. Model implications were discussed, including decisions on dose adjustment in patients with renal impairment.

Methods

Study and Data

PK and efficacy data were collected in ADVANCE, a randomized, double-blind, placebo-controlled phase 3 trial at 183 neurology practices—including hospitals, academic medical centers, and private practices—in 26 countries. The protocol was approved by the institutional review board at each site, and the study was done according to International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki. Investigator information was detailed in a previous publication.[5] Every patient provided written informed consent before entering the study. Study design has been described in detail elsewhere.[3,5,6] Briefly, 1512 patients were randomized (approximately 1:1:1) to receive SC injections of placebo, peginterferon beta-1a at a dose of 125 μg every 2 weeks, or peginterferon beta-1a at a dose of 125 μg every 4 weeks during year 1 of the study. At the end of year 1, patients on placebo were rerandomized to peginterferon beta-1a 125 μg every 2 weeks or every 4 weeks. The intensive PK samples were taken from 25 subjects (every 2 weeks n = 12; every 4 weeks n = 13) at predose, and at 6, 24, 28, 36, 72, 120, 168, and 240 hours postdose during week 4 and week 24 (the first week of the study was designated as week 0). For sparse sampling, 1 sample was taken after each dosing at weeks 4, 12, 24, 56, and 84. The window for drawing a sparse sample was defined as at least 1 hour after the last dose administered at week 4 and no later than 10 days after the scheduled dosing date at weeks 12, 24, 56, and 84. The rationale for this sampling window was that the serum drug concentration may fall under the lower limit of quantification by 10 days postdose, which would render a PK blood draw irrelevant. The sampling window at week 4 was shorter as the patients were in the office during this visit.

For the final model, the following data were excluded: BLQ data (62%) following sensitivity analysis, concentrations beyond 10 days postdose due to missing dose information (4%), 3 sparse PK subjects (0.09%) with positive baseline measurement (due to nonspecific binding), because there were too few data to model the baseline, 1 outlier concentration (14,700 pg/mL), which was considered erroneous, and concentrations with positive anti-IFN antibodies (0.8%). The impact of binding anti-IFN antibody was evaluated during model development.

Determination of Serum Concentrations of Peginterferon Beta-1a

Serum concentrations of peginterferon beta-1a were evaluated using an enzyme-linked immunosorbent assay. The quantitative range was 31.3 to 1500 pg/mL. The accuracy of the assay was in the range of 100.4% to 103.6% during study testing, and the precision of the assay, expressed as percentage coefficient of variation (%CV) and evaluated using assay controls, was less than 7%.

Population PK Model

Analysis based on naive pooled data showed that the PK profiles were well described using a 1-compartment model with a first-order absorption rate and a first-order elimination rate. A 2-compartment model was

tested. Differences of up to 2.5-fold were observed in parameter estimates using various initial values, but the objective function values remained the same, indicating overparameterization.

Because no intravenous PK data were available, the bioavailability was fixed at 1, and the elimination rate was constrained by the absorption rate to avoid flip-flop kinetics as shown below.[7]

$$Ka_i = \theta + \frac{CL_i}{V_i} \quad (1)$$

where Ka_i, CL_i, and V_i represent the absorption rate, clearance, and volume of distribution in subject i, respectively. θ represents the typical value of the difference between absorption rate and elimination rate (CL_i/V_i) for subject i and was constrained to be no less than 0.

Both PK parameter variations and random errors were described by a log-normal distribution. Interindividual variation (IIV) was added to 1 PK parameter at a time, followed by covariance testing once the stochastic model was finalized. IIV estimates, IIV shrinkages, and model stability were examined to determine IIV inclusion.

Covariates were tested using a forward addition ($P \leq .05$) followed by a backward deletion ($P \leq .001$) step, facilitated by the stepwise covariate model procedure in Perl-speaks-NONMEM.[8,9] Covariates were considered based on mechanistic considerations. For clearance, dosing frequency (treatment arm) and time (treatment week) were tested because clearance of peginterferon beta-1a might cause receptor internalization and result in clearance decrease over time[10]; immunogenicity (anti–polyethylene glycol [anti-PEG] antibodies) might increase protein clearance; demographic parameters were tested based on physiological consideration; hepatic and renal function were included because livers and kidneys are among key organs for protein catabolism and excretion, and renal clearance was the major clearance pathway of peginterferon beta-1a[11]; concomitant medications were tested due to drug-protein interaction concerns; the injection site was tested because it might affect bioavailability, which in turn affects clearance (CL), since bioavailability was set as 1. For V and Ka, demographic parameters were tested based on physiological consideration; the injection site was tested because it potentially affected bioavailability and absorption rate; time effect (treatment week) was tested to examine if chronic dosing changed absorption and disposition. The overall tested covariates included:

- For CL: treatment arm, treatment week, injection site, anti-PEG antibody status, weight, BSA, ideal body weight, lean body weight, BMI, age, race, sex, renal function (including creatinine clearance, estimated glomerular filtration rate, serum creatinine concentration, and blood urea nitrogen concentration), hepatic function (including bilirubin concentration, alanine aminotransferase concentration, and aspartate aminotransferase concentration), and concomitant medications (paracetamol, ibuprofen, mepresone, naproxen, modafinil, gabapentin, baclofen, amoxicillin, azithromycin, and zolpidem).
- For V: weight, BSA, ideal body weight, lean body weight, BMI, age, race, sex, injection site, treatment week.
- For Ka: weight, BSA, ideal body weight, lean body weight, BMI, age, sex, injection site, treatment week.

For categorical covariates, an additive relationship was tested; for continuous covariates, linear, exponential, and power relationships were tested, following the default definition of the stepwise covariate model procedure in Perl-speaks-NONMEM.

Statistically significant covariates from Step 1 were tested for confounding effect among correlated covariates ($r > 0.4$).

A conditional estimation method with interaction was used for parameter estimate.

The final PK model was evaluated using bootstrapping ($n = 1000$) and visual predictive check (VPC; $n = 1000$).

Exposure and Efficacy Model

For the exposure-efficacy analysis, monthly cumulative AUC (AUC over 4 weeks for both treatment arms) was used to estimate peginterferon beta-1a exposure; ARR was used as the efficacy endpoint. The AUC was derived from individual posterior Bayes estimates of CL using a sequential analysis approach.[12] The monthly cumulative AUC was selected to represent exposure because interferon reduced acute inflammation as early as within a month of treatment initiation,[13] and acute inflammation was associated with relapses.[14–18] Therefore, reduction of ARR is considered a cumulative effect of peginterferon beta-1a, and the exposure was represented by AUC.

For diagnostic plots, the ARR was calculated as shown in equation 2:

$$ARR_{group} = \frac{\sum_{i=1}^{n} Relapse_i}{\sum_{i=1}^{n} T_i} \times 365 \quad (2)$$

where Relapse_i represents relapse count over duration of T_i days for subject i.

The distribution of placebo relapse count was modeled using 4 distributions, namely, Poisson distribution, log-Poisson distribution, zero-inflated Poisson (ZIP) distribution, and Poisson-gamma distribution. These models are described below in Table 4. The baseline model was selected based on the lowest deviance information criterion, calculated according to equations 3 through 6.

$$D(y,\theta_i) = -2 \times \log\left(p\left[y|\theta_i\right]\right) \tag{3}$$

$$\hat{D}_{avg}(y) = \frac{1}{N}\sum_{i=1}^{N} D(y,\theta_i) \tag{4}$$

$$\hat{p}_D = \frac{1}{2} \times \frac{1}{N-1} \times \sum_{i=1}^{N}\left(D\left[y,\theta_i\right] - \hat{D}_{avg}\left[y\right]\right)^2 \tag{5}$$

$$DIC \approx \hat{D}_{avg}(y) + \hat{p}_D \tag{6}$$

where $D(y, \theta_i)$ represents deviance, y represents the observed data, θ_i represents a given parameter, $p(y|\theta_i)$ represents likelihood, N represents sampling time, $\hat{D}_{avg}(y)$ represents mean deviance, DIC represents deviance information criterion, and $\hat{p}_D$ represents approximated effective number of parameters. Model selection was based on placebo data, as well as placebo and active treatment data.

The effect of drug exposure was evaluated as shown by equation 7.

$$\log(\hat{\lambda}_i) = \log(\lambda_0) + b \times AUC_i \tag{7}$$

where $\hat{\lambda}_i$ represents mean ARR for subject i, and λ_0 represents placebo ARR. An inhibitory E_{max} model was tested as shown by equation 8.

$$\hat{\lambda}_i = \lambda_0 \times \left(1 - \frac{E_{max} \times AUC_i}{EC_{50} + AUC_i}\right) \tag{8}$$

However, the model did not converge. A reparameterization of the inhibitory E_{max} model, also named as the truncated inhibitory E_{max} model, was tested,[19] but also failed to converge.

Covariates tested included baseline Expanded Disability Status Scale scores (scores from 0 [normal] to 10 [death due to MS] with a step 0.5 to grade the degree of neurologic impairment in MS),[20] baseline relapse rate (mean relapse counts in the 3 years before study entry), age, sex, baseline McDonald criteria (a score of 1 through 4 based on number of relapses and MRI lesions; modeled both categorically and continuously), and baseline Gd^+ lesion volume. The covariate was modeled using a log-linear relationship shown in Equation 9.

$$\log(\hat{\lambda}_i) = \log(\lambda_0) + b \times AUC_i + c \times P_i \tag{9}$$

where c represents the covariate coefficient and P_i represents the covariate value for subject i. Covariates that yielded the greatest deviance information criterion decrease were included in the model until no additional covariates produced any further decrease.

The final AUC-ARR model was evaluated using VPC. The patients were grouped either by treatment arms or by monthly cumulative AUC. To plot VPC by treatment arms, the ARR for the observed data and simulated data based on Equation (2) were plotted against group median AUC. The observed data were compared to the [2.5th, 97.5th] percentiles and median of the simulated data. To plot VPC by monthly cumulative AUC, patients in the every-2-weeks and every-4-weeks groups were pooled and divided into 20 subgroups (every 5 quintiles; approximately 50 patients) based on monthly cumulative AUC. The subgroup ARR calculated using Equation (2) was plotted against subgroup median AUC.

Subgroup Analysis in Efficacy and Safety

BMI was used as a surrogate marker for exposure, based on the final PK model, for subgroup analyses for both efficacy and safety. A negative binomial model was run on ARR data for patients in each BMI quintile, adjusted for baseline Expanded Disability Status Scale (<4 vs ≥ 4), baseline relapse rate, and age (<40 vs ≥ 40). The incidence of adverse events (AEs), a safety measure, was summarized by subgroups stratified by BMI quintiles in each treatment arm. Per study protocol, an AE was defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that did not necessarily have a causal relationship with this treatment.

Modeling Software and Hardware

The raw data were assembled using SAS software (SAS Institute Inc, Cary, North Carolina) version 9.3. Subsequent data preparation and diagnostic plots were carried out using R software (R Foundation for Statistical Computing, Vienna, Austria) version 2.15.3. NONMEM (ICON plc, Dublin, Ireland) version 7.2 was used for population PK analyses with the Intel Fortran compiler (Intel Corporation, Santa Clara, California) version 11.1.048 and version 12.1. The stepwise covariate model, bootstrap, and VPC procedures were performed using Perl-speaks-NONMEM software version 3.5.3. The PK model was implemented using the

PREDPP subroutine ADVAN2. WinBUGS (Imperial College and MRC, London, UK) version 1.4.3 was used to develop the exposure-response model.

NONMEM was run using multiple cores on an HP 20-node cluster (each node with 2 quad-core Intel Xeon E5630 at 2.53 GHz). WinBUGS models were run on an HP xw8600 workstation with 4 Intel Xeon E5240 processors at 3.00 GHz.

Results

Patient Demographics

Patient characteristics and demographics that were included in the final PK model are listed in Table 1 and were largely consistent with a general MS population.[5,21] The final population used in the population PK analysis consisted of 239 males and 570 females who were predominantly white (n = 668) and Asian (n = 96). At least 2-fold differences were observed between the 2.5th and 97.5th percentiles for most continuous demographic characteristics, representing a wide range. Demographics of all patients from the trial have been published previously.[4] Excluded patients were all sparse PK population as a result of data exclusion.

Population PK Model

The structural PK model was a 1-compartment linear model with a first-order absorption rate. The stochastic model included IIV for CL and V.

Following the forward addition step, the following covariates were identified for the full model.

$$\mathrm{CL} = \theta_1 \times \left(\frac{\mathrm{BMI}}{23.71}\right)^{\theta_3} \times \exp\left(\theta_4 \times [\mathrm{CRCG} - 109]\right) \times \left(\frac{\mathrm{AGE}}{37.6}\right)^{\theta_5} \quad (10)$$

$$\mathrm{V} = \theta_2 \times \exp\left(\theta_6 \times [\mathrm{BMI} - 23.71]\right) \times (1 + [1 - \mathrm{SEX}] \times \theta_7) \quad (11)$$

where θ_1 represents a typical value of clearance; θ_2 represents a typical value of volume of distribution; θ_3 represents the exponent of BMI as a covariate for clearance; θ_4 represents the coefficient of CRCG (creatinine clearance) as a covariate for clearance with an exponential relationship; θ_5 represents the exponent of age as a covariate for clearance; θ_6 represents the coefficient of BMI as a covariate for volume of distribution with an exponential relationship; and θ_7 represents the coefficient of sex for volume of distribution (SEX = 1 for females). Parameter estimates for the full model are summarized in Table 2. Three covariates were identified for clearance in the full model, including age, BMI, and renal function (represented by creatinine clearance). Clearance increased as the covariate values increased. Two covariates were identified for volume of distribution, including BMI and sex. The volume of distribution increased as BMI increased; compared to females, males had a 26% lower volume of distribution.

During model development, the impact of anti-IFN binding antibody on clearance was evaluated. Using measurable concentrations only, there was a 2.5-fold increase in clearance. The effect was variable and resulted in BLQ concentrations in some subjects. However, the impact was likely due to interference with measurement using enzyme-linked immunosorbent assay because the pharmacological activities of peginterferon beta-1a were not affected, as shown by neopterin, a well-established biomarker for pharmacological activity of interferons. Because of the lack of predictability of the binding antibody interference with assay, the binding antibody data were excluded from the final model. With regard to neutralizing antibody, 26 subjects in the data sets were anti-IFN neutralizing antibodies positive. The PK/PD samples from these subjects were taken either within 2 hours or after 10 days postdose. The concentrations were generally expected to be BLQ or close to be BLQ at these time points. Therefore, the influence of anti-IFN neutralizing antibodies on PK parameters could not be assessed in this study.

The following covariates were significant during the initial screening step but were not significant once other covariates were included:

- For CL: BSA, weight, race, sex
- For V: age, BSA, injection site, weight

No concomitant medication showed any impact on the clearance of peginterferon beta-1a. Following backward deletion, a final model was established. BMI was the only covariate included in the final model, as described by equations 12 and 13:

$$\mathrm{CL} = \theta_1 \times \left(\frac{\mathrm{BMI}}{23.71}\right)^{\theta_3} \quad (12)$$

$$\mathrm{V} = \theta_2 \times \exp\left(\theta_4 \times [\mathrm{BMI} - 23.71]\right) \quad (13)$$

Parameter estimates for the final model are summarized in Table 3. Other body size metrics, such as BSA and weight, were less significant than BMI and did not show statistical significance once BMI was in the model. Both whole-body CL and V increased with BMI. A 50% increase in BMI corresponded to a 37% increase in CL and a 52% increase in V from the typical BMI of 23.71 kg/m^2. The BMI effect on CL and V in turn impacted both steady-state AUC

Table 1. Summary of Baseline Continuous Demographics and Physiological Parameters Included in the Population PK Analysis

Covariate	Formula	Mean (SD); Median	[2.5th, 97.5th]
Age (y)	NA	36.9 (9.7); 36.6	[20.5, 54.7]
ALT (IU/L)	NA	21.2 (12.5); 18.0	[9.0, 52.8]
AST (IU/L)	NA	20.6 (6.8); 19.0	[12.0, 37.0]
Bilirubin (mg/dL)	NA	0.5 (0.3); 0.4	[0.2, 1.3]
Body mass index (kg/m^2)	WT(kg)/HT^2(m^2)	24.0 (4.8); 23.3	[17.4, 35.5]
Body surface area (m^2)[35]	(HT[cm]·WT[kg]/3600)$^{0.5}$	1.8 (0.2); 1.7	[1.4, 2.3]
BUN (mg/dL)	NA	12.7 (3.7); 12.3	[6.8, 20.4]
Creatinine clearance[36] (mL/min)	(140 – Age[y])·(WT[kg]·(0.85 if female)/(72·SCR [mg/dL])	110 (27); 107	[70.0, 169]
Estimated glomerular filtration rate[37] (mL/min/1.73 m^2)	175·SCR(mg/dL)$^{-1.154}$·(Age[y])$^{-0.203}$· (1.212 if black)·(0.742 if female)	92.8 (16.5); 91.4	[64.4, 127]
Height (m)	NA	1.7 (0.1); 1.7	[1.5, 1.9]
Ideal body weight[38] (kg)	45.5 + 0.906·(HT[cm] −152.4) + (4.5 if male)	60.3 (9.8); 58.7	[43.5, 80.4]
Lean body weight[39] (kg)	(1.10 if male; 1.07 if female)·WT(kg) − (128 if male; 148 if female) ·WT^2(kg^2)/HT^2(cm^2)	49.1 (9.3); 46.7	[36.2, 70.9]
Serum creatinine concentration (mg/dL)	NA	0.8 (0.1); 0.8	[0.6, 1.1]
Weight (kg)	NA	67.5 (15.5); 65.0	[46.0, 103]

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; BW, body weight (kg); SCR, serum creatinine concentration; SD, standard deviation.

Table 2. PK Parameter Estimates for the Full PK Model

		NONMEM Results			Bootstrap Results				
Parameter	Definition	Estimated Value	Relative Standard Error (%)	Shrinkage (%)	Mean	Standard Error (%)	Medians	2.5th Percentile	97.5th Percentile
θ_1 (L/h)	Typical value of clearance	3.2	2.9	NA	3.3	4.7	3.3	3.0	3.6
θ_2 (L)	Typical value of volume of distribution	484	6.0	NA	480	13	475	378	620
θ_3	Exponent of BMI as a covariate of clearance	0.28	26	NA	0.25	98	0.26	-0.27	0.72
θ_4	Coefficient of CRCG as a covariate of clearance	0.0046	25	NA	0.0044	37	0.0044	0.0011	0.0078
θ_5	Exponent of age as a covariate of clearance	0.51	47	NA	0.50	35	0.49	0.19	0.85
θ_6	Coefficient of BMI as a covariate of volume of distribution	0.033	11	NA	0.0036	26	0.0036	0.018	0.057
θ_7	Coefficient of sex as a covariate of volume of distribution	-0.26	6.5	NA	-0.25	26	-0.26	-0.38	-0.11
θ_8 (h^{-1})	Difference between absorption rate and elimination rate	0.20	3.1	NA	0.20	13	0.20	0.16	0.26
ω^2_{CL}	Intersubject variance of CL	0.16	0.47	63	0.15	34	0.14	0.055	0.26
ω^2_v	Intersubject variance of V	0.33	18	58	0.35	19	0.34	0.23	0.48
SD1	Standard deviation of random error	0.57	12	NA	0.57	3.8	0.57	0.53	0.61
σ^2	Additive random error for log-transformed data	1, fixed	NA	15	1	0	1	1	1

BMI, body mass index; CL, clearance; CRCG, creatinine clearance based on Cockcroft-Gault equation; NA, not applicable; PK, pharmacokinetics; V, volume of distribution.

(AUC_τ) and C_{max}. For subsequent analysis, patients were stratified into 5 quintile groups based on BMI (kg/m^2), namely, 14.8, 20 kg/m^2, 20.5, 22.6 kg/m^2, 22.6, 24.8 kg/m^2, 24.8, 27.9 kg/m^2, and 27.9, 57.6 kg/m^2, with median BMI of 19.3, 21.7, 23.8, 26.3, and 31.1 kg/m^2, respectively. According to the model, the median AUC_τ was 44.7, 40.8, 38.0, 35.2, and 30.8 h·ng/mL for the 5 quintile groups, respectively. The median C_{max} was 297, 273, 254, 232, and 197 pg/mL, respectively. There was a 45% difference in AUC_τ and a 51% difference

Table 3. PK Parameter Estimates for the Final PK Model

NONMEM Results					Bootstrap Results				
Parameter	Definition	Estimated Value	Relative Standard Error (%)	Shrinkage (%)	Mean	Standard Error (%)	Medians	2.5th Percentile	97.5th Percentile
θ_1 (L/h)	Typical value of clearance	3.28	4.8	NA	3.25	3.9	3.26	2.96	3.52
θ_2 (L)	Typical value of volume of distribution	435	8.1	NA	437	8.8	440	355	535
θ_3	Exponent of BMI as a covariate of clearance	0.779	19	NA	0.804	16	0.770	0.548	1.11
θ_4	Coefficient of BMI as a covariate of volume of distribution	0.0353	26	NA	0.0329	22	0.0353	0.0163	0.0456
θ_5 (h^{-1})	Difference between absorption rate and elimination rate	0.207	9.8	NA	0.206	9.8	0.210	0.164	0.257
ω^2_{CL}	Intersubject variance of CL	0.145	19	63	0.159	39	0.138	0.0490	0.336
ω^2_{V}	Intersubject variance of V	0.352	13	57	0.341	19	0.346	0.182	0.472
SD1	Standard deviation of random error	0.566	2.4	NA	0.569	3.1	0.564	0.534	0.608
σ^2	Additive random error for log-transformed data	1, fixed	NA	14	1	0	1	1	1

BMI, body mass index; CL, clearance; NA, not applicable; PK, pharmacokinetics; V, volume of distribution.

in C_{max} between the bottom and top BMI quintiles in ADVANCE, which provides the basis for subsequent exposure-stratified subgroup analyses for efficacy and safety.

The bootstrap results for the final model are summarized in Table 3. The mean parameter estimates from bootstrapping were almost identical to the NONMEM output. The standard errors from bootstrapping indicated good precision in parameter estimation.

For model evaluation, the VPC plot is shown in Figure 1, which showed that the 2.5th, 50th, and 97.5th percentiles of the observed data all fell in the 95%CI of the respective percentile of the simulated data, indicating that both the structural and stochastic models described the data adequately.

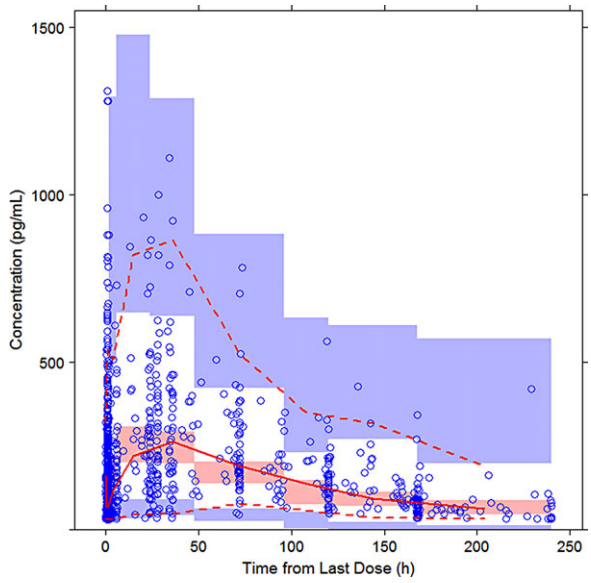

Figure 1. Final pharmacokinetic model visual predictive check. Solid line represents median values of observed data; dashed line represents median values of simulated data; circles represent observed data; shaded area represents 2.5% to 97.5% confidence interval of the 2.5th, 50th, and 97.5th percentiles of the simulated data.

Exposure-Response Model

Descriptions of 4 baseline count models are summarized in Table 4; the parameter estimates for the 4 placebo ARR models and the final exposure-response model are shown in Table 5. For placebo, the Poisson-gamma (negative binomial) model yielded a lower deviance information criterion than the other 3 models, and was thus selected as the baseline model for the exposure-response model and for further covariate selection. Analysis with all data (placebo and active treatment) generated consistent results, supporting Poisson-gamma model as the mode with the best fit. Among all covariates tested, no covariate at baseline produced further deviance information criterion decrease. For the final model, the time-series standard errors were less than 2% of the parameter estimates, indicating adequate estimate precision. A negative slope for AUC (b = –0.00532 1/[ng/mL·h·y]) confirmed that ARR decreased as cumulative AUC increased.

VPC diagnostic plots for the exposure-response model are shown in Figure 2. Both the plot by treatment groups (Figure 2a) and the plot by binned AUC (Figure 2b) showed the alignment between the observed

Table 4. Descriptions of Four Baseline Relapse Count Models

Distribution	Equations	Parameters
Poisson	$n_{relapse,\ i} \sim Poisson(\hat{\lambda} \cdot t_i)$	$n_{replapse,\ i}$: relapse count for subject i; $\hat{\lambda}$: population annualized relapse rate (ARR); t_i: treatment duration for subject i
Log-Poisson	$n_{relapse,\ i} \sim Poisson(\lambda_i \cdot t_i)$ $log(\lambda_i) \sim N(log(\hat{\lambda}),\ \sigma^2)$	$n_{replapse,\ i}$: relapse count for subject i; λ_i: ARR for subject i; $\hat{\lambda}$: population ARR; σ^2: variance
Zero-inflated Poisson	$n_{relapse,\ i} \sim Poisson(\hat{\lambda} \cdot t_i \cdot I_i)$ $I_i \sim Bernoulli(p)$	$n_{replapse,\ i}$: relapse count for subject i; $\hat{\lambda}$: population ARR; t_i: treatment duration for subject I; I_i: 0 or 1, an indicator of relapse count distribution, following Bernoulli distribution; p: the probability of I_i being 1
Negative binomial (Poisson-gamma)	$n_{relapse,\ i} \sim Poisson(\lambda_i \cdot t_i)$ $\lambda_i \sim gamma(\alpha, \frac{\alpha}{\hat{\lambda}})$	$n_{replapse,\ i}$: relapse count for subject i; λ_i: ARR for individual i; t_i: treatment duration for subject i; α: shape factor; $\hat{\lambda}$: population ARR

Table 5. Summary of AUC-Relapse Model Parameter Estimates

		DIC	Parameters	Mean	SD	Time Series SE	Median	2.50%	97.50%
Base model	Poisson	799	λ (1/y)	0.406	0.0305	0.00037	0.406	0.348	0.468
	Zero-inflated Poisson	1799	λ (1/y)	0.572	0.086	0.00301	0.567	0.419	0.753
			p	0.725	0.0967	0.00392	0.716	0.558	0.938
	Log-normal Poisson	789	$\hat{\lambda}$(1/y)	0.343	0.0445	0.00373	0.342	0.259	0.433
			σ	0.585	0.167	0.0193	0.592	0.274	0.892
	Poisson-gamma	783	$\hat{\lambda}$(1/y)	0.405	0.0331	0.00138	0.404	0.343	0.473
			α	5.14	6.25	0.994	2.79	1.06	25.4
Final model	Poisson-gamma model	2316	λ_0 (1/y)	0.37	0.0277	0.00104	0.369	0.318	0.427
			α	0.795	0.121	0.00615	0.782	0.593	1.06
			b (1/[ng/mL·h·y])	–0.00532	0.00148	6.17E-05	–0.00531	–0.00827	–0.00237

DIC, deviance information criteria; SD, standard deviation; SE, standard error

ARR and the model-predicted ARR. In the AUC stratified plot, the slope for ARR reduction in the plot was steep in the every-4-weeks AUC range, especially below the group median AUC. In contrast, the slope started to level off in the every-2-weeks AUC range. This trend was consistent between the observed data and the model-predicted data.

Based on the median model parameter estimates, the median AUCs of the every-4-weeks (38.0 ng/mL·h) and every-2-weeks groups (73.1 ng/mL·h) were associated with ARR reductions of 18% and 32%, respectively, compared with the placebo group.

Subgroup Analysis in Efficacy and Safety by Body Mass Index

The adjusted relapse rate ratio (active/placebo) was calculated for each BMI quintile for each treatment group. For the every-4-weeks group, the ratio was 0.617, 0.678, 0.765, 0.595, and 0.946 for the first (BMI ≤20.5 kg/m^2) through the fifth (BMI >27.9 kg/m^2) quintiles, respectively, trending up as BMI increased except for the fourth quintile. In contrast, the adjusted relapse rate ratio was 0.858, 0.802, 0.474, 0.372, and 0.881 for the first through fifth BMI quintile for the every-2-weeks group, respectively, showing no general trend. Sensitivity analysis using BMI tertile and quartile stratification were consistent with the quintile analysis.

Overall, peginterferon beta-1a showed benign safety profiles. The most common AEs (≥15% in any treatment group, excluding MS relapse) included injection site erythema (7%, 62%, 56% for placebo, every-2-weeks, and every-4-weeks, respectively), flu-like illness (13%, 47%, 47%), pyrexia (15%, 45%, 44%), headache (33%, 44%, 41%), myalgia (6%, 19%, 19%), chills (5%, 17%, 18%), injection site pain (3%, 15%, 13%), and nasopharyngitis (15%, 10%, 14%). The overall AE incidences (excluding MS relapse) were 79%, 93%, and 94% respectively. In general, the AEs were similar between the every-2-weeks group and the every-4-weeks group, despite a 2-fold difference in AUCs, indicating a flat exposure-response relationship.[5] Not surprisingly, within each group, BMI did not appear to have any significant effect on AEs. During year 1, the incidence of AEs for the placebo group was 85%, 81%, 81%, 84%, and 87% for the first through fifth BMI quintiles, respectively; the incidence of AEs for the every-4-weeks group was 98%, 91%, 96%, 93%, and 95%, respectively; the incidence of AEs for the every-2-weeks group was 93%, 92%, 95%, 95%, and 96%, respectively. Because all common AEs showed flat exposure-response relationships, as suggested by the comparable AEs across BMI subgroups and between the 2 active treatment groups despite C_{max} and AUC differences in quintile subgroups and cumulative AUC differences in 2 active treatment

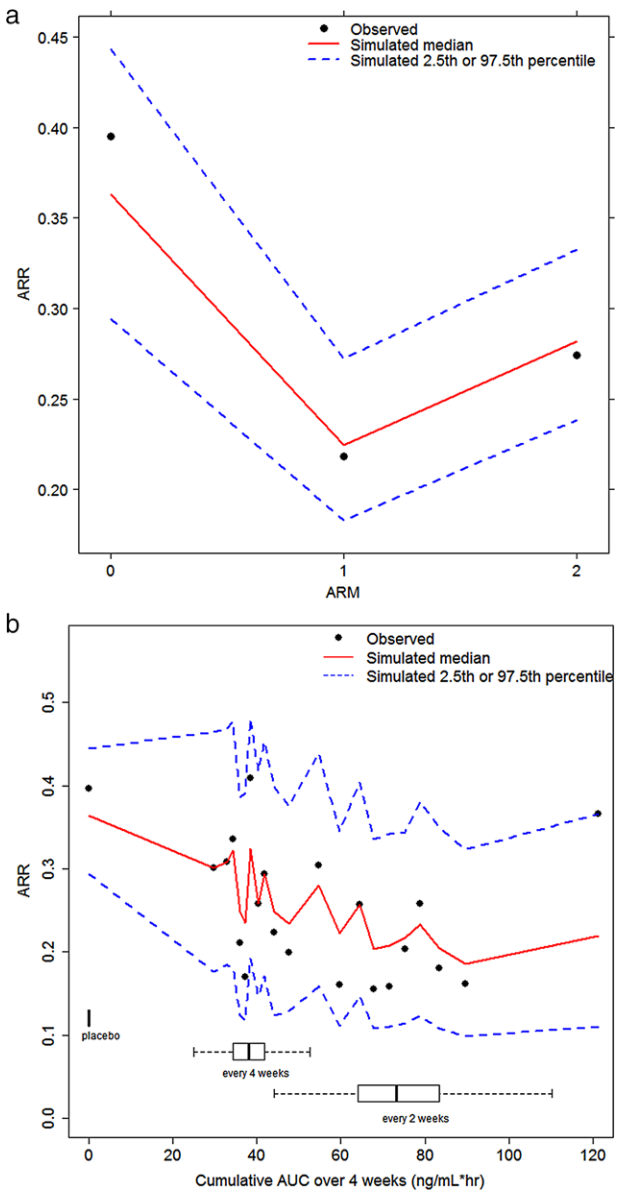

Figure 2. Visual predictive check of AUC-ARR model. a. Plot by treatment arms. b. Plot by cumulative AUC over 4 weeks. Peginterferon beta-1a treated subjects were pooled and divided into 20 subgroups with each subgroup containing 5 percentiles (approximately 50 patients) of monthly cumulatively AUC. AUC, area under the curve; ARR, annualized relapse rate; ARM 0, placebo group; ARM 1, every 2 weeks group; ARM 2, every 4 weeks group. Closed circles represent observed data; solid line represents median of simulated data; dashed lines represent the 2.5th and 97.5th percentiles of simulated data. Bar plots in panel b represent summary statistics of cumulative AUC over 4 weeks for each treatment group.

groups, no further exposure-response analysis was carried out for any specific AEs.

Discussion

By use of a sequential analysis approach, a PK model and an AUC-ARR model were established for peginterferon beta-1a. The PK model identified a significant covariate and provided a basis for further safety and efficacy subgroup analysis; the established exposure-response models described and quantified the relationship between exposure and the primary efficacy endpoint, demonstrating that the better efficacy observed in the every-2-weeks group was not a random event but was a result of greater exposure, as compared with the every-4-weeks group, and provided justification for every-2-weeks dosing as the only recommended regimen in the label.

Although renal function was not included in the final PK model, likely due to the exclusion of patients with renal impairment in ADVANCE, it did show an impact on peginterferon beta-1a PK in a stand-alone renal impairment PK study.[11] A 30%, 40%, and 53% increase in AUC from time 0 to 336 hours postdose (AUC_{336h}) and a 27%, 26%, and 42% increase in C_{max} were observed in subjects with mild, moderate, and severe renal impairment, respectively, as compared with those observed in healthy subjects. The results presented a question during peginterferon beta-1a approval: was it necessary to reduce dosage in patients with renal impairment? The PK model and the BMI stratified subgroup analyses of safety and efficacy provided information to address this question. The BMI-stratified subgroup analyses suggest that efficacy and safety were not sensitive to a 45% change in AUC_{τ} and a 51% change in C_{max} within the every-2-weeks group. Additionally, the AUC-ARR relationship suggests no efficacy concern on further fractional increase in AUC in renally impaired subjects. With regard to safety, the AE incidences were approximately constant among BMI quintiles and did not trend higher as exposure increased. Therefore, the additional fractional AUC increase did not cause safety concerns. Additionally, PK simulations showed minimal accumulation at steady state for patients with severe renal impairment, assuming the same magnitude of clearance decrease in MS patients with severe renal impairment as observed in the stand-alone renal impairment PK study (data not shown). Based on these analyses, dose adjustment was not considered necessary in patients with renal impairment. Such a recommendation was reflected in the Food and Drug Administration and European Medicines Agency labels.[22,23] However, the limitation of this analysis was the limited clinical experience with peginterferon beta-1a in MS patients with renal impairment. The analysis did not consider the other physiological complications in renally impaired subjects. Because of the limited experience, caution should be used when administering peginterferon beta-1a to patients with severe renal impairment.[22,23]

The impact of anti-PEG antibodies was evaluated in the PK model, showing that they do not impact peginterferon beta-1a clearance. This was consistent

with subgroup efficacy analysis in ADVANCE stratified by anti-PEG antibody status, thus alleviating efficacy concerns over anti-PEG antibodies. The incidences of anti-IFN neutralizing antibodies were too low to be assessed in the PK analysis. Anti-IFN binding antibodies interfered with drug measurement but did not reduce neopterin response.[6] This was consistent with antibody-status–stratified analysis of efficacy, which showed that neither neutralizing antibodies nor binding antibodies showed discernible impact on clinical efficacy, with the acknowledgment that the analysis was limited by the low immunogenicity incidence.[23]

The data sets consist of 62% of BLQ data, most of which were collected within 2 hours of dose or later than 10 days postdose, when the concentrations were expected to be BLQ based on phase 1 PK information.[2] Parameter estimates with and without BLQ data using a likelihood-maximization method (M3 method)[24–28] were compared. The parameter estimates were similar, but inclusion of BLQ data resulted in overestimated variability, with the CI of 2.5th percentile and 97.5th percentile of the simulated data totally outside the observed data. Therefore, the BLQ data were excluded for final parameter estimate.

The AUC-ARR model provided more insight into the recommended every-2-weeks dosing regimen. In addition to ARR, exposure-response analyses were carried out for Gd^+ lesions and new or newly enlarged T2 lesions, which showed that a large proportion of subjects with low peginterferon beta-1a exposure in the every-4-weeks group had suboptimal efficacy.[29] Given that the every-2-weeks and every-4-weeks groups showed similar safety profiles and that a large proportion of subjects received suboptimal exposure with every-4-weeks dosing, every-2-weeks was the only proposed dosing regimen, and the recommendation was adopted in the Food and Drug Administration and European Medicines Agency labels.[22,23]

ARR has been commonly used as a primary efficacy endpoint in phase 3 MS clinical trials.[30] Because of data overdispersion, negative binomial regression is often used to analyze the data.[5,31–33] In cases with excessive 0 counts, the ZIP and zero-inflated negative binomial models are gaining popularity.[31–34] However, there are some limitations of zero-inflated models. For instance, the ZIP model cannot explain excess large counts in the data; the parameter estimates can be very sensitive to the distributional assumption because the zero-inflated models depend on the frequency of 0 counts in the population[33]; and the use of zero-inflated models alters the interpretation of relapse rates and model parameters.[34] In the current study, there were 8 patients who had 5 to 10 relapses over 2 years, which was not considered excessive with large counts. Therefore, the ZIP model was tested, but the model did not provide a better fit than the negative binomial model. The zero-inflated negative binomial model was also tested but failed to converge, likely due to overparameterization. The Poisson-gamma model best described the characteristics of the raw data, including the count distribution and number of 0 counts, and provided the best model fit in this study.

One limitation of the PK model was the moderate intersubject variance shrinkage of CL and V due to large percentage of sparse PK samples. In a large phase 3 study, it was operationally challenging to collect more frequent PK samples or design the PK time point based on modeling requirements, a situation often deviating from a simulated exercise. The 2 dosing regimens, which resulted in a 2-fold difference in exposure, counteracted the limitation to some extent and rendered the AUC-ARR relationship more robust.

In summary, peginterferon beta-1a PK profiles were modeled using a 1-compartment model with first-order absorption rate and first-order elimination rate. BMI was identified to impact both clearance and volume of distribution. The PK model provided the basis for safety and efficacy subgroup analysis as well as for the subsequent exposure-response analysis. The established AUC-ARR model demonstrated that the better efficacy of the every-2-weeks group was driven by greater exposure of the dosing regimen, as compared with the every-4-weeks group, and a large percentage of subjects received suboptimal exposure in the every-4-weeks group, supporting every 2 weeks as the only approved dosing regimen.

Conclusions

This manuscript developed quantitative descriptions of peginterferon beta-1a PK and the relationship between PK and response. The exposure-response model illustrates that greater drug exposure was associated with better efficacy, and the every-2-weeks dosing regimen provided more clinical benefit than the every-4-weeks regimen. Therefore, adherence to the recommended every-2-weeks dosing regimen is important for peginterferon beta-1a efficacy in the treatment of relapsing MS.

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Conflict of Interest

X.H., Y.H., Y.C., S.L., A.D., and I.N. are employees and stockholders of Biogen. J.Z. and A.S. are stockholders and former employees of Biogen.

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