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Title Page

In vivo Pharmacology and Toxicology Evaluation of PEGylated Interferon Beta-1a

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Abbreviations: AUC, area under the curve; CL/F, clearance; EMC, encephalomyocarditis; GLP, good laboratory practice; IFN, interferon; MS, multiple sclerosis; NCA, non-compartmental analysis; NOAEL, no-observed-adverse-effect-level; PEG-IFN, Polyethylene glycol-conjugated interferon; PD, Pharmacodynamic; PK, Pharmacokinetic.

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Abstract

Human interferon beta (IFN beta) has well-established beneficial effects in treating relapsing forms of multiple sclerosis (MS), but current first-line treatment requires frequent (from daily to weekly) parenteral administration. A 20 kDa polyethylene glycol conjugated IFN beta-1a (PEG-IFN beta-1a) is being developed to decrease the frequency of administration and improve patient convenience and compliance. This manuscript presents pharmacokinetic (PK) and pharmacodynamic (PD) parameters, immunogenicity, and safety of PEG-IFN beta-1a in Rhesus monkeys in support of a Phase 1 clinical trial. Two single-dose PK/PD studies and one 5-week repeat-dose toxicity study compliant with good laboratory practice (GLP) were conducted. The PK of IFN beta-1a and PEG-IFN beta-1a were modeled with a 2-compartment model and the link between drug concentration and neopterin response (PD marker) was described with an indirect stimulatory model. PEG-IFN beta-1a showed greater exposure, longer half-life, lower clearance, and reduced volume of distribution than unmodified IFN beta-1a. Consistent with the pharmacology of Type I IFNs, PEG-IFN beta-1a resulted in the elevation of neopterin concentration, a transient body temperature increase, and a reversible lymphocyte count decrease. As expected, neutralizing antibodies to PEG-IFN beta-1a formed in almost all monkeys following 5 weeks of treatment, which resulted in significantly reduced drug exposure and abrogation of neopterin induction. There were no drug-related adverse effects at doses up to 100 µg/kg (11 MIU/kg) given subcutaneously or intramuscularly once weekly for five weeks. The no-observed-adverse-effect-level (NOAEL) was determined to be 100 µg/kg (11 MIU/kg), the highest dose tested.

Introduction

Human interferon (IFN) beta has well-established beneficial effects when used to treat patients with relapsing forms of multiple sclerosis (MS), including reducing the development of brain magnetic resonance imaging (MRI) lesions (Jacobs et al., 1996), reducing clinical relapse rates (Jacobs et al., 1996; PRISMS Study Group, 1998), and slowing the advance of physical disability (Jacobs et al., 1996; PRISMS Study Group, 1998). The mechanism of action in MS is not fully understood despite extensive research, due to the heterogeneous nature of the disease and the pleiotropic activities of the protein. The antiproliferative and immunomodulatory activities of IFN beta may be relevant to the therapeutic effect (Axtell and Steinman, 2008). For some patients, injection-related concerns and treatment-associated side effects, such as flu-like symptoms, remain as barriers to compliance and effective treatment (Coyle, 2008). Also, all currently approved parenteral treatments for MS require frequent (from daily to weekly) administration, which can further inconvenience patients already suffering from a debilitating, chronic disease. Thus, there remains a significant unmet need for effective, safe, well-tolerated, and convenient therapies for patients with MS (Mohr et al., 2001; Lugaresi, 2009).

Polyethylene glycol conjugation (PEGylation) is a method to increase the apparent size of a biomolecule and reduce its glomerular filtration rate, thereby extending the biomolecule's half-life, enhancing its *in vivo* efficacy through prolonged systemic drug exposure, and enabling less frequent dosing (Bailon and Won, 2009). Polyethylene glycol (PEG)-conjugated (PEGylated) interferon beta-1a (PEG-IFN beta-1a) was developed by attaching 20 kDa methoxy-PEG-O-2-methylpropionaldehyde to the α-amino group of the N-terminus of IFN beta-1a (Baker et al., 2006), with the aim of providing a less frequent dosing regimen and improved convenience for MS patients. The N-terminus of IFN beta-1a is not critical for binding to the Type 1 IFN receptor (Runkel et al., 2000). *In vitro* evaluation demonstrated that PEG-IFN beta-1a retained significant *in vitro* activity (approximately 50%) in antiviral and antiproliferative assays compared to the

unmodified protein, while PEG-IFN beta-1a *in vivo* efficacy was enhanced compared with IFN beta-1a in a mouse tumor angiogenesis model (Baker et al., 2006).

Rhesus monkeys have been shown to elicit a robust pharmacological response to IFN beta-1a and have been used for pharmacokinetic (PK), pharmacodynamic (PD), and safety evaluation of IFN beta-1a (Biogen Idec, 2007). Rhesus monkey IFN beta is 95% identical in sequence to that of human IFN beta-1a (Arduini et al., 2004). Therefore, the Rhesus monkey was chosen as the most appropriate pre-clinical species to evaluate PEG-IFN beta-1a. No toxicology studies of PEG-IFN beta-1a were conducted in rats or mice because they lack a relevant pharmacologic response to human IFN beta-1a, and thus would not be expected to provide meaningful pharmacological or toxicological data. In Rhesus monkeys, serum neopterin and 2′,5′-oligoadenylate synthetase (OAS) concentrations have been shown to increase following administration of IFN beta-1a (Martin et al., 2002; data on file, Biogen Idec). Neopterin was selected as the pharmacodynamic marker for the studies described in this report since it shows lower inter-animal variability in response based on historic data for IFN beta-1a.

The relationship between IFN beta-1a concentrations and subsequent neopterin response has been characterized with an integrated PK/PD model (Mager and Jusko, 2002; Mager et al., 2003), which was built on simplified biological processes. However, the model included many hypothetical intermediate compartments, was over-parameterized, and final parameter estimates relied on several fixed values derived from the literature. A minimal model was later proposed for a 40 KDa PEG-IFN beta-1a conjugate administered to Cynomolgus monkeys, in which the receptor-mediated drug disposition was simplified to a linear two-compartment PK model with the intermediate compartments between serum drug concentration and stimulatory effect and an intermediate compartment for a neopterin pre-cursor removed (Mager et al., 2005). This simplified PK/PD model was adopted with slight modifications for the current study, including addition of transit compartments to describe neopterin lag time (Savic et al., 2007) and

incorporation of inhibitory feedback of neopterin on the stimulatory effect by IFN beta-1a or PEG-IFN beta-1a (Mager et al., 2003).

Herein we report results of nonclinical studies aimed at evaluating the PK, PD, immunogenicity, and safety of PEG-IFN beta-1a in Rhesus monkeys. The results provided pre-clinical support for two Phase 1 clinical trials of PEG-IFN beta-1a.

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Methods

PEG-IFN beta-1a and IFN beta-1a

Both PEG-IFN beta-1a and IFN beta-1a were manufactured by Biogen Idec (Cambridge, MA, USA), and were formulated in 20 mM acetic acid/sodium acetate pH 4.8, 150 mM arginine.HCl, 0.005% (w/v) Polysorbate 20. Laboratory scale PEGylation, purification, and characterization of PEG-IFN beta-1a is described elsewhere (Baker et al., 2006). The amino acid sequence of IFN beta-1a is available in the DrugBank database (Knox et al. 2011).

In Vitro Potencies of PEG-IFN beta-1a and IFN beta-1a

The in-vitro specific antiviral activity of PEG-IFN beta-1a and IFN beta-1a was determined using a cytopathic effect (CPE) assay that measures the ability of the protein to protect human lung carcinoma A549 cells challenged with encephalomyocarditis (EMC) virus. In the assay, A549 cells were added to wells of a 96-well microtiter plate and incubated for 15-20 h. PEG-IFN beta-1a or IFN beta-1a standards, controls, and serum samples, diluted in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum, were added to each well. Following an additional incubation, EMC virus was added to the assay plates and incubated for 30 h. After the viral incubation, the plates were stained with 0.75% Crystal Violet in formaldehyde fixative. The plates were examined visually to determine the lowest concentration of PEG-IFN beta-1a or IFN beta-1a protecting the cells against virus cytotoxicity. The last positive well (LPW) of the standards was the standard detection limit of the assay. To determine potency of PEG-IFN beta-1a or IFN beta-1a in the samples, the sample dilution in the LPW was multiplied by the standard detection limit and the sample dilution factor. Specific activity for the samples was determined by dividing the sample potency by the sample protein concentration.

Rhesus Monkey PK, PD, Immunogenicity, and Toxicology Studies

Three studies using Rhesus monkeys were conducted to evaluate PK, PD, immunogenicity, and toxicity of PEG-IFN beta-1a. All aspects of the animal studies were fully compliant with the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and the conditions specified in the Guide for the Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press). In Study 1 (PK/PD), four groups of Rhesus monkeys (5 males/per group) received a single-dose of PEG-IFN beta-1a at 10.8 μg/kg (1 MIU/kg) or IFN beta-1a at 5 μg/kg (1 MIU/kg) via subcutaneous (SC) or intramuscular (IM) injection. For assessment of serum drug and neopterin concentrations, blood samples were obtained prior to dosing, and at 1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 h post-dose. In Study 2 (PK/PD/immunogenicity), four groups of Rhesus monkeys (2 male and 2 female per group) received a single-dose PEG-IFN beta-1a treatment at 2, 10, and 100 μg/kg (equivalent to 0.22, 1.1, and 11 MIU/kg, respectively) via SC injection and at 100 μg/kg via IM injection. The blood sampling schedule was the same as Study 1 for assessment of serum drug and neopterin concentrations. In addition, serum samples were collected prior to dosing, and at 14 and 28 days post-dosing for assessment of anti-PEG-IFN beta-1a binding antibodies (BAbs) and neutralizing antibodies (NAbs). Study 3 (PK/PD/immunogenicity /toxicology) was a 5-week repeat dose toxicity study conducted according to Good Laboratory Practice (GLP). This study incorporated SC (all doses) and IM (control and high dose only) routes of administration, since both routes were to be evaluated in the initial Phase 1 clinical study to determine the safety, tolerability, PK, and PD of PEG-IFN beta-1a in humans. Five groups of monkeys received 5 weekly doses of PEG-IFN beta-1a. Group 1 received IM and SC administration of vehicle control; Groups 2-4 received SC administration of PEG-IFN beta-1a at 2, 10, and 100 μg/kg (equivalent to 0.22, 1.1, and 11 MIU/kg); Group 5 received IM administration of PEG-IFN beta-1a at 100 μg/kg. There were 4 male and 4 female monkeys per group for the main study, and an additional 2 males and 2 females per group for recovery for Groups 1, 4, and 5. For assessment of PEG-IFN beta-1a concentration, serum samples were

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obtained prior to dosing, and at 4, 8, 24, 120, and 168 h post-dose for the 1st dose (dosing day defined as Day 1). For the last dose (Day 29), the same schedule was used except that Groups 1 through 4 skipped the 4 h post-dose sample, while Group 5 skipped the 8 h post-dose sample. Additionally, for the 3rd (Day 15) and 4th (Day 22) doses, a pre-dose (Groups 1-5), 4 h (Groups 1-4), and 8 h (Group 5) samples were taken to assess drug exposure. Neopterin concentration was measured from serum samples prior to dosing, at 24 h post-dosing for the 1st, 3rd, and 5th doses, 168 h post-dosing for the 5th dose, and at the end of the study (Day 65) from the recovery animals only. For assessment of anti-PEG-IFN beta-1a BAbs and NAbs, blood samples were collected prior to Day 1 dosing, on Days 15 and 29 at pre-dose, on Day 35, as well as on Day 65 from recovery animals.

The main study necropsy (4/sex/group) was on Day 36 and the recovery necropsy on Day 65 for Groups 1, 4, and 5 (2/sex/group). In addition to PK, PD, and immunogenicity determination, study endpoints included twice daily cage-side observation, daily food consumption, physical and ophthalmic exams (pre-study and once prior to each necropsy), and body weight and ECGs (twice pre-study and weekly thereafter). Blood samples for evaluation of serum chemistry, hematology, and coagulation parameters were collected from all animals twice pre-study (Days –10 and Day –2), on Day 1 (at 8 h post-dose for all groups), on Days 15, 22, and 29 (at 8 h post-dose for Groups 1-4, and at 4 h post-dose for Group 5), and prior to each necropsy. Body temperature was taken twice pre-study (Days –28 and –25), prior to dosing, at 4 and 8 h post-dosing on Days 1, 8, 15, 22, and 29. Blood samples were also taken for neopterin measurement prior to dosing, at 24 h post-dosing for the 1st, 3rd, and 5th doses, 168 h post-dosing for the 5th dose, and at the end of the study (Day 65). Urine samples were collected for analysis (bladder puncture) prior to each necropsy.

At each respective necropsy on Days 36 and 65, a full gross evaluation was conducted. Organs were collected and weighed and a comprehensive panel of tissue samples was taken

from all animals, preserved in neutral-buffered 10% formalin (except for the eyes, which were preserved in Davidson's fixative for optimum fixation), embedded in paraffin, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist.

Determination of Serum Concentration of PEG-IFN beta-1a and IFN beta-1a

The PK assay for PEG-IFN beta-1a and IFN beta-1a was a one step sandwich enzyme-linked immunosorbent assay (ELISA) that utilizes selected kit components from a commercially-available IFN beta Direct ELISA kit (BioSource™, Invitrogen). Affinity-purified goat anti-human IFN beta was pre-coated on a polystyrene 96-well plate by the manufacturer. Assay standards and quality controls prepared from PEG-IFN beta-1a and IFN beta-1a as well as study samples were added to the plate. PEG-IFN beta-1a or IFN beta-1a, captured from the serum, was detected using a mouse anti-human IFN beta monoclonal antibody conjugated to horseradish peroxidase (HRP). Quantitation of quality controls and study samples was carried out by interpolation to the standard curve using a validated Softmax Pro 4.3.1 data reduction software program. The assay was validated for use with Rhesus monkey serum and the quantitation range were 7.8-500 pg/mL for IFN beta-1a and 15.6-1250 pg/mL for PEG-IFN beta-1a for most batches. The precision of both assays, expressed as coefficient of variation (%CV) and evaluated using assay controls, was within 15%.

Determination of Serum Concentration of Neopterin

The neopterin (PD marker) assay was a competitive binding enzyme immunoassay (EIA) wherein neopterin standards, quality controls, and samples compete with a neopterin-HRP conjugate for binding to polyclonal anti-neopterin captured on a microtiter plate. The assay utilizes select Immuchem™ neopterin-MW EIA kit components. Polyclonal anti-neopterin antibodies were pre-coated on a polystyrene 96-well plate by the manufacturer. Assay standards, quality controls, samples, and neopterin-HRP were added to the plate. Quantitation

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of quality controls and study samples was carried out by interpolation to the standard curve using a validated Softmax Pro 4.3.1 data reduction software program. The assay was validated for use with Rhesus monkey serum samples and the quantitation range was 0.521-100 ng/mL, and the precision of the assay (%CV of assay controls) was within 20%.

Immunogenicity Assay

Detection of anti-IFN beta-1a antibodies was carried out using a screening ELISA assay to detect BAbs, and by a reporter gene-based assay to detect NAbs. To detect BAbs, PEG-IFN beta-1a was captured by a murine anti-human IFN beta antibody coated on a 96-well microtiter plate. The plates were then incubated with assay quality controls and study samples. Antibodies to IFN beta-1a were detected using a polyclonal anti-human IgG (cross reacting with monkey IgG) conjugated to HRP. NAbs were characterized by their ability to inhibit the binding of IFN beta-1a to the Type I interferon receptor utilizing a quantitative Neutekbio iLite™ alphabeta human Type I interferon activity detection kit. The binding of IFN beta-1a to its receptor, expressed on stably-transfected human cells, initiates an intracellular cascade of signal transduction events that induces the luciferase gene under the transcriptional control of an interferon-sensitive response element; resulting in the production of the luciferase enzyme in a dose-dependent manner. NAbs, present in the serum, inhibit the binding of IFN beta-1a to its receptor, thereby inhibiting the production of luciferase. The NAb assay was carried out in two steps, a screening assay followed by a titration assay. Samples with a percent inhibition at or above the assay cut point were considered positive, and were further evaluated in a titration assay by serial dilution to determine titer values.

Calculation of PK and PD Parameters Using Non-compartmental Analysis

Non-compartmental PK analyses were carried out using WinNonlin Professional Versions 4.0.1 and 5.0 software (Pharsight Inc., Mountain View, CA). Data were analyzed using non-

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compartmental analysis (NCA) with an extravascular input model (WinNonlin Model 200). The parameters included maximum serum concentration (C_{max}), the time to reach C_{max} (T_{max}), area under the concentration time curve from time zero to infinity (AUC_{inf}), clearance (CL/F), and elimination half-life ($t_{1/2}$). For neopterin, the parameters included post-dose peak concentration (N_{peak}), time to reach N_{peak} (N_{T_max}), and area under the neopterin concentration time curve from time zero to 168 h post-dose (N_{AUC_168h}).

PK Model

The PK/PD model was built sequentially and the integrated PK/PD model is shown in Figure 1. The PK model consists of a two-compartment model with linear absorption rate (Ka), elimination rate (Ke), and inter-compartment rates, K_{12} and K_{21}. The drug concentration is described by the following differential equations:

$$\frac{dA_{DOSE}}{dt} = -Ka \cdot F \cdot A_{DOSE} \quad (1)$$

$$\frac{dA_C}{dt} = Ka \cdot F \cdot A_{DOSE} + K_{21} \cdot A_P - K_{12} \cdot A_C - Ke \cdot A_C \quad (2)$$

$$\frac{dA_P}{dt} = K_{12} \cdot A_C - K_{21} \cdot A_P \quad (3)$$

Re-parameterization:

$$A_C = Cp \cdot V_C \quad (4)$$

$$K_{12} = Q/V_C \quad (5)$$

$$K_{21} = Q/V_P \quad (6)$$

$$Ke = CL/V_C \quad (7)$$

Initial values:

$A_{DOSE}(0)$ = Administered Dose (8)

$A_C(0) = 0$ (9)

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$$A_P(0) = 0 \quad (10)$$

in which A_{DOSE} represents the amount of drug at the injection site, F represents the relative bioavailability, A_C represents the amount of drug in the central compartment, A_P represents the amount of drug in the peripheral compartment, Cp represents the serum drug concentration, Q represents the inter-compartment clearance, V_C represents the central compartment volume of distribution, V_P represents the peripheral compartment volume of distribution, and CL represents the total clearance. The bioavailability of the IM route, F (IM), was fixed as 1. The F (SC), Ka (IM), Ka (SC), CL, V_C, V_P, and Q were to be estimated. Inter-subject variance (ω^2) for the PK parameters was assumed to be log-normal distributed and added one-at-a-time, judged by numerical stability and decrease of objective function value (OFV). The final IFN beta-1a PK model included ω^2 for V_C and Q, with a covariance between the two; the final PEG-IFN beta-1a PK model included ω^2 for CL and Q, with a covariance between the two. The residual error was modeled using an additive error model on the log-transformed PK data.

PD Model

The pharmacodynamic component of the model is an indirect stimulatory response model (Dayneka et al., 1993; Mager et al., 2003; Mager et al., 2005) driven by the fixed individual empirical Bayes PK parameters as input in the dataset. The initial lag time for neopterin response was modeled by 5 transit compartments (Savic et al., 2007). The neopterin concentration was described by the following equations:

$$\frac{dTR1}{dt} = SYN \cdot (1 + EFFECT) - KTR \cdot TR1 \quad (11)$$

$$\frac{dTR2}{dt} = KTR \cdot TR1 - KTR \cdot TR2 \quad (12)$$

…

$$\frac{dTR5}{dt} = KTR \cdot TR4 - KTR \cdot TR5 \quad (13)$$

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$$\frac{dNEOP}{dt} = KTR \cdot TR5 - LOSS \cdot NEOP \quad (14)$$

$$EFFECT = \frac{E_{max} \cdot Cp}{EC_{50} + Cp} \quad \text{for time} \le TAU \quad (15)$$

$$EFFECT = \frac{E_{max} \cdot Cp}{EC_{50} + Cp} \cdot \left(1 - \frac{NEOP}{IC_{50} + NEOP}\right) \quad \text{for time} > TAU \quad (16)$$

Re-parameterization:

$$SYN = BSL \cdot LOSS \quad (17)$$

$$KTR = 5/MTT \quad (18)$$

Assuming stationary initial conditions:

$$\frac{dTR1}{dt} = \frac{dTR2}{dt} = \ldots = \frac{dTR5}{dt} = \frac{dNEOP}{dt} = 0 \quad (19)$$

Initial values:

$$TR1(0) = TR2(0) = \ldots = TR5(0) = \frac{SYN}{KTR} \quad (20)$$

$$NEOP(0) = BSL \quad (21)$$

where TR1 through TR5 represent the neopterin concentration in the respective transit compartment, SYN represents the baseline zero-order neopterin synthesis rate, EFFECT represents the stimulation effect by IFN beta-1a or PEG-IFN beta-1a, NEOP represents the serum neopterin concentration, LOSS represents the first-order elimination rate of serum neopterin, E_{max} represents the maximum stimulation effect, EC_{50} represents the drug concentration to reach 50% of the maximum stimulation effect, KTR represents the first-order rate constant exiting each transit compartment, BSL represents the neopterin baseline concentration prior to dosing, MTT represents the mean time delay in the transit compartment, IC_{50} represents the neopterin concentration that inhibits the stimulation effect by 50%, and TAU represents the time delay for the neopterin inhibitory feedback to occur. Initial modeling without neopterin inhibitory feedback resulted in overestimating the neopterin concentration following

~2-3 days after PEG-IFN beta-1a dosing. It is known that continuous exposure of interferon reduces the magnitude of neopterin response in both humans and monkeys (Rothuizen et al., 1999; Mager et al., 2003). This phenomenon is attributed to either the down-regulation of the Type 1 IFN receptor (Pestka et al., 1987) or inhibitory feedback by prolonged elevation of neopterin concentrations (Liberati et al., 1988). Modeling of the inhibitory effect was attempted by either linking to the drug concentration or to the neopterin concentration, with the latter approach providing more robust parameter estimates, i.e., less sensitive to the initial estimate. BSL, LOSS, E_{MAX}, EC_{50} (IFN beta-1a), EC_{50} (PEG-IFN beta-1a), IC_{50}, MTT (IFN beta-1a), MTT (PEG-IFN beta-1a) were to be estimated. Initial efforts to estimate TAU in the model resulted in zero gradients. To optimize TAU, it was fixed at different values in the range of 2-48 h with a 2 h increment and the model predictions were compared. The TAU was fixed as 22 h in the final model, which showed one of the best predictions and was similar to the estimated delay time (29.3 h) in the literature (Mager et al., 2003). Inclusion of ω^2 followed the same procedure as in the PK analysis and the final model consisted of ω^2 for BSL and E_{max} and a covariance between the two, assuming log-normal distribution. The residual error was modeled using an additive error on the log-transformed neopterin concentration data.

Data Analysis

All PK/PD analyses were carried out using a nonlinear mixed effects modeling program (NONMEM, version VII, level 1.2, ICON Development Solutions, Ellicott City, MD) (Beal et al., 1989-2006; Bauer, 2009) on an Intel-based PC with Intel Visual Fortran Compiler Professional software 11.1.048. A first-order conditional estimation method (CONDITIONAL) was used for parameter estimation.

Visual Predictive Check

To generate a visual predictive check (VPC) plot for model diagnostics, one thousand simulations were carried out in NONMEM with the final PK and PD models. The observed data and simulated data were processed in R (version 2.11.1, R Foundation for Statistical Computing, Vienna, Austria). PK VPC plots were stratified by drug and route and normalized by dose; PD VPC plots were stratified by drug, route, and dose.

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Results

Comparative PK/PD of PEG-IFN beta-1a and IFN beta-1a in Rhesus Monkeys

In Study 1, PEG-IFN beta-1a was compared to IFN beta-1a, following a single-dose injection at 1 MIU/kg via the IM or SC route. The mean serum drug concentration-time curves are shown in Figure 2A. PEG-IFN beta-1a peaked ~4 h post-dose with IM administration, and ~15 h post-dose with SC administration, sustained approximate peak concentrations for about 0.5 to 1 day, and declined with a $t_{1/2}$ of approximately 20 h. In contrast, IFN beta-1a was absorbed more rapidly than PEG-IFN beta-1a, peaking within 2 h post-dose (IM and SC). The concentration of IFN beta-1a declined rapidly with a $t_{1/2}$ of ~7 h after the peak, with no apparent plateau phase. Based on the NCA parameters, both AUC_{inf} and C_{max} increased significantly after incorporation of a 20 kDa PEG at the N-terminus of IFN beta-1a, compared to the unmodified protein (Table 1). For the IM and SC routes of administration, the mean AUC_{inf} (mass normalized) of PEG-IFN beta-1a was approximately 36- to 43-fold greater, respectively, and the mean C_{max} (mass normalized) was approximately 6- to 7-fold greater, respectively. PEGylation reduced CL/F by 35- and 41-fold for the IM and SC routes, respectively.

The VPC of PK models is presented in Figure 3 and the parameter estimates are listed in Table 2. Both IFN beta-1a and PEG-IFN beta-1a were well described using a linear two-compartment model, with well-aligned median values between the observed and simulated drug concentrations (Figure 3). There was a slight overestimation of the inter-subject and/or intra-subject variability for the IFN beta-1a SC group, resulting in a wider 5^{th}-95^{th} percentile range than the observed data at the terminal phase; however, given the small sample size (n=5), the model estimates were considered acceptable. As expected, the Ka of IFN beta-1a was larger than that of PEG-IFN beta-1a, especially for the SC route, where a ~15-fold decrease was observed, equivalent to a 15-fold increase in the absorption half-life. The V_C, V_P, and CL were decreased by 15-, 11-, and 37-fold, respectively, after PEGylation.

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The mean serum concentration-time curves of neopterin following a single injection of PEG-IFN beta-1a or IFN beta-1a via the IM or SC route at 1 MIU/kg are shown in Figure 2B. Single-dose IM or SC administration of PEG-IFN beta-1a or IFN beta-1a to Rhesus monkeys resulted in an increase of serum neopterin concentrations, compared to pre-treatment, confirming the *in vivo* biological activities of both test articles. In general, the neopterin concentration peaked at 24-32 h post-dose in Study 1, declined thereafter, and almost returned to baseline concentrations at 1 week post-dose. Interestingly, despite the significant difference in drug exposure, there were no discernable differences in neopterin profiles over the time course for all four treatment groups. The lack of higher neopterin response after PEGylation was reflected in the EC_{50} estimates from the PD model, where the EC_{50} of PEG-IFN beta-1a was 21-fold higher than that of IFN beta-1a. This result is consistent with previous observations for a 20 kDa mPEG-propionaldehyde-conjugated IFN beta-1a (a predecessor of the current molecule), where no apparent differences were observed in neopterin response between the PEGylated and unmodified proteins, even with an approximately 10-fold difference in AUC (Pepinsky et al., 2001). Overall, the PD model well described the neopterin concentration-time profiles across test articles, routes, and doses (Figure 4). The estimated elimination rate of neopterin LOSS was consistent with value from the literature (Table 4) (Mager et al., 2003; Mager et al., 2005). The variance appeared to be over-estimated, which might be a result of small sample size (n=4-5 for the full PD profiles for each stratified group). Of note, both N_{AUC_168h} and N_{peak} were lower in Study 2 when PEG-IFN beta-1a was administered subcutaneously at 10 μg/kg (1.1 MIU/kg) than those in Study 1 at 10.8 μg/kg (1 MIU/kg). The two batches of materials in the two studies were comparable based on specification tests, including pH, osmolality, in vitro anti-viral potency, peptide map, oxidation, and carbohydrate composition, using pre-specified criteria. The difference is therefore likely to be attributable to inter-study variability, which might partly account for the over-estimated variance in the PD model. Based on the estimated IC_{50} of 1.82

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ng/mL, the stimulatory effect will be inhibited by 92% at the peak neopterin concentration of ~21 ng/mL (Table 4).

Dose and Route Effect on PK/PD of PEG-IFN beta-1a in Rhesus Monkeys

In Studies 2 and 3, PK was characterized over a dose range of 2 to 100 μg/kg (0.22 to 11 MIU/kg). The mean drug concentration-time profiles of the SC groups from Study 2 are shown in Figure 5A. The PK profiles from Study 3 following the first dose were similar to those in Study 2 (data not shown). Overall, PEG-IFN beta-1a showed linear kinetics, as shown by dose-proportional increase in AUC_{inf} and $C_{max,}$ and dose-independent $t_{1/2}$, and CL/F. The linear kinetics was further confirmed by decent linear model predictions (Figure 3).

The N_{AUC_168h} and N_{peak} of neopterin increased with dose in a less-than-dose-proportional manner (Figure 5B). With a 5-fold increase in PEG-IFN beta-1a dose from 0.22 to 1.1 MIU/kg, the N_{AUC_168h} and N_{peak} of neopterin increased approximately 1.5-fold. With a further 10-fold dose increase from 10 to 100 μg/kg, the N_{AUC_168h} and N_{peak} of neopterin increase a further 1.7-fold (Table 3). Based on the final model parameters, the C_{max} of PEG-IFN beta-1a at 0.22, 1.1, and 11 MIU/kg (SC) was equivalent to 8%, 40%, and 400% of the EC_{50}, corresponding to 7%, 28, and 80% of the E_{max}, respectively, accounting at least partly for the less-than-dose proportional response. In addition, the inhibitory feedback of neopterin elevation also contributed to the nonlinearity, with higher neopterin concentration causing a higher inhibitory effect. For instance, the N_{peak} from Study 2 was equivalent to 6 $\times$, 8 $\times$, and 13 $\times$ IC_{50}, at 0.22, 1.1, and 11 MIU/kg (SC), respectively, corresponding to 85%, 89%, and 92% of inhibition of the stimulation by PEG-IFN beta-1a.

The impact of route of administration on the PEG-IFN beta-1a PK parameters was investigated in all three studies. The differences in AUC_{inf} estimated by NCA were small (within ± 25% across the three studies) and no consistent trend was observed; however, the population

F (SC) estimated by the PK model was 0.87 relative to F (IM), which was fixed as 1, equivalent to a 13% lower AUC_{inf} for the SC route. Given the observed CV% in NCA AUC_{inf} (~30%), the difference was considered minimal. More profound differences were observed in the absorption kinetics. PEG-IFN beta-1a was absorbed more quickly following IM injection than following SC injection, resulting in an earlier T_{max} (4.29 vs. 14.6 h) and higher C_{max} (4.79 vs. 2.48 ng/mL/μg), which was consistent across the three studies (Table 1). No consistent differences were identified between the IM and SC routes in $t_{1/2}$ or CL/F. From the modeling approach, the Ka (SC) was 5-fold lower than the Ka (IM), corresponding to a 5-fold longer absorption half-life (7.8 h vs. 1.5 h) for the SC route. Inclusion of route as a covariate for CL, V_C, or V_P did not improve model performance significantly, indicating a lack of impact by route on these parameters.

The effect of route on neopterin response was also evaluated. No apparent trend was identified with regard to route of administration and the magnitude of neopterin elevation. In Study 1, the difference ([IM-SC]/SC%) was 0.2% in N_{AUC_168h} and 8% in N_{peak}. In Study 2, the differences were 21% in N_{AUC_168h} and 19% in E_{max}. In Study 3, neopterin concentration was measured at 24 h only for selected weeks; therefore, the parameters were not calculated. The percentage difference in neopterin concentration was –14% following the first dose prior to immunogenic response. The route did not show significant impact on EC_{50} or E_{max} in the PD model. Overall, the effect of the route of administration on neopterin response was considered minimal.

Immunogenicity and its Impact on PK/PD of PEG-IFN beta-1a in Rhesus Monkeys

Because IFN beta-1a is immunogenic in Rhesus monkeys (Martin et al., 2002; FDA Toxicology Review of Rebif BLA, 1999; data on file, Biogen Idec), anti-drug antibodies were measured following single-dose administration (Study 2), and following repeat-dose administration (Study 3). In Study 2, BAbs and NAbs were detected in 25% and 6% of the monkeys, respectively, at

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14 days post-dosing, and in 50% and 50%, respectively, at 28 days post-dosing. In Study 3, BAbs and NAbs were detected in 85% and 35% of the PEG-IFN beta-1a-treated-monkeys, respectively, after 2 doses; BAbs and NAbs were detected in 100% and 95% of the monkeys, respectively, after 5 doses (Figure 6D).

The impact of immunogenicity on PK/PD of PEG-IFN beta-1a was inferred from the repeat-dose toxicity study. The PEG-IFN beta-1a concentration decreased significantly after multiple weekly doses, secondary to the development of anti-drug antibodies (Figure 6A & 6B). Starting from the 4th dose, drug concentration became undetectable in 86% of the monkeys in the 2 μg/kg dose group, and in 14% of the monkeys in the 10 μg/kg dose group. After the 5th dose, PEG-IFN beta-1a was not detectable in 73% of the drug-treated monkeys. In the 11 monkeys that had detectable PEG-IFN beta-1a following the 5th dose, the AUC was less than 1% (0.0009-0.98%) of the AUC from the 1st dose in 9 monkeys, while the other two had AUC values that were 14.8 and 25% of the respective 1st dose.

The pharmacological response in Rhesus monkeys, as indicated by the serum neopterin concentration, was also affected by the immunogenicity of PEG-IFN beta-1a. Administration of PEG-IFN beta-1a to Rhesus monkeys initially induced the elevation of neopterin. However, the induction of neopterin decreased with successive doses (Figure 6C). The mechanism by which the anti-drug antibodies reduced the pharmacological response is likely due the bound antibody's ability to prevent or diminish the interaction of the IFN portion of the molecule with the Type I IFN receptor, thereby reducing IFN-dependent signaling. However, we cannot rule out the possibility that an increased rate of drug clearance, mediated by the binding of anti-drug antibodies, may also contribute to limiting exposure of the protein to its receptor and therefore reducing neopterin induction.

Safety of PEG-IFN beta-1a in Rhesus Monkeys

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Designed to support Phase I clinical studies, the nonclinical GLP toxicology study evaluated the safety of PEG-IFN beta-1a following repeat-dose SC or IM administration for 5 weeks in Rhesus monkeys with a 4-week treatment-free recovery period. PEG-IFN beta-1a was well tolerated in all dose groups and produced the expected pharmacological effects of transient increases in body temperature, increases in serum neopterin, and a transient decrease in peripheral blood lymphocytes. There were no PEG-IFN beta-1a-related adverse effects on mortality, clinical observations, body weight, food consumption, local irritation, ophthalmology, heart rate, ECGs, respiratory rate, clinical pathology, urinalysis, or macroscopic or microscopic anatomic pathology at doses up to 100 µg/kg (SC or IM) administered once weekly for five weeks.

Treatment with PEG-IFN beta-1a resulted in non-dose-dependent transient increases in body temperature of 1-3°F (Figure 7), and a dose-dependent decrease in lymphocyte counts (Figure 8). The decrease in lymphocyte counts was transient and evident only at Week 1, with the exception of monkeys administered 100 µg/kg IM, where the decrease in lymphocyte counts persisted through Week 5, but not through Week 6. The Week 1 (8 h post-dose) average lymphocyte counts were 1, 28, 33, 59, and 59% lower in monkeys administered vehicle control (SC & IM), 2 µg/kg (SC), 10 µg/kg (SC), 100 µg/kg (SC), and 100 µg/kg (IM) PEG-IFN beta-1a, respectively, compared to lymphocyte counts 10 days prior to the first dose. Monkeys administered 100 µg/kg of PEG-IFN beta-1a IM had lymphocyte counts (collected 4 h post-dose) that were 64, 42, and 36% lower at Week 3, Week 4, and Week 5, respectively, compared to the lymphocyte counts 10 days prior to the first dose. Thus, it appears that dosing of PEG-IFN beta-1a via the IM route perpetuates a reduction in lymphocyte counts during the dosing phase, with a noted recovery upon cessation of dosing. Because the effects on body temperature and lymphocyte counts were mild, reversible, and related to the expected pharmacological activity of Type I IFNs, they were not considered adverse. There were no apparent sex-related differences with any of the findings. The no-observed-adverse-effect-level

(NOAEL) from this study was 100 µg/kg (11 MIU/kg) for both SC and IM routes, the highest dose tested.

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Discussion

PEGylation is a method for extending the half-life of biological therapies, enhancing their *in vivo* efficacy, and enabling less frequent dosing (Bailon and Won, 2009). The mechanism underlying these effects is the increase in the apparent molecular size of the drug, which in turn reduces the glomerular filtration rate. The cut-off size for renal clearance is considered to be approximately the size of albumin (67 kDa). IFN beta-1a has a molecular weight of ~25 kDa and the kidney has been shown to be the major site of interferon degradation based on published animal studies, among which one study quantified the renal clearance contribution as more than 90% of total clearance in rats (Bino et al., 1982a; Bino et al., 1982b; Bocci et al., 1984). The clearance pathway of interferon alpha by the kidneys is described as an initial filtration, followed by absorption by the proximal tubular cells, and subsequent catabolism in cellular lysosomes (Bocci et al., 1981; Bocci et al., 1984). Incorporation of a single 20 kDa PEG resulted in an increase in the apparent M_r of the protein from ~25 kDa to ~320 kDa as measured by size exclusion chromatography (Baker et al., 2006). The increase in the apparent mass compared to the unmodified protein results from a significant increase in the hydrodynamic volume. In addition to decreasing glomerular filtration rate, steric hindrance by the flexible PEG molecule could potentially protect the protein from protease degradation and antibody and receptor-mediated clearance mechanisms.

The N-terminus of IFN beta-1a serves as an attractive site for modification since it does not participate in receptor binding site (Baker et al.,2006; Karpusas et al., 1998; Runkel et al., 2000). Using the same site-specific N-terminal PEGylation procedure, a similar PEG-IFN beta-1a molecule has been synthesized previously with 20 kDa mPEG-propionaldehyde. While 20 kDa mPEG-propionaldehyde-modified IFN beta-1a had improved PK properties in mice, rats, and Rhesus monkeys, and retained full *in vitro* antiviral activity (Pepinsky et al., 2001), direct side-by-side comparison of 20 kDa mPEG-propionaldehyde- and 20 kDa mPEG-O-2-methylpropionaldehyde-modified IFN beta-1a has not been carried out. However, comparisons

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made to unmodified IFN beta-1a in each case can be used to compare the two PEGylated molecules. Both PEGylated molecules showed 2- to 3-fold increase in $t_{1/2}$ over unmodified IFN beta-1a. The AUC increase over IFN beta-1a was much greater (~40-fold) for the current molecule than for the previous molecule (~10-fold), but the comparison may not be relevant, because different bioanalytical methods (ELISA vs. CPE) were used to quantify serum drug concentrations. In terms of pharmacological response, both molecules produced similar neopterin profiles compared to unmodified IFN beta-1a. Overall, the two molecules showed similar PK/PD properties in Rhesus monkeys. Compared to the PEG-IFN beta-1a described in this manuscript, PEGylated IFN alpha-2b (PEG-Intron®), a 12 kDa PEG conjugate, showed a $t_{1/2}$ of 29.5-34.1 h following SC administration in Cynomolgus monkeys, and a 2-3-fold increase in dose-normalized AUC compared to the unmodified protein (FDA Toxicology Review of PEGINTRON BLA, 2000), while PEGylated IFN-alpha-2a (PEGASYS®), a 40 kDa PEG conjugate, provided a $t_{1/2}$ of 143-186 h with AUC values not being available (FDA Preclinical Review of PEGASYS BLA, 2001). The differences in PK parameters may indicate the effect of the size of the conjugated PEG; however, comparison of values is limited by the different monkey species and assays used in each study.

The increase in AUC_{inf} and C_{max} observed for the PEG-IFN beta-1a reported here did not translate into an increase in neopterin exposure. Similar results were reported for the 20 kDa mPEG-propionaldehyde-modified IFN beta-1a (Pepinsky et al., 2001). Possible explanations proposed in that paper included down-regulation of the Type I IFN receptor or downstream signaling components, neopterin-producing cells being less accessible to PEG-IFN beta-1a due to its limited tissue distribution, or saturation of the biological response. The PK/PD model suggests that the stimulatory effect was indeed reduced approximately one day post-dose. The reduced stimulatory effect could be explained by down-regulation of the Type I IFN receptor (Pestka et al., 1987) and/or neopterin inhibitory feedback (Liberati et al., 1988). The latter approach was adopted as the mathematical solution, which linked an inhibitory effect to the

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neopterin concentration, as it provided more robust parameter estimates. Further evidence is necessary to distinguish between these two potential mechanisms. The saturation of the biological response at 1 MIU/kg is less likely, given the fact that the N_{AUC_168h} and N_{peak} increased further when the dose of PEG-IFN beta-1a was increased from 10 μg/kg (1.1 MIU/kg) to 100 μg/kg (11 MIU/kg) and the stimulatory effect in Study 1 was ~60% of the E_{max} even at the C_{max}. Indeed, while the PD parameters increased with dose, they trended towards saturation at 11 MIU/kg (100 μg/kg). For the SC route of administration, for which doses of 2, 10, and 100 μg/kg were tested (Study 2), the neopterin dose-response curves indicated that for both N_{AUC_168h} and N_{peak} the half maximal saturation occurred at approximately 10 μg/kg. At this dose, the C_{max} value was 19.5 ± 6.5 ng/mL, equating to a molar serum concentration of $9.7 \times 10^{-10} \pm 3.2 \times 10^{-10}$ M. While the affinity of PEG-IFN beta-1a for the Rhesus monkey Type I IFN receptor is not known, assuming that the PEG moiety does not significantly affect receptor binding (the K_d for IFN beta-1a and PEG-IFN beta-1a binding to the isolated extracellular portion of the IFNAR2 chain of the human Type I IFN receptor are the same, Biogen Idec data on file), and the affinity of IFN beta-1a for the Rhesus monkey and human receptors is comparable, published data for the affinity of IFN beta for the human receptor can be used to estimate whether the non-linear neopterin response is due to receptor saturation or biological saturation. As the affinity of IFN beta for the Type I IFN receptor on the surface of human Daudi B-cells is 3.2×10^{-10} M (range 0.9-11 $\times 10^{-10}$ M) (Cutrone and Langer, 1997), it suggests that the saturation of the neopterin response in Rhesus monkeys is due to receptor saturation, i.e. 50% response at approximately the K_d. However, as the assumptions made above have yet to be verified, and we do not know whether the neopterin-producing cells are located within the vasculature, from which the C_{max} concentration was measured, or outside of the vasculature, where the concentration is not known, we cannot rule out the possibility that the non-linear neopterin response may be due to saturation of biological responses downstream of the

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receptor. Indeed, IFN beta-dependent biological responses are known to saturate at ligand concentrations well below the K_d i.e. at sub-stoichiometric receptor occupancy. For example, Runkel et al (2000) have shown that the half-maximal antiproliferative activity of IFN beta-1a on human Daudi B-cells occurs at only 0.3% receptor occupancy.

Interestingly, these observations in Rhesus monkeys are in contrast to those in humans, where neopterin elevation was of longer duration following PEG-IFN beta-1a treatment than that following IFN beta-1a treatment at comparable doses (Hu et al., 2009; Hu et al., 2011; Davar et al., 2009). Furthermore, preliminary PK/PD modeling did not indicate reduction of the stimulatory effect in humans over a one week exposure (data not shown). This highlights the limitation of using an animal model to predict human responses. Encouraged by the results from two Phase 1 studies, the efficacy of PEG-IFN beta-1a is currently being tested in a multi-center, randomized, double-blind, placebo-controlled Phase 3 clinical trial in patients with relapsing MS.

Human IFN beta-1a is immunogenic in monkeys (Martin et al., 2002; FDA Toxicology Review of Rebif BLA, 1999; data on file, Biogen Idec). Even though PEGylation has been shown to reduce immunogenicity of some proteins in humans (Hoffman-la Roche, 2006; Hoffman-la Roche, 2009), PEG-IFN beta-1a remained immunogenic in Rhesus monkeys, especially in the repeat-dose study, in which almost all monkeys were BAb- and NAb- positive after 5 weekly doses. Consequently, the PEG-IFN beta-1a concentration decreased dramatically, and the neopterin response diminished almost completely by the end of the study. However, the immunogenicity of PEG-IFN beta-1a in monkeys is not predictive of the human response. When PEG-IFN beta-1a was tested in humans, no subject developed NAbs after single-dose or multiple-dose PEG-IFN beta-1a treatment (Davar et al., 2009; Baker et al., 2010; Hu et al., 2011). This observation is consistent with IFN beta-1a, of which the immunogenicity rate was 2-5% in a study in MS patients (Panitch et al., 2002; Biogen Idec, 2007), in contrast to

100% in studies in Rhesus monkeys (data on file, Biogen Idec). The immunogenicity of PEG-IFN beta-1a over long-term treatment is being tested in the ongoing Phase 3 study.

Pharmacology and safety of unmodified IFN beta-1a have been evaluated in over 10 studies in Rhesus monkeys, including both single- and repeat-dose evaluations (data on file, Biogen Idec). Monkeys developed NAbs to IFN beta-1a after approximately 2 weeks of treatment; for this reason, chronic repeat dose toxicity testing was not feasible. No mortalities and no significant signs of toxicity were observed in the treated animals up to the highest dose of IFN beta-1a tested (50 μg/kg SC). Elevated body temperature, reduced food consumption, decreased platelet counts, and decreased serum albumin and calcium concentrations were observed. No respiratory, cardiovascular, or acute allergic reactions were evident. No overt effects on the central nervous system were noted in any of the treated animals. Comparing to historic data, the safety profile of PEG-IFN beta-1a in Rhesus monkeys is consistent with that of IFN beta-1a. The responses, including a slight increase in body temperature and decreases in lymphocyte counts, are anticipated effects in response to IFN beta-1a treatment and were shown to be reversible following the treatment free period. Similar to IFN beta-1a, monkeys also developed NAbs within two weeks of repeat treatment, resulting in reduced or no exposure in 5 weeks and rendering chronic repeat-dose toxicity testing with PEG-IFN beta-1a not feasible. PEGylating IFN beta-1a did not result in any new toxicities in Rhesus monkeys, even with the changes in PK. Interestingly, other effects seen upon treatment of monkeys with IFN beta-1a include decreased platelets, transient elevation of hepatic transaminases, transient decreases in serum total protein and albumin concentrations, and local irritation and/or inflammation, none of which were seen with PEG-IFN beta-1a treatment in the toxicity study of PEG-IFN beta-1a.

In summary, in Rhesus monkeys PEG-IFN beta-1a showed greater exposure, longer half-life, slower clearance, and reduced volume of distribution compared to IFN beta-1a. Consistent with the pharmacology of Type I IFNs, PEG-IFN beta-1a induced neopterin elevation, caused

transient body temperature increase, and reversible lymphocyte count decrease. However, at comparable 1 MIU/kg doses, PEG-IFN beta-1a did not result in enhancement of neopterin induction compared to the unmodified protein, an observation that contrasts to the situation in humans where the improved PK properties of the molecule translated into improved PD responses. There were no drug-related adverse effects at doses up to 100 µg/kg (11 MIU/kg, SC or IM) administered once weekly for five weeks, and the NOAEL was determined to be 100 µg/kg. These studies were used to successfully enable two Phase 1 clinical trials of PEG-IFN beta-1a in healthy subjects (Hu et al., 2011); data from which in turn enabled a Phase 3 study in patients with relapsing MS.

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Conducted experiments: Hu, Polack, Crossman, Zokowski, and Subramanyam

Contributed new reagents or analytic tools: Baker and Gronke

Performed data analysis: Hu, Olivier, Polack, and Subramanyam

Wrote or contributed to the writing of the manuscript: Hu, Gronke, Parker, Li, Baker, Polack, Ivan, and Subramanyam

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Footnotes

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Legends for Figures

Figure 1. Integrated PK/PD model following a single SC or IM administration in Rhesus monkeys. The PK component consists of a linear two-compartment model. The PD model is a stimulatory indirect response model, where the initial delay of neopterin response is modeled with 5 transit compartments. The legends are: CL=clearance; EC_{50}=drug concentration to achive 50% of the maximum stimulatory effect; E_{max}=maximum stimulatory effect by IFN beta-1a or PEG-IFN beta-1a; IC_{50}=neopterin concentration to inhibit 50% of the stimulatory effect from IFN beta-1a or PEG-IFN beta-1a; Ka=absorption rate; Ke=elimination rate; KTR=1st order rate exiting transit compartment; LOSS=first-order loss rate; NEOP=neopterin concentration; Q=inter-compartment clearance; SYN=baseline synthesis rate; V_C=central compartment volume of distribution; V_P=peripheral compartment volume of distribution; TR1=transit compartment 1; TR5=transit compartment 5.

Figure 2. Pharmacokinetic (A) and pharmacodynamic (B, neopterin) profiles of PEG-IFN beta-1a and IFN beta-1a in Rhesus monkeys following a single IM or SC administration at a dose of 1 MIU/kg (equivalent to 5 μg/kg of IFN beta-1a and 10.8 μg/kg of PEG-IFN beta-1a). Data are from Study 1 and are expressed as mean ± standard error (n=5/group). PEG-IFN beta-1a IM (■); PEG-IFN beta-1a SC (□); IFN beta-1a IM (●); IFN beta-1a SC (○).

Figure 3. Visual predictive check of PK models following a single-dose administration of IFN beta-1a or PEG-IFN beta-1a in Rhesus monkeys. The data was stratified by drug and route of administration. Both the simulated and observed (○) concentrations were normalized by dose (μg). Solid lines represent the median value of the observed concentrations and dashed lines from top to bottom represent the 95th percentile, the median, and the 5th percentile of the simulated concentrations.

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Figure 4. Visual predictive check of PD models following a single-dose administration of IFN beta-1a or PEG-IFN beta-1a in Rhesus monkeys. The data was stratified by drug, route of administration, and dose. The observed concentrations are shown as open circles (○). Solid lines represent the median value of the observed concentrations and dashed lines from top to bottom represent the 95th percentile, the median, and the 5th percentile of the simulated concentrations.

Figure 5. Pharmacokinetic (A) and pharmacodynamic (B, neopterin) profiles of PEG-IFN beta-1a in Rhesus monkeys following a single-dose SC administration at a dose of 2 (●), 10 (○), or 100 μg/kg (■). Each curve is the mean of 4 animals from Study 2. Data are shown as mean ± standard error.

Figure 6. (A) AUC_{168h} of PEG-IFN beta-1a, (B) peak concentrations of PEG-IFN beta-1a, (C) peak concentrations of neopterin, (D) and NAb-positive incidences in percentage following weekly SC administration of PEG-IFN beta-1a at 2, 10, or 100 μg/kg or weekly IM administration at 100 μg/kg in Rhesus monkeys. The inserts are magnified plots of Panel A and Panel B with narrower y-axis ranges. AUC_{168h} is presented instead of AUC_{inf} in panel A because there were insufficient measurable concentrations to estimate AUC_{inf} in the majority of the animals on Day 29. Data are from Study 3.

Figure 7. Body temperature (mean ± standard error) of Rhesus monkeys prior to treatment (mean of Days –28 and –25) and post-treatment (Days 1 through 29) at estimated peak concentration of PEG-IFN beta-1a, following weekly SC and IM administration of placebo (Group 1), or weekly SC administration of PEG-IFN beta-1a at 2 μg/kg (Group 2), 10 μg/kg

(Group 3), 100 μg/kg (Group 4), or weekly IM administration of PEG-IFN beta-1a at 100 μg/kg (Group 5) for 5 weeks. Data are from Study 3.

Figure 8. Lymphocyte count (mean ± standard error) of Rhesus monkeys prior to treatment (mean of Days –10 and –2) and post-treatment following weekly SC and IM administration of placebo (Group 1), or weekly SC administration of PEG-IFN beta-1a at 2 μg/kg (Group 2), 10 μg/kg (Group 3), 100 μg/kg (Group 4), or weekly IM administration of PEG-IFN beta-1a at 100 μg/kg (Group 5). Data are from Study 3.

Tables

Table 1. Mean pharmacokinetic parameters from non-compartmental analysis with standard deviation shown in parentheses. The PK parameters of PEG-IFN beta-1a were consistent across studies (referring to the first dose only for the repeat-dose study), and therefore, pooled parameters are presented.

Drug	Route	n	AUC_{inf}/dose (h•ng/mL/μg)	C_{max}/dose (ng/mL/μg)	CL/F (mL/h/kg)	$t_{1/2}$ (h)	t_{max} (h)
IFN beta-1a	IM	5	3.53 (1.23)	0.829 (0.619)	308 (95)	6.30 (2.95)	1.60 (0.55)
IFN beta-1a	SC	5	2.53 (0.44)	0.347 (0.147)	405 (69.9)	7.42 (1.68)	1.60 (0.55)
PEG-IFN beta-1a	IM	21	126 (35.8)	4.79 (1.64)	8.91 (3.95)	20.7 (5.30)	4.29 (2.10)
PEG-IFN beta-1a	SC	45	108 (31.7)	2.48 (1.05)	10.0 (2.97)	19.1 (4.67)	14.6 (10.0)

Table 2. Population PK model parameters (fixed and random effects), including % relative standard error.

Model Parameter (units)	*Definition*	*Estimated value*	*% Relative standard error*	*Estimated value*	*% Relative standard error*
		IFN beta-1a		*PEG-IFN beta-1a*	
Ka_SC (h^{-1})	Absorption rate following SC administration	1.37	55	0.0892	2
Ka_IM (h^{-1})	Absorption rate following IM administration	1.65	42	0.461	17
V_C (mL/kg)	Central compartment volume of distribution	1610	18	107	0.1
V_P (mL/kg)	Peripheral compartment volume of distribution	1170	28	105	0.1
CL (mL/kg/h)	Clearance	314	8	8.53	0.1
Q (mL/kg/h)	Inter-compartmental clearance between central and peripheral compartment	67.6	40	52.5	0.5
F_SC (unitless)	Relative Bioavailability to IM	0.781	11	0.868	0.02
F_IM (unitless)	Relative Bioavailability	1, fixed	NA[a]	1, fixed	NA[a]
ω^2_{Vc}	Inter-subject variance of V_C	0.127	71	0, fixed	NA[a]
ω^2_{CL}	Inter-subject variance of CL	0, fixed	NA[a]	0.0233	21

Model Parameter (units)	*Definition*	*Estimated value*	*% Relative standard error*	*Estimated value*	*% Relative standard error*
		IFN beta-1a		*PEG-IFN beta-1a*	
ω^2_Q	Inter-subject variance of Q	0.487	117	4.6	0.5
cov_{Vc_Q}	Covariance of V_C and Q	0.106	69	0, fixed	NA[a]
cov_{CL_Q}	Covariance of CL and Q	0, fixed	NA[a]	-0.0353	10
SD	Coefficient of residual error	0.397	10	0.399	0.1
σ^2	Residual error variance	1, fixed	NA[a]	1, fixed	NA[a]

[a]NA, not applicable

Table 3. Mean pharmacodynamic parameters of neopterin from non-compartmental analysis with standard deviation shown in parentheses.

Study #	Test Article	Route	Dose (µg/kg; MIU/kg)	Dose Number	n	N_{AUC_168h} (h*ng/mL)	N_{peak} (ng/mL)	N_{T_max} (h)
1	IFN beta-1a	IM	5; 1	1	5	1970 (689)	21.0 (6.8)	27.2 (4.38)
1	IFN beta-1a	SC	5; 1	1	5	1680 (327)	21.4 (3.71)	25.6 (3.58)
1	PEG-IFN beta-1a	IM	10.8; 1	1	5	2050 (266)	21.8 (3.29)	24.0 (0)
1	PEG-IFN beta-1a	SC	10.8; 1	1	5	2050 (441)	20.2 (3.76)	28.8 (4.38)
2	PEG-IFN beta-1a	SC	2; 0.22	1	4	1090 (539)	10.4 (4.1)	36.0 (13.9)
2	PEG-IFN beta-1a	SC	10; 1.1	1	4	1560 (462)	14.8 (4.7)	24.0 (0.0)
2	PEG-IFN beta-1a	SC	100; 11	1	4	2640 (573)	22.8 (5.4)	42.0 (12.0)
2	PEG-IFN beta-1a	IM	100; 11	1	3[a]	3190 (625)	27.0 (3.4)	32.0 (13.9)
3	Control	IM & SC	0	1	12	NA[b]	5.02 (2.60)[b]	24 (0)[b]
3	Control	IM & SC	0	3	12	NA[b]	3.66 (3.06)[b]	24 (0)[b]
3	Control	IM & SC	0	5	12	NA[b]	3.39 (2.64)[b]	24 (0)[b]

3	PEG-IFN beta-1a	SC	2; 0.22	1	8	NA[b]	9.27 (6.01)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	2; 0.22	3	8	NA[b]	8.28 (7.80)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	2; 0.22	5	8	NA[b]	5.28 (5.55)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	10; 1.1	1	8	NA[b]	13.4 (2.88)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	10; 1.1	3	8	NA[b]	9.27 (3.38)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	10; 1.1	5	8	NA[b]	5.85 (5.69)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	100; 11	1	12	NA[b]	26.9 (5.95)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	100; 11	3	12	NA[b]	16.4 (6.90)[b]	24 (0)[b]
3	PEG-IFN beta-1a	SC	100; 11	5	12	NA[b]	7.32 (3.89)[b]	24 (0)[b]
3	PEG-IFN beta-1a	IM	100; 11	1	12	NA[b]	23.2 (5.12)[b]	24 (0)[b]
3	PEG-IFN beta-1a	IM	100; 11	3	12	NA[b]	16.7 (6.23)[b]	24 (0)[b]
3	PEG-IFN beta-1a	IM	100; 11	5	12	NA[b]	4.81 (3.43)[b]	24 (0)[b]

[a]Data from one monkey was excluded because of unusually high pre-dose and post-dose peak concentration (25.8 and 100 ng/mL, respectively).

[b]One post-dose sample was collected at 24 h as an approximation of N_{peak} after administration of Dose # 1, 3, and 5, respectively; hence, N_{AUC_168h} was not available and N_{T_MAX} was 24 h for all animals in Study 3.

Table 4. Final population PD model parameters (fixed and random effects), including % relative standard error.

Model Parameter (units)	Definition	Estimated value	% Relative standard error	Estimated value	% Relative standard error
		IFN beta-1a		PEG-IFN beta-1a	
BSL (ng/mL)	Baseline neopterin serum concentration	2.97	6	Same as IFN beta-1a	
LOSS (h^{-1})	First-order elimination rate of serum neopterin	0.0174	7	Same as IFN beta-1a	
E_{max} (unitless)	Maximum stimulatory effect	198	31	Same as IFN beta-1a	
MTT (h)	Mean transit time in transit compartments	7.13	6	5.60	4
EC_{50} (pg/mL)	Drug concentration to achieve 50% of E_{max}	2980	44	62200	26
IC_{50} (ng/mL)	Neopterin concentration to inhibit 50% of the stimulatory effect	1.82	29	Same as IFN beta-1a	
ω^2_{BSL}	Intersubject variance of V_C	0.264	27	Same as IFN beta-1a	
ω^2_{Emax}	Intersubject variance of Q	0.185	31	Same as IFN beta-1a	
Cov_{BSL_Emax}	Covariance of BSL and E_{max}	-0.0859	38	Same as IFN beta-1a	
SD	Coefficient of residual error	0.173	8	Same as IFN beta-1a	
σ^2	Residual error variance	1, fixed	NA[a]	1, fixed	NA[a]

[a]NA, not applicable

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Figures

Figure 1

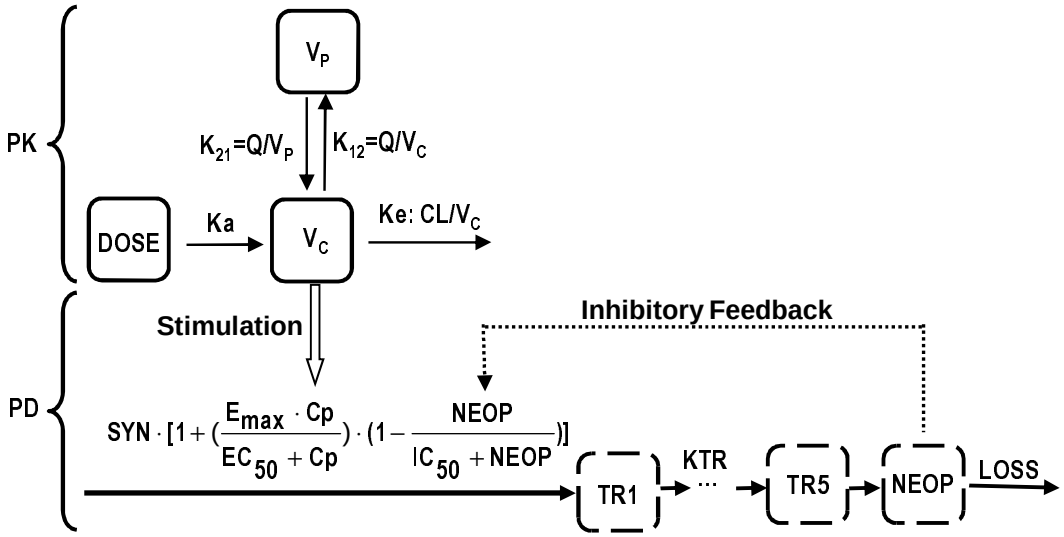
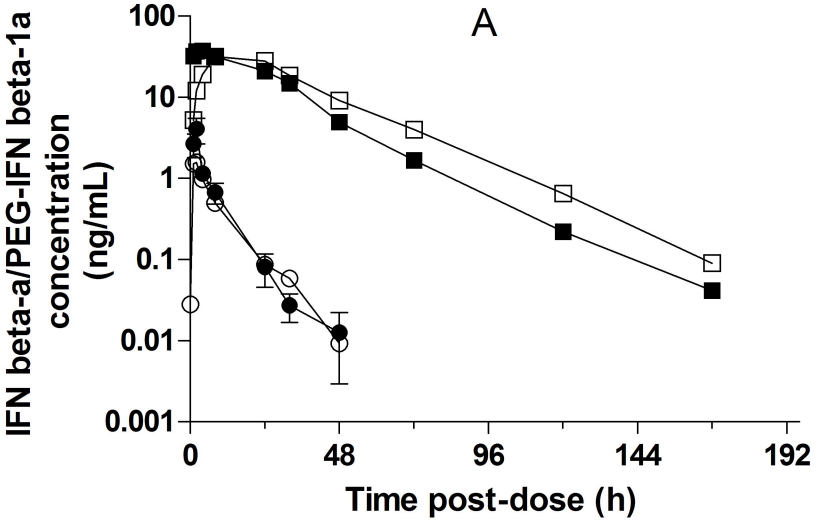


Figure 2.


A
IFN beta-a/PEG-IFN beta-1a concentration (ng/mL)
100
10
1
0.1
0.01
0.001
0
48
96
144
192
Time post-dose (h)

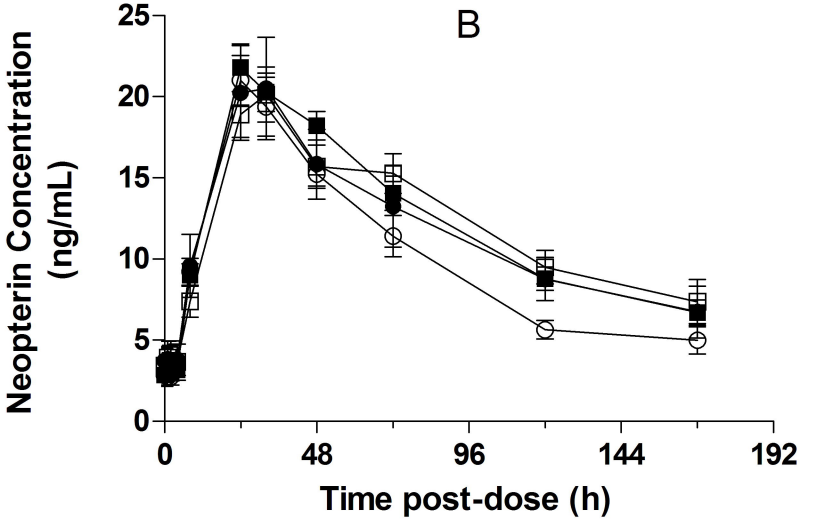

B
Neopterin Concentration (ng/mL)
25
20
15
10
5
0
0
48
96
144
192
Time post-dose (h)

Figure 3

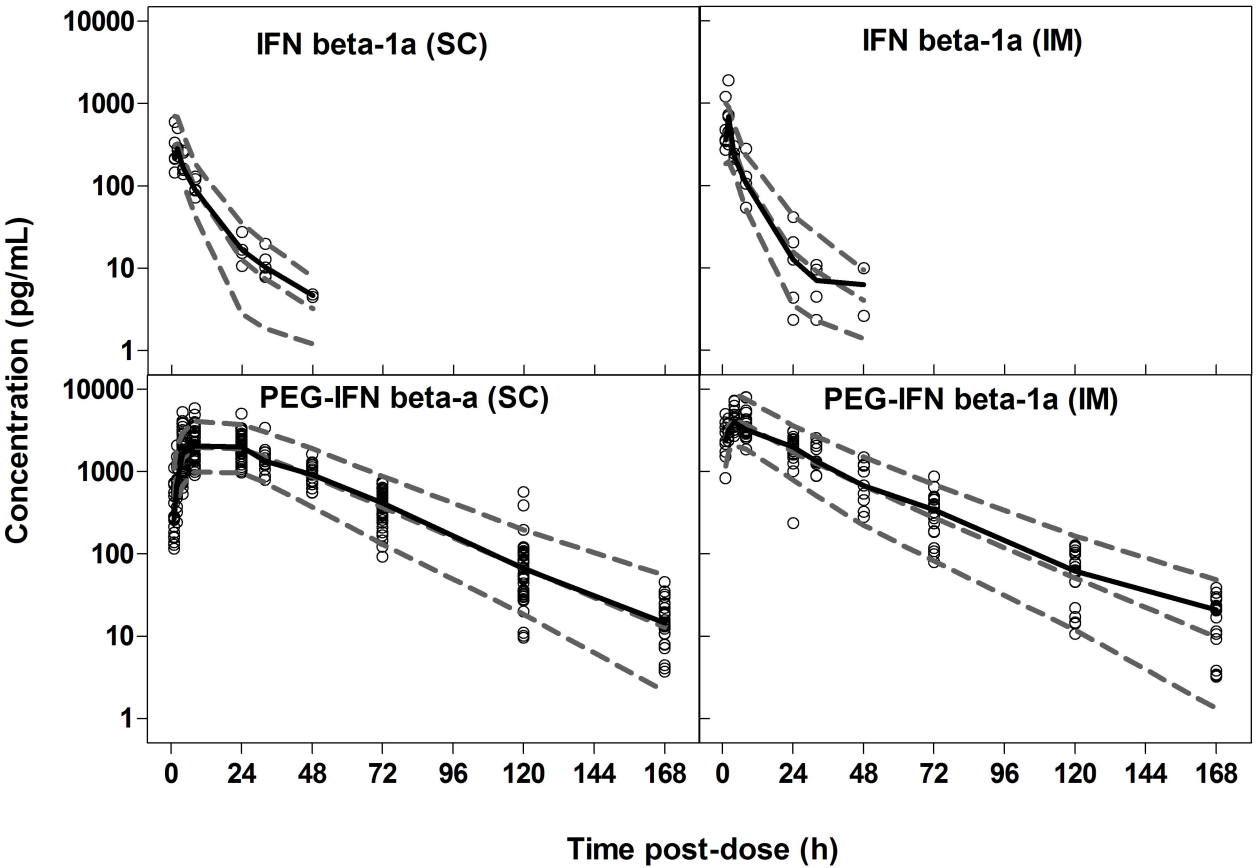

IFN beta-1a (SC)
IFN beta-1a (IM)
PEG-IFN beta-a (SC)
PEG-IFN beta-1a (IM)
Concentration (pg/mL)
10000
1000
100
10
1
0
24
48
72
96
120
144
168
Time post-dose (h)

Figure 4

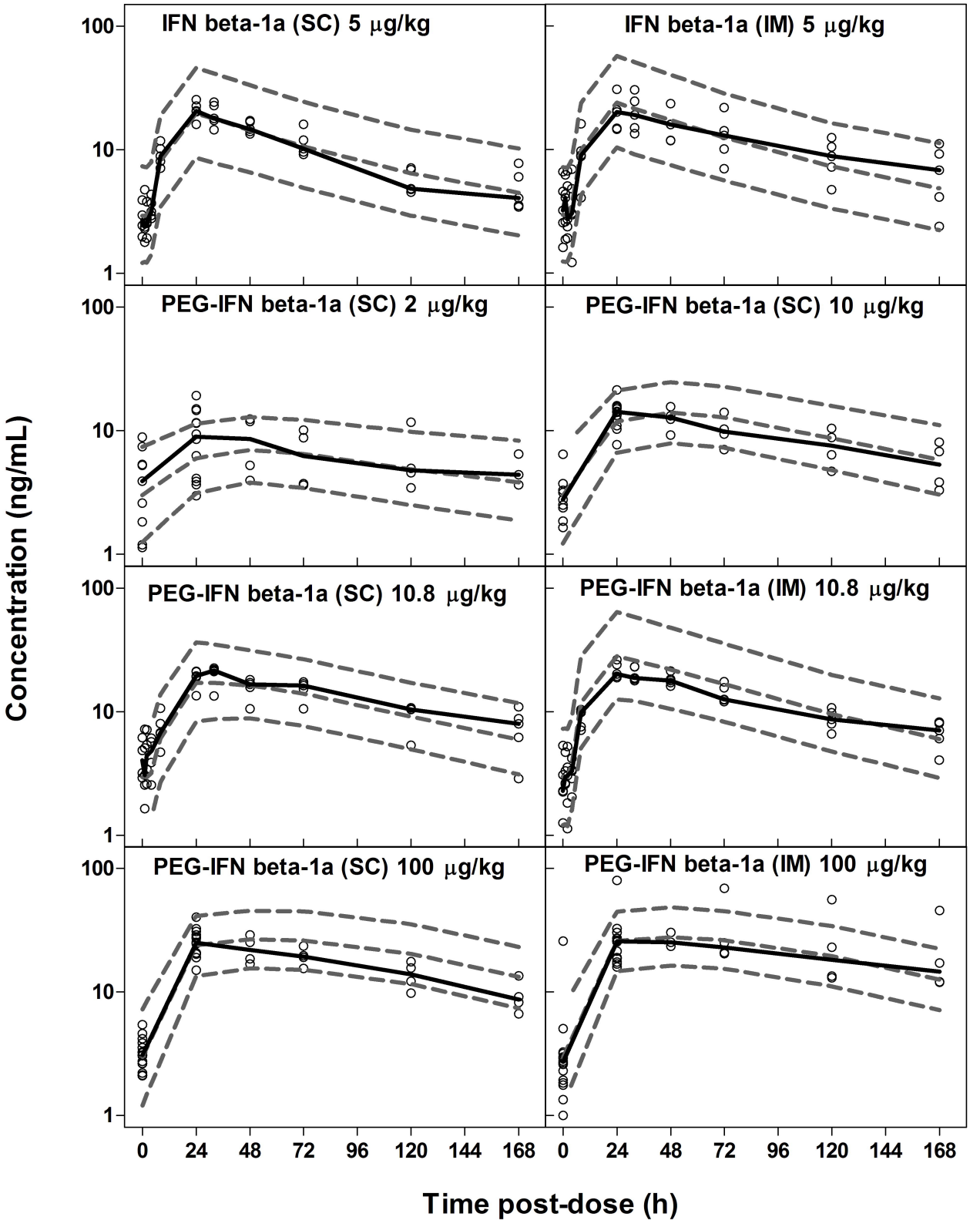
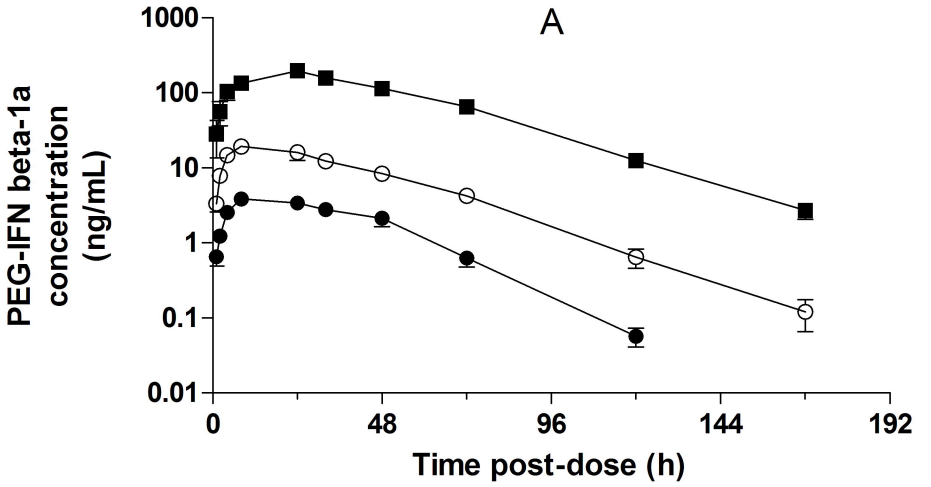

IFN beta-1a (SC) 5 μg/kg
IFN beta-1a (IM) 5 μg/kg
PEG-IFN beta-1a (SC) 2 μg/kg
PEG-IFN beta-1a (SC) 10 μg/kg
PEG-IFN beta-1a (SC) 10.8 μg/kg
PEG-IFN beta-1a (IM) 10.8 μg/kg
PEG-IFN beta-1a (SC) 100 μg/kg
PEG-IFN beta-1a (IM) 100 μg/kg
Concentration (ng/mL)
100
10
1
0
24
48
72
96
120
144
168
Time post-dose (h)

Figure 5.


A
PEG-IFN beta-1a concentration (ng/mL)
1000
100
10
1
0.1
0.01
0
48
96
144
192
Time post-dose (h)

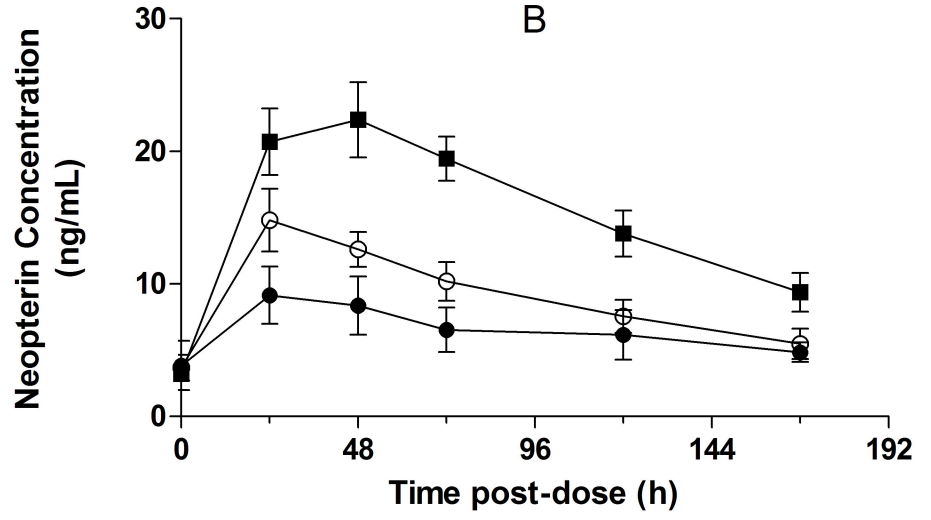

B
Neopterin Concentration (ng/mL)
30
20
10
0
0
48
96
144
192
Time post-dose (h)

Figure 6.

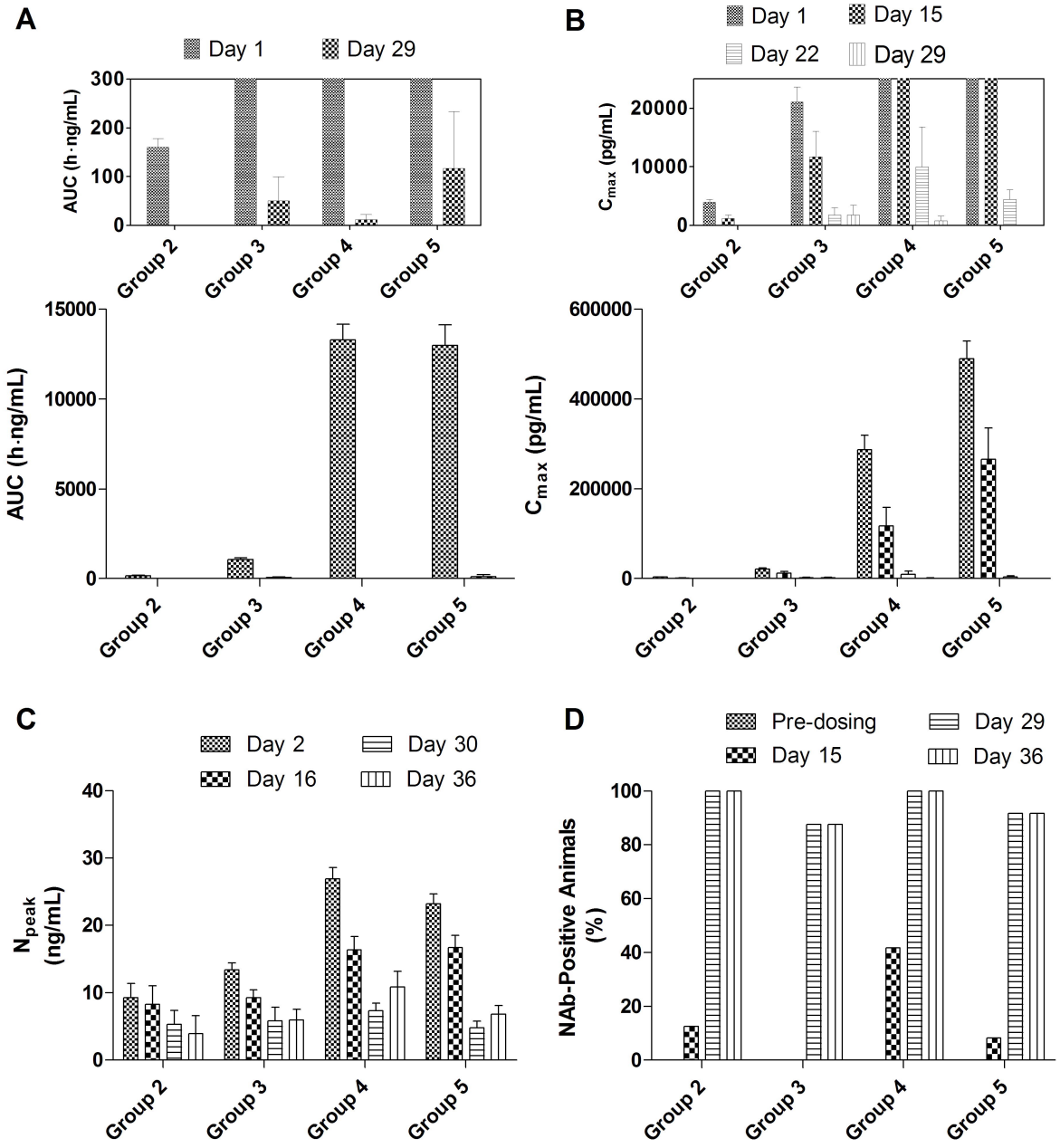

A
Day 1
Day 29
AUC (h·ng/mL)
300
200
100
0
Group 2
Group 3
Group 4
Group 5
AUC (h·ng/mL)
15000
10000
5000
0
Group 2
Group 3
Group 4
Group 5
B
Day 1
Day 15
Day 22
Day 29
Cmax (pg/mL)
20000
10000
0
Group 2
Group 3
Group 4
Group 5
Cmax (pg/mL)
600000
400000
200000
0
Group 2
Group 3
Group 4
Group 5
C
Day 2
Day 16
Day 30
Day 36
Npeak (ng/mL)
40
30
20
10
0
Group 2
Group 3
Group 4
Group 5
D
Pre-dosing
Day 15
Day 29
Day 36
NAb-Positive Animals (%)
100
80
60
40
20
0
Group 2
Group 3
Group 4
Group 5

Figure 7.

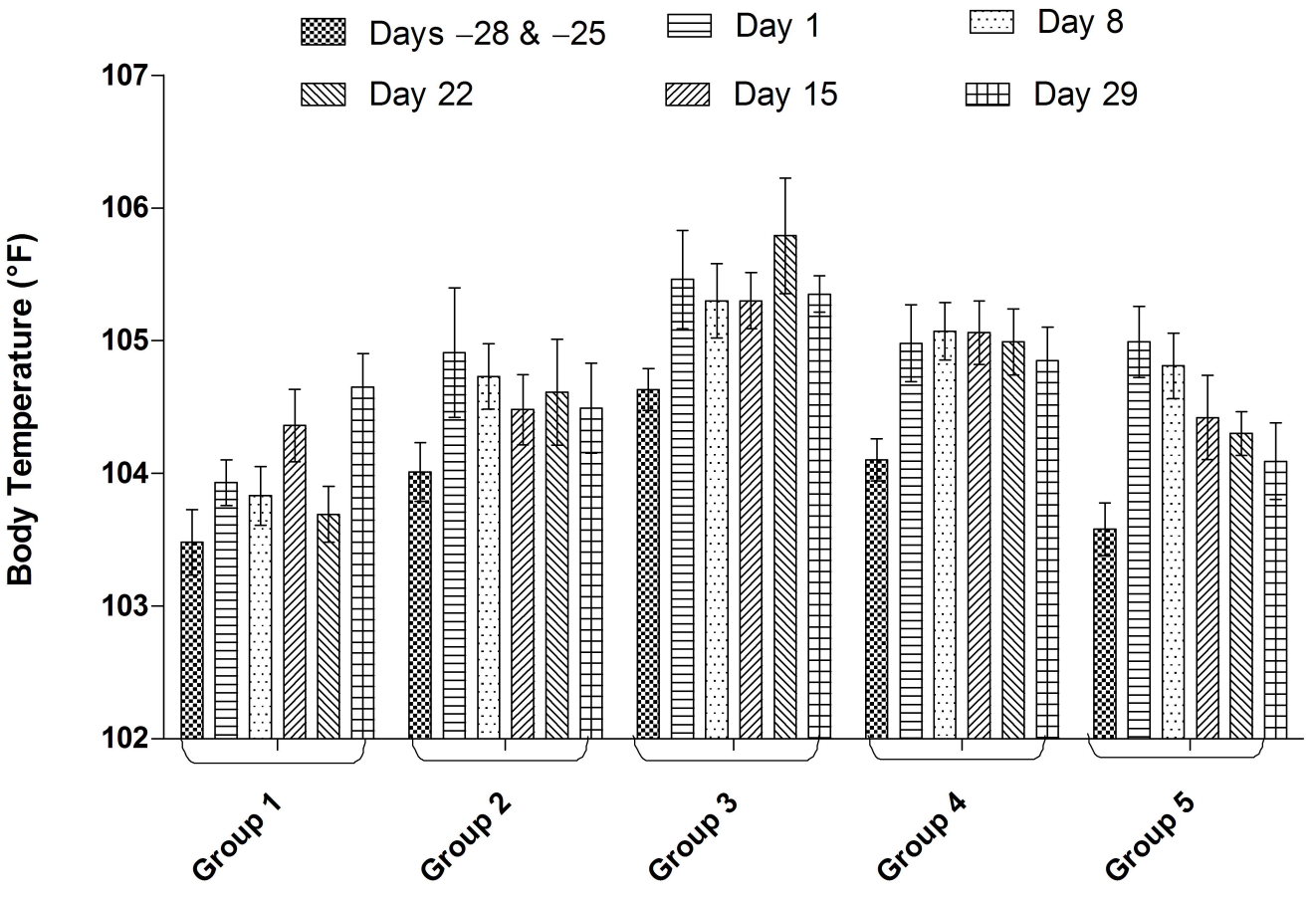
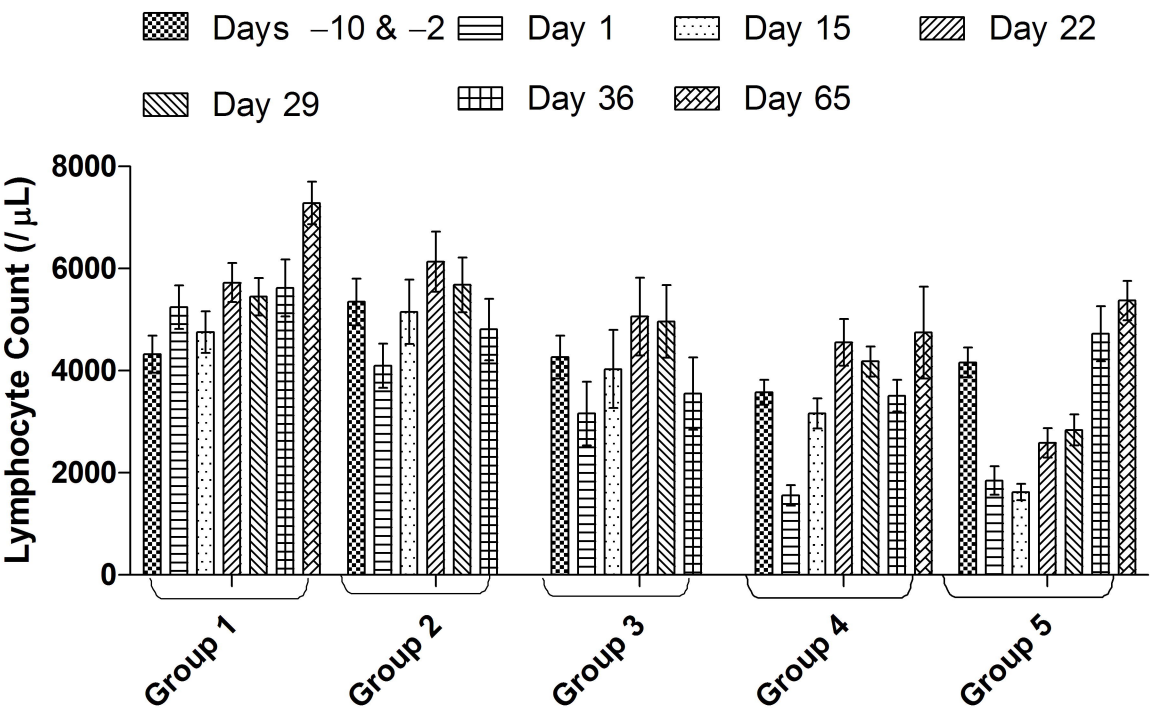

Days −28 & −25
Day 1
Day 8
Day 22
Day 15
Day 29
Body Temperature (°F)
107
106
105
104
103
102
Group 1
Group 2
Group 3
Group 4
Group 5

Figure 8.


Days −10 & −2
Day 1
Day 15
Day 22
Day 29
Day 36
Day 65
Lymphocyte Count (/μL)
8000
6000
4000
2000
0
Group 1
Group 2
Group 3
Group 4
Group 5