

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**125499Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**BLA: 125499**

**Submission date:** 5/15/2013

**Drug:** Plegridy (Peginterferon beta-1a)

**Applicant:** Biogen Idec, Inc.

**Indication:** Relapsing-Remitting Multiple Sclerosis

**Reviewing Division:** Division of Neurology Products

### **Discussion:**

The pharmacology/toxicology reviewer and supervisor concluded that this BLA could be approved from a nonclinical perspective for the indication listed above.

The nonclinical program assessing this product was limited because peginterferon beta-1a was not active in rodents and anti-drug antibodies developed fairly rapidly in the monkey. Nonetheless, the effects observed were consistent with the expected pharmacologic effects of the molecule.

A linking moiety was used in the pegylation of this molecule. Genotoxicity studies of the linking moiety were not conducted but the level of exposure to this moiety is expected to be low enough so as not to pose a risk. This is further discussed in the supervisor's secondary review.

The applicant also conducted studies with the un-pegylated form of interferon beta-1a. Some of the information from these studies is relevant to peginterferon beta-1a and may be included in labeling.

### **Conclusions:**

The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this BLA may be approved for the above indication.

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PAUL C BROWN  
07/31/2014

**MEMORANDUM**

**DEPARTMENT OF HEALTH & HUMAN SERVICES  
Public Health Service  
Food and Drug Administration**

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**Division of Neurology Products (HFD-120)  
Center for Drug Evaluation and Research**

Date: February 6, 2014

From: Lois M. Freed, Ph.D.  
Supervisory Pharmacologist

Subject: BLA 125499 (BIIB017; peginterferon  $\beta$ -1a; Plegridy)

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BLA 125499 was submitted by the sponsor (Biogen Idec, Inc.) on May 15, 2013 for BIIB017 as a treatment for patients with relapsing forms of multiple sclerosis (MS). BIIB017 is a pegylated form of human interferon  $\beta$ -1a, with a (b) (4) human interferon  $\beta$ -1a and a single 20 kDa methoxy poly(ethyleneglycol)-O-2-methylpropionaldehyde. BIIB017 contains the same human interferon  $\beta$ -1a found in Avonex (Biogen Idec, Inc.) (b) (4). The proposed recommended human dose (RHD) of BIIB017 is 125  $\mu$ g every 2 weeks; the RHD for Avonex is 30  $\mu$ g once a week. (Doses of both BIIB017 and Avonex are expressed as the amount of interferon  $\beta$ -1a per dose.)

To support approval of BIIB017 for MS, the sponsor conducted the following nonclinical studies of BIIB017:

- in vitro and in vivo pharmacology studies
- PK/ADME studies in guinea pig and rhesus monkey
- five-week SC/IM toxicity studies in rhesus monkey
- menstrual cycle and hormone study in rhesus monkey
- in vitro Ames assay and in vitro chromosomal aberration in human peripheral blood lymphocytes

These studies were reviewed by Dr. Houghtling (*Pharmacology/Toxicology BLA Review and Evaluation, BLA 125499, Rick A. Houghtling, 1/24/2014*).

The ability to assess BIIB017 in nonclinical studies was limited by the lack of biological activity in animal species typically used for nonclinical safety testing (i.e., mouse, rat) and fairly rapid development of neutralizing anti-drug antibodies (ADAs) in a biologically relevant species (rhesus monkey). In addition to the studies conducted for BIIB017, the sponsor also submitted an embryo-fetal development (EFD) study of interferon  $\beta$ -1a in rhesus monkey, previously submitted in support of marketing approval

for Avonex (BLA 103628). This study was considered by the sponsor and the division to be relevant to BIIB017 since the (b) (4) interferon  $\beta$ -1a in Avonex has been determined to be comparable to that in BIIB017 (BLA 103628/5249 Approval letter, 12/9/2013).

Based on his review of the nonclinical studies, and taking into consideration the issues limiting the extent of nonclinical testing, Dr. Houghtling has concluded that the nonclinical data are adequate and recommends approval of the BLA.

### Pharmacology

The sponsor conducted in vitro and in vivo studies to compare the pharmacological activity of interferon  $\beta$ -1a and BIIB017. In in vitro receptor binding assays assessing affinity for IFNAR2, interferon  $\beta$ -1a and BIIB017 demonstrated similar affinity. However, in cell-based assays, in which both IFNAR1 and IFNAR2 were present, interferon  $\beta$ -1a exhibited ~2-fold greater activity than BIIB017. BIIB017 was not tested in a relevant animal model of efficacy (e.g., experimental autoimmune encephalomyelitis models in rodent) because of the lack of biological activity in mouse or rat.

### Toxicology

Toxicity studies were conducted in rhesus monkey, a biologically relevant species. In the pivotal 5-week study (P017-06-02), BIIB017 was administered at doses of 0, 2, 10, and 100  $\mu$ g/kg/week SC and 100  $\mu$ g/kg/week IM for 5 weeks (4/sex/group); additional animals were followed for a 4-week recovery period (2/sex C and HD). Findings were consistent with the expected pharmacological effects of an interferon  $\beta$ -1a, e.g., increased body temperature, reduced circulating lymphocytes. Development of neutralizing ADAs was observed from approximately 2 weeks of dosing on, resulting in decreases in plasma BIIB017 exposure throughout the dosing period. Mean serum levels (8 hrs post dose) were reduced 50% after the 3<sup>rd</sup> dose and >90% after the 4<sup>th</sup> dose.

### Reproductive Toxicology

No reproductive and developmental toxicology studies were conducted for BIIB017. As previously noted, the EFD study in rhesus monkey conducted for Avonex (interferon  $\beta$ -1a) was considered relevant to BIIB017. The findings from that study are reviewed by Dr. Houghtling and are described in Avonex labeling, which notes that “Abortifacient activity was evident following 3 to 5 doses...” at a dose 100 times (but not 2 times) the recommended weekly dose of (non-pegylated) interferon  $\beta$ -1a.

In considering the relevance of the EFD study of interferon  $\beta$ -1a to BIIB017, the findings of a tissue distribution study in guinea pig are of note. (The sponsor characterized the guinea pig as a biologically relevant species.) In that study, the tissue distribution of <sup>125</sup>I-interferon  $\beta$ -1a and <sup>125</sup>I-BIIB017 were compared following a single IV dose of 12.4 and 23.8  $\mu$ g/kg, respectively. (The higher dose of BIIB017 was selected because of the 2-fold decrease in activity observed in the in vitro studies.) Serum AUC was markedly higher (~70-fold) for BIIB017, as expected. While the serum-to-tissue AUC ratio was markedly lower with <sup>125</sup>I-BIIB017 (0.00647-0.630 vs 0.0198-15 for <sup>125</sup>I-interferon  $\beta$ -1a), the absolute tissue exposure was substantially higher with <sup>125</sup>I-BIIB017 in multiple tissues

(~2-23-fold; 18-fold for brain, 13-fold for testis). According to the sponsor, SDS-PAGE conducted in selected tissues indicated that radioactivity detected in spleen, kidney, and liver (up to 24, 24, and 6 hrs post dose, respectively) was “primarily associated” with intact BIIB017. In humans, the serum AUC<sub>168 hr</sub> following a 125 µg SC dose of PEG was 9 times that following a 30 µg IM dose of interferon β-1a; with repeated dosing, the V<sub>d</sub> for BIIB017 was ~480 L, suggesting “wide distribution in the body” (Wu T-C *et al.*, *Clinical Pharmacology Review, BLA 125499, 1/24/2014*). (The volume of distribution in humans for Avonex is similar to total body water, i.e., 0.6 L/kg or 36 L for a 60 kg individual.) Therefore, it is possible that higher tissue exposure to interferon β-1a will be achieved in humans following BIIB017 administration, compared to Avonex.

From the data provided by the sponsor, it is unclear what pharmacological or toxicological effects would result from an increase in tissue exposure. Potential differences could result from a variety of factors, including the location of the products and of Type 1 interferon receptors in tissues. Considering the history of the use of interferons in the treatment of MS and for other indications, there is a surprising paucity of information on the tissue distribution of interferon receptors (IFNAR1 and IFNAR2) and on the distribution of interferons in tissues following systemic administration.

Rosenfeld *et al.* (Rosenfeld *et al.* *Biol Reprod* 67:847-853, 2002) suggest that “...scant data exist on their expression patterns within various tissues, possibly because it has been assumed that the receptors...are ubiquitously expressed.” A recent review (de Weerd NA, Nguyen T. *Immunol Cell Biol* 90:483-491, 2012) lists tissue-specific locations of interferon receptors, including in CNS, liver, lung, and GI, but few supportive studies are referenced. And, for some tissues, it appears that indirect effects cannot be ruled out. Data do, however, suggest that there is some support for presence of interferon β binding sites in certain cells within the CNS (e.g., astrocytes) (Okada K *et al.* *J Neuroimmunol* 159:48-54, 2005; Prinz M *et al.* *Immunity* 2/:675-686, 2008) and in uterus (Rosenfeld *et al.*, 2002). de Weere and Nguyen (2012) conclude that “...there is a need for detailed studies of the relative levels of IFNAR1 and IFNAR2 protein expression in particular cell types/organs and during different cellular processes.” Regardless of the location of receptors, it is possible that the increased tissue levels of BIIB017 reflect greater sequestration, e.g., uptake into macrophages, which would not be expected to result in increased activity. Although of uncertain relevance, Webster *et al.* (Webster R *et al.* *Drug Metab Disp* 35(1):9-16, 2007) reported a “Greater incidence and/or severity of the findings...noted in...high-dosed monkeys given PEG-Intron compared with those given Intron A...in accordance with the prolonged exposure and higher AUC values obtained using PEG-Intron...” No unique toxicities were identified with the pegylated product.

A direct comparison between interferon β-1a and BIIB017 was not assessed in toxicity studies conducted by the sponsor. The pivotal 28-day toxicity study in rhesus monkey conducted for Avonex cannot be directly compared to the 5-week monkey study of BIIB017 because of differences in study design; however, the sponsor stated that the only toxicities observed with BIIB017 are similar to those observed with interferon β-1a in animals and humans. In addition, the safety of BIIB017 has been assessed in clinical trials. The only remaining concern is how the possible increase in tissue distribution of

BIIB017 might affect its reproductive and development toxicity, which is more difficult to assess in humans. However, considering the rapid development of neutralizing ADAs in rhesus monkey, it would also be difficult to assess any differences between interferon  $\beta$ -1a and BIIB017 in that species. The sponsor discussed the additional challenges of conducting an EFD study in rhesus monkey, which are seasonal breeders. (The sponsor provided no data in the BLA demonstrating that BIIB017 is not biologically active in a monkey species more amenable to testing, e.g., cynomolgus monkeys, which are receptive year round. Published studies have reported cynomolgus monkey to be “biologically responsive to HuIFN- $\beta$ 1a” [Martin PL et al. *J Interferon Cytokine Res* 22:709-717, 2002; also, Mager DE et al *JPET* 306:262-270, 2003]. And, EFD studies have been conducted in cynomolgus monkey for another interferon  $\beta$ -1a product [*cf.* Rebif labeling].) Other approved pegylated interferon products (PEG-Intron, Pegasys, Sylatron) have successfully relied on EFD studies of the non-pegylated interferon products. Taking this and possible feasibility issues into consideration, it seems that additional studies in monkey may not provide sufficiently useful information. If a pregnancy registry is established for Plegridy, it may allow for an assessment in humans of the potential risk from fetal exposure.

The sponsor conducted a study (P017-09-02) in rhesus monkey to assess the effects of BIIB017 (0, 2.5, and 125  $\mu$ g/kg/week) on menstrual cycle length and hormone (progesterone, 17- $\beta$ -estradiol) levels; animals were treated through one menstrual cycle ( $\leq 5$  doses). Dr. Houghtling concluded, based on his review, that the variability in the data compromised study interpretation. What is notable is that an apparent increase in menstrual cycle duration was observed in only one HD animal (#33): cycle lengths were 32, 36, 47, 28, and 33 days during the 1<sup>st</sup> acclimation cycle (AC), 2<sup>nd</sup> AC, the dosing cycle, 1<sup>st</sup> recovery cycle (RC), and 2<sup>nd</sup> RC, respectively. The other HD animal (#31) noted by the sponsor to have exhibited an increase in menstrual cycle length (61 days) during the dosing cycle had an even longer menstrual cycle length during the 2<sup>nd</sup> RC (70 days). With only one animal possibly affected, it is impossible to interpret the hormone data. The sponsor concluded that “Administration of BIIB017 to rhesus monkeys at 125  $\mu$ g/kg/week may have had a mild effect on serum E2 and Prog concentrations resulting in altered menstrual cycles.”

The Avonex labeling describes the results of a menstrual cycle and hormone study in monkey (doses of 1.25 or 50  $\mu$ g/kg SC). According to summary data provided in the Toxicologist’s Review of PLA #95-0979 (*Toxicologist’s Review, Anne Pilaro, Ph.D., 4/30/96*), there were 2 saline controls, 1 placebo control, 3 LD, and 6 HD female rhesus monkeys. Menstrual cycle duration was increased in 2 HD females (>40 days and 45 days); in the other 4 HDF, menstrual cycle duration was slightly shorter (16, 19, 19, and 19 days) than in both control groups (21-22 days). There was no apparent correlation between peak progesterone levels and cycle duration; for example, peak progesterone levels associated with menstrual cycle durations of 19 days in three animals ranged from 0.44 to 8.85 ng/mL, both lower and higher than those in the two HD animals (0.07 and 3.17 ng/mL) with longer menstrual cycles. With such variability, the difference in the number of control and HD animals complicates interpretation of the data. Dr. Pilaro

concluded that there was an increase in “the frequency of individual cycles falling outside of the normal range” at the HD.

While I agree with Dr. Houghtling that the results of the BIIB017 study do not warrant inclusion in labeling, some mention of potential effects of interferon  $\beta$ -1a may need to be added; exclusion of any discussion of potential effects of BIIB017 on the menstrual cycle and hormones would suggest less toxicity for Plegridy, which would be incorrect.

#### Genetic Toxicology

Standard genetic toxicology studies are not considered appropriate for assessing the genotoxic potential of biologic products, except under certain conditions, e.g., the presence of an organic linker in a conjugated protein product. BIIB017 contains an organic linker (mPEG-O-2-methylpropionaldehyde); a DEREK QSAR assessment of this compound was positive for genotoxicity. Therefore, two genetic toxicology studies (Ames assay, in vitro chromosomal aberration assay in human peripheral blood lymphocytes) of BIIB017 were conducted by the sponsor. Although BIIB017 was negative in both assays, neither was an adequate test of the genotoxic potential of the linker since it is unlikely that there was any exposure to the linker in these systems.

According to the CMC review team, a conservative estimate of the amount of (b) (4) in the clinical product, based on the highest levels of (b) (4) in DS process validation batches (b) (4)  $\mu\text{g/mL}$ , is (b) (4)  $\mu\text{g}$  per dose of Plegridy (125  $\mu\text{g}$ ) (also see OBP CMC Review of BLA125499, Bernstein RM et al., 1/16/2014). This amount is well below the allowable limit for a potentially genotoxic impurity. The sponsor provided no in vivo mass balance data in humans. However, according to published literature (Webster et al. *Drug Metab Disp* 35:9-16, 2007), nonclinical and clinical data, although limited, suggest that “PEG metabolism appears to be similar in animals and humans...with high molecular weight PEGs (>5000 [kDa]; typical of those used to PEGylate proteins) showing little or no metabolism.” Therefore, it is unlikely that humans would be exposed in vivo to the linker in a form that could enter the cell and induce genotoxic effects, at least at a level that would raise a safety concern.

#### Recommendations

A standard battery of nonclinical studies could not be conducted for BIIB017, primarily because it is biologically active only in a few species typically used for nonclinical safety testing. The nonclinical studies conducted by the sponsor do not adequately characterize the toxicity of BIIB017 in rhesus monkey, a biologically relevant species; however, fully adequate studies could not be conducted because of the fairly rapid development of neutralizing ADAs. Additional nonclinical studies would not be useful for evaluating potential differences between interferon  $\beta$ -a1 and BIIB017 because of these limitations. The data provided by the sponsor demonstrate that BIIB017 exerts effects consistent with its pharmacological activity, and provide sufficient information for labeling. From a nonclinical standpoint, there is no objection to approval of the BLA, with appropriate labeling.

Labeling recommendations will be provided separately.

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LOIS M FREED  
02/07/2014

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION**

Application number: 125499  
Supporting document/s: SDN 001 [eCTD #0000]  
Applicant's letter date: May 15, 2013  
CDER stamp date: May 15, 2013  
Product: BIIB017  
Indication: Relapsing-Remitting Multiple Sclerosis  
Applicant: Biogen Idec, Inc.  
Review Division: Neurology Products  
Reviewer: Rick A. Houghtling, Ph.D.  
Supervisor: Lois M. Freed, Ph.D.  
Acting Division Director: Billy Dunn, M.D.  
Project Manager: Nicole Bradley, Pharm.D.

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Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125499 are owned by Biogen Idec, Inc. or are data for which Biogen Idec, Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125499 that Biogen Idec, Inc. does not own or have a written right to reference constitute one of the following: (1) published literature or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Biogen Idec, Inc. do not own (or from FDA reviews or summaries of a previously approved application) are for descriptive purposes only and are not relied upon for approval of BLA 125499.

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# 1 Executive Summary

## 1.1 Recommendations

### 1.1.1 Approvability

From a nonclinical perspective, this application is approvable; the studies submitted and reviewed were adequate to assess the nonclinical safety profile of peginterferon  $\beta$ -1a.

### 1.1.2 Brief Summary of Nonclinical Findings

Peginterferon beta-1a (PLEGRIDY) is recombinant human interferon beta-1a that has been modified by a 20 kDa-mPEG group at the N-terminal amino acid residue. Peginterferon beta-1a (PEGIFN  $\beta$ -1a) was developed to alter the pharmacokinetic profile of human interferon beta-1a (Avonex) to permit changes to the route of administration and dosing frequency. PEGIFN  $\beta$ -1a was formulated for subcutaneous injection at a dosing frequency of once every two weeks as a treatment for patients with relapsing forms of multiple sclerosis. In support of this licensing application, limited pharmacology studies, multiple PK/PD studies, and an abbreviated toxicology package were submitted.

Pharmacologically, PEGIFN  $\beta$ -1a binds to the human interferon receptor subunit, IFNAR2, with an apparent affinity similar to that demonstrated for interferon beta-1a (IFN  $\beta$ -1a). The evaluation of human PEGIFN  $\beta$ -1a is limited to nonclinical species in which biological activity, such as, increased body temperature, induction of 2',5'-oligoadenylate synthetase activity, or increased serum neopterin can be demonstrated. Three species were identified as biologically relevant, guinea pig, dog, and monkey. The Sponsor selected guinea pig and rhesus monkey; these species are considered appropriate for the evaluation of human PEGIFN  $\beta$ -1a.

PK studies for PEGIFN  $\beta$ -1a were conducted in monkey and the results obtained were consistent with the expected effects of pegylation of a protein biologic. Compared to IFN  $\beta$ -1a, PEGIFN  $\beta$ -1a had a significantly higher systemic exposure ( $C_{max}$  and  $AUC_{inf}$ ), a reduced total body clearance (CL/F), a reduced volume of distribution ( $V_d/F$ ), and a prolonged  $t_{1/2}$ . Administration of increasing doses of PEGIFN  $\beta$ -1a was associated with a dose-proportional increase in systemic exposure ( $C_{max}$  and  $AUC_{inf}$ ), as well as a dose-related increase in  $t_{1/2}$ . As expected, the  $T_{max}$  was faster following intramuscular than after subcutaneous injection. Overall, a similar pattern of distribution was observed for PEGIFN  $\beta$ -1a and the non-pegylated IFN  $\beta$ -1a. Tissues with the highest concentration of both pegylated and non-pegylated IFN  $\beta$ -1a were spleen, kidney, liver, and lung; whereas, muscle, brain, and spinal cord had the lowest concentrations. Although, the distribution

pattern is similar, the tissue:serum  $AUC_{72h}$  Ratios of PEGIFN  $\beta$ -1a was reduced compared to non-pegylated interferon.

In single- and repeated-dose studies of IFN  $\beta$ -1a and PEGIFN  $\beta$ -1a conducted in rhesus monkey, antidrug antibodies (ADAs) have been found and demonstrated to be binding and neutralizing. The immunogenicity of IFN  $\beta$ -1a was significant; detection of ADAs that were binding and neutralizing in a single dose PK study of PEGIFN  $\beta$ -1a suggested chronic repeat dose toxicity studies were not feasible in rhesus monkey. Most of the neutralizing antibodies targeted the IFN  $\beta$ -1a protein; whereas most of the antibodies that targeted PEG were binding but not neutralizing. These data suggest that the pegylated drug did not lessen the immunogenicity of IFN  $\beta$ -1a.

A GLP 5-week repeat-dose toxicity study was submitted to evaluate the safety of PEGIFN  $\beta$ -1a, since longer duration studies were not feasible. Rhesus monkeys were treated once weekly for 5 weeks. PEGIFN  $\beta$ -1a was administered at doses of 0, 2 mcg/kg (0.22 MU/kg), 10 mcg/kg (1.1 MU/kg), and 100 mcg/kg (11 MU/kg) by subcutaneous injection and 100 mcg/kg (11 MU/kg) by intramuscular injection. On Day 1, mean lymphocyte counts measured at  $\approx T_{max}$  were reduced at each dose compared to baseline values (Day -10) by 1%, 28%, 33%, 59%, and 59% for control, and PEGIFN  $\beta$ -1a at 2 mcg/kg, 10 mcg/kg, 100 mcg/kg sc, and 100 mcg/kg im, respectively. Two pharmacodynamic effects of PEGIFN  $\beta$ -1a administration were observed—febrile response and induction of serum neopterin. PEGIFN  $\beta$ -1a induced a febrile response that occurred 4 hr postdose on both Day 1 and Day 8 at doses levels of 10 and 100 mcg/kg [sc, im]. On Day 22 (3<sup>rd</sup> weekly dose), a febrile response was achieved at 4 hr postdose, however, this was limited to the 10 and 100 mcg/kg doses administered by sc injection. PEGIFN  $\beta$ -1a induced serum neopterin responses at 24 hr postdose. Maximal neopterin responses to PEGIFN  $\beta$ -1a treatment were observed on Days 2, 16, and 30; whereas placebo had no effect. Maximal neopterin responses were achieved on Day 2, at all doses tested (sc and im). On Day 15 (3<sup>rd</sup> dose), serum neopterin increased on Day 16; however, the magnitude of the increase was less than that achieved on Day 2. Throughout the dosing period, the trend of a reduced responsiveness of neopterin to PEGIFN  $\beta$ -1a occurred at both weeks 4 and 5; this reduction was attributed to the presence of neutralizing antibodies. Due to immunogenicity, TK parameters were determined following single dose administration; mean estimated  $C_{max}$  values were calculated for each dose group after the 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> dose. Day 1 systemic exposure ( $C_{max}$  and  $AUC_{168h}$ ) was approximately dose proportional. The half-lives were similar among the treatment groups, ranging from 15-24.3 hr. On Day 29 (5<sup>th</sup> dose), the estimated mean  $C_{max}$  at 2, 10, and 100 mcg/kg (sc) was 0.00, 1.76, and 0.80 ng/mL, respectively. At the HD (im), the estimated mean  $C_{max}$  was 0.04 ng/mL. Clearly, exposure was markedly reduced by the 5<sup>th</sup> dose. Consistent with this, ADAs were detected by the 3<sup>rd</sup> dose (Day 15); a substantial number of dosed animals were positive for binding antibodies and a few animals were positive for neutralizing activities. However, by Day 29 (5<sup>th</sup> dose), almost all animals were positive for

neutralizing antibodies. The NOAEL was established at the high dose (100 mcg/kg). The reduction in lymphocyte counts, generation of a febrile response, and the induction of serum neopterin represent pharmacologic effects of PEGIFN  $\beta$ -1a treatment. The NOAEL is approximately 31.1 times the maximum recommended human biweekly dose (MRHD) of 125 mcg based on a  $\text{mg}/\text{m}^2$  basis; plasma exposure ( $\text{AUC}_{168\text{hr}}$ ) at the NOAEL is 471 times that anticipated in humans at the MRHD, based upon single-dose  $\text{AUC}_{168\text{hr}}$  in the monkey and 24-week  $\text{AUC}_{168\text{hr}}$  data from clinical study 105MS301.

It has been previously established that IFN  $\beta$ -1a was not mutagenic as is noted in the label for Avonex. However, PEGIFN  $\beta$ -1a is a pegylated-interferon beta and the potential genotoxicity of the pegylated-interferon was unknown. Therefore, an *in silico* DEREK evaluation of 20-kDa mPEG-O-2-methylpropionaldehyde was assessed; this analysis revealed a structural alert for potential genotoxicity. PEGIFN  $\beta$ -1a was not genotoxic in an *in vitro* bacterial reverse mutation (Ames) test or in an *in vitro* cytogenetic assay in human lymphocytes.

Standard reproductive and developmental toxicity studies of PEGIFN  $\beta$ -1a were not performed; the Sponsor's rationale was based upon the known abortifacient, but not teratogenic effects, of IFN  $\beta$ -1a that is described in the label for Avonex. Additional factors, such as a short breeding season, a higher spontaneous abortion rate of approximately 17%, and the possibility that PEGIFN  $\beta$ -1a might increase the abortion frequency in dosed animals suggest that only a small number of fetuses would be available for evaluation. For these reasons, the Sponsor argues that an EFD and PPND study are not feasible. Since an EFD study was conducted to support the licensure of IFN  $\beta$ -1a, these data from the Avonex label could be used to inform labeling of PEGIFN  $\beta$ -1a. Considering the variability and uncertainty in the interpretation of the hormone and menstrual cyclicity study results conducted for PEGIFN  $\beta$ -1a, it is recommended that these data not be included in the prescribing information. Instead, the fertility data for IFN  $\beta$ -1a as provided in the Avonex label should be included in the prescribing information for PEGIFN  $\beta$ -1a.

As demonstrated in the PK and repeat-dose toxicity studies conducted in monkey, the effects of PEGIFN  $\beta$ -1a were similar to those achieved with IFN  $\beta$ -1a. Due to the robust immunogenicity that occurred following single- or repeated dosing of BIIB017 in rhesus monkeys, it is unclear that a PPND study, if conducted, would generate results that were not compromised due to the presence of neutralizing ADAs resulting in reduced and/or ablated systemic exposure.

### 1.1.3 Labeling

The following recommended labeling revisions to the nonclinical sections of the Prescribing Information (PI) proposed by the Sponsor.

1. INDICATIONS AND USAGE: PLEGRIDY is (b) (4) indicated for the treatment of patients with relapsing forms of multiple sclerosis (1)

2. 8.1 Pregnancy: (b) (4)  
Abortifacient activity was evident following 3 to 5 doses (b) (4)

3. 12.1 Mechanism of Action: The mechanism of action by which PLEGRIDY exerts its effects in patients with multiple sclerosis is unknown.  
(b) (4)

4. 13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility:

*Carcinogenesis*

The carcinogenic potential of PLEGRIDY has not been tested in animals.

*Mutagenesis*

PLEGRIDY was not mutagenic when tested in an *in vitro* bacterial reverse mutation (Ames) test (b) (4)

*Impairment of Fertility*

In monkeys administered interferon beta by subcutaneous injection (b) (4) over the course of one menstrual cycle, menstrual irregularities, anovulation, and decreased serum, progesterone levels were observed (b) (4). These effects were reversible after discontinuation of drug. (b) (4)

5. (b) (4)

## **2 Drug Information**

### **2.1 Drug**

- Peginterferon beta-1a

#### **2.1.2 Generic Name**

- Peginterferon beta-1a

#### **2.1.3 Code Name**

- BIIB017

#### **2.1.4 Chemical Name**

- mPEG-O-2-methylpropionaldehyde-modified interferon beta-1a

#### **2.1.5 Molecular Formula/Molecular Weight**

- Molecular weight: 20 kDa

#### **2.1.6 Structure (sponsor's figure)**



#### **2.1.7 Pharmacologic class**

- Interferon beta

### **2.2 Relevant INDs, BLAs, and DMFs**

- IND 100,110 DNP Treatment of relapsing-remitting multiple sclerosis, Active
- BLA 103628 DNP AVONEX® treatment for relapsing-remitting multiple sclerosis (Approved 1995)

## 2.3 Clinical Formulation

- BIIB017 is supplied as a sterile, clear, colorless solution for injection in a pre-filled syringe.

### 2.3.1 Drug Formulation

- BIIB017 is formulated as a liquid solution for injection in a (b) (4) sodium acetate/ acetic acid (b) (4) L-arginine HCL, (b) (4) polysorbate 20, pH 4.8.

### 2.3.2 Comments on Novel Excipients

- There are no novel excipients used in the BIIB017 formulation.

### 2.3.3 Comments on Impurities/Degradants of Concern

- There are no impurities/degradants of concern.

## 2.4 Proposed Clinical Population and Dosing Regimen

- BIIB017 (125 mcg) is to be administered once every 2 weeks (q2wk) by subcutaneous injection to patients with relapsing multiple sclerosis.

## 2.5 Regulatory Background

BIIB017 is human interferon beta-1a (Avonex, made by process B) that has been pegylated at the alpha-amino group of the N-terminal amino acid residue by addition of a linear molecule of mPEG-O-2-methylpropionaldehyde.

For licensure of BIIB017, reproductive toxicology studies (embryofetal development and pre- and post-natal development) were needed. The Sponsor's plan was to submit general toxicity data and a hormone and menstrual cycle study in rhesus monkey; however, these were considered inadequate. The Division recommended that a scientific justification be provided for their planned approach; the justification should address potential differences in plasma exposure due to PEGylation, the relevance of general toxicity findings to reproductive toxicology, and issues regarding the feasibility of conducting these studies in nonhuman primate. The Sponsor proposed addressing the need for an EFD study by using the study conducted for Avonex. The Sponsor's plan was considered acceptable, however, this was contingent on the Agency's determination that the drug substances manufactured using two different processes (b) (4) were reviewed and comparability between these two drug substances established.

The comparability data for the two human IFN  $\beta$ -1a drug substances was submitted to the Agency for review as a CMC supplement to the BLA for Avonex (s5429). The review team decided that there was sufficient evidence to support the comparability of these two drug substances manufactured by different processes. Therefore,

some of the nonclinical data, such as the embryofetal study, conducted during the development of Avonex may be relied upon for the safety of PEGIFN  $\beta$ -1a; likewise, the extensive human experience with Avonex in the treatment of relapsing forms of multiple sclerosis was relied upon to waive the need for safety pharmacology studies of BIIB-017.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

| TYPE OF STUDY           | STUDY NO.     | TITLE   |
|-------------------------|---------------|---|
| <b>Pharmacology</b>     |               |   |
| <i>Primary PD</i>       | CHR-83-07-13  | Interaction of PEGylated-Interferon Cycle 1 DS with the extracellular portion of the human IFNAR2 receptor chain  |
|                         | P91-004       | Effect of in vitro exposure to rhIFN-B on 2'5'-oligoadenylate synthetase levels in peripheral blood leukocytes derived from seven animal species  |
| <b>Pharmacokinetics</b> |               |   |
| <i>Absorption</i>       | P017-06-01    | A comparative single-dose pharmacokinetic and pharmacodynamic study of BIIB017 and Avonex® administered by the intramuscular or subcutaneous route to rhesus monkeys  |
|                         | P017-06-03    | BIIB017: A single-dose pharmacokinetic and pharmacodynamic study of PEG-Interferon $\beta$ -1a administered by subcutaneous or intramuscular injection to rhesus monkeys  |
|                         | P017-07-01    | a comparative pharmacokinetic and pharmacodynamic evaluation of cycle 1 and cycle 2 material for BIIB017 (pegylated interferon $\beta$ -1a) in rhesus monkeys following a single subcutaneous or intramuscular dose |
| <i>Distribution</i>     | P017-10-01    | Distribution of [ $^{125}$ I]Interferon beta-1a and PEGylated-[ $^{125}$ I]Interferon beta-1a in selected tissues following intravenous administration to guinea pigs   |
| <i>Distribution</i>     |               |   |
|                         | P9418-02-01   | BG9418: Collection of samples for determining bioactivity of hIFN- $\beta$ -1a in male guinea pigs  |
| <i>Metabolism</i>       | RsCh-2011-037 | <i>Ex vivo</i> stability of BIIB017 in rhesus monkey and human serum  |

| TYPE OF STUDY                         | STUDY NO.  | TITLE   |
|---------------------------------------|------------|---|
| <b>Toxicology</b>                     |            |   |
| <i>Repeat-dose</i>                    | P017-06-02 | BIIB017: A 5-week subcutaneous or intramuscular toxicity study in rhesus monkeys with a 4-week treatment-free recovery period |
| <i>Genotoxicity</i>                   | P017-11-02 | BIIB017: Bacterial reverse mutation test in <i>Salmonella typhimurium</i> and <i>Escherichia coli</i>                         |
|                                       | P017-11-01 | BIIB017: In vitro mammalian chromosome aberration test in human peripheral blood lymphocytes                                  |
| <i>Reproductive and Developmental</i> | P017-09-02 | BIIB017: A Study for the Effects on the Menstrual Cycle of Rhesus Monkeys when Administered BIIB017 Subcutaneously            |
|                                       | P926-93-10 | BG9216 (r-Hu IFN-B) rhesus monkey reproduction study (development evaluation)   |

**3.2 Studies Not Reviewed**

| TYPE OF STUDY       | STUDY NO. | TITLE |
|---------------------|-----------|-------|
| <b>Pharmacology</b> |           |       |
| <i>Secondary PD</i> |           |       |

(b) (4)

| TYPE OF STUDY                           | STUDY NO.     | TITLE  |
|---|---------------|--|
|   |               | (b) (4)  |
| <b>ADME</b><br><i>Absorption</i>        | Rsch-2012-003 | Pharmacokinetic analysis of BIIB017 in non-tumor bearing nude mice following subcutaneous administration   |
|   | P017-11-04    | A pharmacokinetic comparison of IFN molecules with different percentage of sialylation and pegylation with mpeg from different sources following a single subcutaneous dose to Sprague Dawley rats |
| <b>Toxicology</b><br><i>Repeat-dose</i> |               |  |
|   | P9588-98-02   | BG9588/BG9418: A 6-month chronic toxicity and drug interaction study in rhesus monkeys followed by a 6-month recovery period   |

### 3.3 Previous Reviews Referenced

Anne M. Pilaro, Ph.D. Pharmacology/Toxicology Review of BLA 103628 4/30/1996, referenced for review and evaluation of toxicology study P9588-98-02, entitled "BG9588/BG9418: A 6-Month Chronic Toxicity and Drug Interaction Study in Rhesus Monkeys followed by a 6-Month Recovery Period" and reproductive toxicology Study No. P926-93-10, entitled "BG9216 (r-Hu IFN-B) Rhesus Monkey Reproduction Study (Development Evaluation)."

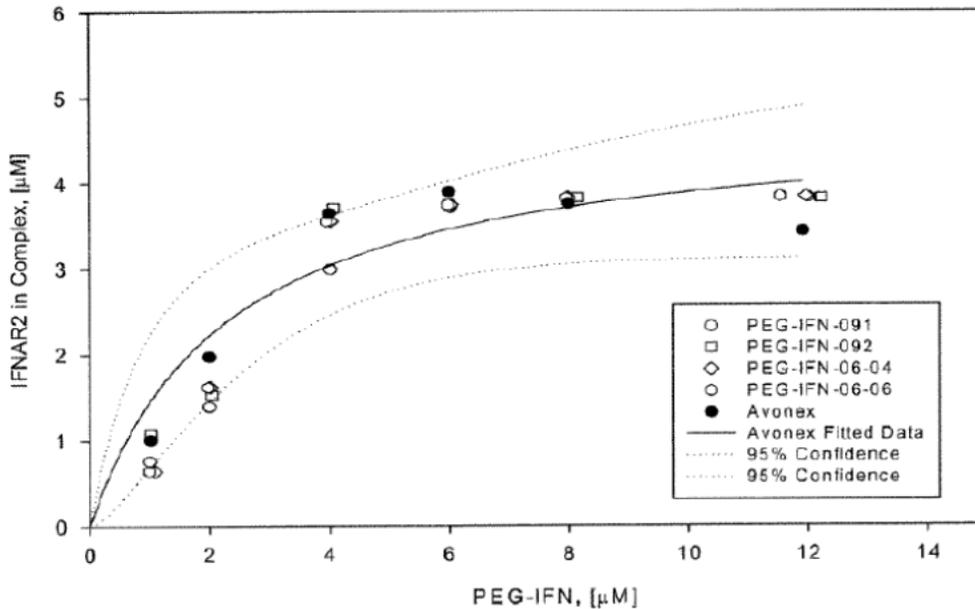
Richard A. Houghtling, Ph.D. Pharmacology/Toxicology Review BLA 103628 supplement 5249, Review of Study No. P017-11-04, December 6, 2013.

## 4 Pharmacology

### 4.1 Primary Pharmacology

Two primary pharmacology studies were conducted. The first (No. P91-004; non-GLP, 02/1992) tested whether recombinant human interferon beta (rhIFN  $\beta$ ) was pharmacologically active in any of the animal species routinely used in nonclinical toxicology studies. Peripheral blood lymphocytes isolated from seven animal species (mouse, rat, guinea pig, rabbit, dog, rhesus monkey, and cynomolgus monkey) were treated *in vitro* with 0, 100, or 250 U rhIFN- $\beta$ . After a 20-hr incubation, the induction of 2',5'-oligoadenylate synthetase (OAS) was measured. Three species demonstrated a dose-related increase in OAS activity—guinea pig, beagle dog, and rhesus monkey. In the second (CHR-83-07-13; non-GLP, 12/2012), the binding of interferon beta-1a (IFN- $\beta$  1a, drug substance in Avonex) or BIIB017-A (pegylated-IFN- $\beta$  1a made by the same process used to generate the drug substance used in Avonex) to the ligand binding domain of the human interferon receptor (IFNAR2) was studied. Using size exclusion chromatography, the formation of a stable binary complex of IFN- $\beta$ /IFNAR2 was used to determine the apparent affinity of BIIB017-A (4 different batches) to IFNAR2. In Figure 1 (Sponsor's), the binding isotherm for IFN- $\beta$  1a or 4 batches of BIIB017-A to IFNAR2, is shown; the binding data for IFN- $\beta$  1a were fit and the 95% confidence intervals were shown. The binding of BIIB017-A to IFNAR2 occurred within the 95% confidence intervals for IFN- $\beta$  1a (Avonex, in the figure); therefore, the apparent binding affinities were the same. Pegylation of IFN- $\beta$  1a did not affect the binding characteristics to IFNAR2.

**Figure 1: Isotherm for the binding of interferon beta-1a to the extracellular portion of the IFNAR2 chain**



Data are shown for interferon beta-1a reference standard RS005-003 (referred to in the key as Avonex) and for BIIB017-A drug substance batches RECD-13976-06-091, RECD-13976-06-092, PSE-pIFN-06-04, and PSE-pIFN-06-06 (referred to in the key as PEG-IFN-091, PEG-IFN-092, PEG-IFN-06-04, and PEG-IFN-06-06, respectively). The solid line represents the fit to the interferon beta-1a data, and the dotted lines show the 95% confidence limits for the interferon beta-1a data in which the data for BIIB017 lie.

## 4.2 Secondary Pharmacology

Ten secondary pharmacology study reports and one scientific article were submitted; the article was authored by Baker et al. (2006), an affiliate/employee of the Sponsor. (b) (4)

Since BIIB017 is intended for treatment of patients with multiple sclerosis, these data are of limited relevance.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

The PK of BIIB017 was evaluated and compared to that of Avonex in 3 absorption studies conducted in rhesus monkey, a biologically relevant species. (A study in rat was also submitted; however, rat is not a biologically relevant species.) The distribution of BIIB017 and IFN  $\beta$ -1a was tested in guinea pig, a biologically relevant species. Finally, an in vitro stability study was performed for BIIB017 in serum from rhesus monkey and human.

#### **Absorption**

1) Study no. P017-06-01

Title: A Comparative Single-Dose Pharmacokinetic and Pharmacodynamic Study of BIIB017 and Avonex<sup>®</sup> Administered by the Intramuscular or Subcutaneous Route to Rhesus Monkeys

GLP/QA, [REDACTED] (b) (4)  
[REDACTED]; Study Initiation: 2/2006; Final Report: 11/2006

Avonex (interferon  $\beta$ -1a, Lot No. P35054, 95%; Syringe Lot V35076/2) and BIIB017 (pegylated-interferon  $\beta$ -1a, Lot 11863-30A, 99.5%)

The routes of administration were subcutaneous and intramuscular based upon the anticipated clinical route for BIIB017 and the approved route of administration for Avonex, respectively. The dose of 1 MU/kg was selected based upon previous studies (P9418-98-01 and P9418-94-01). This dose is a high multiple of the expected human dose and, furthermore, serum IFN- $\beta$  1a was measurable and PD responses were obtained at this dose. Study P9418-98-01 was not found in the present submission nor was it found in Dr. Pilaro's review. In Study P9418-94-01, IFN- $\beta$  1a was administered at doses of 1 MU/kg (5 mcg/kg; iv) and 10 MU/kg (50 mcg/kg; sc) to rhesus monkey, as reviewed by Dr. Pilaro.

Rhesus monkey (5M/group), experimentally naive, 2 to 5.5 years of age, weighing 3.5 to 6.1 kg (Day -1) were assigned to one of four groups; Grp 1, Avonex (sc), Grp 2, Avonex (im), Grp 3, BIIB017 (sc), and Grp 4, BIIB017 (im). The animals were evaluated twice daily for clinical signs (predose, Day -7 to -1; postdose Days 1-8), food consumption (once daily), body weight (predose only), and body temperature (twice predose, and postdose at 4, 12, and 168 hr). Blood samples for PK and PD analyses were collected predose and postdose (1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 hr). At the end of the study, these animals were returned to the colony.

No treatment-related clinical signs were observed. The slight bruising observed in the femoral area was related to venipuncture. Other clinical signs (bruises, alopecia, reddened areas, and watery stool) were sporadic, and these were also attributed to study procedures. Although the study report mentions an assessment of food consumption, these data were not found in the study report.

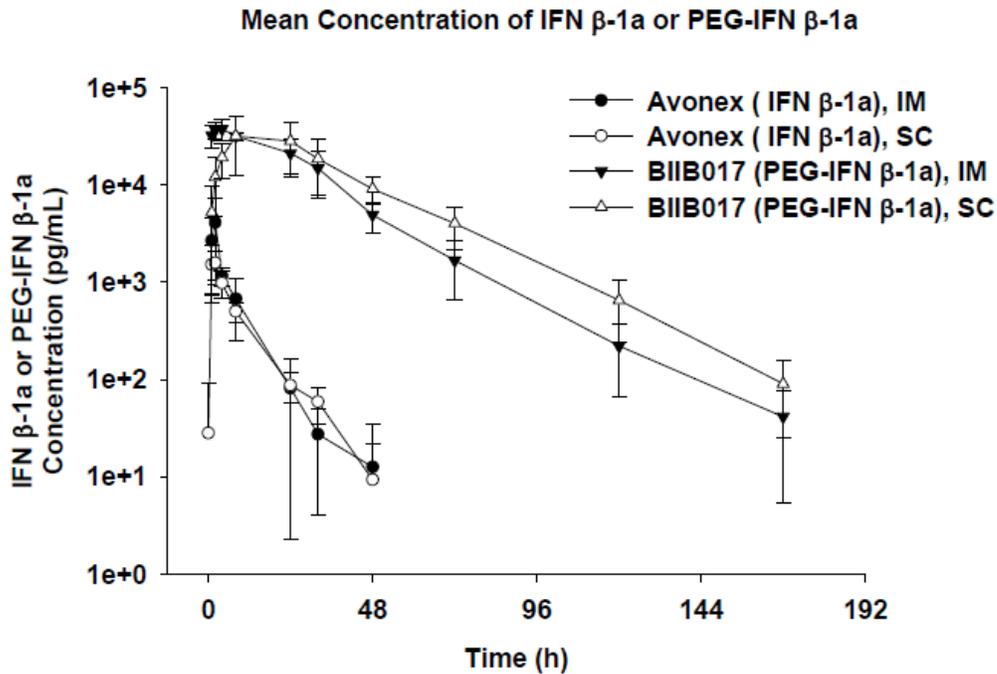
The study report claims there was no body temperature effect attributed to treatment because of higher than normal predose temperatures resulting from the stress of immobilization and handling; however, evaluation of Day 1 predose and 4 hr postdose temperatures suggest a treatment-related effect in all groups. Treatment-related increases in body temperature occurred at 4 hr postdose on Day 1 after Avonex by 1.6°F (sc) and by 1.3°F (im) and after BIIB017 by 0.9°F (sc) and by 1.4°F (im).

### *Pharmacokinetics*

The mean pharmacokinetic parameters of Avonex and BIIB017 were summarized in the Sponsor's table below and the mean concentration-time profiles were illustrated in Sponsor's Figure 2.

- BIIB017 demonstrated greater exposure ( $AUC_{last}$  and  $C_{max}$ ), longer  $t_{1/2}$ , lower total body clearance (CL/F), and a smaller volume of distribution (Vd/F) than Avonex.
- A longer  $T_{max}$  was observed for BIIB017 administered by sc injection compared to im injection; this difference was not apparent following Avonex treatment.

**Figure 2:** The serum concentration-time profiles of BIIB017 and Avonex® in rhesus monkeys following a single IM or SC dose.



BIIB017 and interferon beta-1a serum concentrations in rhesus monkeys were determined using an ELISA. Data are represented as mean ± S.D. (n=5)

| Test Article | Group | Route | AUC <sub>last</sub> <sup>1</sup> (h*ng/mL) | C <sub>max</sub> (ng/mL) | t <sub>1/2</sub> <sup>2</sup> (h) <sup>2</sup> | T <sub>max</sub> (h) | CL/F (mL/h/kg) | Vd/F (mL/kg) |
|--------------|-------|-------|--|--------------------------|--|----------------------|----------------|--------------|
| Avonex®      | 1     | IM    | 17.4                                       | 4.1                      | 6.3  | 1.6                  | 308            | 2559         |
|              | 2     | SC    | 12.2                                       | 1.7                      | 7.4  | 1.6                  | 405            | 4254         |
| BIIB017      | 3     | IM    | 1070                                       | 40.1                     | 17.1   | 3.4                  | 10.4           | 256          |
|              | 4     | SC    | 1259                                       | 32.1                     | 16.8   | 10.4                 | 9.3            | 235          |

<sup>1</sup> AUC<sub>last</sub> = area-under-the-concentration- time-curve from time zero to the last measurable time point

<sup>2</sup> h = hour

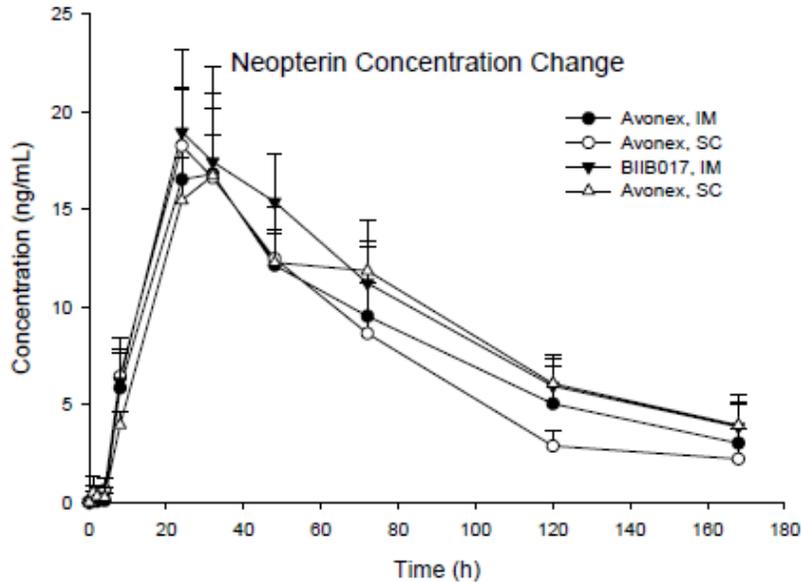
IM = intramuscular; SC = subcutaneous

**Pharmacodynamic response**

The serum neopterin response was similarly induced by Avonex and BIIB017 and it was independent of route of administration. The exposure (AUC and C<sub>max</sub>) and T<sub>max</sub> values achieved were of a similar magnitude. The mean serum concentration-time profiles of neopterin were illustrated in Sponsor’s Figure 4; the mean pharmacokinetic parameters (AUC, C<sub>max</sub>, and T<sub>max</sub>) are summarized in Sponsor’s table, below. It is notable that despite greater serum BIIB017 exposure, the

neopterin response was of a similar magnitude compared to that achieved with Avonex.

**Figure 4.** The mean serum concentration-time profiles of neopterin (after correcting for baseline level) in rhesus monkeys following a single IM or SC dose of Avonex or BIIB017 at 1 MU/kg.



| Test Article | Group | Route | AUC <sub>last</sub> (h*ng/mL) | C <sub>max</sub> (ng/mL) | T <sub>max</sub> (h) |
|--------------|-------|-------|-------------------------------|--------------------------|----------------------|
| Avonex®      | 1     | IM    | 1967                          | 21.0                     | 27.2                 |
|              | 2     | SC    | 1679                          | 21.4                     | 25.6                 |
| BIIB017      | 3     | IM    | 2049                          | 21.8                     | 24.0                 |
|              | 4     | SC    | 2045                          | 20.2                     | 28.8                 |

Study No. P017-06-03

Title: BIIB017: A Single-Dose Pharmacokinetic and Pharmacodynamic Study of PEG-Interferon β-1a Administered by Subcutaneous or Intramuscular Injection to Rhesus Monkeys

GLP, Yes; QA, No. (b) (4)  
Study Initiation: 7/2006; Final Report: 4/2007

BIIB017 (pegylated-interferon β-1a, Lot MFG-83-06-063, 98%)

The routes of administration were subcutaneous (the proposed clinical route) and intramuscular as it was a potential route of administration to humans and for

examining the bioavailability of BIIB017 by this route. The dose selection was based on information from previously conducted studies. The high dose (11 MU/kg) was approximately the highest dose of non-pegylated IFN  $\beta$ -1a administered to rhesus monkey without adverse effects. The low dose (0.22 MU/kg) was approximately equivalent to the highest anticipated clinical dose level and the mid dose (1.1 MU/kg) was five times the clinical dose.

Rhesus monkey (2/sex/group), experimentally naïve, males 2.8 to 4.0 years of age weighing 2.8 to 5.4 kg and females 2.8 to 4.0 years of age weighing 3.0 to 5.5 kg were assigned to one of four dose groups. BIIB017 was administered to all 4 groups, Groups 1, 2, and 3 were administered a single dose of 0.22 MU/kg (2 mcg/kg), 1.1 MU/kg (10 mcg/kg), and 11 MU/kg (100 mcg/kg), respectively, by subcutaneous injection; Group 4 was administered a single dose of 11 MU/kg (100 mcg/kg) by intramuscular injection. Dose volume was 0.4 mL/kg.

The following in-life observations and measurements were performed:

- Clinical signs twice daily (AM/PM) commencing 7 days prior to initiation of dosing and through the termination of the in-life phase, included postdose observations on Day 1 (2 and 4 hr);
- Food consumption (once daily) commencing 7 days prior to initiation of dosing continuing until return of animal to colony on Day 29;
- Body weight (prestudy and once weekly thereafter) over a 28-day postdose period;
- Blood samples were collected for PK (predose, and at 1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 hr postdose), PD (predose, and at 24, 48, 72, 120, and 168 hr postdose), and antibody analyses (predose and on Days 15 and 29).

BIIB017 was well tolerated at all doses. No mortality occurred and no test article-related clinical signs were observed.

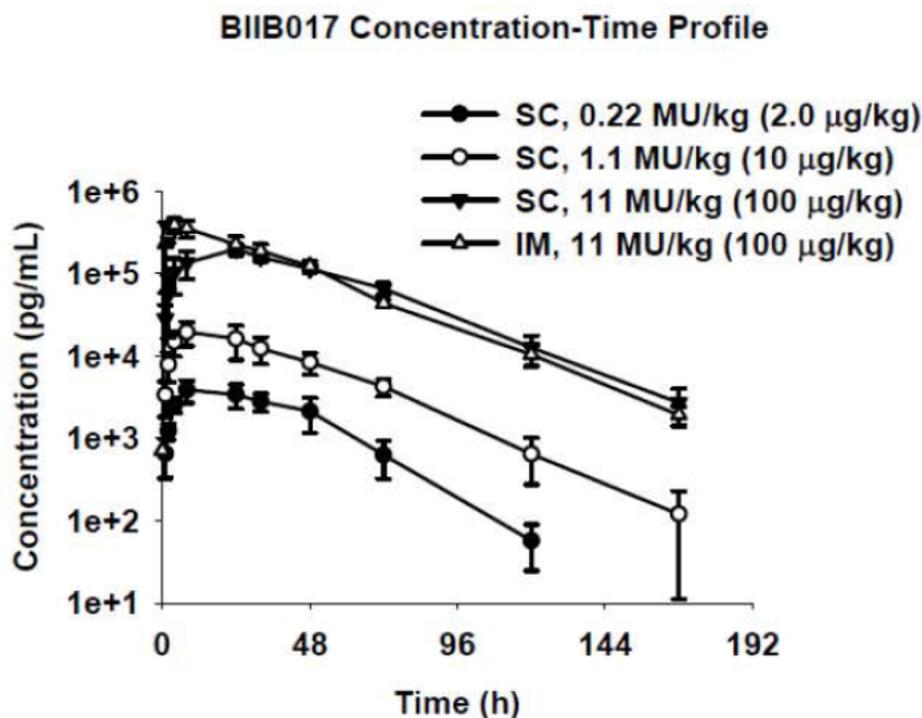
#### *Pharmacokinetics*

The PK parameters following either subcutaneous or intramuscular injection of BIIB017 were summarized in Sponsor's Table 15 (below) and the serum concentration-time profiles are shown in Sponsor's Figure 3.

(Sponsor's)

**Table 15: PK Parameters of BIIB017 following Single SC and IM Dose to Rhesus Monkeys**

| Gender | Route | Dose                    |                       | $C_{max}$             | $AUC_{inf}$                         | $T_{max}$ | $t_{1/2}$ | $V_dF$                | $CL/F$                         |
|--------|-------|-------------------------|-----------------------|-----------------------|-------------------------------------|-----------|-----------|-----------------------|--------------------------------|
|        |       | $\mu\text{g}/\text{kg}$ | $\text{MU}/\text{kg}$ | $\text{ng}/\text{mL}$ | $\text{ng}\times\text{h}/\text{mL}$ | h         | h         | $\text{mL}/\text{kg}$ | $\text{mL}/\text{h}/\text{kg}$ |
| 2M/2F  | SC    | 2                       | 0.22                  | 3.8                   | 176                                 | 22.0      | 14.1      | 242                   | 11.9                           |
| 2M/2F  |       | 10                      | 1.1                   | 19.5                  | 912                                 | 12.0      | 19.7      | 326                   | 11.6                           |
| 2M/2F  |       | 100                     | 11                    | 197                   | 10905                               | 24.0      | 20.9      | 277                   | 9.2                            |
| 2M/2F  | IM    | 100                     | 11                    | 403                   | 14361                               | 4.3       | 21.2      | 218                   | 7.1                            |

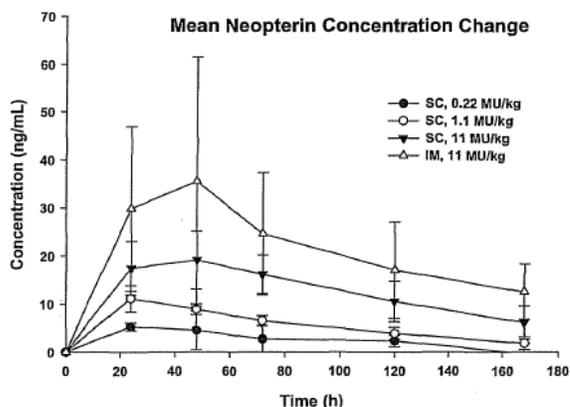
**Figure 3: The serum concentration-time profiles of BIIB017 in rhesus monkeys following a single SC dose at 2, 10, and 100  $\mu\text{g}/\text{kg}$  or a single IM dose at 100  $\mu\text{g}/\text{kg}$ .**

BIIB017 serum concentration in rhesus monkeys was determined using an ELISA. Data are represented as mean  $\pm$  S.D. (n=4)

### Pharmacodynamics

Mean serum neopterin baseline-corrected concentration-time profiles (baseline values were removed) were illustrated in Figure 5 (Sponsor's).

**Figure 5.** The mean serum concentration change-time profiles of neopterin (by subtracting pre-dose neopterin concentration) in rhesus monkeys following a single SC dose at 0.22, 1.1 or 11 MU/kg, or a single IM dose at 11 MU/kg.



*Immunogenicity*

Serum was collected on observational Days 15 and 29 to screen for antidrug antibodies. Binding and neutralizing antibodies were evaluated. The results are shown in Sponsor’s Table 4, below.

**Table 4.** List of BAB or NAB positive monkeys on Days 15 and 29.

|              | Day 15       |              | Day 29       |              |
|--------------|--------------|--------------|--------------|--------------|
|              | BAB Positive | NAB Positive | BAB Positive | NAB Positive |
| Animal ID*   | -            | -            | -            | 1002         |
|              | -            | 1501         | 1501         | 1501         |
|              | 3001         | -            | 3001         | -            |
|              | -            | -            | 3002         | 3002         |
|              | -            | -            | 3501         | 3501         |
|              | -            | -            | 3502         | 3502         |
|              | 4001         | -            | 4001         | 4001         |
|              | 4501         | -            | 4501         | 4501         |
|              | 4502         | -            | 4502         | 4502         |
| <b>Total</b> | <b>4</b>     | <b>1</b>     | <b>8</b>     | <b>8</b>     |

\*Animal numbers beginning in the 1000s, 2000s, 3000s and 4000s are associated with Groups 1 to 4, respectively.

- By Day 29, binding and in some cases neutralizing antibodies directed against BII017 were observed following a single dose; these data indicate that pegylated-interferon β-1a is immunogenic in rhesus monkeys, a finding, which is well established for Avonex.

Study No. P017-07-01

Title: A Comparative Pharmacokinetic and Pharmacodynamic Evaluation of Cycle 1 and Cycle 2 Material for BII017 (Pegylated Interferon β-1a) in Rhesus Monkeys Following a Single Subcutaneous or Intramuscular Dose

GLP/QA. [redacted] (b) (4)

[redacted] Study Initiation: 9/2007; Final Report: 5/2008

BIIB017 Cycle 1 (pegylated-interferon  $\beta$ -1a, Lot RECD13976-06-091, 95.4%); same manufacturing process of the interferon  $\beta$ -1a starting material used to produce Avonex.

BIIB017 Cycle 2 (pegylated-interferon  $\beta$ -1a, Lot PSE-pIFNc2-07-03); new manufacturing process of the interferon  $\beta$ -1a starting material [redacted] (b) (4).

The routes of administration were subcutaneous (the proposed clinical route) and intramuscular as it was the route of administration developed for Avonex. A single dose of 1 MU/kg (10 mcg/kg) was tested; this dose was justified based upon previous studies using Cycle 1 BIIB017, in which 1 MU/kg produced detectable drug concentrations and a pharmacologic response in rhesus monkey (Study Nos. P017-06-01 and P017-06-03).

Rhesus monkey (6 males/group), experimentally naïve, 2.1 to 5.3 years of age, weighing 2.5 to 7.3 kg were assigned to one of four dose groups by stratified randomization to achieve similar group mean body weights. Groups 1 and 3 tested BIIB017 Cycle 1 and Groups 2 and 4 tested BIIB017 Cycle 2 effects on PK/PD parameters by two parenteral routes of administration, subcutaneous (Groups 1 and 2) and intramuscular (Groups 3 and 4). Dose volume was 0.125 mL/kg.

The following in-life observations and measurements were performed:

- Mortality and morbidity was evaluated twice daily (AM/PM) on Days -7 (prestudy) to Day 9 (observation period);
- Cageside observation and food consumption were performed once daily (AM) on Days -7 (prestudy) to Day 9 (observation period);
- Injection site observations were performed on Day 1 at 6, 24, 48, 72, and 168 hr postdose; a qualitative assessment for local irritation was determined using a modified Draize score, see Sponsor provided tables, below.

| <b>Evaluation of Injection Site Reactions – Erythema (Redness)</b> |   |              |
|--|---|--------------|
| <b>Evaluation</b>  | <b>Observation</b>  | <b>Score</b> |
| Erythema   | No redness  | 0            |
|  | Very slight redness, barely perceptible (edges of area not defined)                               | 1            |
|  | Well defined redness, slight (pale-red in color)  | 2            |
|  | Moderate to severe redness (definite red in color)  | 3            |
|  | Severe redness (beet or crimson red in color) and/or slight eschar (dry scab or slough) formation | 4            |

| Evaluation of Injection Site Reactions – Edema (Swelling) |  |       |
|---|--|-------|
| Evaluation  | Observation  | Score |
|   | No swelling  | 0     |
|   | Barely perceptible, very slight swelling (edges of area not defined) | 1     |
| Edema   | Slight swelling (edges of area not definable, but definite raising)  | 2     |
|   | Moderate swelling (area well defined and raised approximately 1 mm)  | 3     |
|   | Severe swelling (raised more than approximately 1 mm)                | 4     |

- Postdose observations were performed on Day 1, at 6, 24, 48, 72, and 168 hr.
- Body weight was measured prior to the first dose (Week -1) and on Day 7; food was withheld before body weights were measured.
- Body temperature was measured within 1 hr of dosing and at 4, 8, 24, and 168 hr postdose on Day 1. All blood collections at these time points were collected prior to the body temperature measurement.
- Blood samples were collected for PK (predose, and at 1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 hr postdose), PD (prestudy, predose, and at 8, 24, 32, 48, 72, 120, and 168 hr postdose), and antibody analyses (Day 1 predose and on Day 8).

BIIB017 (Cycle 1 and Cycle 2) was well tolerated in rhesus monkey by either route of administration at a dose of 1 MU/kg (10 mcg/kg). No treatment-related deaths, clinical signs, food consumption, postdose observations, body weights, or injection site observations occurred.

Mean body temperature (change from predose value) was elevated on Day 1, 4 hr postdose, by both Cycle 1 and Cycle 2 treatment; at 8, 24, and 168 hr postdose, mean body temperature was decreased compared to predose values.

- Injection (sc): +1.5°F (Cycle 1) and +1.4°F (Cycle 2)
- Injection (im): +1.2°F (Cycle 1) and +1.9°F, (Cycle 2)

### *Pharmacokinetics*

Summarized in Table 16 (Sponsor's) are the mean PK parameters for BIIB017-A (Cycle 1) and BIIB017-B (Cycle 2) following single-dose subcutaneous or intramuscular injection.

**Table 16: PK parameters of BIIB017-A and BIIB017-B in Rhesus Monkeys following a Single Dose IM or SC Administration**

| Test Article | Gender | Route | Dose  |       | C <sub>max</sub> | AUC <sub>inf</sub> | T <sub>max</sub> | t <sub>1/2</sub> | V <sub>d</sub> F | CL/F    |
|--------------|--------|-------|-------|-------|------------------|--------------------|------------------|------------------|------------------|---------|
|              |        |       | µg/kg | MU/kg | ng/mL            | ngxh/mL            | h                | h                | mL/kg            | mL/h/kg |
| BIIB017-A    | 6M     | SC    | 10    | 1     | 10.5             | 547                | 16.0             | 16.0             | 428              | 18.8    |
| BIIB017-B    | 6M     | SC    | 10    | 1     | 15.1             | 612                | 8.3              | 16.0             | 383              | 16.7    |
| BIIB017-A    | 6M     | IM    | 10    | 1     | 26.7             | 679                | 3.2              | 20.1             | 464              | 15.6    |
| BIIB017-B    | 6M     | IM    | 10    | 1     | 21.3             | 596                | 3.0              | 21.0             | 559              | 18.7    |

PK/PD parameter ratios between Cycle 1 and Cycle 2 BIIB017 for sc and im routes were summarized in Sponsor’s Table 4.

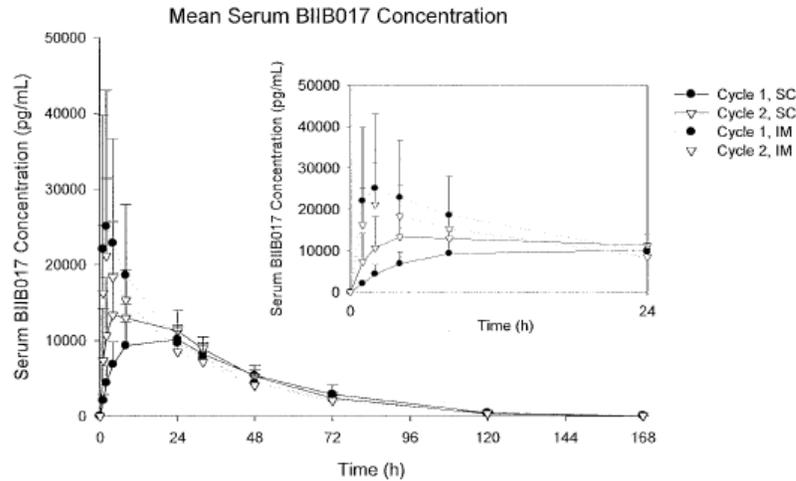
**Table 4.** PK/PD parameter ratios between Cycle 1 and Cycle 2 BIIB017 for the SC and IM routes. The mean values of each treatment group were used to calculate the ratios.

|                        | Treatment Groups          | AUC <sub>168h</sub> | AUC <sub>inf</sub> | C <sub>max</sub> | T <sub>max</sub> | CL/F | t <sub>1/2</sub> | Vd/F |
|------------------------|---------------------------|---------------------|--------------------|------------------|------------------|------|------------------|------|
| BIIB017<br>Parameter   | Cycle 2 (SC)/Cycle 1 (SC) | 1.1                 | 1.1                | 1.4              | 0.5              | 0.9  | 1.0              | 0.9  |
|                        | Cycle 2 (IM)/Cycle 1 (IM) | 0.9                 | 0.9                | 0.8              | 0.9              | 1.2  | 1.0              | 1.2  |
| Neopterin<br>Parameter | Cycle 2 (SC)/Cycle 1 (SC) | 1.2                 | NA                 | 1.2              | 0.9              | NA   | 0.7              | NA   |
|                        | Cycle 2 (IM)/Cycle 1 (IM) | 1.0                 | NA                 | 1.1              | 1.0              | NA   | 1.1              | NA   |

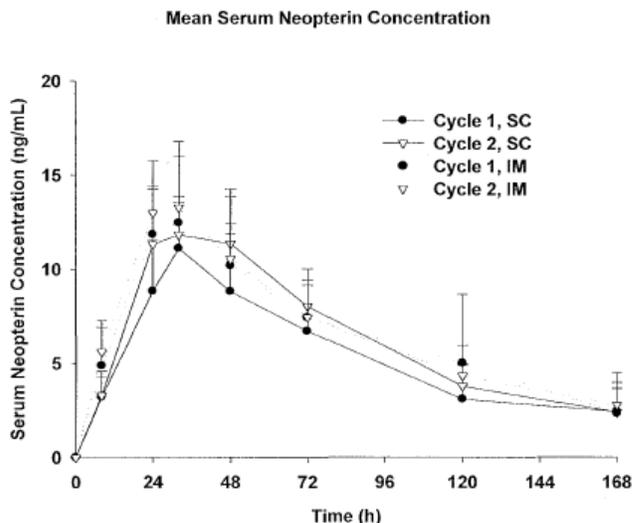
NA, not applicable

Mean serum concentration-time profiles of BIIB017 and neopterin are illustrated in Sponsor’s Figures 2 and 4, respectively.

**Figure 2.** The mean serum concentration-time profiles of BIIB017 by treatment group.



**Figure 4.** The mean serum concentration-time profiles of neopterin after baseline correction by treatment group.



### **Distribution**

Study No. P017-10-01

Title: Pharmacokinetic analysis of PEGylated-[<sup>125</sup>I]Interferon beta-1a (<sup>125</sup>I-BIIB017) and [<sup>125</sup>I]Interferon beta-1a (<sup>125</sup>I-Avonex®) in serum and selected tissues following intravenous administration to guinea pigs

### **Key findings:**

- [<sup>125</sup>I]IFN β-1a and [<sup>125</sup>I]BIIB017 were measured in all the tissues examined; the distribution pattern was similar for these two test articles. Tissue uptake was high in spleen, kidney, liver, and lung, whereas, tissues with the lowest exposure were muscle, brain, and spinal cord.
- Mean %TCA-precipitated PEG [<sup>125</sup>I]IFN β-1a radioactivity in most tissues examined ranged from 76.4% [kidney] to 106% [heart], except the pancreas, in which 31.6% was found. In contrast, mean %TCA-precipitated [<sup>125</sup>I]IFN β-1a radioactivity ranged from 67.8% to 163% [heart].
- PEG [<sup>125</sup>I]IFN β-1a had lower tissue-to-serum ratios as measured by AUC<sub>72hr</sub> suggesting poor tissue uptake; in contrast, serum PEG [<sup>125</sup>I]IFN β-1a at 72 hr postdose was considerably more stable than [<sup>125</sup>I]IFN β-1a in serum at 1 hr postdose.

Non-GLP, (b) (4); Study Initiation: 9/2007; Final Report: 4/2012

The radiolabeled IFN b-1a and BIIB017 tested were listed (below).

(Sponsor's table)

|                       |   |
|-----------------------|---|
| Test article:         | [ <sup>125</sup> I]Interferon beta-1a           |
| Lot No.:              | 134-068-001                                     |
| (b)(4) ID:            | BG9418 (RS031-002-T), [ <sup>125</sup> I]       |
| Specific activity:    | 11.8 µCi/µg                                     |
| Total batch activity: | 1.93 mCi as of 11 February 2011                 |
| Storage conditions:   | Approximately -70°C                             |
| Test article:         | PEGylated-[ <sup>125</sup> I]Interferon beta-1a |
| Lot No.:              | 134-068-002                                     |
| (b)(4) ID:            | BIIB017 (RS028-001), [ <sup>125</sup> I]        |
| Specific activity:    | 7.2 µCi/µg                                      |
| Total batch activity: | 1.33 mCi as of 11 February 2011                 |
| Storage conditions:   | Approximately -70°C                             |

[<sup>125</sup>I]IFN β-1a and [<sup>125</sup>I]BIIB017 were administered by bolus intravenous injection into the jugular vein via a dose cannula; 0.5 mL of saline was flushed into the cannula to ensure each animal received the full dose. Hartley albino guinea pigs CrI:((HA) BR) (15 males/group) were tested; at dosing, the animals were 4 weeks of age and weighed 278 to 352 g.

Mean administered dose of [<sup>125</sup>I]IFN β-1a was 0.0124 ± 0.000560 mg/kg, equivalent to 82.4 ± 3.68 µCi/kg, and a mean dose of [<sup>125</sup>I]PEG-IFN β-1a was 0.0238 ± 0.000434 mg/kg, equivalent to 95.9 ± 1.70 µCi/kg.

The study design is shown in Sponsor's table, below.

| Group          | Number of Male JVC Animals | Dose Route | Target Dose Level (mg/kg) | Target Dose Volume (mL/kg) | Samples Collected         |
|----------------|----------------------------|------------|---------------------------|----------------------------|---------------------------|
| 1 <sup>a</sup> | 15                         | IV         | 0.01                      | 2.0                        | Blood, Urine, and Tissues |
| 2 <sup>b</sup> | 15                         | IV         | 0.02                      | 2.0                        | Blood, Urine, and Tissues |

IV Intravenous (given as a bolus injection via the jugular cannula).

JVC Jugular vein-cannulated.

a Animals in Group 1 received [<sup>125</sup>I]Interferon beta-1a.

b Animals in Group 2 received PEGylated-[<sup>125</sup>I]Interferon beta-1a.

Notes: The doses were approximately 80 to 100 µCi/kg.

One Group 1 animal (Animal No. E29077) was observed to have a prolapsed rectum at approximately 8 hours post-dose. This animal was euthanized and a replacement animal was dosed. The data and samples collected from the original animal will not be reported but will be archived in accordance with the protocol, as applicable.

Prior to dose administration, 3 animals/group (Group 1: E29077, E29078, E29079; Group 2: #29092, E29093, and E29094) were acclimated to individual Nalgene cages for separation and collection of urine.

- Urine samples were collected from the 3/grp designated for blood and tissue collections at 72 hr postdose for analysis of excretion of radioactivity. Urine samples were collected at the following intervals 0-8 hr and 8-24 hr postdose and at 24, 48, and 72 hr postdose.

Blood, serum, and tissue collection (3 animals/time point: 0.25, 1, 6, 24, and 72 hr postdose). Bladder, brain, fat (mesenteric), heart, kidney(s), large intestine (including colon and cecum), liver, lung(s), lymph node(s) [of close proximity to kidney(s) and or liver], muscle, pancreas, prostate, skin, small intestine, spinal cord, spleen, stomach, testes, and thyroid/parathyroid gland were collected.

- Radioactivity was measured by solid scintillation counting of samples that were divided into triplicate weighed aliquots to determine the concentration of radioactivity and homogeneity.
- Triplicate aliquots of the dose formulations prior to and following dose administration were used for SDS-PAGE and TCA precipitation analysis.

#### **Unscheduled death:**

Group 1: E29077 presented with a prolapsed rectum and red discharge at approximately 8 hr postdose. The animal was euthanized and a replacement animal was dosed.

#### **Results:**

The PK parameters associated with [<sup>125</sup>I]IFN β-1a and [<sup>125</sup>I]PEG-IFN β-1a in blood, serum, and tissues following intravenous administration in guinea pig (male) are summarized in Sponsor's Table 2.

- Tissue distribution of IFN β-1a and PEG-IFN β-1a was similar. The tissues with the highest concentrations of each of these test articles include spleen, kidney, liver, and lung; whereas, the lowest concentrations occurred in the muscle, brain, and spinal cord.
- A notable difference between IFN β-1a and PEG-IFN β-1a was the tissue uptake. PEG-IFN β-1a had lower tissue:serum ratios (AUC<sub>72hr</sub>) than IFN β-1a; these differences were greatest in highly perfused tissues (spleen and kidney) and less so in poorly perfused tissues (brain and spinal cord).
- PEG-[<sup>125</sup>I]IFN β-1a (94-99.2%) was more stable than [<sup>125</sup>I]IFN β-1a (47.3-96.3%) as determined by amount of radioactivity associated with TCA-precipitated protein from serum (up to 72 hr).
- SDS-PAGE of TCA-precipitates showed bands at expected molecular weights in serum, spleen and kidney, and liver for PEG-[<sup>125</sup>I]IFN β-1a (72 hr, 24 hr, and 6 hr, respectively) and for [<sup>125</sup>I]IFN β-1a (up to 1 hr—serum, spleen, and liver; and 0.25 hr kidney). These data demonstrate that the PEG-[<sup>125</sup>I]IFN β-1a is more stable in tissue than [<sup>125</sup>I]IFN β-1a.
- Urinary excretion was a major elimination route for radioactivity associated with both [<sup>125</sup>I]IFN β-1a and PEG-[<sup>125</sup>I]IFN β-1a; SDS-PAGE analysis of urine showed an absence of intact proteins and presence of lower molecular weight molecules of ≤12.3 kDa, presumably due to catabolism.

**Table 2.** The PK parameters of [<sup>125</sup>I]interferon beta-1 and PEGylated-[<sup>125</sup>I]interferon beta-1a in serum and tissues based on total radioactivity.

| TISSUE               | [ <sup>125</sup> I]interferon beta-1 |                  |                    |  | PEGylated-[ <sup>125</sup> I]interferon beta-1a |                  |                    |  |
|----------------------|--------------------------------------|------------------|--------------------|--|---|------------------|--------------------|--|
|                      | C <sub>max</sub>                     | T <sub>max</sub> | AUC <sub>72h</sub> | Tissue/Serum<br>AUC <sub>72h</sub> Ratio | C <sub>max</sub>                                | T <sub>max</sub> | AUC <sub>72h</sub> | Tissue/Serum<br>AUC <sub>72h</sub> Ratio |
|                      | (ng/g)                               | (h)              | (h*ng/g)           |  | (ng/g)  | (h)              | (h*ng/g)           |  |
| Blood                | 12.0                                 | 0.250            | 45.0               | 0.526                                    | 175   | 0.250            | 2680               | 0.440                                    |
| Serum                | 22.5                                 | 0.250            | 85.5               | 1.00                                     | 385   | 0.250            | 6090               | 1.00                                     |
| Bladder (urinary)    | 4.53                                 | 1.00             | 37.8               | 0.442                                    | 9.94  | 1.00             | 378                | 0.0620                                   |
| Brain                | 0.630                                | 0.250            | 3.65               | 0.0427                                   | 4.63  | 0.25             | 67.3               | 0.0111                                   |
| Fat (mesenteric)     | 2.04                                 | 1.00             | 13.8               | 0.161                                    | 11.0  | 1.00             | 184                | 0.0302                                   |
| Heart                | 7.13                                 | 0.250            | 38.1               | 0.446                                    | 28.0  | 1.00             | 477                | 0.0783                                   |
| Kidney(s)            | 158                                  | 1.00             | 1280               | 15.0                                     | 127   | 1.00             | 2700               | 0.444                                    |
| Large intestine      | 1.95                                 | 6.00             | 77.2               | 0.904                                    | 6.69  | 6.00             | 275                | 0.0452                                   |
| Liver                | 206                                  | 0.250            | 735                | 8.60                                     | 117   | 0.250            | 1290               | 0.212                                    |
| Lungs                | 63.8                                 | 0.250            | 397                | 4.64                                     | 47.0  | 0.250            | 724                | 0.119                                    |
| Lymph nodes          | 3.34                                 | 1.00             | 25.4               | 0.297                                    | 10.8  | 1.00             | 318                | 0.0522                                   |
| Muscle               | 0.902                                | 0.250            | 6.98               | 0.0817                                   | 2.51  | 1.00             | 52.7               | 0.00866                                  |
| Pancreas             | 3.47                                 | 0.250            | 25.0               | 0.293                                    | 16.0  | 1.00             | 282                | 0.0464                                   |
| Prostate gland       | 1.33                                 | 1.00             | 9.47               | 0.111                                    | 3.53  | 0.250            | 83.4               | 0.0137                                   |
| Skin (dorsal shaved) | 2.15                                 | 1.00             | 18.3               | 0.214                                    | 3.22  | 1.00             | 94.1               | 0.0155                                   |
| Small intestine      | 10.9                                 | 1.00             | 59.0               | 0.691                                    | 15.7  | 1.00             | 198                | 0.0325                                   |
| Spinal cord          | 0.415                                | 0.250            | 1.69               | 0.0198                                   | 3.04  | 0.250            | 39.4               | 0.00647                                  |
| Spleen               | 295                                  | 0.250            | 1170               | 13.7                                     | 347   | 1.00             | 3830               | 0.630                                    |
| Stomach              | 4.63                                 | 1.00             | 66.5               | 0.779                                    | 10.6  | 1.00             | 228                | 0.0374                                   |
| Testis(es)           | 1.73                                 | 1.00             | 19.8               | 0.232                                    | 12.8  | 1.00             | 258                | 0.0424                                   |
| Thyroid/parathyroid  | 3.94                                 | 0.250            | 70.2               | 0.822                                    | 13.4  | 0.250            | 274                | 0.0450                                   |

### Dose Solution Analysis

Predose and postdose solution analysis was performed; total dosing solution radioactivity was determined. Measurement of TCA precipitate (pellet) was determined and calculated as a percentage of total dose solution radioactivity. The results are shown in the reviewer-generated table below for Group 1: [<sup>125</sup>I]IFN β-1a and Group 2: PEG-[<sup>125</sup>I]IFN β-1a.

| DOSE SOLUTION ANALYSIS          |          |  |                                     |
|---------------------------------|----------|--|-------------------------------------|
| Treatment                       | Sampling | % TCA Precipitated<br>Radioactivity<br>(Mean ± SD) | Protein<br>Concentration<br>(mg/mL) |
| [ <sup>125</sup> I]IFN β-1a     | Predose  | 96 ± 1.2%  | 0.00554                             |
|                                 | Postdose | 95.3 ± 0.4%  |                                     |
| PEG-[ <sup>125</sup> I]IFN β-1a | Predose  | 97.5 ± 0.2%  | 0.00946                             |
|                                 | Postdose | 97.4 ± 0.2%  |                                     |

### Potencies of iodinated interferons—non-pegylated and pegylated (BIIB017)

Iodination of IFN  $\beta$ -1a and BIIB017 was performed using the Bolton-Hunter method; *in vitro* antiviral activity was determined for each IFN  $\beta$ -1a and BIIB017 following iodination (for antiviral assay, nonradioactive iodine incorporation was used). As shown in Sponsor's Table 3, both proteins had slightly reduced potency compared to its respective reference standard; an effect mediated by the chemical reaction conditions (without Bolton-Hunter reagent, "non-iodinated"). Comparison of "iodinated" proteins (in presence of Bolton Hunter reagent) revealed a 2-fold decrease in the antiviral potency of PEG-interferon  $\beta$ -1a compared that of IFN  $\beta$ -1a.

**Table 3. EC<sub>50</sub> values, and fold potency differences compared to assay standards for non-iodinated and iodinated interferon  $\beta$ -1a and BIIB017 from the second Bolton-Hunter iodination study.**

| Sample                               | Mean EC <sub>50</sub> (pg/mL)* | Fold potency difference from assay standard |
|--------------------------------------|--------------------------------|---|
| Interferon $\beta$ -1a standard      | 3.1 (3.7, 2.6, 3.0)            | 1   |
| Non-iodinated interferon $\beta$ -1a | 3.6 (3.0, 4.1)                 | 1.2   |
| Iodinated interferon $\beta$ -1a     | 3.7 (3.9, 3.4, 3.8)            | 1.2   |
| BIIB017 standard                     | 6.5 (6.5, 7.2, 5.9)            | 1   |
| Non-iodinated BIIB017                | 12.6 (12.9, 12.3)              | 1.9   |
| Iodinated BIIB017                    | 15.5 (15.1, 15.3, 16.2)        | 2.4   |

\*Mean values are shown with the duplicate or triplicate values shown in parentheses.

- The iodination reactions above using non-radioactive iodine were conducted in parallel with [<sup>125</sup>I]; the radiolabeled proteins were used for the distribution study. To accommodate the approximately 2-fold difference in potency, the dose of PEG-[<sup>125</sup>I]IFN  $\beta$ -1a was approximately twice that of [<sup>125</sup>I]IFN  $\beta$ -1a (see Dose Solution Analysis, above).

## 6 General Toxicology

During the development of Avonex, it was determined that repeated dosing of human IFN  $\beta$ -1a in rhesus monkey was highly immunogenic; binding and neutralizing antibodies against IFN  $\beta$ -1a occurred and loss of drug exposure and PD markers occurred. In P017-06-03 (previously discussed), single-dose BIIB017 administered by either sc or im injection resulted in the formation of binding antibodies by Day 15 and both binding and/or neutralizing antibodies by Day 29. Collectively, these data indicated that the standard 6-month repeated-dose toxicology study would not be feasible. Therefore, the Sponsor submitted one 5-week study to assess the toxicology of BIIB017 in rhesus monkey.

### 6.2 Repeat-Dose Toxicity

Study title: BIIB017: A 5-Week Subcutaneous or Intramuscular Toxicity Study in Rhesus Monkeys with a 4-Week Treatment-Free Recovery Period

|                                     |   |
|-------------------------------------|---|
| Study no.:                          | P017-06-02  |
| Study report location:              | EDR 4.2.3.2   |
| Conducting laboratory and location: | (b) (4)   |
| Date of study initiation:           | June 11, 2006   |
| GLP compliance:                     | Yes   |
| QA statement:                       | Yes   |
| Drug, lot #, and % purity:          | 20 kDa mPEG-O-2-methylproprionaldehyde-modified human interferon- $\beta$ -1a (Pegylated interferon- $\beta$ -1a [PEG IFN $\beta$ -1a]) |

#### Methods

|                          |  |
|--------------------------|--|
| Doses:                   | 0 (C), 2 mcg/kg [0.22 MU/kg] (LD), 10 mcg/kg [1.1 MU/kg] (MD), 100 mcg/kg [11 MU/kg]                     |
| Frequency of dosing:     | Once weekly for 5 consecutive weeks on study Days 1, 8, 15, 22, and 29                                   |
| Route of administration: | Subcutaneous: intrascapular region (all doses)<br>Intramuscular: outer thigh (C and HD)                  |
| Dose volume:             | 0.4 mL/kg  |
| Formulation/Vehicle:     | (b) (4) acetic acid/acetate pH 4.8, (b) (4) Arginine HCl, (b) (4) Polysorbate 20 (Lot No. MFG-83-06-099) |
| Species/Strain:          | rhesus monkeys ( <i>Macaca mulatta</i> )   |
| Number/Sex/Group:        | Main study: 4/sex/group<br>Recovery: 2/sex/group, Control and HD (SC and IM)                             |
| Age:                     | M, 2.1 to 4.3 years; F, 2.8 to 3.8 years   |

Weight: M, 2.5 to 6.0 kg; F, 2.4 to 5.3 kg  
Satellite groups: None  
Unique study design: Intramuscular administration was administered concurrently to parallel the clinical route used for Avonex

### **Justification for route, dose levels, and dosing schedule**

BIIB017 was administered by subcutaneous or intramuscular injection; in controls, both subcutaneous and intramuscular injections were performed in the same animals. Two high dose (HD) groups were used, one each for subcutaneous and intramuscular injection. In humans, Avonex is administered by intramuscular injection, and BIIB017 is administered by subcutaneous injection. The high dose selection was based upon a 50 times multiple of the proposed highest maximum clinical dose. The selection of the LD was based upon the anticipated human clinical dose. The MD was selected based upon a dose multiple of 5 times the highest anticipated clinical dose. The duration of the study was shortened due to the presence of ADAs and reduced or ablated exposure.

### **Observations and Results**

**Mortality** Animals were checked for morbidity/mortality twice daily (AM/PM) from prestudy (Day -7) to the end of the recovery period (Day 65).

- No mortality/morbidity occurred during this study.

**Clinical Signs** Cage side observations were conducted twice daily (AM/PM) from Study Day -7 (predose) to Day 65 of the recovery period; during the dosing phase; observations were made approximately 10 min postdose to observe the dosing site and perform a subjective respiratory evaluation (shallow vs. deep breathing) for all animals. During the dosing period, injection site evaluations were performed prior to and at 30 min and 4 hr post dose (modified Draize scoring criteria used).

- None of the clinical signs present were considered test article-related.

**Body Weights** were measured prestudy (Days -5 and -1), once weekly during dosing (Days 7, 14, 21, 28, and 35), on day of terminal sacrifice (main study: Day 36; recovery: Day 65), and once weekly during the recovery period (Days 42, 49, 56, and 63).

- No test article-related effect on body weight was observed.

**Food Consumption** was measured once daily from predose (Day -7) to recovery (Day 65); however, no measurement was performed on days of fasting prior to study procedures.

- No test article-related effect on food consumption was observed.

**Physical Examinations** were performed once prestudy and on the day of necropsy for all animals on study to evaluate the physical condition, body temperature, respiration, and heart rate by a staff veterinarian.

- No test article-related effects on any of the parameters assessed.

**Body Temperature** was measured twice prestudy, predose, and postdose at 4 and 8 hr ( $\pm$  1 hr), for each dose administered. Food was withheld prior to body temperature measurement.

- Dose-dependent increase in body temperature occurred at 4 hr post BIIB017 administration of 10 mcg/kg and 100 mcg/kg (sc and im) on Days 1 and 8 (statistically significant  $p < 0.05$  at both doses and routes). On Day 22, 10 and 100 mcg/kg (subcutaneous) a statistically significant ( $P < 0.05$ ) elevation of body temperature was observed following 10 and 100 mcg/kg sc; 8 hr postdose, body temperature nearly returned to predose values.
- Day 29, no change in body temperature occurred at any dose tested.

**Ophthalmoscopy** was performed once prestudy and at the physical exam prior to necropsy.

- No test article-related ocular abnormalities were identified.

**ECG** was performed twice prestudy and weekly thereafter (at approximately 4 hr postdose  $\pm$  1 hr) and prior to terminal necropsy. Qualitative examination of each tracing for abnormalities was performed by [REDACTED]<sup>(b) (4)</sup>, DVM, DACVIM, a veterinary consultant in cardiology. The interpretation of these results and the narrative were signed and dated by Dr. [REDACTED]<sup>(b) (4)</sup> and included in the study report.

- No treatment-related findings were observed; the ECGs were qualitatively considered normal for rhesus monkeys.

**Clinical Pathology** was performed to evaluate serum chemistry, hematology, and coagulation parameters from all animals prestudy (Days -10 and -2), during Week 1 (8 hr postdose), Weeks 3 and 5 (4 hr post dose from Group 5; 8 hr postdose from Groups 1-4), and prior to the terminal and recovery necropsies. Animals were fasted for at least 8 hr prior to blood collections for serum chemistry. Urine samples were collected for at least 8 hr prior to blood collections for serum chemistry; a 5 mL urine sample was collected by bladder puncture during necropsy.

**Hematology** was performed and the following standard parameters were measured as shown in the Sponsor’s table, below.

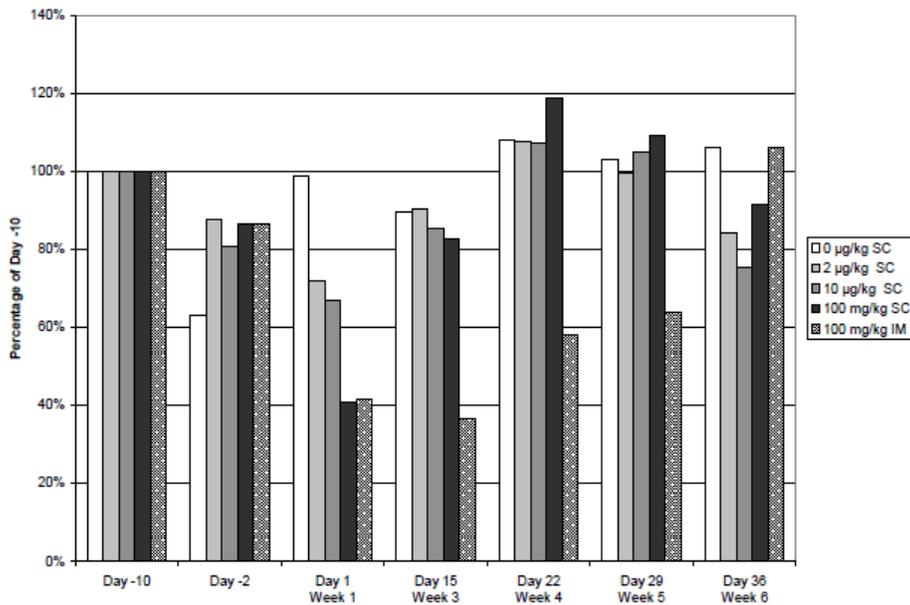
| Hematology Parameters         |  |
|-------------------------------|--|
| Red blood cell (RBC) count    | Mean corpuscular hemoglobin (MCH)                |
| White blood cell (WBC) count* | Mean corpuscular volume (MCV)                    |
| Hemoglobin concentration      | Mean corpuscular hemoglobin concentration (MCHC) |
| Hematocrit                    | Platelet count                                   |
| Reticulocyte count            | Blood cell morphology**                          |

\* Included total white blood cell, polysegmented neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, basophile, and other cell counts, as appropriate.

\*\* The blood smear from all animals was examined at each time point (including prestudy).

As illustrated in Sponsor’s Figure 1 (below), at all doses of BIIB017 treatment on Day 1 of dosing, circulating lymphocyte counts were reduced in a dose-dependent manner; at the HD, both sc and im injection were equally effective at decreasing circulating lymphocyte counts. Mean lymphocyte counts (measured 8 hr postdose) were decreased on Day 1 relative to Day -10 [prestudy] by 1%, 28%, 33%, 59%, and 59% for control, 2 mcg/kg, 10 mcg/kg, 100 mcg/kg (sc and im), respectively. Lymphocyte counts were measured at 4 hr postdose (Weeks 3-5) following once weekly BIIB017 (100 mcg/kg; im) treatment; im injection at Weeks 3-5 decreased circulating lymphocytes to 64%, 56%, and 36% less than the prestudy cell counts.

Figure 1: Change in Average Lymphocyte Counts Compared to Day -10



**Clinical Chemistry** was performed and the following standard parameters were measured as shown in the Sponsor's table, below.

| Serum Chemistry Parameters       |                        |
|----------------------------------|------------------------|
| Sodium                           | Calcium                |
| Potassium                        | Phosphorus             |
| Chloride                         | Urea nitrogen (BUN)    |
| Carbon dioxide                   | Creatinine             |
| Total bilirubin                  | Total protein          |
| Alkaline phosphatase (ALP)       | Albumin                |
| Lactate dehydrogenase (LD)       | Globulin               |
| Aspartate aminotransferase (AST) | Albumin/globulin ratio |
| Alanine aminotransferase (ALT)   | Glucose                |
| Gamma-glutamyltransferase (GGT)  | Cholesterol            |
|                                  | Triglycerides          |

- At the end of dosing, there were no treatment-related changes in clinical chemistry parameters.

**Urinalysis** was performed on urine that was collected from the bladder at necropsy; the following standard parameters were evaluated: color, pH, specific gravity, prot, gluc, ket, bili, occult blood, and microscopic examination of sediment.

- There were no treatment-related changes in urinalysis parameters.

**Gross Pathology** On Day 36, necropsy was performed on 40 animals (4/sex/group 1-5); food was withheld overnight prior to necropsy. On Day 65, necropsy was performed on 12 animals (2/sex/group 1, 4, and 5). Terminal body weights were collected and used to calculate organ:body weight ratios. A complete gross necropsy was performed; no treatment-related abnormalities were described.

**Organ Weights** were recorded prior to fixation; paired organs were weighed together unless gross abnormalities were present and the organs were then weighed separately. The following organ weights were recorded: adrenals, prostate, kidneys, lungs, pituitary, testes, thyroid with parathyroids, brain, heart, liver, ovaries, spleen, thymus, and uterus (including cervix). Ratios of organ weights were calculated relative to terminal body weight or brain weight.

- At terminal sacrifice (Day 36), there were no treatment-related changes in organ weight (absolute or relative) for either sex.

**Histopathology**

- Adequate Battery: Yes, an adequate battery of tissues and organs were collected, fixed, and prepared for histology. The following Sponsor-provided table lists the tissues collected.

| Tissues Collected  |   |
|--|---|
| Cardiovascular   | Urogenital  |
| Aorta (thoracic)   | Kidneys   |
| Heart  | Urinary Bladder   |
| Digestive  | Testes  |
| Salivary Gland (mandibular)  | Epididymides  |
| Esophagus  | Prostate  |
| Stomach (glandular and nonglandular mucosa) – cardiac, fundic, pyloric | Seminal Vesicles  |
| Small Intestine  | Ovaries   |
| Duodenum   | Uterus  |
| Jejunum  | Cervix  |
| Ileum (w/Peyer’s Patches) <sup>c</sup>                                 | Vagina  |
| Large Intestine  | Endocrine   |
| Cecum  | Adrenals  |
| Colon  | Pituitary   |
| Rectum   | Thyroid/Parathyroids <sup>a</sup>                               |
| Pancreas   | Skin/Musculoskeletal  |
| Liver  | Skin/Mammary Gland  |
| Gallbladder  | Bone (femoral head, including articular cartilage) <sup>d</sup> |
| Respiratory  | Joint (femoral-tibial) <sup>e</sup>                             |
| Trachea  | Bone (7th rib)  |
| Lung   | Skeletal Muscle (psoas, biceps, femoris and diaphragm)          |
| Lymphoid/Hematopoietic   | Nervous/Special Sense   |
| Bone Marrow (sternum)  | Eyes with Optic Nerve (in Davidson’s fixative)                  |
| Thymus   | Sciatic Nerve   |

| Tissues Collected                     |   |
|---------------------------------------|---|
| Spleen                                | Brain (cerebrum, midbrain, cerebellum and brain stem) |
| Lymph Nodes                           | Spinal Cord (cervical, thoracic, lumbar)              |
| Bronchial, Mesenteric, Sub-mandibular | Other   |
|                                       | Animal Number Tattoo                                  |
|                                       | Gross Lesions   |
|                                       | Injection Sites <sup>o</sup>                          |

<sup>a</sup> The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.  
<sup>b</sup> The most recent injection site (used on Day 29) for both IM and SC routes  
<sup>c</sup> The occasional absence of Peyer’s patches in the sections of small intestine did not require a recut of the section.  
<sup>d</sup> In addition to the histologic sections, bone marrow smears were prepared from the seventh rib from all animals.  
<sup>e</sup> The femoral-tibial joint was evaluated macroscopically, collected and retained for possible histologic processing and evaluation.

- Bone marrow smears were collected from the 7<sup>th</sup> rib; however, since there was no test article-related effect observed in the bone marrow section evaluated microscopically, these were not examined.
- No treatment-related findings were observed at terminal or recovery sacrifice in either sex at any dose; a signed and dated Pathology Peer Review Certificate was provided. Peer Review was conducted on Control (Grp 1) and HD (Grp 4 and 5) monkeys by (b) (6), DVM, PhD, DACVP, a pathologist at Biogen Idec Inc.

## Toxicokinetics

- TK parameters following the 1<sup>st</sup> dose (sc or im) of BIIB017 administered to male and female rhesus macaques are summarized in the reviewer-generated table below.

| BIIB017 DOSE   | TK PARAMETERS (DAY 1) |                          |                               |                              |                      |                      |                 |              |
|----------------|-----------------------|--------------------------|-------------------------------|------------------------------|----------------------|----------------------|-----------------|--------------|
|                | Sex                   | C <sub>max</sub> (ng/mL) | AUC <sub>168h</sub> (h*ng/mL) | AUC <sub>0-∞</sub> (h*ng/mL) | t <sub>1/2</sub> (h) | T <sub>max</sub> (h) | CL/F (mL/hr/kg) | Vd/F (mL/kg) |
| 2 mcg/kg, sc   | M                     | 14.3 ± 1.5               | 146 ± 36.5                    | 150 ± 36.6                   | 15 ± 1.4             | 16 ± 9.2             | 14 ± 3.7        | 303 ± 78.2   |
|                | F                     | 4.0 ± 1.3                | 175 ± 62                      | 192 ± 47                     | 24.3 ± 11.4          | 16 ± 9.2             | 11 ± 3.1        | 409 ± 257    |
| 10 mcg/kg, sc  | M                     | 26.1 ± 6.8               | 1185 ± 290                    | 1188 ± 291                   | 17.6 ± 1.7           | 11 ± 8.9             | 8.8 ± 2.1       | 221 ± 48.8   |
|                | F                     | 19.4 ± 2.7               | 932 ± 180                     | 939 ± 178                    | 19.9 ± 3.5           | 16 ± 9.2             | 10.9 ± 18       | 307 ± 29.8   |
| 100 mcg/kg, sc | M                     | 326 ± 140                | 13235 ± 3670                  | 13284 ± 3677                 | 20.4 ± 1.3           | 10.7 ± 10.3          | 8.2 ± 3         | 242 ± 91     |
|                | F                     | 317 ± 108                | 13316 ± 2775                  | 13395 ± 2773                 | 21.3 ± 2.1           | 12 ± 9.5             | 7.7 ± 1.5       | 238 ± 51.8   |
| 100 mcg/kg, im | M                     | 648 ± 183                | 13476 ± 5078                  | 13548 ± 5057                 | 24.1 ± 7.6           | 5.3 ± 2.1            | 9.5 ± 7         | 392 ± 465    |
|                | F                     | 449 ± 120                | 12493 ± 2943                  | 12564 ± 2897                 | 20 ± 5               | 4 ± 0                | 8.3 ± 1.9       | 248 ± 108    |

- Following Day 1 administration of BIIB017, systemic exposure was approximately dose proportional.
- As summarized in Sponsor's Table 6, BIIB017 exposure was achieved up to Week 3. By the third dose, serum BIIB017 concentrations were reduced by approximately 50% compared to the first dose (sc and im injections, similarly affected). By the 4<sup>th</sup> dose, the mean BIIB017 concentration for the LD group was markedly reduced. At the MD and the HD administered sc, BIIB017 increased in a dose-related manner, albeit not dose proportional; the serum concentration at each dose, however, was markedly reduced (10 and 28 times, respectively) when compared to Day 1 dosing. After the 5<sup>th</sup> dose, BIIB017 was not detected in LD animals; BIIB017 was measurable in MD and HD animals, but the levels were not dose-related. Similar to sc injections, repeated im administration of BIIB017 demonstrated systemic exposure; by the 3<sup>rd</sup> dose, a reduction of the estimated peak mean concentration (C<sub>max</sub>) was 54% lower than achieved following the 1<sup>st</sup> dose. By the 4<sup>th</sup> dose, the estimated peak mean C<sub>max</sub> was reduced by 99.1% and by the 5<sup>th</sup> dose, it was negligible (reduced by 99.99%).

**Table 6.** Estimated peak concentrations of BIIB017 following the 1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> doses. The peak concentration was estimated by the concentration at 8 hours and 4 hours post-dose for SC and IM routes, respectively.

| Group No. | Dosing Route | Dose<br>(MU/kg; µg/mL) | BIIB017 Serum Concentration (ng/mL) |          |          |          |        |
|-----------|--------------|------------------------|-------------------------------------|----------|----------|----------|--------|
|           |              |                        | 1st Dose                            | 3rd Dose | 4th Dose | 5th Dose |        |
|           |              |                        | 1d, 8h                              | 15d, 8h  | 22d, 8h  | 29d, 8h  |        |
| 2         | SC           | 0.22; 2                | Mean                                | 3.95     | 1.14     | 0.00751  | 0.00   |
|           |              |                        | SD                                  | 1.37     | 1.80     | 0.01988  | 0.00   |
| 3         | SC           | 1.1; 10                | Mean                                | 21.1     | 11.7     | 1.78     | 1.76   |
|           |              |                        | SD                                  | 7.3      | 12.3     | 3.23     | 4.79   |
| 4         | SC           | 11; 100                | Mean                                | 287      | 117      | 10.0     | 0.80   |
|           |              |                        | SD                                  | 112      | 145      | 21.5     | 2.68   |
| Group No. | Dosing Route | Dose<br>(MU/kg; µg/mL) | BIIB017 Serum Concentration (ng/mL) |          |          |          |        |
|           |              |                        | 1st Dose                            | 3rd Dose | 4th Dose | 5th Dose |        |
|           |              |                        | 1d, 4h                              | 15d, 4h  | 22d, 4h  | 29d, 4h  |        |
| 5         | IM           | 11; 100                | Mean                                | 490      | 266      | 4.40     | 0.0419 |
|           |              |                        | SD                                  | 138      | 241      | 5.26     | 0.0910 |

**Serum Neopterin** was measured in all animals at the following time points: prestudy (Day -7), Day 1 (24 hr postdose), Day 15 (24 hr postdose), Day 29 (predose and 24 hr postdose), and Day 36 (prior to necropsy).

- Serum neopterin levels are summarized in Sponsor’s Table 9.

**Table 9.** The mean neopterin concentration change from the pre-study level at selected time points in rhesus monkeys following 5 weekly dose of control vehicle or BIIB017 at 0.22, 1.1 or 11 MU/kg via SC route, or at 11 MU/kg via IM route.

| Study Day | Time Point          | SC/IM, Control |      | SC, 0.22 MU/kg |      | SC, 1.1 MU/kg |      | SC, 11 MU/kg |      | IM, 11 MU/kg |      |
|-----------|---------------------|----------------|------|----------------|------|---------------|------|--------------|------|--------------|------|
|           |                     | Mean           | SD   | Mean           | SD   | Mean          | SD   | Mean         | SD   | Mean         | SD   |
| 1         | Pre-dose (baseline) | 0.00           | 0.00 | 0.00           | 0.00 | 0.00          | 0.00 | 0.00         | 0.00 | 0.00         | 0.00 |
| 2         | 24 h Post-dose      | 0.09           | 1.05 | 6.51           | 4.29 | 11.8          | 2.9  | 23.9         | 5.6  | 20.9         | 4.9  |
| 16        | 24 h Post-dose      | -0.55          | 1.56 | 5.52           | 5.30 | 7.65          | 3.33 | 13.4         | 6.7  | 14.4         | 5.8  |
| 29        | Pre-dose            | -1.30          | 1.98 | 7.02           | 11.3 | 2.27          | 2.58 | 9.6          | 7.7  | 6.61         | 9.87 |
| 30        | 24 h Post-dose      | -0.82          | 2.66 | 2.12           | 3.09 | 4.22          | 5.35 | 4.34         | 4.34 | 2.53         | 3.13 |
| 36        |                     | -0.13          | 2.12 | 0.91           | 4.14 | 4.08          | 4.50 | 7.9          | 8.5  | 4.52         | 4.26 |
| 65        |                     | 0.03           | 3.13 |                |      |               |      | 0.80         | 2.18 | 1.00         | 1.90 |

- During dosing, Control animals do not induce the production of serum neopterin.

- A dose-dependent increase of serum neopterin levels was induced by PEGIFN β-1a at 24 hr postdose on Day 1; the maximal mean neopterin response was achieved at the high dose (sc and im).
- A reduction in the levels of neopterin induced by PEGIFN β-1a occurred in a dose-related manner by the 3<sup>rd</sup> dose. On Day 16, neopterin levels were reduced by 15%, 35%, and 44%, for PEGIFN β-1a at doses of 2, 10, and 100 mcg/kg/week (sc) and by 32% at a dose of 100 mcg/kg (im).
- By the 5<sup>th</sup> dose, measurement of neopterin induction on Day 30 was markedly reduced in a dose-related manner. Day 30 neopterin levels were reduced by 68%, 66%, 82%, for PEGIFN β-1a doses of 2, 10, and 100 mcg/kg/week (sc) and by 88% at a dose of 100 mg/kg (im).

**Immunogenicity** Samples were collected from all animals prestudy (Day -7), predose on Days 15, 29, 36, and 65 (recovery group). Serum was evaluated for antibodies against BIIB017 (PEG-interferon-beta-1a); ADA antibodies were then evaluated for binding ( (b) (4) ) and neutralizing activity (Biogen Idec CST).

**Table 2: Number of Binding and Neutralizing Ab positive monkeys**

| Group No.    | Route | Dose (MU/kg; μg/kg) | Binding Ab Positive |          |           |           |           |           |           |           |          |          | Neutralizing Ab Positive |          |          |          |           |           |           |           |          |          |
|--------------|-------|---------------------|---------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|----------|--------------------------|----------|----------|----------|-----------|-----------|-----------|-----------|----------|----------|
|              |       |                     | Pre-study           |          | Day 15    |           | Day 29    |           | Day 36    |           | Day 65   |          | Pre-study                |          | Day 15   |          | Day 29    |           | Day 36    |           | Day 65   |          |
|              |       |                     | M                   | F        | M         | F         | M         | F         | M         | F         | M        | F        | M                        | F        | M        | F        | M         | F         | M         | F         | M        | F        |
| 1            | SC/IM | 0                   | 0                   | 0        | 0         | 0         | 1         | 0         | 1         | 0         | 0        | 0        | 0                        | 0        | 0        | 0        | 0         | 0         | 0         | 0         | 0        | 0        |
| 2            | SC    | 0.22; 2             | 0                   | 0        | 4         | 3         | 4         | 4         | 4         | 4         | -        | -        | 0                        | 0        | 0        | 1        | 4         | 4         | 4         | 4         | -        | -        |
| 3            | SC    | 1.1; 10             | 0                   | 0        | 2         | 3         | 4         | 4         | 4         | 4         | -        | -        | 0                        | 0        | 0        | 0        | 3         | 4         | 3         | 4         | -        | -        |
| 4            | SC    | 11; 100             | 0                   | 2        | 5         | 6         | 6         | 6         | 6         | 6         | 2        | 2        | 0                        | 0        | 3        | 2        | 6         | 6         | 6         | 6         | 2        | 2        |
| 5            | IM    | 11; 100             | 0                   | 0        | 6         | 5         | 6         | 6         | 6         | 6         | 2        | 2        | 0                        | 0        | 1        | 0        | 6         | 5         | 6         | 5         | 2        | 2        |
| <b>Total</b> |       |                     | <b>0</b>            | <b>2</b> | <b>17</b> | <b>17</b> | <b>21</b> | <b>20</b> | <b>21</b> | <b>20</b> | <b>4</b> | <b>4</b> | <b>0</b>                 | <b>0</b> | <b>4</b> | <b>3</b> | <b>19</b> | <b>19</b> | <b>19</b> | <b>19</b> | <b>4</b> | <b>4</b> |

MU: Mega (10<sup>6</sup>) International units; Ab: antibody; SC: subcutaneous; IM: intramuscular

- By Day 15 (3<sup>rd</sup> dose), a substantial number of dosed animals were positive for binding antibodies; however, there were a limited number of animals that had tested positive for neutralizing antibodies.
- By Days 29 and 36, all animals dosed with PEGIFN β-1a were positive for neutralizing antibodies.
- On Day 65, all animals in the high dose groups [sc and im] remained positive for neutralizing antibodies to PEGIFN β-1a.

**Stability and Homogeneity**

Dose solution analysis is summarized in Sponsor’s table, below.

| Group Number | Nominal Conc. ( $\mu\text{g/mL}$ ) | Mean Assayed Conc. Week 1 ( $\mu\text{g/mL}$ ) <sup>a</sup> | Mean Assayed Conc. Week 5 ( $\mu\text{g/mL}$ ) <sup>a</sup> | Average % Difference from Nominal Conc. <sup>b</sup> |
|--------------|------------------------------------|---|---|--|
| 1            | 0                                  | 0   | 0   | 0  |
| 2            | 5                                  | 4.0465  | 3.889   | -20.65   |
| 3            | 25                                 | 23.705  | 24.47   | -3.66  |
| 4/5          | 250                                | 218.65  | 215.5   | -13.17   |

Conc. = concentration

<sup>a</sup> Numbers represent an average of four determinations (pre- and post dose aliquots) at each week.

<sup>b</sup> Numbers represent an average of eight determinations (pre- and post dose aliquots) at Weeks 1 and 5.

## 7 Genetic Toxicology

The Sponsor conducted an *in vitro* genotoxicity assessment (Ames assay, *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes) on BIIB017 since the sponsor's DEREK analysis for 20kDa mPEG-O-2-methylpropionaldehyde, which is reacted with interferon beta-1a to produce BIIB017, revealed a structural alert for genotoxicity.

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: BIIB017: Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

|                                     |  |
|-------------------------------------|--|
| Study no.:                          | P017-11-02   |
| Study report location:              | eCTD 0000; 4.2.3.3.1   |
| Conducting laboratory and location: |  (b) (4) |
| Date of study initiation:           | May 11, 2011   |
| GLP compliance:                     | Yes (US)   |
| QA statement:                       | Yes  |
| Drug, lot #, and % purity:          | BIIB017, RECD16283-ENG-01, 97.7%   |

**Methods**

Strains: *S. typhimurium*: TA1535, TA1537, TA98, and TA100  
*E. coli*: WP2uvrA

Concentrations in definitive study: 0, 11.2, 35.4, 112, 354, and 1120 mcg/plate  
 Basis of concentration selection: Highest concentration selected was based upon the maximum concentration that was achievable due to the employment of the maximum volume of drug at the highest concentration available.

S9 Mix: SD rat liver fraction, Aroclor-induced

Negative control: Untreated and 3 vehicle controls of 100 µL, 316 µL, and 1000 µL

Positive control: (-S9): Sodium azide (NaAz), 9-Aminoacridine (9AC), 2-Nitrofluorene (2NF), 4-Nitroquinoline N-oxide (NQO)

(+S): 2-Aminoanthracene (2AA)

Formulation/Vehicle: BIIB017 Placebo: (b) (4) sodium acetate, (b) (4) arginine-HCl, (b) (4) Polysorbate 20, pH 4.8

Vehicle for positive controls: DMSO

Incubation & sampling time: Plate Incorporation Method: 67 h 37 min  
 Pre-incubation Method: 67 h 37 min

**Study Validity**

As conducted, this assay has met the criteria for a valid study as the spontaneous mutant frequency and the positive control responses for each strain were within the range of the historical controls for this laboratory.

**Results**

In the absence or presence of metabolic activation, BIIB017 did not increase the mutant frequency in any of the tested bacterial strains.

## 7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: BIIB017: In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes

Study no.: P017-11-01  
 Study report location: EDR 4.2.3.3.1.  
 Conducting laboratory and location: [REDACTED] (b) (4)  
 Date of study initiation: May 13, 2011  
 GLP compliance: Yes (US)  
 QA statement: Yes  
 Drug, lot #, and % purity: BIIB017, RECD16283-ENG-01, 97.7%

### Methods

Cell line: Human peripheral blood lymphocytes isolated from a healthy donor  
 Concentrations in definitive study: 50, 112, 224 mcg/mL  
 Basis of concentration selection: Highest concentration tested was based upon the maximum practical based upon the supplied concentration of the drug product and maximum dosing volume compatible with this test system.  
 S9 Preparation: Sprague Dawley rat liver, Aroclor-induced  
 Negative control: Untreated and Vehicle control  
 Positive control: -S9: Mitomycin C (MMC: 0.05 and 0.1 mcg/mL)  
 +S9: Cyclophosphamide monohydrate (CP: 8 mcg/mL)  
 Formulation/Vehicle: Vehicle: [REDACTED] (b) (4) sodium acetate, [REDACTED] (b) (4) arginine-HCl, [REDACTED] (b) (4) Polysorbate 20, pH 4.8  
 Incubation & sampling time: ±S9: 4 hr treatment  
 -S9: 21 hr treatment

### Study Validity

As conducted, this study met the criteria for a valid assay as the number of cells with spontaneous chromosomal aberrations was consistent with historical control data and the positive controls (mitomycin C and cyclophosphamide) induced a clear increase in the number of cells with chromosomal aberrations.

### Results

In the absence (4 hr and 21 hr) or in the presence of metabolic activation (4 hr), BIIB017 did not induce chromosomal aberrations (chromosome gaps) nor produce any evidence of increased polyploidy in human peripheral blood lymphocytes.

## **8 Carcinogenicity**

BIIB017 is not pharmacologically active in rat or mouse; therefore, the carcinogenic potential of BIIB017 has not been evaluated.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

Study title: BIIB017: A study for the effects on the menstrual cycle of rhesus monkeys when administered BIIB017 subcutaneously

|                                     |                                 |
|-------------------------------------|---------------------------------|
| Study no.:                          | P017-09-02                      |
| Study report location:              | EDR 4.2.3.5.1                   |
| Conducting laboratory and location: | (b) (4)                         |
| Date of study initiation:           | September 30, 2009              |
| GLP compliance:                     | Yes                             |
| QA statement:                       | Yes                             |
| Drug, lot #, and % purity:          | BIIB017, Lot VVIB12, 98%        |
|                                     | Specific Activity: 1 mg = 84 MU |

**Methods**

Doses: 0, 2.5, 125 mcg/kg/week  
 Frequency of dosing: Once, weekly  
 Dose volume: 0.5 mL/kg  
 Route of administration: Subcutaneous injection, bolus  
 Formulation/Vehicle: (b) (4) acetic acid, (b) (4) arginine HCL,  
 (b) (4) Tween 20 pH 4.8  
 Species/Strain: Monkey/rhesus macaque (*Macaca mulatta*)  
 Number/Sex/Group: 5F/group  
 Study design: Menstrual cycles were evaluated for two acclimation periods (AC1 and AC2), the mean baseline AC was calculated. Animals were dosed by sc injection once weekly commencing on menses day 2 during the dosing cycle (DC). Dosing was stopped if the animal experienced either the next menses cycle or administration of up to 5 doses of a once weekly sc bolus injection, whichever occurred first. This study was limited to 5 weeks, because immunogenicity develops in the monkey within this period. Following DC, the animals were monitored for two recovery cycles (RC1 and RC2).

Deviation from study protocol:

- Pre-study Test Article characterization detailed in a certificate of analysis and QC-51 (stability analysis for drug product)
- Sample bio-analysis for anti-PEG Immunogenicity
- Potency analysis was conducted under GMP regulations
- Long-term stability of 17-beta estradiol and progesterone in rhesus monkey serum was not tested.
- Premature signing of the TK/Immunogenicity reports by the PI prior to the signature of each bioanalytical report

**Dose Justification**

The dose levels were selected based on the results of the fertility (P9216-93-11) and embryo-fetal development toxicity studies (P9216-93-10) that were conducted for the licensing of Avonex (interferon beta-1a, BLA 103628), in which the highest dose administered reduced progesterone concentrations. The high dose (125 mcg/kg) was selected, since this dose was equivalent to the high dose administered in the previous study, in which progesterone levels were affected. The low dose (2.5

mcg/kg) was selected as it had a similar specific activity to the low dose from the previous studies, in which progesterone levels were unaffected.

**Observations and Results**

**Mortality/Clinical Signs** were checked twice daily (AM, then at least 4 hr post AM observation).

- No treatment-related effect was observed at either dose.

**Menses Check** Each female was swabbed daily for menstrual bleeding, beginning on the second day of the animal selection phase. Any cycle duration ≥70 days was recorded as 70, and it was interpreted as amenorrhea.

- Baseline mean menstrual cycle lengths were similar among Groups 1-3 during the acclimation periods.
- At the HD, mean menstrual cycle length was increased (2/5 animals, 47 days and 61 days) compared to the baseline mean cycle length for this group, as well as compared to placebo and LD groups.
- The group means are summarized in the following Sponsor-provided data table.

Sponsor Reference Number: P017-09-02  
Summary Menstrual Cycle Data

(b) (4)  
Confidential

(b) (4) 205.11

| Group / Dose Level  |            | First Acclimation Cycle (AC1) | Second Acclimation Cycle (AC2) | Average of Acclimation Cycles (Baseline) | Dosing Cycle (DC)   |               | First Recovery Cycle (RC1) |               | Second Recovery Cycle (RC2) |               |
|---------------------|------------|-------------------------------|--------------------------------|--|---------------------|---------------|----------------------------|---------------|-----------------------------|---------------|
|                     |            | Cycle Length (days)           | Cycle Length (days)            | Cycle Length (days)                      | Cycle Length (days) | % to Baseline | Cycle Length (days)        | % to Baseline | Cycle Length (days)         | % to Baseline |
| Group 1 (0 µg/kg)   | Group Mean | 28                            | 34                             | 31                                       | 27                  | 92%           | 22                         | 75%           | 25                          | 83%           |
|                     | Group SD   | 6                             | 10                             | 6  | 1                   | 21%           | 4                          | 24%           | 4                           | 24%           |
|                     | Min.       | 22                            | 26                             | 26                                       | 25                  | 67%           | 17                         | 45%           | 19                          | 59%           |
|                     | Max.       | 38                            | 50                             | 38                                       | 29                  | 112%          | 26                         | 100%          | 30                          | 115%          |
| Group 2 (2.5 µg/kg) | Group Mean | 28                            | 26                             | 27                                       | 26                  | 98%           | 28                         | 104%          | 35                          | 130%          |
|                     | Group SD   | 4                             | 3                              | 3  | 4                   | 14%           | 8                          | 18%           | 20                          | 73%           |
|                     | Min.       | 24                            | 23                             | 24                                       | 20                  | 74%           | 23                         | 85%           | 20                          | 85%           |
|                     | Max.       | 34                            | 30                             | 32                                       | 31                  | 108%          | 42                         | 131%          | 70                          | 259%          |
| % to Group 1 Mean   |            | 100%                          | 75%                            | 86%                                      | 96%                 | 107%          | 126%                       | 139%          | 140%                        | 156%          |
| Group 3 (125 µg/kg) | Group Mean | 26                            | 29                             | 28                                       | 37                  | 133%          | 23                         | 84%           | 20                          | 133%          |
|                     | Group SD   | 3                             | 6                              | 3  | 16                  | 43%           | 6                          | 22%           | 35                          | 50%           |
|                     | Min.       | 22                            | 23                             | 25                                       | 23                  | 94%           | 15                         | 48%           | 20                          | 106%          |
|                     | Max.       | 29                            | 36                             | 32                                       | 61                  | 194%          | 28                         | 104%          | 20                          | 222%          |
| % to Group 1 Mean   |            | 94%                           | 85%                            | 89%                                      | 138%                | 145%          | 103%                       | 112%          | 81%                         | 159%          |

**Hormone Analysis** Blood samples were collected in serum separator tubes on MDs 2, 7, 9, 11, 13, 15, 17, 21, 23, 27, and 30, as well as every 5 days thereafter until the onset of the next menstruation in each cycle. Progesterone (Prog) and 17-beta estradiol (E2) concentrations were measured using a solid-phase chemiluminescent immunoassay and an extraction method followed by a RIA.

*Acclimation cycles (AC1, AC2); establishment of baseline Groups 1-3*

- Menstrual cycle length ranged from 22 to 38 days. In Group 1 (1/5), animal #SSAN 9, had an AC2 of 50 days; however, analysis of serum hormone levels, suggested that during this period, 2 menstrual cycles occurred—each of 25 days. The calculated baseline AC was between 23.5 to 37.5 days; this period was considered within the range of normal for rhesus monkeys (Gilardi et al., 1997).
- The peak E2 concentration occurred on menses days 11 and 12 (MD11, Group 3) and MD12 (Groups 1 and 2). The mean peak E2 concentration was similar for Groups 1, 2, and 3 with values of 96 pg/mL, 97 pg/mL, and 92 pg/mL, respectively.
- The peak progesterone concentration occurred on MD22, MD19, or MD18 in Groups 1, 2, and 3 respectively. The mean peak progesterone concentration was similar for Groups 1, 2, and 3 with values of 5.1 ng/mL, 5.9 ng/mL, and 5.3 ng/mL, respectively. The hormone data from AC2 are summarized in Sponsor's Text Table 4.

**Text Table 4 Summary hormone parameters in the second acclimation cycle**

| Group<br>(Dose)       | Cycle Length<br>(Days):<br>Mean<br>(Range) | MD of the Peak:<br>Mean (Range) |                   | Intervals (Days):<br>Mean (Range) |  | Peak Concentration:<br>Mean (Range) |                      |
|-----------------------|--|---------------------------------|-------------------|-----------------------------------|--|-------------------------------------|----------------------|
|                       |  | E2                              | Prog              | E2 and<br>Prog<br>Peaks           | Prog Peak<br>and the<br>Next<br>Menses | E2<br>(pg/mL)                       | Prog<br>(ng/mL)      |
| 1<br>(Control)        | 34<br>(26 – 50)*                           | 12<br>(9 – 15)                  | 22<br>(17 – 30)   | 10<br>(4 – 15)                    | 9<br>(4 – 14)                          | 96<br>(49 – 176)                    | 5.1<br>(2.3 – 11.6)  |
| 2<br>(2.5 µg/kg/week) | 26<br>(23 – 30)                            | 12<br>(7 – 23)                  | 19<br>(13 – 27)   | 7<br>(4 – 10)                     | 8<br>(4 – 13)                          | 97<br>(40 – 190)                    | 5.9<br>(1.9 – 11.2)  |
| 3<br>(125 µg/kg/week) | 29<br>(23 – 36)                            | 11<br>(9 – 15)                  | 18**<br>(15 – 23) | 7**<br>(4 – 8)                    | 11**<br>(9 – 14)                       | 92<br>(16 – 202)                    | 5.3**<br>(3.0 – 8.1) |

\* SSAN 9 had a 50-day cycle but this was considered to consist of two 25-day cycles based upon hormone data.

\*\* A Prog peak was not detected in SSAN 31.

*Dosing cycle (DC); Group 1: Control, Group 2: LD, Group 3: HD*

- At the HD (2/5), prolonged menstrual cycles (47 days, SSAN33 and 62 days, #SSAN31) were observed; in these animals, 5 doses of BIIB017 were administered and on Day 29, systemic exposure ( $AUC_{0-24h}$ ) was 34-44% that observed on Day 1. One HD animal (SSAN 23) had systemic exposure ( $AUC_{0-24h}$ ) at Day 22 that was 38% that observed on Day 1; however, no increase in mean cycle duration was observed.
- Mean day of menses, in which the peak level of E2 or Prog occurred, was delayed following BIIB017 administration (2.5 and 125 mcg/kg). Notably, at the HD, the mean delay between the two hormonal peaks was greatest, and the mean and range of the peak E2 and Prog levels were decreased compared to the Control and LD groups. These hormone data are summarized in Sponsor's Text Table 5, below.

**Text Table 5 Summary hormone parameters in the dosing cycle**

| Group (Dose)          | Cycle Length (Days): Mean (Range) | MD of the Peak: Mean (Range) |                   | Intervals (Days): Mean (Range) |                               | Peak Concentration: Mean (Range) |                      |
|-----------------------|-----------------------------------|------------------------------|-------------------|--------------------------------|-------------------------------|----------------------------------|----------------------|
|                       |                                   | E2                           | Prog              | E2 and Prog Peaks              | Prog Peak and the Next Menses | E2 (pg/mL)                       | Prog (ng/mL)         |
| 1<br>(Control)        | 27<br>(25 – 29)                   | 11<br>(9 – 13)               | 18<br>(13 – 21)   | 6<br>(4 – 10)                  | 10<br>(7 – 13)                | 68<br>(37 – 96)                  | 4.0<br>(1.6 – 6.5)   |
| 2<br>(2.5 µg/kg/week) | 26<br>(20 – 31)                   | 13<br>(9 – 21)               | 22*<br>(17 – 27)  | 8*<br>(4 – 12)                 | 7*<br>(5 – 12)                | 50<br>(19 – 72)                  | 4.4*<br>(2.7 – 6.1)  |
| 3<br>(125 µg/kg/week) | 37<br>(23 – 61)                   | 14**<br>(9 – 17)             | 25**<br>(15 – 40) | 11**<br>(6 – 25)               | 8**<br>(1 – 13)               | 54**<br>(26 – 89)                | 3.6**<br>(1.2 – 5.6) |

\* A Prog peak was not detected in SSAN 21.

\*\* E2 and Prog peaks were not detected in SSAN 31.

*Recovery cycles (RC1, RC2); Group 1: Control, Group 2: LD, Group 3: HD*

- In RC1, mean menstrual cycle lengths and mean menstrual day of peak E2 concentrations was similar amongst the treatment groups; however, at the HD, a delay in the mean menstrual day of peak progesterone occurred that was 5 days longer than placebo; expectedly, the interval between E2 and Prog peaks was increased. In contrast, a reduction in the interval between peak progesterone and the next menses was observed. These hormone data are summarized in Sponsor's Text Table 6, below.
- At the HD, SSAN 31 had mean menstrual cycle lengths that were within the range of normal (28 to 33 days) during the recovery cycles; however, SSAN 33 had a shorter abnormal 15 day cycle (RC1), which was followed by amenorrhea during RC2.

**Text Table 6 Summary hormone parameters in the first recovery cycle**

| Group (Dose)          | Cycle Length (Days): Mean (Range) | MD of the Peak: Mean (Range) |                    | Intervals (Days): Mean (Range) |                               | Peak Concentration: Mean (Range) |                       |
|-----------------------|-----------------------------------|------------------------------|--------------------|--------------------------------|-------------------------------|----------------------------------|-----------------------|
|                       |                                   | E2                           | Prog               | E2 and Prog Peaks              | Prog Peak and the Next Menses | E2 (pg/mL)                       | Prog (ng/mL)          |
| 1<br>(Control)        | 22<br>(17 – 26)                   | 11<br>(9 – 11)               | 17*<br>(15 – 21)   | 7*<br>(4 – 10)                 | 8*<br>(4 – 10)                | 99<br>(7 – 211)                  | 2.1*<br>(1.8 – 2.3)   |
| 2<br>(2.5 µg/kg/week) | 28<br>(23 – 42)                   | 10<br>(9 – 11)               | 17**<br>(17)       | 8**<br>(8)                     | 9**<br>(8 – 10)               | 77<br>(15 – 121)                 | 6.4**<br>(3.4 – 8.4)  |
| 3<br>(125 µg/kg/week) | 23<br>(15 – 28)                   | 10<br>(9 – 11)               | 22***<br>(21 – 23) | 12***<br>(10 – 14)             | 5***<br>(5)                   | 51<br>(9 – 195)                  | 5.6***<br>(3.6 – 7.6) |

\* A Prog peak was not detected in SSAN 27.

\*\* Prog peaks were not detected in SSAN 19 and SSAN 21.

\*\*\* Prog peaks were not detected in SSAN 29, SSAN 31, and SSAN 33.

**Body Weight** was measured for each animal every two weeks during the acclimation period, and once weekly during the dosing and recovery periods.

- No test-article related effects were observed on body weight during the acclimation, dosing, or recovery periods.

**Food Consumption** was measured once daily for each animal in the morning prior to feeding. Food consumption was based upon the amount of biscuits remaining from the prior feeding.

- No test-article related effects were observed on body weight during the acclimation, dosing, or recovery periods.

**Toxicokinetics** were evaluated from each animal pre-dose and 4, 8, 24, 72, 120, and 168 hr after the first dose; 8 hr after the 3rd dose, and 4, 8, and 24 hr after the 4th dose, when a 5th dose occurred, blood was collected at 4, 8, and 24 hr postdose.

- The individual subject and group mean TK data are summarized in Sponsor's Table 2 (below) for Days 1, 15, 22, and 29.

**Table 2. Individual Subject and Group Mean BIIB017 Toxicokinetic Summary Data; Sorted by Day, Group, Dose, and Subject ID**

| Day | Group | Dose (µg/kg/week) | Subject ID | C <sub>max</sub> (pg/mL) | T <sub>max</sub> (h) | AUC <sub>0-24</sub> (h*pg/mL) | AUC <sub>0-168</sub> (h*pg/mL) | HL <sub>A,Z</sub> (h) |
|-----|-------|-------------------|------------|--------------------------|----------------------|-------------------------------|--------------------------------|-----------------------|
| 1   | 2     | 2.5               | 11         | 3660                     | 24.0                 | 69600                         | 220000                         | 18.2                  |
|     |       |                   | 17         | 7480                     | 8.00                 | 128000                        | 272000                         | 22.7                  |
|     |       |                   | 19         | 5310                     | 24.0                 | 109000                        | 278000                         | 17.3                  |
|     |       |                   | 21         | 6390                     | 8.00                 | 125000                        | 297000                         | 19.3                  |
|     |       |                   | 37         | 6240                     | 24.0                 | 115000                        | 344000                         | 19.9                  |
|     |       |                   | N          | 5                        | 5                    | 5                             | 5                              | 5                     |
|     |       |                   | Mean       | 5820                     | 17.6                 | 109000                        | 282000                         | 19.5                  |
|     |       |                   | SD         | 1430                     | 8.76                 | 23500                         | 44600                          | 2.06                  |
|     | 3     | 125               | 23         | 411000                   | 8.00                 | 7360000                       | 17900000                       | 24.7                  |
|     |       |                   | 29         | 347000                   | 24.0                 | 5600000                       | 16800000                       | 21.9                  |
|     |       |                   | 31         | 461000                   | 8.00                 | 9140000                       | 23100000                       | 22.4                  |
|     |       |                   | 33         | 264000                   | 8.00                 | 5180000                       | 17200000                       | 20.3                  |
|     |       |                   | 49         | 423000                   | 8.00                 | 7660000                       | 18400000                       | 21.4                  |
|     |       |                   | N          | 5                        | 5                    | 5                             | 5                              | 5                     |
|     |       |                   | Mean       | 381000                   | 11.2                 | 6990000                       | 18700000                       | 22.2                  |
|     |       |                   | SD         | 77300                    | 7.16                 | 1610000                       | 2550000                        | 1.64                  |
|     |       |                   | CV%        | 20.3                     | 63.9                 | 23.1                          | 13.6                           | 7.4                   |

**Table 1. Individual Subject and Group Mean BIIB017 Serum Concentration versus Time Data; Sorted by Day, Group, Dose, and Subject ID (continued)**

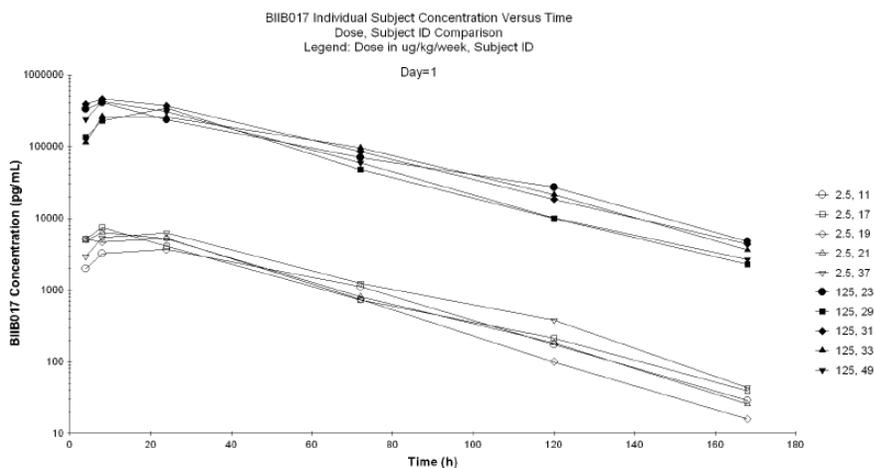
| Day  | Group | Dose (µg/kg/week) | Subject ID | Time (h) |
|------|-------|-------------------|------------|----------|
|      |       |                   |            | 8        |
| 15   | 1     | 0.0               | 5          | 0.00     |
|      |       |                   | 7          | 0.00     |
|      |       |                   | 9          | 0.00     |
|      |       |                   | 27         | 0.00     |
|      |       |                   | 39         | 0.00     |
|      |       |                   | N          | 5        |
|      |       |                   | Mean       | 0.00     |
|      |       |                   | SD         | NC       |
|      | 2     | 2.5               | 11         | 17.8     |
|      |       |                   | 17         | 7270     |
|      |       |                   | 19         | 195      |
|      |       |                   | 21         | 2790     |
|      |       |                   | 37         | 860      |
|      |       |                   | N          | 5        |
|      |       |                   | Mean       | 2230     |
|      |       |                   | SD         | 3030     |
|      | 3     | 125               | 23         | 368000   |
| 29   |       |                   | 58800      |          |
| 31   |       |                   | 704000     |          |
| 33   |       |                   | 352000     |          |
| 49   |       |                   | 2900       |          |
| N    |       |                   | 5          |          |
| Mean |       |                   | 297000     |          |
| SD   |       |                   | 281000     |          |
| CV%  | 94.7  |                   |            |          |

Variable = Concentration (pg/mL)

| Day  | Group  | Dose (µg/kg/week) | Subject ID | C <sub>max</sub> (pg/mL) | T <sub>max</sub> (h) | AUC <sub>0-24</sub> (h*pg/mL) |
|------|--------|-------------------|------------|--------------------------|----------------------|-------------------------------|
| 22   | 2      | 2.5               | 11         | 0.00                     | NC                   | NC                            |
|      |        |                   | 17         | 5690                     | 8.00                 | 108000                        |
|      |        |                   | 19         | 0.00                     | NC                   | NC                            |
|      |        |                   | 37         | 38.8                     | 4.00                 | 410                           |
|      |        |                   | N          | 4                        | 2                    | 2                             |
|      |        |                   | Mean       | 1430                     | 6.00                 | 54300                         |
|      |        |                   | SD         | 2840                     | NA                   | NA                            |
|      | CV%    | 198.2             | NA         | NA                       |                      |                               |
|      | 3      | 125               | 23         | 138000                   | 8.00                 | 2580000                       |
|      |        |                   | 29         | 9610                     | 4.00                 | 155000                        |
|      |        |                   | 31         | 261000                   | 24.0                 | 4050000                       |
|      |        |                   | 33         | 105000                   | 24.0                 | 1750000                       |
|      |        |                   | 49         | 2950                     | 4.00                 | 31000                         |
|      |        |                   | N          | 5                        | 5                    | 5                             |
| Mean |        |                   | 103000     | 12.8                     | 1710000              |                               |
| SD   | 106000 | 10.4              | 1690000    |                          |                      |                               |
| CV%  | 102.6  | 80.9              | 98.8       |                          |                      |                               |
| 29   | 3      | 125               | 31         | 21300                    | 8.00                 | 369000                        |
|      |        |                   | 33         | 44700                    | 24.0                 | 764000                        |
|      |        |                   | N          | 2                        | 2                    | 2                             |
|      |        |                   | Mean       | 33000                    | 16.0                 | 566000                        |
|      |        |                   | SD         | NA                       | NA                   | NA                            |
|      |        |                   | CV%        | NA                       | NA                   | NA                            |

- Day 1, systemic exposure was achieved following subcutaneous administration of BIIB017 (2.5 and 125 mcg/kg); T<sub>max</sub> values ranged from 11.2 to 17.6 hr following subcutaneous dosing, and half-life values were 19.5 and 22.2 hr, respectively. Mean exposure to BIIB017 (C<sub>max</sub>, AUC<sub>0-24</sub>, and AUC<sub>0-168</sub>) increased in a dose-related manner. The BIIB017 concentration-time profile for the individual animals from Groups 2 and 3 are illustrated in Sponsor's Figure 1A.

Figure 1A. BIIB017 Individual Subject Concentration versus Time—Dose and Subject ID Comparison; Day 1

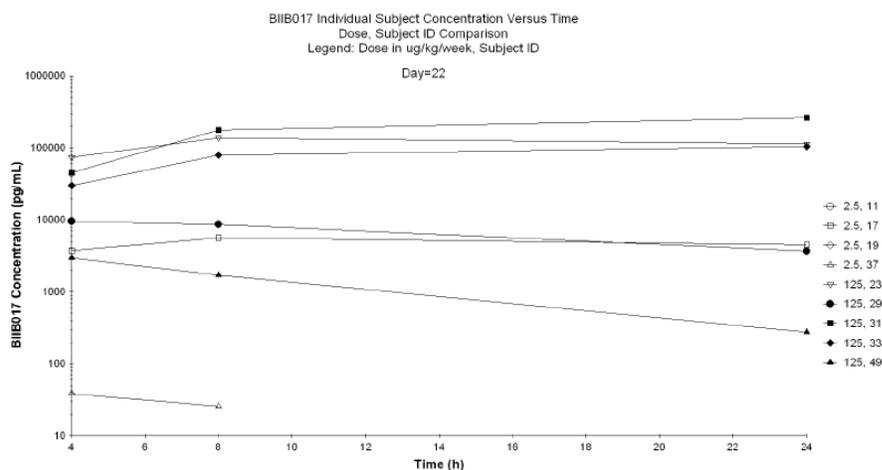


- Day 15 [8 hr postdose], at the LD, marked reduction in exposure (C<sub>max</sub>) occurred in 4/5 animals compared to Day 1 values. As a percent reduction from Day 1 C<sub>max</sub>, the values for each animal were 2.9% [SSAN 11], 56.3%

[SSAN 21], 86.2% [SSAN 37], and 96.3% [SSAN 19]. In contrast, at the HD, reduction in exposure ( $C_{max}$ ) occurred in 2/5 animals compared to Day 1 values. As a percent reduction from Day 1  $C_{max}$  values, the most affected were by 83.1% [SSAN 29] and 99.3% [SSAN 49]; in contrast 3/5 had a slight reduction or an increased exposure ( $C_{max}$ ) compared to Day 1 values, including -10.5% [SSAN 23] or +153% [SSAN 31] and +133% [SSAN 33].

- Day 22, at the LD, 1/4 animals had systemic exposure comparable to Day 1 [SSAN #17]; at the HD, 3/5 animals [SSAN 23, SSAN 31, and SSAN 33] had systemic exposures based on  $AUC_{0-24hr}$  that were approximately 34-44% those achieved on Day 1. The BIIB017 concentration-time profiles for the individual animals are illustrated in Sponsor's Figure 2A

Figure 2A. BIIB017 Individual Subject Concentration versus Time—Dose and Subject ID Comparison; Day 22



- Day 29, HD #SSAN 31 and #SSAN 33 were administered the 5<sup>th</sup> dose of BIIB017; however, following this dose, systemic exposure ( $AUC_{0-24hr}$ ) was limited to approximately 4% and 14.7% of the Day 1  $AUC_{0-24hr}$  exposure, respectively.
- As noted below, the reduction in BIIB017 exposure correlated with the appearance of anti-drug antibodies.

**Immunogenicity** was evaluated from each animal pre-dose on Days 1, 22, and 29 for the presence of antidrug antibodies directed against interferon beta-1a and/or PEG; these data are summarized in the Sponsor's Tables 3 and 4, respectively. It is noted, however, that the Sponsor did not request titers for the neutralizing antibodies.

- Following repeated sc administration of BIIB017, animals dosed at 2.5 mcg/kg and 125 mcg/kg were positive for interferon beta-1a antibodies (4/5 and 5/5, respectively).

- Likewise, animals dosed at 2.5 mcg/kg (5/5) and 125 mcg/kg (2/5) were positive for PEG antibodies on at least one TK collection interval.
- The reduction or ablation of BII017 systemic exposure achieved on Days 22 and 29 of the Dosing Cycle, clearly demonstrated the neutralizing effect of the ADAs, even though, titers to quantify the magnitude of the neutralizing antibody response was not evaluated.

Table 3. Summary of Individual ADA Status (Anti-interferon ADA); Sorted by Group, Dose and Subject ID

| Group | Dose (µg/kg/week) | Subject ID | Day        |            |            |            |
|-------|-------------------|------------|------------|------------|------------|------------|
|       |                   |            | 1          | 22         | 29         | 41         |
| 1     | 0.0               | 5          | NEG        | NEG        | NEG        | —          |
|       |                   | 7          | NEG        | NEG        | NEG        | —          |
|       |                   | 9          | NEG        | NEG        | NEG        | —          |
|       |                   | 27         | NEG        | NEG        | —          | —          |
|       |                   | 39         | NEG        | NEG        | NEG        | —          |
| 2     | 2.5               | 11         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 17         | NEG        | NEG        | NEG        | —          |
|       |                   | 19         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 21         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 37         | NEG        | <b>POS</b> | <b>POS</b> | —          |
| 3     | 125               | 23         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 29         | <b>POS</b> | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 31         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 33         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 49         | NEG        | <b>POS</b> | —          | <b>POS</b> |

Variable = ADA Status

Table 4. Summary of Individual ADA Status (Anti-PEG ADA); Sorted by Group, Dose and Subject ID

| Group | Dose (µg/kg/week) | Subject ID | Day        |            |            |            |
|-------|-------------------|------------|------------|------------|------------|------------|
|       |                   |            | 1          | 22         | 29         | 41         |
| 1     | 0.0               | 5          | NEG        | NEG        | NEG        | —          |
|       |                   | 7          | NEG        | NEG        | NEG        | —          |
|       |                   | 9          | NEG        | NEG        | NEG        | —          |
|       |                   | 27         | NEG        | NEG        | —          | —          |
|       |                   | 39         | <b>POS</b> | NEG        | NEG        | —          |
| 2     | 2.5               | 11         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 17         | NEG        | <b>POS</b> | NEG        | —          |
|       |                   | 19         | NEG        | <b>POS</b> | NEG        | —          |
|       |                   | 21         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 37         | NEG        | <b>POS</b> | <b>POS</b> | —          |
| 3     | 125               | 23         | NEG        | NEG        | NEG        | —          |
|       |                   | 29         | NEG        | <b>POS</b> | <b>POS</b> | —          |
|       |                   | 31         | NEG        | NEG        | NEG        | —          |
|       |                   | 33         | NEG        | NEG        | NEG        | —          |
|       |                   | 49         | NEG        | <b>POS</b> | —          | <b>POS</b> |

Variable = ADA Status

### Stability and Homogeneity

- All dose formulations that were measured for mean BII017 concentrations were within the acceptable limits of  $\pm 10\%$  of nominal concentration for the low range and  $\pm 15\%$  of nominal concentration for the high range.
- Homogeneity was acceptable at  $\leq 5\%$  RSD for all dose formulations.

- The specific activity of BIIIB017 was within the acceptable range, based upon a validated assay.

## 9.2 Embryonic Fetal Development

The Sponsor re-submitted their embryo fetal development study (P9216-93-10) that evaluated the effects of interferon beta-1a (BG9216) in rhesus monkey as support for BIIIB017. This study was previously reviewed for licensure of Avonex®, (*cf, Anne M. Pilaro, Ph.D., Toxicologists Review of BLA 103628*). The initial study details are provided as well as a summary of the data considered relevant for the purpose of licensure and product labeling of BIIIB017. In addition, some of Sponsor's graphical data are provided.

Study title: BG9216 (r-HuIFN- $\beta$ ) rhesus monkey reproduction (developmental evaluation)

|                                     |   |
|-------------------------------------|---|
| Study no:                           | P9216-93-10                                       |
| Study report location:              | EDR 4.2.3.5.1                                     |
| Conducting laboratory and location: | (b) (4)<br>(in-life)                              |
|                                     | (b) (4)<br>(Serum progesterone measurements)      |
| Date of study initiation:           | October 1, 1993                                   |
| GLP compliance:                     | Yes   |
| QA statement:                       | Yes   |
| Drug, lot #, and % purity:          | interferon-beta-1a, BG9216 Lot 16Z02Q and 16Z03Q, |

## Methods

Doses: C1: Saline; C2: Placebo; LD: BG9216, 0.25 MU/kg; HD: BG9216, 10 MU/kg  
 Frequency of dosing: Every other day, 15 doses in total  
 Dose volume: Grp 1, 2, and 4: 0.83 mL/kg  
 Grp 3: 0.17 mL/kg  
 Route of administration: Subcutaneous injection, dorsal region  
 Formulation/Vehicle: Saline = 0.9% sterile saline for injection  
 placebo= 1.5% HAS in PBS  
 Species/Strain: Monkey/rhesus (*Macaca mulatta*)  
 Number/Sex/Group: 35 F, pregnant (total); 5/saline control; 10/group  
 in each: INF-beta placebo, INF-beta dose groups (2)

## Study Design

- Treatments were administered by sc injection every other day to pregnant animals during GD21-GD49, for 15 doses.
- Fetal monitoring occurred by ultrasound examinations that were performed on GD19 (predose), 25, 30, 40, 50, 70, and 100.
- On GD100  $\pm$  2 days, fetuses were obtained by Caesarean delivery; standard teratology evaluations were performed, including physical measurements, whole body, organ, and placental weights, amniotic fluid volumes, and gross evaluation of the viscera, and a complete evaluation of the heart. Following gross evaluation, all tissues and carcasses were preserved in appropriate fixative, stained with Alizarin red S, and evaluated for skeletal and soft tissue abnormalities.
- Female reproductive capacity was monitored by assessments of serum progesterone levels (determined by RIA), serum interferon-beta levels (ELISA for BG9216 and cytopathic effect bioassay to detect interferon beta activity), and antidrug BG9216 antibodies (binding and neutralizing; measured on GD19 and once weekly until GD100). Serum progesterone was measured predose on GD19, and then once every 3 days during the dosing period GD23-41 ( $\pm$  1 day).
- Maternal toxicity was evaluated by assessing physical signs (AM and 4 hr postdose), body weights (prior to dosing, then once weekly), rectal body temperature (immediately prior to dosing and 4 hr postdose on GD21, 23, 27, 31, 33, 41, and 49), and serum hematology and biochemistry profiles (prior to dosing on GD19, 30, 40, 50, 70, and 100).

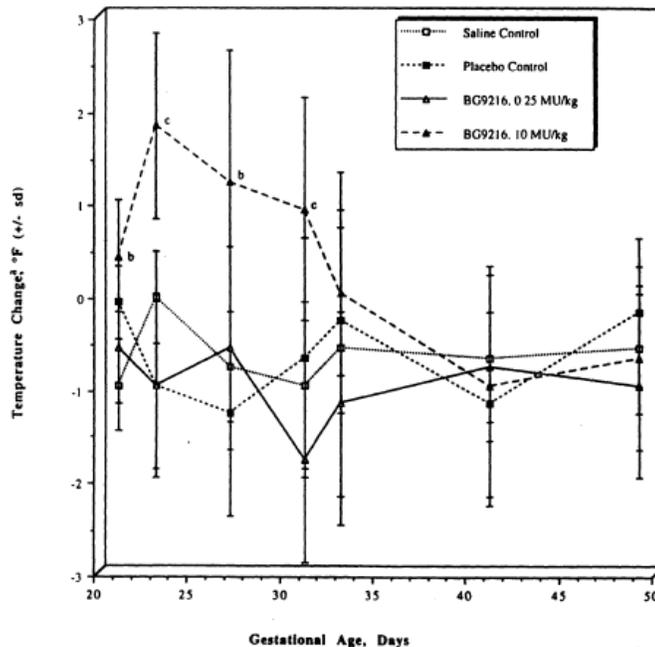
## Key Study Findings

- No evidence of maternal toxicity was identified in this study; loss of body weight occurred in all dose groups and clinical signs were variable (loose or mucus stools observed, in all groups). At the HD, 2 animals developed rashes on the arms and backs, #23230 (GD25 and GD27) and #22956

(GD47-48). The rashes observed on #22956 resolved by GD49 and on #23230 by GD28 following dose discontinuation.

- Elevated body temperatures measured 4 hr postdose compared to predose occurred in interferon beta-1a treated animals; temperature elevation is a known effect of Type 1 interferon treatment in monkey as well as in human. A dose-related increase in temperature elevation occurred on GD21, GD27, and GD31; elevations were markedly decreased on GD33 and were no longer significant from saline or placebo control (see Sponsor's Figure 1, below).

Figure 1. Maternal Body Temperature Change



a. Temperature change = four hrs postdose temperature minus predose temperature, averaged by group.  
 b. Indicates timepoints which are different statistically ( $p < 0.05$ ).  
 c. Indicates time points which were not evaluated statistically due to unbalanced data.

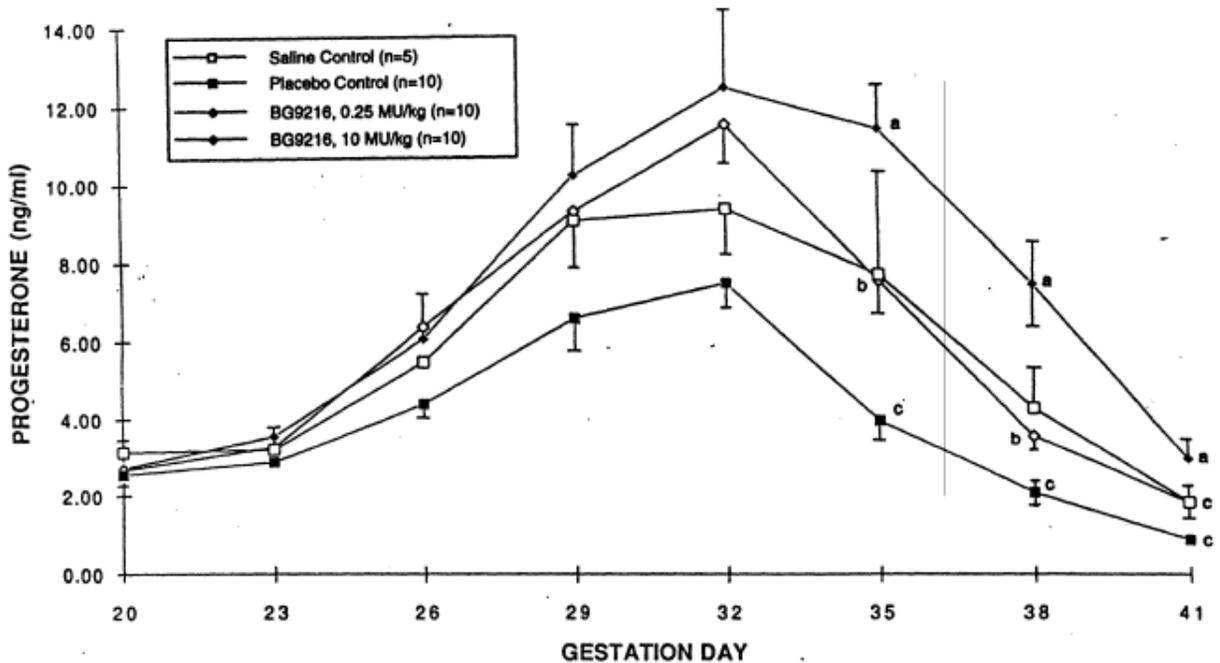
- One placebo animal (#23665) aborted on GD50. Ultrasound evaluations on GD20, 24, 30, and 40 showed a normal viable fetus; however, on GD50, an incomplete abortion was observed and on GD55, a nongravid uterus was present.
- At the HD, 2/10 animals #23318 (GD26) and #23230 (GD28) aborted following 3 to 5 doses, respectively. Ultrasounds performed on GD21 and GD23 showed normal viable fetuses in both animals. Although maternal toxicity was not observed in this study, Dr. Pilaro concluded that the finding of 2 abortions occurring at the HD (GD26 and GD28) was considered evidence of adequate dosing and no additional studies were requested for licensure.
  - This information is documented in the product labeling for Avonex:

In pregnant monkeys given interferon beta at 100 times the recommended weekly human dose (based upon a body surface area [ $\text{mg}/\text{m}^2$ ] comparison),

no teratogenic or other adverse effects on fetal development were observed. Abortifacient activity was evident following 3 to 5 doses at this level. No abortifacient effects were observed in monkeys treated at 2 times the recommended weekly human dose (based upon  $\text{mg}/\text{m}^2$ ).

- 
- One maternal death (#22135) occurred in the placebo group on GD95; the cause of death was hypoxia secondary to severe pulmonary edema, and not treatment-related.
- Although the rate of spontaneous abortion between placebo and BG9216 were similar, Dr. Pilaro concluded that the abortions that occurred at the HD were consistent with similar findings of abortifacient effects of other interferons.
- Fetal examinations showed no gross malformations in either control article or either dose of BG9216; however, fetal adrenal weights were significantly smaller at both doses of BG9216. No pathologic effects were observed by histology relative to controls. Skeletal abnormalities included extra rudimentary ribs or missing rib pairs, and additional or missing thoracic, cervical or lumbar vertebrae; however, these findings were observed at a low incidence and occurred in all groups (saline control, 2 fetuses; placebo control, 1 fetus, LD, 1 fetus, and HD, 1 fetus) and were considered within normal limits for rhesus macaques. Placentas were found normal; no other treatment-related differences between control and BG9216 were found in fetal weights, measurements, skeletal or soft tissue anomalies noted.
- In all animals on study, serum progesterone levels were measured on GD20, 23, 26, 29, 32, 35, 38, and 41. Serum progesterone increased in a time-dependent manner, peaking around GD32 and declining until GD41; it is noted, at most time points measured after GD29, serum progesterone levels were noticeably higher in animals at the HD (see Sponsor's Figure 3, below). In the 2 HD animals that aborted (#23318 and #23230), normal progesterone levels were measured on GD20 and GD23; however, on GD26 a marked reduction in serum progesterone (10-fold and 17-fold, respectively) was observed.

Figure 3. Mean Serum Progesterone Concentrations, GD20-GD41



Points with letters are significantly different ( $p < 0.01$ ) from each other by ANOVA and Duncan's multiple range test. The saline control group was not tested in the ANOVA. Vertical bars indicate SEM.

- At the LD, serum interferon beta activity was detected 4 hr postdose between days GD21 and GD33 in 4/5 animals tested; animal #23372 had detectable interferon beta present on GD49, 4 hr after the final dose. At the HD, serum interferon-beta activity was present after the first dose, and at subsequent dosing, it was observed predose and 4 hr postdose (between GD21 and GD33; in 2 animals this was observed up to GD49).
- By GD49, LD and HD animals were positive for antidrug antibodies detected by ELISA; serum samples remained positive for anti-interferon-beta throughout the remainder of the study. Neutralizing antibody titers were first detected on GD49 at the LD (4/5) and on GD33 at the HD (5/5); at the HD, titers were  $<1:4$  at GD33, but continued to increase to  $>1:972$  in 3/5 animals by the end of treatment at GD49.
- Neither fetal malformations nor evidence of teratogenicity were found following treatment of pregnant rhesus macaques with BG9216 at either 0.25 or 10 MU/kg; however, at 10 MU/kg, spontaneous abortion occurred in 2/10 animals following 3 to 5 injections. No significant maternal toxicities were noted. The NOAEL for interferon-beta-1a is 0.25 MU/kg administered by subcutaneous injection every other day from GD21 to GD49.

## 11 Integrated Summary and Safety Evaluation

Peginterferon beta-1a (PLEGRIDY) is recombinant human interferon beta-1a (IFN  $\beta$ -1a), a glycoprotein that has been chemically modified on the alpha-amino group of the N-terminal amino acid residue by addition of a 20 kDa-mPEG group, which has been submitted as a parenteral treatment for patients with relapsing forms of multiple sclerosis. Interferon beta is one of the first line therapies for the treatment of patients with relapsing forms of multiple sclerosis. Several licensed products are on the market and each one is formulated for parenteral administration by intramuscular or subcutaneous injection at a dosing frequency ranging from 1 to 4 times per week. Although interferon beta is a first line therapy, patient compliance is often low, which has been attributed to frequency of injection and presence of flu-like side effects. To alter the PK profile, the Sponsor pegylated human IFN  $\beta$ -1a; this chemical modification altered the physiochemical characteristics so that the drug product could be formulated for subcutaneous injection at a more favorable dosing frequency of once every two weeks. In the present application, the Sponsor submitted a complete and adequate package of nonclinical studies to make a determination regarding the safety profile of PEGIFN  $\beta$ -1a (BIIB017), as well as, to write the label of prescribing information.

### ***Pharmacology***

Interferon beta is a member of the type 1 family of interferons; it is an endogenous protein produced by fibroblasts, epithelial cells, and macrophages in response to the presence of viral and foreign nucleic acids (for review see, Tyring, S.K., 1995). Type 1 interferons act on their target cells by binding to a common cell surface receptor to initiate a signaling cascade that induces transcription of many IFN-inducible genes (cf., Darnell, J.E., et al., 1994) resulting in many activities that include antiviral, antiproliferative, antitumor, and immunomodulatory effects. The pharmacology of interferon beta is reasonably well established; however, the mechanism by which interferon beta exerts its effects to provide clinical benefit in patients with relapsing forms of multiple sclerosis is unknown. In this application, studies were not conducted to establish a mechanism of action for PEGIFN  $\beta$ -1a either in established animal models of multiple sclerosis (e.g., experimental autoimmune encephalomyelitis) or in patients with multiple sclerosis. However, the Sponsor demonstrated that IFN  $\beta$ -1a and PEGIFN  $\beta$ -1a bound to the same human, Type 1, high affinity interferon receptor subunit (IFNAR2). In fact, the apparent binding affinity of each interferon tested was the same for IFNAR2; taken together, these data suggested that pegylation of IFN  $\beta$ -1a did not alter its binding characteristics to IFNAR2. The selection of the pegylation site was so chosen based upon the x-ray crystallography structure of IFN  $\beta$ -1a bound to the high affinity receptor, IFNAR2 to avoid disruption of its pharmacologic activity (Pepinsky et al., 2001).

### ***Species Selection***

Binding of human interferon to its target receptor localized on human peripheral blood lymphocytes initiates an antiviral response in the lymphocyte that is characterized by the induction of 2',5'-oligo A Synthetase (OAS). In an *ex-vivo* study

conducted to support licensing of Avonex, a concentration-dependent induction of OAS in response to human interferon beta was evaluated in peripheral blood lymphocytes isolated from several nonhuman species, including: mouse, rat, dog, guinea pig, rabbit, rhesus monkey, and cynomolgus monkey. Pharmacologic activity of IFN  $\beta$ -1a was identified in three nonhuman species—guinea pig, dog, and rhesus monkey. In monkey, several pharmacologic and pharmacodynamic effects of IFN  $\beta$ -1a administration have been observed, including: febrile responses, decreased circulating lymphocyte counts, and elevated serum neopterin (biological response marker). Similarly, in the clinic, subjects treated with IFN beta-1a had febrile responses, flu-like sickness, and an elevation of serum neopterin; taken together, these data suggest that the monkey is a good toxicity model to establish the safety profile of PEGIFN  $\beta$ -1a. Based on the above considerations, the guinea pig and rhesus monkey were considered acceptable and adequate models.

### ***PK/ADME***

Several single-dose studies to characterize the PK/ADME of PEGIFN  $\beta$ -1a were conducted in monkey, while others were conducted in rat and mouse. Since human IFN  $\beta$ -1a has no pharmacological activity in the rodent, these studies were considered of limited value.

PK parameters were determined from an absorption study conducted in male monkeys following subcutaneous and intramuscular injection of either PEGIFN  $\beta$ -1a or human IFN  $\beta$ -1a. The dose of PEGIFN  $\beta$ -1a and of IFN  $\beta$ -1a administered was normalized to antiviral activity; therefore, the final dose administered was 1 MU/kg, which corresponds to  $1 \times 10^6$  International units of antiviral activity/kg body weight. The results were consistent with the expected effects of pegylation on biological drugs; PEGIFN  $\beta$ -1a had significantly higher systemic exposure for both  $C_{\max}$  (10 times [im] and 16 times [sc]) and  $AUC_{\text{inf}}$  (63 times [im] and 100 times [sc]) compared to that achieved following IFN  $\beta$ -1a administration. Due to the increase in molecular weight of PEGIFN  $\beta$ -1a, glomerular filtration was reduced and thus total body clearance (CL/F) was reduced (30 times [im] and 44 times [sc]). The reduction in total body clearance likely contributed, at least in part, to the prolonged half-life ( $t_{1/2}$ ) that was 2.1 times [im] and 6.5 times [sc] greater than that of IFN  $\beta$ -1a. Finally, the increased molecular size of PEGIFN  $\beta$ -1a restricted the distribution of PEGIFN  $\beta$ -1a, as evidenced by the reduced volume of distribution ( $V_d/F$ ) that was 8.8-times [im] and 18-times [sc] lower in PEGIFN  $\beta$ -1a treated monkeys. A second study focused on dose-related effects of PEGIFN  $\beta$ -1a administration by subcutaneous injection at doses of (2, 10, and 100 mcg/kg) as well as at by intramuscular injection at the high dose of 100 mcg/kg. Exposure ( $C_{\max}$  and  $AUC_{\text{inf}}$ ) increased in a dose proportional manner; likewise, the  $t_{1/2}$  increased in a dose-related manner (14-21 hr). As expected, the  $T_{\max}$  achieved following subcutaneous injection ranged from 12-24 hr; whereas, the an intramuscular injection resulted in a faster  $T_{\max}$  of 4.3 hr, which is attributed to a more rapid absorption from an intramuscular depot. For the most part, there was not much of a difference observed in either the  $V_d/F$  or the CL/F among the different dose groups and route administered.

Comparison of the tissue distribution of radiolabeled interferons, [ $^{125}$ I]PEGIFN  $\beta$ -1a and [ $^{125}$ I]IFN  $\beta$ -1a was conducted in guinea pigs. Overall, a similar pattern of distribution was achieved; the tissues with the highest and lowest concentration of PEGIFN  $\beta$ -1a were distributed in the same tissues identified when the non-pegylated interferon was administered. The tissues with the highest concentrations were spleen, kidney, liver, and lung; the lowest concentrations were identified in muscle, brain, and spinal cord. Although, the distribution pattern is similar, the actual tissue exposure ( $AUC_{72h}$ ) to PEGIFN  $\beta$ -1a based on is minimal compared to non-pegylated interferon. Comparison of the tissue:serum ratios for each interferon tested in the spleen and kidney illustrates the magnitude of this difference; the exposure ratio of tissue to serum ( $AUC_{72h}$ ), in spleen is 0.63 (PEGIFN  $\beta$ -1a) and is 13.7 (IFN  $\beta$ -1a) and in kidney is 0.44 (PEGIFN  $\beta$ -1a) and is 15.0 (IFN  $\beta$ -1a).

### **Immunogenicity**

In single- and repeated-dose studies of IFN  $\beta$ -1a and PEGIFN  $\beta$ -1a conducted in rhesus monkey, antidrug antibodies (ADAs) have been found. Characterization of the ADAs demonstrated a presence of binding as well as neutralizing antibodies. During the development of IFN  $\beta$ -1a, the immunogenicity response of rhesus monkeys to human interferon beta was demonstrated. ADAs were identified within 2-3 weeks of IFN  $\beta$ -1a administration; characterization of the antibodies showed presence of both binding and neutralizing antibodies. Upon repeated dose administration, there was a reduction in the systemic exposure of drug as well as a concomitant loss of PD effects in response to dose administration.

Similarly, in a single dose PK study (P017-06-03) of PEGIFN  $\beta$ -1a administered on Day 1, presence of ADAs was confirmed by Days 15 and 29. Characterization of these ADAs revealed the presence of binding antibodies and neutralizing antibodies. On Day 15, binding antibodies (4/9) predominated and neutralizing (1/9) were detected; however, by Day 29, almost all animals were shown to have binding and neutralizing antibodies (8/9). Further evaluation of the antibody specificity demonstrated that in animals administered PEGIFN  $\beta$ -1a the majority of the antibodies (binding and especially neutralizing) were directed against the human protein; whereas, some of the antibodies targeted the PEG molecule. Antibodies that targeted the PEG molecule often were binding but did not account for the neutralizing effects. Taken together, these data suggest that the pegylated drug did not lessen the immunogenicity of IFN  $\beta$ -1a. In terms of the nonclinical study results, it is imperative that reduction of PD effects and of systemic exposure be considered in interpreting the toxicology studies. However, the immunogenicity response in rhesus monkey should not be considered evidence of a safety risk to humans.

### **Toxicology**

Since a chronic, 6-month repeat dose toxicology study of PEGIFN  $\beta$ -1a was not feasible due to immunogenicity concerns, loss of PD, and exposure, the Sponsor submitted a pivotal (GLP/QA) 5-week repeat dose toxicity study. Rhesus monkeys (4/sex/group; 2/sex/control and HD [sc and im]) were treated once weekly for 5-weeks. Placebo or PEGIFN  $\beta$ -1a at doses of 0, 2 mcg/kg (0.22 MU/kg), 10 mcg/kg (1.1 MU/kg), and 100 mcg/kg (11 MU/kg) was administered by subcutaneous

injection. A second HD group was included that was administered by intramuscular injection (once weekly for 5-weeks). The standard battery of parameters was tested; there were no test article-related findings associated with mortality, clinical signs, food consumption, body weight, ophthalmoscopy, ECG, clinical chemistry, urinalysis, necropsy, organ weight changes, and histopathology. A dose dependent reduction in circulating lymphocyte counts was found. At the HD, both routes of administration produced similar results. On Dosing Day 1, mean lymphocyte counts measured at  $\approx T_{max}$  were reduced at each dose compared to baseline values (Day -10) of 1%, 28%, 33%, 59%, and 59% for control, and PEGIFN  $\beta$ -1a at 2 mcg/kg, 10 mcg/kg, and 100 mcg/kg (sc and im), respectively. Lymphocyte counts were measured at 4 hr postdose during Weeks 3-5 following the once weekly intramuscular PEGIFN  $\beta$ -1a (100 mcg/kg/week) administration; this treatment reduced lymphocytes by 64%, 56%, and 36% relative to the prestudy baseline values.

Two pharmacodynamic effects of PEGIFN  $\beta$ -1a administration were observed—febrile response and induction of serum neopterin. PEG-IFN  $\beta$ -1a administration induced a treatment-related febrile response that occurred 4 hr postdose on both Day 1 and Day 8 at doses levels of 10 and 100 mcg/kg [sc, im]. On Day 22, a similar response was achieved at 4 hr postdose, however, this was observed only in animals following subcutaneous injection at dose levels of 10 and 100 mcg/kg. Following PEGIFN  $\beta$ -1a administration, the induction of serum neopterin responses was evaluated after dosing on Days 1 and 29 (at 24 hr postdose). Maximal neopterin responses to PEGIFN  $\beta$ -1a treatment were observed on Days 2, 16, and 30; whereas placebo did not induce neopterin responses. Maximal neopterin responses were achieved on Day 2, at all doses tested (sc and im). Following the third dose (Day 15), serum neopterin was increased on Day 16; however, the magnitude of the increase was less than that achieved on Day 2. The trend toward reduced responsiveness of neopterin to repeated doses of PEGIFN  $\beta$ -1a at weeks 4 and 5 continued throughout the dosing period. This reduction was considered most likely due to the generation of neutralizing antibodies.

Toxicokinetic parameters were determined on Day 1 of dosing; systemic exposure ( $C_{max}$  and  $AUC_{168h}$ ) was approximately dose proportional. For subcutaneous administration of BII017,  $T_{max}$  ranged from 12-16 hr; whereas following intramuscular injection,  $T_{max}$  occurred more rapidly (4-5.3 hr). The half-lives were similar among the treatment groups ranging from 17.6-24.3 hr at doses  $\geq 10$  mcg/kg (100 mcg/kg, [sc, im]). At the LD (2 mcg/kg, sc),  $t_{1/2} = 15$  hr, which was shorter than the other dose groups. As described previously in the PK section, similar values for mean total body clearance and mean volume of distribution for the different PEGIFN  $\beta$ -1a treatment groups or parenteral routes of administration were similar. The mean serum concentration of PEGIFN  $\beta$ -1a was measured on Day 29 (5<sup>th</sup> dose; postdose at 4 hr [im] and at 8 hr [sc]). The estimated peak mean PEGIFN  $\beta$ -1a concentration at 2, 10, and 100 mcg/kg (sc) was 0.00, 1.76, and 0.80 ng/mL, respectively. At the HD (im), the estimated peak mean concentration was 0.04 ng/mL.

From all dosed animals, serum was screened for the presence of ADAs. By Day 15 (3<sup>rd</sup> dose), a substantial number of dosed animals were positive for binding antibodies. Neutralizing antibodies were detected at the low dose (F 1/4) and the high dose (F, 2/4; M, 3/4 [sc]; M, 1/4 [im]). Although few animals were positive for neutralizing antibodies, the estimated peak mean  $C_{max}$  (ng/mL) values at each dose tested showed an approximate 50% reduction compared to the first dose administered. Likewise, the neopterin response to the 3<sup>rd</sup> dose administered on study Day 15 was also blunted in comparison to that achieved on Day 1. These findings demonstrate that not only exposure, but also pharmacologic and physiological responses to PEGIFN  $\beta$ -1a were markedly affected by the robust immunogenicity observed in rhesus monkey. By Days 29 and 36, all animals dosed with PEGIFN  $\beta$ -1a were positive for neutralizing antibodies; in fact, all Day 65 high dose recovery animals remained positive for neutralizing antibodies to PEGIFN  $\beta$ -1a.

Taken together, the NOAEL is considered the high dose of 100 mcg/kg/week. The reduction in lymphocyte counts, generation of a febrile response, and the induction of serum neopterin are each considered a PD effect of PEGIFN  $\beta$ -1a treatment and thus, not adverse. The no adverse effect level is approximately 31 times the maximum recommended human biweekly dose (MRHD) of 125 mcg on a  $mg/m^2$  basis and 471 times the MRHD based upon single-dose  $AUC_{168hr}$  in the monkey and 24-week  $AUC_{168hr}$  exposure data from clinical study 105MS301.

#### *Mutagenicity*

As noted in the prescribing information for Avonex, IFN  $\beta$ -1a was previously evaluated and was not mutagenic when tested in an *in vitro* bacterial reverse mutation (Ames) test or in an *in vitro* cytogenetic assay in human lymphocytes. For PEGIFN  $\beta$ -1a, the 20 kDa mPEG-O-2-methylpropionaldehyde had not been evaluated for genotoxic potential; an *in silico* DEREK analysis identified a potential genotoxic structural alert in the pegylation molecule. PEGIFN  $\beta$ -1a was not mutagenic when tested in an *in vitro* bacterial reverse mutation (Ames) test and an *in vitro* cytogenetic assay in human lymphocytes.

#### *Carcinogenicity*

The carcinogenic potential of PEGIFN  $\beta$ -1a has not been tested in animals.

#### *Reproductive and Developmental Toxicity*

Standard reproductive and developmental toxicity studies of PEGIFN  $\beta$ -1a were not performed; the Sponsor's rationale was based upon the known abortifacient, but not teratogenic effects of IFN  $\beta$ -1a that is described in the prescribing information label for Avonex. In addition, the Sponsor argued that the nonhuman primate "pose unique challenges" compared to the studies conducted using rodents and rabbits; the availability of pregnant animals, a small N size, and a single fetus per pregnancy were suggested as difficulties. Further, the rhesus monkey, in which PEGIFN  $\beta$ -1a is pharmacologically active, is a seasonal breeder; and therefore, the short breeding season and a higher spontaneous abortion rate of approximately 17% when

combined with the likelihood that PEGIFN  $\beta$ -1a would induce abortions in some of the dosed animals, the number of fetuses for evaluation would be even smaller. For these reasons, the Sponsor argues that an EFD and PPND study are not feasible. Since, an EFD study was conducted to support the licensure of IFN  $\beta$ -1a, the Sponsor submitted this study as support for the presumed abortifacient effect of PEGIFN  $\beta$ -1a. The EFD study results are summarized in the prescribing information label for Avonex, as noted below:

### 8.1 Pregnancy

In pregnant monkeys given interferon beta at 100 times the recommended weekly human dose (based upon a body surface area [ $\text{mg}/\text{m}^2$ ] comparison), no teratogenic or other adverse effects on fetal development were observed. Abortifacient activity was evident following 3 to 5 doses at this level. No abortifacient effects were observed in monkeys treated at 2 times the recommended weekly human dose (based upon  $\text{mg}/\text{m}^2$ ).

In addition to the EFD study for IFN  $\beta$ -1a, a hormone and menstrual cyclicity study was conducted in non-pregnant, female rhesus monkeys ( $n=5/\text{group}$ , ages 5-8 years old); the effects of placebo (vehicle) and PEGIFN  $\beta$ -1a at doses of 2.5 mcg/kg and 125 mcg/kg were tested. The high dose selection was based upon the dose associated with the reduction of serum progesterone that was correlated with the abortifacient effect observed in the EFD study for IFN  $\beta$ -1a. The low dose for this study was a dose equivalent for PEGIFN  $\beta$ -1a in which no effects were observed on sex steroid hormones. In each animal, five menstrual cycles were evaluated; the first 2 cycles determined the individual animal cycle baseline (acclimation periods), a dosing cycle in which animals were injected subcutaneously with placebo or PEGIFN  $\beta$ -1a, and finally 2 cycles were analyzed as recovery from dosing. During the dosing cycle, sc injection was administered once weekly for a maximal duration of 5-weeks; the dosing duration was limited based upon previous experience with extensive immunogenicity associated with the repeated dosing of PEGIFN  $\beta$ -1a in rhesus monkey. Dosing was discontinued in females following demonstration of menses or after the 5<sup>th</sup> weekly dose. It is notable, that the dosing regimens used in these hormone and menstrual cyclicity studies were considerably different. The initial evaluation of IFN  $\beta$ -1a was assessed following 8-15 doses administered every other day during in a single menstrual cycle; in contrast, the dosing regimen employed for PEGIFN  $\beta$ -1a was once a week administration for up to a maximum of 5 doses during a single menstrual cycle.

During each cycle, data were collected to determine cycle length (days), day of peak hormone (17- $\beta$  estradiol (E2) and progesterone (Prog)) level and serum concentration of E2 and Prog, interval between E2 and Prog peaks, peak E2 to next menses, and peak Prog to next menses. Samples were collected for TK and ADA determinations; in the dosing cycle, Day 1 administration of PEGIFN  $\beta$ -1a resulted in dose-related increase in exposure ( $C_{\text{max}}$  and  $\text{AUC}_{0-24\text{h}}$ ) consistent with systemic exposure. Throughout the dosing and recovery periods, PEGIFN  $\beta$ -1a was well tolerated, at both doses tested.

Group mean menstrual cycle lengths were determined during the two acclimation (AC) periods that were variable among the 3 dose groups. The baseline menstrual cycle length was calculated as the mean of AC1 and AC2 that ranged from 23.5 to 37.5 days, which is considered within the range of normal for rhesus monkey. Peak E2 concentrations occurred on menses days (MD) 11 and 12; the peak E2 concentration among the three dose groups was similar (96, 97, and 92 pg/mL for Groups 1, 2, and 3, respectively). The peak Prog concentration occurred on MD22, MD19, and MD18 for Groups 1, 2, and 3, respectively; among these 3 groups, the peak Prog concentration was 5.1, 5.9, and 5.3 ng/mL for Groups 1, 2, and 3, respectively.

During the dosing cycle (DC), the mean menstrual cycle duration at the high dose was increased compared to placebo and LD groups; however, the reason for the increase was driven by 2/5 high dose females that had prolonged menstrual cycles of 47 and 61 days (each of these animals had 5 dose administrations). There was no effect of the low dose on mean menstrual cycle duration. Mean day of menses associated with the peak E2 and peak Prog concentrations were delayed in BII017-treated animals. The delay to peak Prog was observed at both doses; however, the delay to peak E2 was limited to the HD. Finally, at the HD there was a reduction in the interval between peak Prog and the start of the next menses cycle.

The recovery cycles (RC1 and RC2) were highly variable and thus, the interpretation of the study results is limited. During RC1, the mean menstrual cycle length and the mean MD to peak E2 were similar among the treatment groups. At the HD, the mean MD to peak Prog concentration was delayed by 5 days compared to placebo. Thus, the interval was increased between peak E2 and peak Prog. One of the two affected HD females that had a prolonged menstrual cycle duration of 62 days recovered during both RC1 and RC2 with menstrual cycle durations of 29 and 33 days, respectively. In contrast, female with the DC prolonged menstrual cycle that was 47 days had a shorter cycle of 15 days during RC1 and amenorrhea ( $\geq 70$  days). Thus, it is unclear based on these results if there is in fact recovery at the HD.

The TK data demonstrated a clear dose-related increase in serum PEGIFN  $\beta$ -1a at the two doses tested on Day 1. However, repeated-dosing of PEGIFN  $\beta$ -1a in these monkeys was associated with a rapid and robust ADA response that was detected as early as Day 15; this response must have included formation of neutralizing antibodies since the exposure (concentration at 8 hr) was reduced in some of individual low dose and high dose animals. Unfortunately, the Sponsor did not perform titers on these samples to semi-quantify the magnitude of the neutralizing antibody response. At the low dose, the percent reduction in exposure (at 8 hr) relative to that achieved on Day 1 was 2.9% [SSAN 11], 56.3% [SSAN 21], 86.2% [SSAN 37], and 96.3% [SSAN 19]. At the HD, the percent reduction in exposure (at 8 hr) relative to that achieved on Day 1 was 83.1% [SSAN 29] and 99.3% [SSAN 49]; in contrast, three high dose animals had TK exposures that were slightly reduced by 10.5% [SSAN 23] or increased by +153% [SSAN 31] and +133% [SSAN

33]. By Day 22, at the LD, one animal SSAN 17 had a systemic exposure ( $AUC_{0-24h}$ ) that approximated the Day 1 exposure; likewise, at the HD, 3 animals had measurable PEGIFN  $\beta$ -1a levels that ranged between 34% and 44% of the Day 1  $AUC_{0-24h}$  values. By Day 29, HD animals SSAN 31 and SSAN 34 had exposures that were reduced to 4% and 14.7% of Day 1 exposure. Taken together, these data are difficult to interpret due to the extensive immunogenicity and the unknown confounding factors associated with such a robust ADA response.

The Sponsor interprets these data to suggest that there was a dose-related increase in PEGIFN  $\beta$ -1a exposure ( $C_{max}$  and AUC) at the 2.5 and 125 mcg/kg on both Days 1 and 22. The Day 1 assessment by the Sponsor is evident, a clear dose-related increase is observed for both  $C_{max}$  and AUC values. On Day 22, however, the exposure values were highly variable, for example, Group mean  $\pm$  SD values for  $C_{max}$  and AUC at the low dose were  $1430 \pm 2840$  pg/mL and  $54,300$  pg\*h/mL, respectively and at the high dose were  $103,000 \pm 106,000$  pg/mL and  $1,710,000 \pm 1,690,000$  pg\*h/mL, respectively. The extreme variability in the Day 22 results (possibly related to the presence of neutralizing ADAs) seems to negate the Sponsor's claim to a dose-related increase. Based on the results presented, at the high dose, 2/5 animals had prolonged menstrual cycles (47 and 61 days) during the dosing phase of this study; and at least one of the two affected animals appeared to recover.

As demonstrated in the PK, repeat-dose toxicity, and hormone and menstrual cyclicity study conducted in monkey, the effects of PEGIFN  $\beta$ -1a were similar to those achieved with IFN  $\beta$ -1a. Due to the robust immunogenicity that occurs following single- or repeated-dosing of PEGIFN  $\beta$ -1a in rhesus monkeys, it is unclear that a PPND study, if conducted, would generate interpretable results.

### *Genotoxicity*

It has been previously established that interferon-beta was not mutagenic when tested in an *in vitro* bacterial reverse mutation (Ames) test or in an *in vitro* cytogenetic assay in human lymphocytes; however, BIIB017 is a pegylated-interferon beta and the potential genotoxicity of the pegylated-interferon was unknown. Therefore, the Sponsor used an *in silico* DEREK evaluation of the pegylation molecule, 20-kDa mPEG-O-2-methylpropionaldehyde; this analysis revealed a structural alert for potential genotoxicity. Therefore, BIIB017 was evaluated in an *in vitro* bacterial reverse mutation (Ames) test and in an *in vitro* cytogenetic assay in human lymphocytes, and BIIB017 was neither mutagenic nor clastogenic at any of the concentrations tested.

For the overall conclusions and recommendations, please see the Executive Summary.

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/s/  
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RICHARD A HOUGHTLING  
01/24/2014

LOIS M FREED  
01/24/2014

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**BLA Number: 125499**

**Applicant: Biogen Idec**

**Stamp Date: May 16, 2013**

**Drug Name: PLEGRIDY™  
peginterferon beta-1a  
BIIB017**

**BLA Type: BLA**

On **initial** overview of the NDA/BLA application for filing:

|   | Content Parameter  | Yes | No | Comment   |
|---|--|-----|----|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?  | X   |    |   |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?   |     | X  | eCTD Study Title labels are missing   |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin?   | X   |    |   |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?        |     | X  | The EFD study is missing; however, this is a matter of review pending the decision by CMC regarding the comparability of Avonex and BIIB017 starting material.  |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). |     |    | The to-be-marketed formulation is BIIB017-B. The pivotal toxicology study used BIIB017-A; however, some of the nonclinical assessments (e.g., menstrual cycle evaluation) were conducted using BIIB017-B. |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?   | X   |    |   |
| 7 | Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?  | X   |    |   |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?  |     | X  | No, but this is a matter of review; depending on the outcome of the comparability analysis by CMC.  |

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA/BLA or Supplement**

|    | <b>Content Parameter</b>  | <b>Yes</b> | <b>No</b> | <b>Comment</b>   |
|----|---|------------|-----------|--|
| 9  | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | X          |           |  |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)   | X          |           | The Sponsor addressed potential poly(ethyleneglycol)-related impurities in the Nonclinical Overview Section 1.4.2. |
| 11 | Has the applicant addressed any abuse potential issues in the submission?   |            |           | Abuse potential issues are addressed by CSS.   |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?   |            |           | N/A  |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

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Reviewing Pharmacologist Date

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Team Leader/Supervisor Date

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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RICHARD A HOUGHTLING  
07/05/2013

LOIS M FREED  
07/05/2013