

TOXICOLOGIST'S REVIEW

STN BLA #: 103628\5021

SPONSOR: BIOGEN, INC.

PRODUCT: recombinant, human interferon- β 1a (AVONEX[®], Chinese hamster ovary cell-derived)

FORMULATION: sterile solution containing 11.6 mM sodium acetate and 150 mM arginine hydrochloride buffer, plus 0.005% polysorbate 20 (Tween[®]-20), pH 4.8; in pre-filled glass syringes

RELATED DOCUMENTS: BB IND # — PLA #95-0979

PROPOSED INDICATION: treatment of relapsing-remitting multiple sclerosis

ABBREVIATIONS: IFN- β , recombinant, human interferon-beta 1a; HSA, human serum albumin; i/m, intra-muscular; C_{max}, maximal serum concentration; AUC, area under the serum concentrations vs. time curve; T_{1/2}, elimination half-life; CHO, Chinese hamster ovary; kD, kilodaltons; MS, multiple sclerosis; IU, international anti-viral units of activity (by cytopathic effect bioassay); CPE, cytopathic effect;

ELISA, enzyme-linked, immunosorbent assay; NOAEL, no observable adverse effect level

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ABSTRACT:

The pharmacokinetic and pharmacodynamic profiles, antigenicity, and local irritation potential of several different formulations of IFN- β , either with or without HSA were determined in male — monkeys and — rabbits after a single i/m dose. Interferon- β was well-tolerated in all test species, regardless of dose or formulation. One pharmacokinetic study in — monkeys was determined to be invalid, since the data presented were not the original observations and represented values that had been corrected for an increased dose of the product. While 30 to > 90% increases in pharmacokinetic parameters including C_{max}, AUC_(0-inf.) and T_{1/2} were observed in — monkeys treated with IFN- β formulated in buffer without HSA and at a pH of — these were not statistically significantly different from values obtained in

animals treated with the licensed AVONEX[®] formulation, containing 15 mg/ml HSA. There were no remarkable differences in local tolerability between the commercial AVONEX[®] formulation and the liquid, HSA-free formulation after *i/m* injection of male rabbits. Taken together, these data support the hypothesis that the liquid, HSA-free formulation of AVONEX[®] proposed for marketing in the present supplemental biologics licensing application is comparable in terms of exposure, time to elimination, and local tolerability to the currently marketed, lyophilized form of the product containing HSA, and support its safety for use in patients with multiple sclerosis.

INTRODUCTION:

AVONEX[®] [recombinant human interferon-beta 1a (IFN- β)] is a natural protein that is secreted by genetically engineered, mammalian CHO cells, with an amino acid sequence identical to that of naturally occurring human interferon- β . IFN- β is a single chain, glycosylated polypeptide 166 amino acid residues in length, and with an approximate molecular weight of 22.5 kD.

The intended clinical use of IFN- β is in the treatment of MS, a disease that is characterized by progressive loss of motor and sensory neural functions. Multiple sclerosis is believed to result from an abnormal autoimmune response occurring within the central nervous system, which leads to destruction of the myelin sheaths covering nerve fibers, and a resulting deterioration in nerve signal conduction. Interferons are postulated to produce clinical effects in MS by an immunodulation of the aberrant immune response against the myelin sheath, and by antagonizing the immune activating effects of interferon- β , which has been demonstrated in pilot clinical studies in MS to enhance exacerbation frequencies. *In vitro*, IFN- β decreases mitogen-stimulated T lymphocyte proliferation and interferon- β synthesis and secretion.

AVONEX[®] is indicated for the treatment of relapsing forms of MS. In patients with MS, IFN- β has been demonstrated to decrease the progression of physical disability, decrease the frequency of clinical exacerbations, and reduce the number and volume of active brain lesions identified on MRI scans. The dose and schedule of AVONEX[®] currently licensed for chronic administration to MS patients is 30 μ g, which is equivalent to 6×10^6 IU of antiviral biological activity, given once weekly by *i/m* injection.

The current licensed AVONEX[®] product is formulated as a sterile, off-white to white powder for reconstitution with supplied diluent or sterile water for injection, USP. Each 1.0 ml of reconstituted AVONEX[®] contains 30 μ g IFN- β , 15 mg HSA, USP, 5.8 mg sodium chloride, USP, 5.7 mg dibasic sodium phosphate buffer, USP, and 1.2 mg monobasic sodium phosphate, USP, at a pH of approximately 7.3.

The present submission addressed the re-formulation of AVONEX[®] into a liquid, HSA-free buffering system. Each 0.5 ml of the new formulation of AVONEX[®] contains 30 μ g IFN- β , 0.79 mg sodium acetate trihydrate, USP/EP, 0.25 mg glacial acetic acid, USP/EP, 15.8 mg arginine hydrochloride, USP/EP, 0.025 mg polysorbate 20, and water for injection, USP/EP at a pH of 4.8. For clinical use, the product is provided in pre-filled, glass syringes, containing 30 μ g IFN- β per 0.5 ml. The IFN- β used for all preclinical pharmacology, pharmacokinetics, and toxicology studies was produced at commercial scale, was greater than 99% pure, was formulated according to clinical procedures and was either of clinical grade, or representative of that which is to be marketed.

PRECLINICAL PHARMACOLOGY AND PHARMACOKINETICS:**Pharmacology Study Summary and Review:**

No preclinical pharmacology studies were included in the present, supplemental BLA submission. For a complete review of preclinical pharmacology studies conducted in support of IFN- β , please see the toxicologist's review for PLA #95-0979.

Pharmacokinetics Study Summary:

1. Interferon beta-1a: Reformulation vs. PK/PD in _____ monkeys. Study #P9418-97-01. _____ 4 σ /group, weight range 5.0-8.0 kg; 30 μ g/monkey BG9418 IFN- β , formulated in 15 mg/ml HSA, pH 7.2, i/m (current marketed AVONEX[®] formulation); 30 μ g/monkey BG9418 IFN- β , formulated in _____ mg/ml HSA, pH _____ i/m; or 30 μ g/monkey BG9418 IFN- β , formulated in HSA-free buffer, pH 5.0, i/m; non-GLP; 6/3 – 7/18/97 (in-life); study completed 9/24/98; _____
2. BG9418: A comparative evaluation of the pharmacokinetics, pharmacodynamics, and antigenicity of lyophilized, liquid, and liquid serum-free BG9418 formulations in _____ monkeys. Study #P9418-01-01. _____ 6 σ /group, 1-5 years old, weight range 2.1 – 3.4 kg; 30 μ g/monkey BG9418, i/m from either lyophilized product containing 15 mg/ml HSA (current marketed AVONEX[®] formulation, lot # _____), liquid, HSA-free formulation (lot # _____), or liquid, HSA-free formulation produced in cells cultured in _____ (lot # _____); GLP (in-life phase and analysis of pharmacodynamic parameters); 8/6 – 11/2/01 (in-life and analyses); study completed 2/13/02; _____

Pharmacokinetics Study Review:

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Study #P9418-01-01. BG9418: A Comparative Evaluation of the Pharmacokinetics, Pharmacodynamics, and Antigenicity of Lyophilized, Liquid, and Liquid Serum-Free BG9418 Formulations in — monkeys.

The pharmacokinetic and pharmacodynamic profiles following injection of three different formulations of IFN- β were evaluated in male — monkeys after a single dose of the product. Six monkeys per group received a single, i/m dose of 30 μ g IFN- β in either 1.0 ml of sodium phosphate buffer containing 1.5% HSA (BG9418, current AVONEX[®] licensed formulation); 0.5 ml of a liquid reformulation of BG9418 in HSA-free buffer (labeled "liquid"); or 0.5 ml of liquid IFN- β produced from a — and formulated in HSA-free buffer (labeled "serum-free"). The buffer composition of the two liquid formulations was identical to that proposed for the current reformulation change, and consisted of 11.6 mM sodium acetate, 150 mM arginine hydrochloride, and 0.005% polysorbate 20 (Tween-20), buffered with 8.4 mM glacial acetic acid to a pH of 4.8

Peripheral blood samples for pharmacokinetic and pharmacodynamic analyses and determination of serum neutralizing antibody activity were collected pre-dose, and at designated time points out to 29 days (672 h) after dosing for analysis of serum IFN- β , neopterin, and anti-IFN- β antibody levels. Serum levels of IFN- β were analyzed using an anti-viral cytopathic effect (CPE) assay, and are expressed as international units (IU) of anti-viral activity, per ml of serum. Antibodies against IFN- β were measured using an ELISA developed by the sponsor. The contracting laboratory conducted the measurements of serum neopterin by EIA, using a —

— The lower limit of detection for the serum IFN- β — assay was not specified, while the lower limit of quantitation was — ng/ml for the neopterin — Interferon- β and neopterin pharmacokinetic and pharmacodynamic parameters were calculated by non-compartmental analysis of the raw data, using the computer software —

All animals survived until study termination on d 29, and were returned to the colony at the contract facility following completion of the study. There was no clinical evidence of toxicity of IFN- β in any of the monkeys tested, and all groups maintained or gained weight over the course of the study. Individual animals in each group did demonstrate slight (0.1 to 0.2 kg) increases or decreases in body weight over the course of the study, but there was no apparent relation to treatment. No differences in mean food consumption were observed between the dose groups.

Occasional incidences of watery to liquid and/or black stools were reported in individual animals in all three treatment groups, and were likely related to the stress of repeated handling for blood sample collections. Black stool was attributed to the use of oral bismuth subsalicylate (Pepto-Bismol[®]) as treatment for diarrhea. One monkey (animal number not specified) in the group treated with the commercial formulation of AVONEX[®] exhibited rectal prolapse at 1 and 4 h post-dosing, which was attributed to the animal resisting the restraint procedures. The attending veterinarian re-inserted the exteriorized tissue following the 1 h observation; the second event at 4 h post-dosing resolved without additional intervention prior to the next observation point.

Other incidental findings included sores or lacerations, and swelling and/or bruising in the femoral areas attributed to blood collection procedures in all animals except monkey #R17063M, in the group treated with the HSA-free liquid IFN- β formulation. Two additional monkeys in the group treated with the commercial AVONEX[®] formulation had either moderate bruising over the right arm (animal #R17204M) or minor lacerations on the 3rd and 4th digits of the left foot (animal #R17190M). Both of these findings were attributed to traumatic injury.

After i/m injection, the time to peak serum IFN- β levels was variable between individual monkeys, but was approximately 1 to 2 h. Mean values for AUC and peak concentrations, although not significantly different between the three test articles, demonstrated an approximate 93% increase in C_{max} and 34% increase in $AUC_{(0-inf)}$ between the group dosed with the licensed formulation of AVONEX[®], and the group receiving liquid IFN- β in HSA-free buffer. A similar 49% increase in C_{max} and 21% increase in $AUC_{(0-inf)}$ were observed between the licensed AVONEX[®]-treated group and the animals dosed with the liquid IFN- β derived from _____ Elimination half-lives, although not significantly different between the three groups, were increased in the group treated with the liquid IFN- β in HSA-free buffer, as compared to the groups receiving either the liquid IFN- β from _____ or the licensed AVONEX[®] formulation. Mean residence times, clearance, and steady-state volume of distribution were not determined for this route of administration.

The mean values and standard deviations for each of the pharmacokinetic parameters for the three groups are presented in table III, below.

Table III – Pharmacokinetic Parameters of IFN- β Levels Following IM Administration of BG 9418 Commercial Lyophilized, Liquid, or Liquid Serum-Free Formulations

Group	BG9418 Lyophilized ^{a,b}	BG9418 Liquid	BG9418 Liquid, Serum-Free
Parameter	Mean Value + Standard Deviation		
C _{max} (U/ml)	1310 + 627	2533 + 1063	1950 + 1031
T _{max} (h)	2.0 + 1.1	1.4 + 1.4	1.9 + 1.2
AUC _(0-inf) (U*h/ml)	11858 + 4274	15912 + 4012	14392 + 5601
T _{1/2} (h)	4.7 + 1.3	6.5 + 2.1	4.7 + 1.2

^a monkey #R169109M had values that were set to zero by the sponsor since the pre-dose value was greater than zero (*i.e.* 380 IU/ml). Any values that were below the determined pre-dose value were therefor also set to zero, to give greater power of analysis with the computer software program.

^b the 336 hour time point for monkey #R171105M was changed to zero since the serum IFN- β levels had returned to zero by the 168 h timepoint.

Analysis of bioactivity of IFN- β in each of the different formulations was determined by measuring serum neopterin levels, which is an accepted pharmacodynamic marker of interferon activity. Peak neopterin levels varied between individual animals, but overall these values were less variable than those obtained for serum IFN- β levels. There were no remarkable differences between the three different formulations for the induction of interferon-related bioactivity. The pharmacodynamic parameters calculated for serum neopterin are presented in Table IV, below.

Table IV – Pharmacodynamic Parameters for Serum Neopterin Levels Following Intra-Muscular Administration of Three Different Formulations of IFN- β

Group	BG9418 Lyophilized	BG9418 Liquid	BG9418 Liquid, Serum-Free
Parameter	Mean Value + Standard Deviation		
E _{max} (ng/ml)	18.4 + 3.8	24.7 + 7.5	22.8 + 2.5
T _{max} (h)	22.0 + 4.9	24.0 + 13.2	22.0 + 4.9
E-AUC _(0-inf) (U*h/ml)	6725 + 5097	7200 + 2090 ^a	5967 + 1246
T _{1/2effect} (h)	345 + 132	323 + 89	252 + 24

^a n = 5; animal #R17206M had aberrantly high value for E-AUC_(0-inf), and was eliminated from this calculation as an outlier.

Comment: Independent analyses of the pharmacokinetic and pharmacodynamic parameters were conducted by this reviewer, using the _____ package, _____ While the calculated values for the pharmacokinetics parameters were slightly different from those obtained by the sponsor, this finding may reflect differences in how the values were rounded up or down to the nearest decimal prior to entering into the software program. The sponsor had not

calculated $AUC_{(0-inf)}$ or $T_{1/2effect}$ for the pharmacodynamic parameters in the — spreadsheet provided; the values presented in Table IV represent this reviewer's independent results.

Analysis of serum levels of anti-IFN- β antibodies was conducted by the sponsor. Three monkeys (animals #R171105M and #R17204M in the AVONEX[®] commercial formulation group and animal #R17565M in the group treated with the IFN- β liquid, HSA-free formulation) had positive titers at baseline. Monkey #17561M was negative for anti-IFN- β antibody at all subsequent timepoints after treatment, while the other two animals remained positive over the duration of the study. One other monkey, animal #R17178M in the group treated with a single, i/m injection of liquid IFN- β derived from serum-free cell culture, demonstrated a transient positive titer at study d 15, which had resolved back to baseline (titer <20) by the next timepoint at study d 22. No neutralizing antibody was detected in any of the treated monkeys at any time point after a single 30 μ g injection of IFN- β , regardless of formulation. These data are presented in the Table V, below.

Table V – Serum Anti-IFN- β Antibody Titers in Rhesus Monkeys Following a Single IM Injection of Three Different Formulations of BG9418

Lyophilized	Individual Animal Number, Titers					
	R17076M	R171105M	R17190M	R169109M	R17204M	R17207M
Timepoint						
Pre	< 20	Positive/<10	< 20	< 20	Positive/<10	< 20
Day 15	< 20	< 20	< 20	< 20	Positive/<10	< 20
Day 22	< 20	< 20	< 20	< 20	Positive/<10	Retest/< 10
Day 29	< 20	< 20	< 20	< 20	Positive/<10	< 20
Liquid						
Timepoint	R17061M	R17063M	R17565M	R16398M	R17206M	R16965M
Pre	< 20	< 20	Positive/<10	< 20	< 20	< 20
Day 15	< 20	< 20	Retest/< 10	< 20	< 20	< 20
Day 22	< 20	< 20	< 20	< 20	< 20	< 20
Day 29	< 20	< 20	Retest/< 10	< 20	< 20	< 20
Serum-free						
Timepoint	R17561M	R17563M	R17564M	R17091M	R17178M	R17239M
Pre	Retest/< 10	< 20	< 20	< 20	< 20	< 20
Day 15	< 20	< 20	< 20	< 20	Positive/<10	< 20
Day 22	< 20	< 20	< 20	< 20	< 20	Retest/< 10
Day 29	< 20	< 20	< 20	< 20	< 20	< 20

Comment: These data were abstracted directly from the final study report, p. 95 and represent the — spreadsheet prepared by the sponsor to describe the results of this study. There is no further description in the study report of how these data were obtained, or what a "positive" or "retest" sample mean.

In summary, the pharmacokinetic, pharmacodynamic, and antigenicity profiles of the liquid IFN- β formulated in HSA-free buffer were not appreciably different from the currently marketed,

lyophilized AVONEX[®] product, containing 1.5% HSA. Although differences in serum IFN- β parameters (*i.e.* C_{max} , $AUC_{(0-inf)}$, and $T_{1/2}$) were observed in the groups treated with either of the two liquid formulations of IFN- β as compared to the marketed formulation, these were not statistically significant and were felt to represent the high degree of variability between the small number of animals in each test group.

PRECLINICAL TOXICOLOGY:

Toxicology Study Summary:

1. BG9418: Intramuscular toxicity study in rabbits. Study #P9418-99-02. Rabbit, _____ strain, 3 σ /group; 30 μ g/rabbit BG9418 liquid formulation (lot # _____ IFN- β in 20 mM acetate, 150 mM arginine buffer, pH 4.8); 30 μ g/rabbit AVONEX[®] liquid formulation (lot #929004, IFN- β in 20 mM acetate, 150 mM arginine buffer plus 0.005% polysorbate 80, pH 4.8) or 30 μ g/rabbit AVONEX[®] lyophilized (lot # _____ current marketed formulation), i/m with equivalent volume of saline in contra-lateral flank; GLP; 9/27 – 9/3/99 (in-life); study completed 3/28/00; _____

Toxicology Review:

Study #P9418-99-02. BG9418: Intramuscular toxicity study in rabbits.

The local irritation effects of three different formulations of IFN- β were evaluated in _____ rabbits after a single i/m injection. Three animals per group were dosed i/m with IFN- β (30 μ g), formulated in either 0.5 ml acetate:arginine buffer, pH 4.8, with or without 0.005% polysorbate 20, or in 1.0 ml of buffer containing 15 mg/ml HSA, pH 7.2 into the right *gluteus medius* muscle group. The contralateral side was injected with 0.5 or 1.0 ml of sterile, 0.9% saline as a vehicle control. Macroscopic observations for local irritation were performed prior to dosing on study d 1, and at approximately 0.5, 6, and 24 h post-dose. Any findings were rated numerically on a score of 0 to 4 for evidence of local irritation, erythema, and/or edema, according to the method of Draize¹. Rabbits were euthanized following the final observations on study d 3; the injection sites examined for evidence of gross pathologic lesions, preserved in _____ neutral-buffered formalin, and embedded in paraffin. Three tissue blocks were prepared for each injection site. Samples obtained for injection site analysis included the needle injection tract, where possible, and blocks prepared from tissue on each side of the needle entry site. Tissue blocks were sectioned and stained with _____ and examined microscopically for evidence of irritation or inflammation.

All animals survived until study termination, and there was no clinical evidence of overt toxicity related to IFN- β treatment. Weight loss (mean values ranging from -8 to -102 g from baseline) was observed in all three treatment groups; however, animals were fasted overnight prior to euthanasia, which may account for this finding. Rabbit #F56781 (group 3, AVONEX[®] and saline-injected) displayed very slight erythema (recorded as "barely perceptible") at the IFN- β

¹ Draize, J.H. 1959. In: *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics*, pp. 46-49; The Association of Food and Drug Officials of the United States, Austin, TX.

injection site 24 h after injection, with no other grossly observable evidence of local irritation (*i.e.* edema, atonia, fissuring, desquamation, or eschar formation). There were no remarkable findings of local irritation on Draize evaluation in any of the other animals at any time point, regardless of formulation of IFN- β tested. All saline injected sites appeared normal on Draize evaluation.

At necropsy, one darkened area of approximately 2 cm x 1 cm was observed on the right leg of rabbit #F56779, in the group treated with liquid IFN- β in acetate:arginine buffer and 0.005% polysorbate 20, pH 4.8. One gross pathologic evaluation, this area was associated with two apparent needle marks at the site of IFN- β injection. Histologically, this finding was correlated with hemorrhage into the subcutis and hematoma formation, both of moderate severity, as well as evidence of subacute inflammation (severity reported as "slight") in the surrounding tissue. The reviewing pathologist attributes the hematoma formation to trauma associated with the injection procedure, and unrelated to the test article. There were no other grossly evident lesions at either the saline or IFN- β injection sites in any of the other animals treated.

Microscopically, evidence of subacute inflammation, hemorrhage, focal areas of muscle degeneration and/or granuloma formation, surface exudates, and presence of foreign material including hair was present in injection site samples from all animals, regardless of whether the site received saline or IFN- β in any of the three test formulations. Severity of these lesions ranged from minimal to moderate, and was not appreciably increased at the sites of IFN- β injection as compared to those injected with saline control. The mean values for Draize irritation scores for each of these findings are listed in Table IX, below:

Table VI – Mean Draize Evaluation Scores for Rabbits Injected with IFN- β Formulations

Group	BG9418 acetate:arginine		BG9418 acetate:arginine +0.005% polysorbate 20		Lyophilized Avonex [®] (marketed formulation)	
	Left (saline)	Right (IFN- β)	Left (saline)	Right (IFN- β)	Left (saline)	Right (IFN- β)
Subacute inflammation	2.0 ^a	1.3	0.7	1.3	0.7	1.3
Hemorrhage	1.7	0.7	0	1.0	0	1.0
Hair shaft	0	0	0	0.7	0	1.0
Focal muscle degeneration	1.0	1.0	0.7	1.0	1.0	0.7
Surface Exudate	0.3	0	0.7	0.3	0.3	0.3
Granuloma	0	0.3	0.3	0	0.3	0.7
Hematoma	0	0	0	1.0	0	0
Foreign material	0	0.3	0.3	0	0	0.3

^a Draize evaluation scores as follow:

- 1 minimal – the least amount of change that can be observed with the light microscope
- 2 slight – less than average amount of change, but readily discernible as abnormal
- 3 moderate – the average amount of change that is expected for a lesion

- 4 moderately severe (marked) – a marked amount of change with possible loss of function of the affected cells or organ
- 5 severe – a great amount of change with probable loss of function of the affected cell or organs, and frequently involves large areas of the organ

In conclusion, intramuscular injection of 0.5 ml to 1.0 ml of either 0.9% sterile saline or 30 µg formulated IFN-β was not associated with any, acute, local irritation effects. A single animal had findings consisting of localized areas of hemorrhage (Grade 2 severity) at the IFN-β-injected site. This effect was apparently due to the acute trauma associated with the injection procedure, and does not appear to be test article-related. The NOAEL for local irritation of IFN-β after i/m injection is therefore 30 µg given in 0.5 ml acetate:arginine buffer, pH 4.8, with or without 0.005% polysorbate 20, or in buffer containing 15 mg/ml HSA, pH 7.2 (the currently marketed formulation). These doses are approximately equal to the currently licensed dose of AVONEX® used in the treatment of multiple sclerosis.

Comment: The duration of follow-up for these animals after injection of the different IFN-β formulations was only 48 hours. In clinical use, AVONEX® is injected weekly. Therefore, it would have been preferable for the sponsor to have included additional groups of animals with both macroscopic evaluations and sacrifice times at later time points, *i.e.* one and two weeks post-injection, to determine if any late inflammatory changes were observed.

Comment: Typically, local irritation studies conducted by the Draize method include a group of animals treated with a known irritating substance, such as low concentrations (*e.g.* 1.5%) of acetic acid as a positive control. A positive control group was not included in the present study. However, the two liquid formulations of IFN-β were found to be no more irritating at the injection site than the currently marketed formulation of AVONEX®.

SUMMARY AND CONCLUSION:

Study #P9418-01-01 in _____ monkeys demonstrated that the pharmacokinetic, pharmacodynamic, and antigenicity profiles of the liquid IFN-β formulated in HSA-free buffer were not appreciably different from the currently marketed, lyophilized AVONEX® product, containing 1.5% HSA. Although differences in serum IFN-β parameters (*i.e.* C_{max}, AUC_(0-inf), and T_{1/2}) were observed in the groups treated with either of the two liquid formulations of IFN-β as compared to the marketed formulation, these were not statistically significant and were felt to represent the high degree of variability between the small number of animals in each test group. Intramuscular injection of _____ rabbits with 0.5 ml of HSA-free buffer containing 30 µg formulated IFN-β was not associated with any, acute, local irritation effects, as compared to the currently licensed formulation of AVONEX® containing 1.5% (15 mg/ml) of HSA. A single animal had findings consisting of localized areas of hemorrhage, related to acute trauma associated with the injection procedure. The NOAEL for local irritation of IFN-β after i/m injection is therefore 30 µg given in 0.5 ml acetate:arginine buffer, pH 4.8, with or without 0.005% polysorbate 20. These doses are approximately equal to the currently licensed dose of AVONEX® used in the treatment of multiple sclerosis. Taken

together, these data support the hypothesis that the liquid, HSA-free formulation of AVONEX[®] proposed for marketing in the present supplemental BLA is comparable in terms of exposure, time to elimination, and local tolerability to the currently marketed, lyophilized form of the product containing HSA, and support its safety for use in patients with multiple sclerosis.

COMMUNICATIONS TO THE SPONSOR:

The following comments were noted during the review process and should be communicated to the sponsor for information only:

"1. We note from our review that all of your preclinical pharmacokinetic, pharmacodynamic, and local tolerability studies were conducted in male _____ monkeys and _____ rabbits. Since multiple sclerosis is a disease that affects male and female patients at approximately equal incidence but tends to be more severe in female subjects, we recommend that in future preclinical studies groups of both male and female animals be treated with your interferon-beta 1a product.

4. An independent analysis of the pharmacokinetic and pharmacodynamic parameters described for Study #P9418-01-01 was conducted by this reviewer, using the _____ package, _____ While the calculated values for the pharmacokinetics parameters were slightly different from those obtained by your evaluation, this finding may reflect differences in how the values were rounded up or down to the nearest decimal prior to entering into the software program. We note from our review that you had not calculated E-AUC_(0-inf.) or T_{1/2} for the pharmacodynamic parameters; therefore, the values presented in this review (Table IV) represent this reviewer's independent results, and may not be in agreement with your own calculations.

5. The antigenicity data contained in this review were abstracted directly from the final study report for Study #P9418-01-01, and represent the _____ spreadsheet prepared by your laboratory

to describe the results of this study. There is no further description in the study report of how these data were obtained, or what a "positive" or "retest" sample mean. In future studies we recommend that tabulated summaries, with an adequate description of study methodology, results, and qualifying identifiers be included in the final study report, for ease and understanding of review.

6. The duration of follow-up for rabbits in Study #P9418-99-02 after injection of the different interferon-beta 1a formulations was only 48 hours. In clinical use, AVONEX® is injected weekly. Therefore, it would have been preferable for you to have included additional groups of animals with both macroscopic evaluations and sacrifice times at later time points, *i.e.* one and two weeks post-injection, to determine if any late inflammatory changes were observed. We recommend that you include later time points for follow-up of local irritation in any future studies of this product.

7. Typically, local irritation studies conducted by the Draize method include a group of animals treated with a known irritating substance, such as low concentrations (*e.g.* 1.7%) of acetic acid as a positive control. A positive control group was not included in the present study, #P9418-99-02. However, the two liquid formulations of interferon-beta 1a were found to be no more irritating at the injection site than the currently marketed formulation of AVONEX®, despite the decreased pH of the sample buffer. In future studies evaluating local tolerance, we recommend that you include at least one group of animals treated with a known positive control."



Anne M. Pilaro, Ph.D., Toxicologist

Key Words: interferon-beta 1a; formulation; multiple sclerosis; neopterin; pharmacokinetics

conurrences: OTRR/C,P-T/MGreen ^{MDG 8/29/02}

cc: OTRR/C,P-T/MGreen