

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**103471**

**PHARMACOLOGY REVIEW**

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

PLA #: 92-0495

BB-IND #: 1846, —

Sponsor: Chiron Corporation, Berlex (preclinical and clinical safety and efficacy information)

Name of Product: Betaseron (recombinant human interferon beta ser17) derived from E. coli. The product is supplied lyophilized. Each vial contains —. Dextrose (15 mg) and Albumin Human, USP (15 mg) are added as stabilizers. The diluent is sodium chloride, 0.54%. The specific activity of Betaseron is —. The reconstituted clinically used material contains a maximum concentration of SDS of —.

Documents reviewed: Vols. 1, 4, 5, 27-33, 37, 173

Intended Clinical Use: Treatment of patients 18 years or older with relapsing-remitting multiple sclerosis (MS). The pivotal double blind placebo controlled trial included 338 subjects: Betaseron at 45 MIU (115); Betaseron at 9 MIU (111) and placebo (112). 81% of subjects (273) completed the two years of therapy. The recommended human dose is 45 MIU injected via subcutaneous administration (s.c.) every other day. No data exist that support continued efficacy if decreased dosages are administered for prolonged periods.

Species specificity: Human recombinant beta interferon is species specific therefore studies examining the biological activity and mechanism of action have relied on studies using human-derived cells in culture and ex vivo studies using human cells. Pivotal in vivo studies examining activity and toxicity have been restricted primarily to non-human primates.

Abstract: Beta interferon is a natural human protein. Recombinant human E. coli derived interferon beta ser17 (Betaseron) is intended to be administered chronically by s.c. administration for the treatment of relapsing-remitting MS. Although the mechanism of action of Betaseron is not fully understood, it has been hypothesized that the immunomodulatory effects of Betaseron play a major role in modulating the regulatory components of the immune system implicated in the exacerbation of MS. In early clinical studies in MS patients investigating the potential therapeutic activity of interferon gamma, the number of exacerbations in MS actually increased suggesting that gamma may play an active role in the pathogenesis of MS. The rationale for using interferon beta to treat MS patients was supported by the above observation and data from in vitro studies which showed that Betaseron modulated immune activation by down regulating the secretion of gamma interferon activated T cells. Additionally, the anti-viral and anti-proliferative effects of Betaseron were also thought to play a role in MS by limiting immune activation during viral infections and by limiting clonal expansion or immune effector cells believed to be responsible for central nervous system damage observed in MS.

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Data from pharmacology/pharmacodynamic studies, pharmacokinetic and toxicology studies, and literature references, form the basis of the preclinical evaluation of Betaseron. In vivo antiviral pharmacologic activity was demonstrated in a Simian Varicella Virus (SVV) model of infection in African Green monkeys and in a primary and recurrent genital herpes simplex virus (HSV-2) infection model in guinea pigs. No animal models of MS were evaluated. Pharmacokinetic studies measured systemic absorption in normal human volunteers, patients with various malignancies and patients with AIDS. Pharmacokinetic parameters were also evaluated in African Green monkeys infected with SVV. Assays of blood levels of Betaseron did not discriminate between bound or unbound or endogenous levels of natural beta interferon. Bioavailability following s.c. dosing was calculated and ranged between 30-50%. There was no accumulation of Betaseron following every day dosing. No studies were performed to evaluate tissue distribution or protein binding. Despite undetectable serum levels of IFN following every other day s.c. injection at the recommended human dose of 45 MIU, interferon-induced gene products were induced in a dose-dependent manner. Subcutaneous administration appears to be as effective as intravenous administration in modulating the biologic responses.

Pivotal toxicity studies (defined as such because the test material used in these studies was presumed to be representative of the clinical material) were performed in Rhesus macaques to support the proposed indication and patient population. These included a 28 day repeat dose toxicity study, reproductive/developmental toxicity studies (Segment II) and a study to evaluate the effects of Betaseron on female reproductive potential. Results of the latter study are expected in September of 1993. To assess the potential for hypersensitivity reactions following dosing, one group of animals (low dose, 10 IU/kg) in the 28 day repeat dose toxicity study were maintained on study up to 90 days for weekly dosing. The repeat dose toxicity study included complete clinical, gross and histomorphological evaluations as well as periodic analyses for antibody response to Betaseron. In vitro mutagenicity studies and special toxicity evaluations (e.g. ocular, skin and vaginal irritation studies) were also performed to further characterize the safety of Betaseron. No safety pharmacology studies were performed to assess effects on cardiovascular, CNS, renal or GI systems. No studies were performed assessing effects on male fertility. No chronic bioassay(s) was performed, due to species specificity, to assess in vivo carcinogenic potential.

Serum-binding antibodies were measured in the 28 day repeat dose study and were shown to increase with increasing dosing levels. Administration (via s.c.) produced higher peak levels than i.v. administration. While neutralizing antibodies were detected in MS subjects receiving Betaseron, there was no apparent affect on the ability of Betaseron to modulate the in vivo induction of

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biological markers.

The preclinical safety profile suggests that Betaseron should be adequately tolerated following chronic administration. The major clinical systemic events associated with effective doses of Betaseron dose included flu-like symptoms i.e. fever, chills, myalgia, malaise and sweating. Neutropenia was also reported in MS subjects following Betaseron treatment and increased in incidence in subjects who entered the study with  $WBC \leq 1500/mm^3$ ). Injection site reactions have also been noted. Major toxicities observed in non-human primate studies included anemia, neutropenia, thrombocytopenia and hyperfibrinogenemia. These toxicities however were predominantly seen in the reproductive toxicology study which evaluated much higher doses. Doses were uncharacteristically higher in this study than in the repeat dose toxicity study. Additional preclinical treatment related findings included transient elevations in lymphocytes and body temperatures and local reactogenicity reactions at the injection site. Betaseron caused abortions in non-human primates which is a consistent (i.e. class specific) finding with all interferons tested to date. No teratologic findings were noted. A study is ongoing to assess effects on female reproductive potential.

The clinical and preclinical data are similar to the effects observed with other licensed interferons. In general, while intolerance to the 'flu-like' side effects observed in subjects may preclude adequate or chronic treatment with interferons, life-threatening complications have been infrequent.

#### Pharmacology:

Human- Beta interferon is one of three major serotypes of interferons. The other two that have been described are alpha and gamma. One natural and two recombinant alpha interferons and one recombinant gamma interferon have been licensed. Interferons beta and alpha are classified as type I because they compete for binding to the same receptor and demonstrate similar biological activities. Despite a 70% homology in amino acid composition, they are immunologically distinct. More than 20 human alpha interferon subtypes have been produced by lymphocytes. In contrast, only one human beta interferon subtype, produced in fibroblasts, has been identified. (Because expression of the human fibroblast interferon gene in *E. coli* resulted in a less active and less stable protein than the natural interferon produced in fibroblasts, one of the cystine residues, located at position 17 was replaced by serine. This substitution resulted in a molecule with enhanced activity and stability presumably due to the decreased likelihood of forming aggregates).

Despite differences between type I and type II (i.e. gamma) interferons and their receptors, some of their biologic activities

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(and adverse effects) overlap, suggesting the possibility that similar second messenger systems might be triggered by formation of type I or type II interferon-receptor complexes.

Numerous in vitro studies in human-derived cells were referenced which described the in vitro activities of Betaseron. These included affects- on gamma interferon synthesis, antigen processing, antigen presentation, on immune cell functions e.g. suppressor T cells and antigen-specific cytotoxic T cells. In addition studies supporting anti-proliferative and anti-viral activities were also summarized.

Human peripheral blood mononuclear cells (PBMCs) obtained from MS patients treated with Betaseron showed a decreased ability to produce IFN gamma upon ex vivo stimulation with mitogen and antigens. Interferon-induced gene products were detectable in cellular and plasma fractions of blood collected from individuals treated with Betaseron (i.e., neopterin, beta2-microglobulin and soluble IL 2 receptor). These products were induced in a dose-dependent manner despite undetectable serum levels. Doses of at least 9 MIU were required to achieve significant changes in most biological markers (e.g. cellular 2-5A synthetase, serum beta2-microglobulin and neopterin, and HLA class I and II antigens).

**Comment-** Formation of neutralizing antibodies to Betaseron in MS subjects did not appear to affect the ability of Betaseron to modulate the in vivo induction of biological markers.

**Animal-** The species specificity of Betaseron precluded the use of rodent models of MS to evaluate the in vivo pharmacological activity. Studies listed below (evaluating anti-viral potential) were therefore performed in non-human primates (African green monkeys infected with SVV). One study was also performed in guinea pigs (infected with HSV-2).

**Title:** Comparison of Intravenous, Intramuscular and Subcutaneous Injection of Recombinant Human Beta Interferon in the Treatment of Simian Varicella Infection (TBP01-31) (\*PK)

**Title:** Effect of Dosing Interval of rHUIFNS on Simian Varicella Virus Infection in Monkeys (TBP01-32)

**Title:** Results of an Experiment to Compare Two Different Formulations of Human Recombinant Beta Interferon for Antiviral Efficacy in the Treatment of Simian Varicella Infection in the African Green Monkey (TBP01-33) (\*PK)

**Title:** Effect of 9-(1,3-Dihydroxy-2 Propoxymethyl) Guanine and Recombinant beta Interferon Alone and in Combination in Simian Varicella Virus Infection in Monkeys (TBP01-34)

Title: Efficacy of Parenteral Recombinant Interferon-beta in Primary and Recurrent Genital HSV-2 Infection of Guinea Pigs (TBP01-39)

Route-dependent differences were not noted with i.v. or s.c. administration with respect to activity at 2 MIU/kg/day or hematologic toxicities at doses ranging from 2 to 50 MIU/kg/day. Hyperthermic responses, however, were observed in monkeys after i.v. admin. at 2 MIU or greater while doses of 50 MIU/kg/day via s.c. were needed to induce similar responses. After 7 days admin. hyperthermic responses became insignificant in all animals regardless of route. Daily injections of 2 MIU/kg/day were required to induce a sustained biological response in monkeys without major toxicities. More frequent administration at lower doses was more effective than less frequent at higher doses. Despite the limited bioavailability following repeated daily s.c. injections, the level of the biological effect was maintained and appeared protective. Similar activity was observed via s.c. injections with Laureth or HSA formulations while Laureth formulations appeared more effective than HSA formulations given i.v. Reduction in clinical disease was therefore dose and schedule dependent but not ROA dependent.

In G.P. there was a reduction in lesion severity but no decrease in HSV-2 titers. No effect on primary infection or recurrent episodes or in the frequency of recurrent episodes were observed. Animals received repeat doses t.i.d. at 5 MIU/kg/day.

Pharmacokinetics:

Human- A list of human PK studies was presented in vol. 5 section 5.1.2. 17 studies were presented (2 were combined) with a total of 264 subjects analyzed including 18 normal healthy volunteers and 246 subjects with various malignancies and AIDS subjects. Studies in the latter population were limited because of high background IFN levels which are known to be present in the serum of AIDS pts. Doses ranged from 5 to 1350 MIU. Routes of administration included i.v., i.m., and s.c. Not all ROA were evaluated at all dose levels. When compared to natural beta interferon, PK profiles of Betaseron were notably different (e.g. the AUC of natural was shown to be 5 times greater than that of Betaseron in one study). These differences are presumably due to the lack of carbohydrate moieties on Betaseron.

PK studies in pt were not performed. This is because serum levels of Betaseron are low or not detectable following S.C. administration of 45 MIU.

Following single and multiple daily (8 days) s.c. administration of 90 MIU in healthy volunteers, serum concentrations were generally

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below 100 IU/ml (assay sensitivity \_\_\_\_\_). Peak serum concentration occurred between 1 to 8 hrs, with a mean peak serum interferon concentration of 40 IU/ml. No accumulation was seen following daily dosing for 8 days. Serum neopterin and other biologic response marker levels peaked at 24 or 48 hrs after s.c. injection and remained detectable at 72 hrs.

Using non-compartmental analysis methods, the (CL) and (Vdss) were calculated to be  $12.7 \pm 4.7$  L/min/kg and  $2.88 \pm 1.81$  L/kg respectively with a mean terminal t<sub>1/2</sub> of  $257 \pm 137$  minutes ( $4.49 \pm 2.29$  hrs) in normal healthy volunteers. Using a two compartment analysis model (PCNONLIN), calculated PK parameters were comparable to those obtained when using non-compartmental analysis. No accumulation was seen following dosing for 8 days or s.c. admin. t.i.w. for 2 weeks.

No apparent accumulation after every other day s.c. dosing of 9 or 45 MIU was evident in MS subjects when either serum interferon conc. or other biologic response markers were measured. Bioavailability was evaluated in healthy volunteers who received two s.c. injections for a total dose of 90 MIU. Bioavailability was approximately 50%.

In patients, other than MS, receiving i.v. doses up to 300 MIU, increases in serum half-life were dose proportional. (At doses of 100 MIU or higher the terminal t<sub>1/2</sub> ranged from 2-5 hrs). The PK parameters calculated in subjects with cancer or AIDS (via i.v. inj.) were generally comparable to those obtained in normal volunteers, with CL values of 9.4 to 28.9 mL/min-kg, Vdss of 0.35-1.56 L/kg and t<sub>1/2</sub> values of 25 to 155 min at dose levels ranging from 3 MIU to 300 MIU. Betaseron did not accumulate in the serum of subjects with various malignancies receiving 3 to 100 MIU t.i.w. for 2 wks.

**Comment-** Most of the human PK studies were performed early in the development phase (i.e. 'pre-current test material'). Results were presented from a number of not very well-defined/detailed protocols, data sets, insufficient numbers of evaluable pts (selected statistical analyses), studies were combined (post hoc), there were different performance evaluations and dosing regimens, different product lots used etc. In general there were insufficient data provided for reliable 'traditional' clinical pharmacology labelling. Caution is therefore recommended in extrapolation of the data to meaningful labelling information.

**Animal-** PK parameters of Betaseron were evaluated using different routes of administration and different dose levels in African green monkeys (assessed in vivo pharmacological models referred to above).

Following a single i.v. administration of 1 MIU/kg, serum interferon conc. generally peaked at the first sampling time (5 min. post-dose). The average clearance (CL) values at steady-state

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and volume of distribution ( $V_{dss}$ ) were  $0.36 \pm 0.08$  L/hr-kg and  $0.65 \pm 0.09$  L/kg, respectively, and mean terminal half-life ( $t_{1/2}$ ) was  $1.90 \pm 0.43$  hrs. There were some differences in PK parameters following administration of 2 MIU/kg. These differences were attributed to intersubject variability; and the small numbers of animals studied.

Betaseron was slowly absorbed following s.c. injection. As doses increased (from 1 to 6 MIU/kg), the time to peak serum conc. also increased from  $2.7 \pm 1.2$  hrs to  $4.7 \pm 1.2$  hrs. Bioavailability was calculated to be 31% and 43.7% after a single 1 MIU/kg and 2 MIU/kg/dose, respectively. At steady state the AUC increased proportionally with increasing dose from 1 to 10 MIU/kg, indicating linear kinetic characteristics at this dose range. There was significant accumulation in serum following s.c. b.i.d. dosing. No accumulation was seen if dosing frequency was less than once per day.

**Comment-** Interpretation of animal PK data is complicated since the evaluation of parameters was incidental to SVV infection. Results should therefore be considered as 'best estimates'.

#### Safety/Toxicity:

**Human-** No subject died while receiving therapy or within 30 days of last dose at the cut off time for study analysis. Dose reductions occurred in 4% of subjects (13). Ten of these subjects subsequently completed the study.

Adverse events associated with Betaseron therapy are consistent with events observed with other licensed interferons i.e. "flu-like" symptoms including fever, chills, myalgia, malaise and sweating. Reactions at the injection site included inflammation and pain. Less than 5% of subjects receiving Betaseron experienced cardiovascular events (i.e. arrhythmia, tachycardia, angina, myocardial infarction and congestive heart failure. Neutropenia ( $<1500/mm^3$ ) occurred in 8% of MS subjects who entered studies with adequate WBCs ( $>4500/mm^3$ ). When WBCs were ( $<1500/mm^3$ ) the incidence increased to 63%. In the pivotal studies, elevated ALT/SGPT occurred in 12% of Betaseron subjects compared to 4% of placebo subjects. Spontaneous abortions were reported in subjects with MS. Dose independent increase in neutralizing antibody activity was detected in 40% of subjects. No evidence of anaphylaxis was observed despite the presence of antibody titers. (Based on data submitted in the original PLA submission).

#### Animal-

None of the PK or toxicology studies were (reportedly) previously submitted to BB-IND 1846                     

No single-dose acute studies were performed.

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**Pivotal preclinical GLP safety studies:**

**Title: A 28-Day Subacute Toxicity Study of Recombinant Human Interferon Beta (IFN-B ser17) Given Daily by Subcutaneous or Intravenous Injection to Rhesus Monkeys (Study # TBT01-22 and TBT01-23).(GLP)** (Lot # DP-100, 180 MIU/ml) (Dosing 9/86- Final report 1/90).

**Study Design:** Forty-eight animals were assigned to 8 groups of three male and three females per group. Three dose levels (2 MIU, 10 MIU and 50 MIU/kg) of Betaseron were administered qd for 28 days via s.c. or i.v. routes (0.2 mL/kg). Control animals received 28 consecutive doses of excipient containing HSA administered s.c. or i.v. Parameters evaluated included clinical observations, body weights, loss of appetite, mortality, rectal body temperature, systolic blood pressure, heart rate, ophthalmic examinations, hematology, chemistry and urinalysis. All i.v. high dose and two excipient control monkeys were sacrificed on study day 29 and examined (including gross and histopathologic morphology). Dosing was continued at 10 MIU/kg (Groups 2 and 6). These animals received weekly injections of Betaseron starting on study day 53 and were maintained under study conditions until day 90 day for dosing (last day of dosing was Day 84) through Day 174 for hypersensitivity testing and observations.

DTH testing was conducted predose and on Days 14 and 25 for all dose groups. ID injections into the eyelids were monitored for 60 min post inj. for edema, induration or erythema. Injection sites were scored at 24, 48 and 72 hrs. DTH testing was repeated on Days 50, 90 and Day 118 for animals receiving 10 MIU/kg. Follow up abdominal testing was performed on Days 95 and/or 103 due to immune reactions on Day 90. Animals which showed reactions following abdominal testing on Days 95 and/or 103 received additional abdominal testing on Days 118 and 124.

Serum samples were collected for antibody determinations ( IgG antibodies that bind Betaseron) predose and on Days 14 and 28 for all animals and during the subsequent dosing period for animals maintained on study.

**Results:** No animals died during the study. Clinical observations were unremarkable. On day one of treatment, a mild hyperthermic response was observed in all i.v. treated groups but only in the HD s.c. group. This response, still present in the higher dose i.v. group at day 3, was insignificant by day 7. Hematologic changes were similar following s.c. and i.v. administration. These changes occurred generally from day 7 to day 14 of treatment and included decreased segs, increased lymphs and decreased plts. All counts returned to normal by day 28, minor changes observed only in the HD i.v. group included transient increases in retics and decreases in total protein and albumin concentrations.

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No gross changes were observed at necropsy or following histopathological evaluation.

There were no positive DTH reactions up to study day 59. From day 90 onward mild to moderate reactions were observed with equivalent severity following i.v. or s.c dosing but with a higher frequency following i.v. dosing. Hypersensitivity reactions were however considered equivocal since reactions also occurred in response to the excipient control. By day 124 all animals reacted negatively to excipient and Betaseron.

Serum Betaseron binding antibodies were measured by ELISA methods. Results were presented in terms of monkey IgG titer, OD x DILN (optical density times dilution factor). In general, animals receiving Betaseron via s.c. showed higher peak levels of IgG binding than via i.v. route. For animals that continued on study beyond day 28 IgG antibody levels generally decreased after day 28 and approached baseline by day 90. Antibody levels generally increased with increasing dose levels and decreased with time upon less frequent dosing or after discontinuation of dosing. Overall, s.c. admin. produced higher peak levels of binding antibodies than i.v. admin.

Title: Range-Finding and Developmental Toxicity Studies of Recombinant Human Interferon- $\beta$  ser (Betaseron) in Pregnant Rhesus Macaques (macaca mulatta) Study # TBT01-30 and 31 (GLP)  
#GLP (Lot #s BAP-009, BAP-G10A, BAP-011A, BAP-013B) (Day 1 of dosing 1/11/91 and 3/27/91-final report 12/9/91).

**Study Design:** Rhesus monkeys were treated early to mid-pregnancy (days 20 to 70) of gestation via daily s.c. injections. 4 females/gp were evaluated in the range-finding study at 5, 45, 90 or 135 MIU/kg. In the main study there were 10 females/ treatment gp with 9 in the excipient control group. Dosage evaluated were 5, 45 or 75 MIU/kg/day. Hysterotomies were scheduled for GD 100 $\pm$ 2 for all dose groups. Dosing was scheduled to cease immediately upon detection of a nonviable embryo or presence of a nongravid uterus (indicating completed abortion) by ultrasound. Blood samples were obtained for serum progesterone and 17 beta estradiol assays, serum markers, antibody titer and interferon concentration. Fetal necropsies consisted of standard teratological evaluation (external measurements, gross exams, selected organ weights). Skeletal structures were examined post staining. Placentas were weighed, examined for gross abnormalities and measured.

**Results:** The number of animals receiving the full course of treatment included 6/10 at 5 MIU/kg/day; 8/10 at 45 MIU/kg/day; 5/10 at 75 MIU/kg/day; 2/4 at 90 MIU/kg/day; and 1/4 at 135 MIU/kg/day. Poor appetite was observed in all dose groups with an increased incidence at equal to or greater than 45 MIU/kg. Severe

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hematologic toxicity was observed at  $\geq 75$  MIU/kg/day. Compound related changes in hem/chem parameters included anemia, neutropenia, thrombocytopenia and hyperfibrinogenemia. Hematologic toxicity at higher doses warranted treatment termination and/or treatment intervention. One female required a blood transfusion, 3 required supplemental iron and one was euthanized due to severe necrosis of distal limb. While there was no statistically significant effects on BP, there was slight elevation during the treatment period when compared to concurrent controls. Sporadic elevations in rectal temperatures were also noted. The increase in temps did not however correlate with other test substance findings. There were no effects of treatment on serum hormone levels measured in the range-finding study.

Maternal toxicity was documented by increases in ALT, AST and CPK. There was a statistically significant increase in abortion rates (embryonic loss) at 90 and 135 MIU/kg/day. Incidence of prenatal mortality per dosage groups were as follows: control- 1/9 (11%); 5 MIU/kg/day- 4/10 (40%), 45 MIU/kg/day- 3/10 (30%); 75 MIU/kg/day -4/10 (40%); 90 MIU/kg/day- 2/4 (50%); and 135 MIU/kg/day -3/4 (75%). Most of the abortions occurred prior to GD 40. All fetuses appeared normal following gross anatomical evaluation. All fetal external measurements and organ weights were normal. There were no significant effects on skeletal structures nor any effects on placental weights and measurements, umbilical cord length or amniotic fluid volume.

**Comment-** Doses selected in the above studies were uncharacteristically higher than those used in the repeat dose toxicity study. No PK parameters were evaluated in pregnant animals. While serum samples were obtained for measurement test article and antibody, no data were provided.

**Title:** An Evaluation of IFN-B ser17 Effects of Menstrual Cycles in Rhesus Monkeys (Study # BLT 01-32). (GLP) (            )  
(Lot #s BAP-024C, BAP-031A and BAP-121D). ONGOING.

**Study Design:** It is not known whether Betaseron can affect human reproductive capacity. The purpose of this study is therefore to characterize the effect of Betaseron on menstrual cycle and hormone profiles following daily s.c. injection to rhesus monkeys for 3 menstrual cycles. The animals will be followed for 2 menstrual cycles following the treatment period.

**Results:** Twenty-four females (6/gp) were dosed at 0 (placebo control) 5, 30 or 60 MIU/kg/day Betaseron. Treatment of the last animal was initiated on 11/28/92. Last post-treatment bleeding is scheduled for 5/1/93. No data have been submitted to date for review. The proposed date for submission of the audited draft report for this study is 9/15/93.

**Title: Mutagenicity Test on Recombinant Human Interferon Beta (IFN-B Ser17) in the Salmonella/Reverse Mutation Assay (AMES Test). Preincubation Method (Study # TBT01-28) (GLP)**  
(Lot # BAP-928F) (1/31/90-5/16/90).

**Study Design:** Five tester strains of histidine dependent Salmonella typhimurium designed to detect both frameshift mutations or base-pair substitutions were used (TA 98, TA 100, TA 1535, TA 1537, TA 1538). Concentrations up to 99.9 MIU/plate were tested in the presence or absence of metabolic activation prepared from Aroclor 1254 induced rat liver microsomes.

**Results:** No evidence of mutagenicity. The test material was not cytotoxic at the dose levels tested.

**Title: Morphological Transformation of BALB/3T3 Mouse Embryo Cells. Test Article: Recombinant Human Interferon Beta (IFN-B ser17) (Study # TBT01-29) (GLP)**  
(lot # BAP-928F) (5/23/90-9/26/90).

**Study Design:** Non-cytotoxic concentrations up to 30 MIU were tested in BALBc/3T3 mammalian cell line to evaluate carcinogenicity potential (3 day exposure).

**Results:** No statistically significant increase in the morphological transformation frequency was observed. The cloning efficiency for the negative control was 45%. Cell survival at the highest treatment dose was 87% and the osmolality was 386 mOsm/kg.

NO CHRONIC IN VIVO STUDIES WERE PERFORMED TO ASSESS CARCINOGENIC POTENTIAL DUE TO LACK OF RELEVANT ANIMAL MODEL. Carcinogenic potential is usually evaluated in mice and/or rats for 'traditional' pharmaceutical products. A maximum tolerated dose is selected as the high dose. This dose is generally determined from data obtained following evaluation of a 90 day repeat dose toxicity and/or from consideration of (a multiple) of the proposed human exposure. The species specificity of Betaseron precluded the estimation of MTD and the rationale for chronic dosing in rodents. While no antibody measurements were performed in the repeat dose rat studies it is expected that antibodies are elicited following repeat dosing based on results from non-human primate repeat dose study.

**Preliminary/Exploratory (non-GLP studies):**

A number of exploratory studies were performed during the early product development stages. These studies included evaluation of the related molecule IFN- Bcys (IFN-B1 or Hu IFN B1). These studies were generally low powered in terms of the number of animals.

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Title: Toxicity Study of Intravenously Administered Human Interferon Beta (IFN-B ser17) in Mice (Study # TBT01-12) (non-GLP)  
(Lot #8B130AB-2) (Dosing 1/83-revisions to final report 8/90).

**Study Design:** Ten male mice received 0.24 MIU/mouse/day (ranging from 7.7 to 13.3 MIU/kg/day) via daily i.v. injection for 10 days (five consecutive days per week for an interval of two weeks). Ten mice received sodium chloride for injection and served as control. Animals were observed for an additional 2 weeks after administration. BW and clinical observations were monitored. No postmortem exams were performed.

**Results:** No adverse effects were observed.

Title: Toxicity Study of Intravenously-Administered Recombinant Human Interferon Beta (IFN-B ser17) in Rabbits and Mice (Study # TBT01-16) (non-GLP)  
(Lot #BP-102) (10/83-8/90).

**Study Design:** Five rabbits and 10 mice received daily i.v. injections for 10 days at doses of 10 MIU/kg/day and 50 MIU/kg/day respectively. Animals were observed for an additional 2 weeks after administration. BW and clinical observations were monitored.

**Results:** No adverse effects were observed in mice. Two deaths occurred in rabbits receiving placebo control attributed to intercurrent disease (coccidiosis infection).

Title: Fourteen-Day Subacute Intravenous Study with Laureth 12-Formulated Recombinant Human Interferon Beta (IFN-Bser17) in Rats (Study #TBT01-24) (GLP)  
(Lot #s TS-1EA, TS-1A, BP-1610) (12/86-3/87).

**Study Design:** Thirty male rats were placed on study (5/gp). Animals received daily i.v. injection for 14 days at doses of 1, 10 or 100 MIU/kg/day (0.2ml/100 g BW). Control animals received NaCl. Animals were evaluated for changes in clinical signs, BW, clinical pathology, organ weights, gross and microscopic changes in selected organs. No antibody measurements were obtained in this study.

**Results:** No adverse effects were observed.

Title: Toxicity Study of Intravenously-Administered Recombinant Human Interferon Beta (IFN-Bser17) in Rabbits (Study #TBT01-11)(non-GLP)  
(lot #8B130AB-1) (Dosing 1/83- revised final report 8/90).

**Study Design:** Ten animals (5 males/gp) were dosed with either NaCl or 1.5 MIU/kg/day test material daily via i.v. injection for 10 days (5 consecutive days for 2 weeks). Animals were observed

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for an additional 2 weeks after administration. BW and clin obs were monitored. No information was provided on gross obs or organ weights.

**Results:** No adverse effects were observed that were attributable to test compound. There was one death on Day 9. Death and clinical obs were consistent with spontaneous intercurrent disease.

**Title:** A 14-Day Dose Range Finding Study of Recombinant Human Interferon Beta (IFN-B ser17) Given Daily by Intravenous Injection to Rhesus Monkeys (Study # TBT01-21) (non-GLP)  
(Lot #BP-161B) (Dosing 5/18-approved amendment final report 1/90).

**Study Design:** Range-finding study. Betaseron was administered at 0.44, 2.5, 12.5 or 33.3 MIU/kg/day via i.v. injection for 14 days (2/sex/gp). Clinical signs, appetite, BW and BT were monitored. Clinical path was performed pretreatment, and on days 3, 7 and 14. No antibody was measured in this study. There was no scheduled necropsy.

**Results:** No adverse effects were observed. The only test compound-related change was a dose-related decrease, starting at 12.5 MIU/kg/day, in segmented neutrophils and an increase in lymphocytes at 14 days. Values however were still close to normal ranges. Serum cholesterol was slightly increased above baseline in all interferon groups related to excipient control.

**Supporting studies (different formulations and/or routes of administration):**

**Title:** 28-Day Subacute Intravenous and Subcutaneous Toxicity Study of Human Interferon Beta (IFN-B ser17) Formulated with Laureth 12 in Rabbits (Study # TBT01-26) (GLP)  
(Lot # DP-106) (6/87-2/88).

**Study Design:** Sixty animals were placed on study (3 males and 3 females/gp). Animals received daily i.v. or s.c. injections for 28 days at doses of 0 (NS or Laureth 12 placebo), 0.1, 1 or 10 MIU/kg/day (0.2 mL/kg). The conc. of Laureth 12 was not provided. Animals were monitored for changes in clinical signs, BW, clinical pathology, urinalysis, gross and histopathological changes. No antibody was measured in this study.

**Results:** No adverse effects were observed.

**Title:** Primary Skin Irritation of Recombinant Human Interferon Beta (IFN-B ser17) in Pluronic Gel in Guinea Pigs (Study # TBT01-18) (GLP)

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(Lot # BP-110) (10/84-10/84).

**Study Design:** The test substance was a non-injectable dosage form of the HSA formulation of Betaseron and consisted of 5 MIU per mL of Pluronic gel base with Thimerosal. One half (0.5) mL was applied on each of two sites (intact non-abraded and abraded skin) on the backs of G.P. and occluded (semi-occlusive dressing) for 24 hours (single exposure). Daily observations were made for clinical toxicity and evaluations for skin irritation were performed at 24 and 72 hours. (Evaluated by Draize scoring)

**Results:** No observable adverse effects were noted. The test substance was determined to be non-irritating.

**Title:** Eye Irritation of Recombinant Human Interferon Beta (IFN-B ser17) in Pluronic Gel (Study # TBT01-17) (GLP) (Lot # BP 110) (Dosing 9/84-revisions to report 6/90).

**Study Design:** Twelve rabbits were placed on study. 0.1 mL of the test substance formulated at 5 MIU/mL in pluronic gel was instilled into the eyes of 9 rabbits. Nine rabbits were treated with the test substance and 3 control animals received pluronic gel alone. Animals were observed for signs of clinical toxicity and for ocular irritation at 1, 24, 48 and 72 hours after treatment. (Scoring range: nonirritating, practically non-irritating, minimally irritating, mildly, moderately, severely, extremely).

**Results:** Betaseron was determined to be minimally irritating to unwashed eyes and practically non-irritating to washed eyes. Treatment with Pluronic gel alone was determined to be practically non-irritating to unwashed eyes.

**Title:** Vaginal Irritation Study of Recombinant Human Interferon Beta (IFN-B ser17) in Pluronic Gel (Study # TBT01-19) (GLP) (Lot # BP-110)  
\*Dosing 11/84-revisions to report 6/90).

**Study Design:** Eleven rabbits were evaluated. Three were treated with pluronic gel and served as vehicle controls, 2 animals were sham treated (bell-tipped needle insertion), 6 animals received 0.5mL of Betaseron (5 MIU/mL).

**Results:** Transient slight erythema was noted in 2 Betaseron animals. Test material was determined to be non-irritating.

**Title:** Eye Irritation of Recombinant Human Interferon Beta (IFN-B ser17) in Saline (Study # TBT01-20) (Lot #BP143) (Dosing 8/85-revisions to report 6/90).

**Study Design:** Twelve animals were placed on study. 0.1 mL of

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an ophthalmic solution of Betaseron in saline with thimerosal (50 MIU/mL) was instilled into the eyes 6 times per day (over 10 hr period) for 10 consecutive days. Animals were sacrificed on study day 13.

**Results:** A higher incidence of slight conjunctival redness was observed in the treated animals. No other clinical signs of toxicity were observed. The test material was considered to be non-irritating to the eyes of rabbits after multiple treatments.

**Title:** Ten Day Toxicity Study of Intramuscularly-Administered Recombinant Human Interferon Beta (IFN-B cys) in Female Rats (Study # TBT01-15) (GLP)  
 (Lot # 81-114-1, 81-114-2) (Dosing 10/82-corrections to final report 6/90).

**Study Design:** Fifty-six rats were placed on study. Forty-eight rats (8/gp) were dosed daily for 10d (dose vol. 0.1 ml/kg) with conc. of SDS ranging from 0.04 to 5 mg/ml. Eight rats received WFI and served as controls. Animals were observed for changes in clinical signs. BW, BT, local tissue reactions, clinical pathology, complete gross necropsy, organ wts and histomorphological evaluation of selected tissues.

**Results:** At the highest conc. of SDS enhanced degeneration and necrosis plus interfiber edema/ inflammatory changes were observed.

**Title:** Ten Day Toxicity of Intramuscularly-Administered Recombinant Human Interferon (IFN-Bcys) in Female Rabbits (Study # TBT01-14) (GLP)  
 (Lot #81-114-1 and -2) (Dosing 12/82- report revised 6/90).

**Study Design:** Fifty-six animals were assigned to study (8/gp). Dose of IFN Bcys was 0.5 MIU/kg. Dose groups included WFI (control), HSA + dextrose (excipient), IFN + excipient, excipients + 0.04 mg SDS/mL, IFN + excipient + 0.5 mg SDS/mL, excipient + 0.5 mg SDS/mL, excipient + 5.0 mg SDS/mL. Dose vol. 0.1 mL/kg.

**Results:** Notable treatment related effects occurred in both IFN groups and included injection site reactions, slight elevations in BT postinjection (increased incidence compared to other groups). Slight BW increase in relative spleen and liver weights. Clin hem changes reflected local tissue rxns. at inj. site. (e.g. dec. plt, inc. total leucocytes, inc. globulin and dec. erythron. Formulations with SDS conc.  $\geq$  0.04 mg/mL resulted in minimally toxic reactions.

**Title:** Nine Day Toxicity Study of Intravenously-Administered Recombinant Human Interferon Beta (IFN-Bcys) in Male Rabbits (Study #TBT01-13) (GLP)

\_\_\_\_\_ (Lot #81-114-1; 81-114-2) (Dosing 10/82-revisions to final report 6/90).

**Study Design:** Fifty-six rabbits were placed on study (8/gp). Each group received one of the following treatments; HSA + dextrose (excipient), IFN-Bcys + excipient, 0.04mg SDS/mL + excipient, IFN-Bcys + excipient + 0.5 mg/mL SDS, excipient + 0.5 mg SDS/mL, excipient + 5 mg/mL (dose vol. 0.3-0.4 mL). Observations were similar to those listed in previous study.

**Results:** Mean post-injection temps were increased consistently (but only slightly). Rhinorrhea was observed in animals receiving IFN. Animals receiving IFN had a lower mean %segs and higher mean %lymphs. There were no microscopic correlates to localized reaction site edema and erythema.

**Title:** Acute Toxicity Study of Various Concentrations of SDS and of Formulations of Recombinant Human Interferon Beta (IFN-Bcyc) in Solubilizers SDS or HSA in Female NZW Rabbits (Study # TBT01-10) (non-GLP) \_\_\_\_\_ (N/A)

**Study Design:** Forty-eight female rabbits were infused daily with increasing conc. of SDS (4/gp). Single i.m. inj. of 3 conc. SDS (0.5, 2.5, and 5.0 mg/mL in NS) each at 3 diff. pH (7.4, 8.5, and 9.5). IFN dose 0.3 MIU/kg.

**Results:** Injection site necrosis was noted with increasing SDS conc. Intense local reactions were observed in 10 mg/ml SDS formulation admin. i.v. or i.m at 0.1 ml/kg. IFN  $\leq$  2.5 mg SDS/mL PBS were comparable to controls. Rabbits receiving IFN developed mild hyperthermia responses irrespective of SDS conc.

**Title:** Twenty-Eight Day Subacute Subcutaneous Toxicity Study with Laureth 12 in Rats (Study# TBT01-25) (GLP) \_\_\_\_\_  
 \_\_\_\_\_ (Lot #N/A) (5/87-9/87)

**Study Design:** Thirty male rats were placed on study (6/gp). Animals received daily s.c. inj. for 29 days of 0.07, 0.7 or 7.0 mg/kg/day Laureth 12. One group received 7.0 mg/kg/day via i.v. Animals were observed for clinical signs, changes in BW, clinical pathology, gross-organ and histomorphological evaluation on selected organs.

**Results:** Treatment related effects were limited to injection site reactions in the i.v. group.

**Title:** Acute Toxicity Study of Varying Concentrations of Sodium Dodecyl Sulfate (SDS) in Female NZW Rabbits (Study # TBT01-09) (non-GLP) \_\_\_\_\_ (Lot # N/A)  
 (Dosing 4/82- report revised 6/90)

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**Study Design:** Thirty two animals were placed on study (8/gp). Animals were treated with 0.1, 2.5 or 10 mg/mL (dose vol. 0.1mL/kg) via i.v. and i.m. inj. Animals were observed for clinical signs, BW, clinical pathology, gross and histomorphological evaluation on selected organs.

**Results:** Severe reactions were noted at the injection site at  $\geq 2.5$  mg/mL or 0.5 mg/kg/day. Minimal effective conc. of SDS in PBS is 0.1 mg/mL at dose vol. of 0.1 ml/kg (total dose of 0.02 mg/kg/day).

**Title:** Acute Toxicity Study of Varying Concentrations and pH of Sodium Dodecyl Sulfate (SDS) Following Single Intramuscular Injection in Female NZW Rabbits (Study # TBT01-08) (non-GLP) (Lot # N/A) (5/82-report revised 6/90)

**Study Design:** Forty-four rabbits were placed on study (4/gp). Each received a single i.m. injection of one of three conc. (0.5, 2.5 and 5.0 mg/ml NS) of SDS at one of 3 pH ranges (7.4, 8.5 or 9.5) at 0.1 mL/kg. One group of rabbits received IFN-B1 "current production sample" (0.3 MIU/kg). Rabbits were closely monitored for 4 days, then euthanized and examined for possible pathologic changes.

**Results:** Injection site muscle necrosis increased with SDS conc. and with pH.

#### Conclusions and Integrated Summary:

The preclinical studies in support of the safety of Betaseron represent over a decade of product development. Over the course of this development the final product and clinical formulation 'evolved'. Most of the earlier exploratory studies performed were non-GLP. In general these studies were not considered scientifically rigorous enough i.e. to conform to the principles of GLP (e.g. sufficient data was not provided to allow for a full review of the conclusions especially with regards PK analysis, full tabulations of original data were not always included, reports to some studies performed in 1982 were revised in 1990). Those studies performed according to GLPs and using test material equivalent to the clinical material were considered pivotal to the assessment of safety. Despite the shortcomings in many of the earlier studies there are no significant preclinical safety issues that would preclude product approval.

The predictive value of preclinical studies assessing the safety of interferons have historically been of questionable relevance due to the strict species specificity of the interferons. While non-human primates have been the species of choice, and have shown adverse reactions to high doses of interferons, no species to date has been

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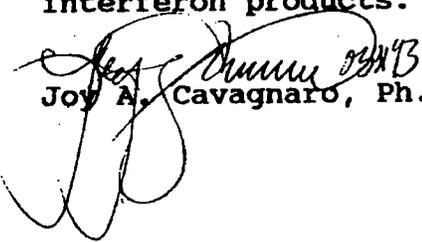
able to mimic the "flu-like" symptoms which has been the major dose-limiting toxicity. Studies reported in this submission suggest that the rabbit may also be useful in assessing some aspects of interferon toxicity e.g. neutropenia and hyperthermia. Interestingly, only the non-human primate and the rabbit showed sensitivity to the hypotensive response of IL 2. Unfortunately the high incidence of intercurrent diseases in the rabbit studies submitted compromised the evaluation of these studies.

Both the rabbit and the rat were useful in assessing local reactogenicity exhibited by changes in formulation.

Although Betaseron will be administered chronically the restricted species specificity precludes testing in the traditional rodent model. While antibody was not measured in the rodent studies antibodies are expected to develop based on the development of an antibody response in the repeat dose non-human primate study. Curiously the doses used in the reproduction toxicology study were higher than in the repeat dose study. While sera were taken no data were presented on antibody level. Most of the adverse effects including abortions occurred within the first 3 weeks of dosing during GD 20-40. Unfortunately there is not sufficient information to date to determine clinically or preclinically the significance of the antibody response to human proteins. In some cases there is abrogation of activity and safety coincident with measurable antibody levels especially neutralizing antibodies. In other cases and in the clinical studies reported in this submission, while antibodies were measured in 40% of the subjects there was no affect either on efficacy or safety.

Based on a conservative NOAEL (no adverse effect level) of 10 MIU/kg in the repeat dose primate study and converting to human equivalents (body surface area, est. 60kg person), the highest dose in the clinical study has a safety factor of at least 13 X. (Animals received daily injections). Mild to moderate changes principally in hematological parameters were noted at a safety factor of 67 x. Moderate to severe toxicity including anemia, neutropenia, thrombocytopenia and hyperfibrinogenemia were noted in the reproductive toxicology studies at equal to or greater than 100 x the highest clinical dose.

The toxicities observed following Betaseron administration were consistent with those observed with currently licensed interferon products.

  
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