MEMORANDUM

TO: The file
FROM: Anne M. Pilaro, Ph.D., Clinical Pharmacology/Toxicology Branch, DCTDA
THROUGH: M. David Green, Ph.D., Chief, Clin. Pharmacology/Toxicology Branch, DCTDA
RMS BLA #: 103964
SPONSOR: Hoffmann-La Roche
PRODUCT: pegylated interferon-α 2a (PEG-IFN, PEGASYS®, Hoffmann-La Roche)
PROPOSED INDICATION: treatment of chronic hepatitis C infection

DATE: February 19, 2001

SUBJECT: Summary Pharmacology/Toxicology Review of PEGASYS® for hepatitis C

INTRODUCTION

The incidence of hepatitis C infection in the United States and worldwide is increasing. In a recent report from the Centers for Disease Control and Prevention, it was estimated that hepatitis C infection was responsible for approximately 150,000 new cases of acute hepatitis each year, in the United States alone. Approximately 1.6% of the population, or 3.5 million patients are estimated to be infected with the virus, with an estimated annual mortality of 8000 to 10,000 patients.

The hepatitis C virus is unique in that it is a single-stranded, RNA-based virus that targets hepatocytes for infection and replication of new virions. About 4 to 8 weeks after the initial HCV infection, acute elevations of hepatic transaminase levels in serum are often noted, signaling that inflammation in the liver is occurring. During this stage of the disease, the majority of patients are asymptomatic, although using sensitive PCR-based assays, HCV RNA may be detected in the serum.

Approximately 80% of patients with acute HCV infection progress to more chronic liver disease. This stage is manifested by persistent elevations in serum levels of hepatic transaminases and HCV RNA. Histologic evidence of chronic inflammatory changes may or may not be present in biopsied liver samples. Further progression of the disease leads to scarring, fibrosis, and cirrhosis in the affected regions of the liver in approximately 20 to 50% of infected patients between 10 to 20 years after the initial infection. At this stage, other clinical features commonly associated with cirrhotic liver disease become evident, such as ascites, jaundice, esophageal varices, and encephalitis. A number of patients with chronic HCV infection may also progress to primary hepatocellular carcinoma.

Treatment options for HCV infection are limited. Consistent decreases in serum transaminase levels, a surrogate marker for hepatic inflammation, have been observed in approximately 50% of patients treated with 3×10^6 IU of recombinant, type I interferons three times weekly for a duration of 6 to 12 months. However, between 50 and 75% of the responding patients relapsed after cessation of interferon treatment, resulting in durable response rates of < 25%. Lower doses of interferon, even when administered on a daily schedule were demonstrated to be ineffective when compared to either untreated or placebo-treated control patients. Taken together, these data

suggest that continuous, high levels of exposure to type I interferons are necessary for the anti-viral effects in HCV infection.

Pegylated interferon alpha 2a (PEGASYS®, PEG-IFN) is comprised of recombinant, *Escherichia coli*-derived interferon-α 2a (ROFERON®-A), chemically conjugated to a single, branched polyethylene glycol moiety with an average molecular weight of 40,000 daltons. The *in vitro* and *in vivo* properties of PEG-IFN are similar to those of other, recombinant type I interferons. Specifically, PEG-IFN can induce intracellular antiviral activity, inhibit the proliferation of several tumor cell lines, activate natural killer cell-mediated tumor cytolysis, and induce cytokine synthesis and release by immune effector cells similarly to other interferon-α preparations. Its advantages, however, are that it is very slowly cleared after s/c injection, leading to longer terminal half-lives and higher exposure levels (AUC and C_{max}) in both preclinical and clinical pharmacokinetic studies. The resulting increase in exposure is thought to be a major factor in the increased efficacy seen in patients with HCV infection treated with PEG-IFN in the phase 2 and phase 3 pivotal trials.

The intended clinical use of PEG-IFN is for the treatment of patients with chronic HCV infection. In two phase 3 pivotal trials, the safety and efficacy of different doses of PEG-IFN after 48 weeks of treatment and 24 weeks of treatment-free follow-up were compared to those attained in patients treated with the currently licensed, interferon-α 2b regimen (ROFERON®-A, Hoffmann-La Roche). Efficacy in the two studies study was evaluated by measurement of serum ALT levels over time, as well as quantitation of HCV RNA levels by --- at baseline, at the end of 24 and 48 weeks of treatment, and at the end of follow-up. The percentages of patients exhibiting normalization of serum ALT levels at the end of the 24 week follow-up period were 23% and 31% for patients treated with 180 µg/week PEG-IFN in trials NV15496 and NV15497, respectively, as compared to 13% and 19% complete responders, respectively for the two trials in the groups treated with ROFERON®-A. Comparable decreases in serum HCV RNA levels to less than the detectable limits of the assay (considered "HCV RNA-negative") were obtained in both groups of patients (22% and 31 % of patients had undetectable RNA levels in trials NV15496 and NV15497, respectively when treated with 180 µg/week PEG-IFN, as compared to 9% and 15% virologic responders in patients treated with 3 MIU ROFERON®-A, t.i.w.). Combined response rates for both normalization of ALT and negative HCV RNA responses were 20%, 28%, and 12%, for patients treated with 180 µg/week PEG-IFN in the two studies, or ROFERON®-A, respectively.

The dose and schedule of PEGASYS® intended for administration to chronically-infected HCV patients is 180 µg, injected subcutaneously, once weekly for 48 weeks. PEGASYS® is formulated with sodium chloride, benzyl alcohol, sodium acetate trihydrate, acetic acid, and polysorbate 80, pH 6.0, and provided as a colorless to light yellow, clear solution. The PEG-IFN used for all preclinical pharmacology, pharmacokinetics, and toxicology studies was produced at commercial scale, was greater than ----- pure, was formulated according to clinical procedures and was either of clinical grade, or representative of that used in the clinic.

PRECLINICAL PHARMACOLOGY, SAFETY PHARMACOLOGY, AND PHARMACOKINETICS

No information is included in the submission that describes preclinical pharmacologic activity of PEGASYS® against the hepatitis C virus (HCV), due to the absence of either a suitable animal model, or a cell-based replication system for detection of HCV. Results from both *in vitro* and *in vivo* pharmacology studies using previously established, and/or validated assays confirm that PEG-IFN exhibits improved pharmacokinetic and pharmacodynamic properties *in vivo*, while retaining the *in vitro* activities characteristic of alpha interferon(s).

In Vitro Pharmacology of PEGASYS®

The interaction of PEGASYS® with the type I interferon receptor(s) was determined using *in vitro*, ---------- to ---------- and ---------- cells, and ---------- unconjugated interferon-α 2a (ROFERON®-A, IFN-α). The results indicate that while PEG-IFN did not bind to ----- cells, competition for binding of unmodified, ------IFNα to ---- cells was observed at PEG-IFN concentrations of greater than 100 ng/ml. The IC_{50} for inhibition of binding of the unmodified, parent interferon by PEGASYS® was approximately 1000 ng/ml.

Induction of known DNA binding proteins associated with IFN-α mediated signal transduction was measured by ---------- assays of nuclear extracts from treated ----- or ---------- cell lines after exposure to PEG-IFN. Treatment of cells with 5 ng/ml unmodified, IFN-α resulted in induction and subsequent DNA binding of the nuclear transcription factors *Stat* 1:1, *Stat* 1:3, and *Stat* 3:3 homo- and hetero-dimers in both -------- and ---------- cell lines. By contrast, PEG-IFN at the same concentration resulted in detectable transcription factor binding only in ----- cells, and was markedly lower than that observed with the unmodified, parent compound. Similar effects on nuclear binding of the ------- protein complex (the interferon-stimulated response element of the 2', 5'-oligoadenylate synthetase promoter) were observed in ---------- cells exposed to either IFN-α or PEG-IFN at concentrations of 5 to 20 ng/ml, in a dose-related fashion. Treatment of ------ cells with PEGASYS® at all concentrations tested did not induce DNA binding of the ----- protein complex, while the unmodified, parent compound was active at concentrations as low as 5 ng/ml. The human gene ---- was used as a marker of IFN-α induced gene expression in---------- cells after exposure to either PEG-IFN or the unmodified, parent compound. Using a ribonuclease protection assay, both the unmodified, IFN-α and PEG-IFN were found to induce human ---- gene expression two to 15-fold over the untreated control cells, in a dose-related fashion. At concentrations of 1000 ng/ml, unmodified IFN-α resulted in a 10-fold increase in gene expression as compared to baseline, while ----- gene expression in this system was increased approximately 11-fold after PEG-IFN treatment at the same concentration.

Anti-viral activity of PEGASYS® against ---------- virus ---------- was evaluated in ---------- cells. As compared to unmodified IFN-α, the anti-viral activity of PEG-IFN against ---------- cells was approximately 7-fold lower, with a specific activity (IC_{50}) of 2×10^8 U/mg protein for IFN-α and 1.4×10^7 U/mg for PEGASYS®, respectively. Interferon activity against ---------- virus was assayed in ---------- after incubation with 0-1000 ng/ml PEG-IFN or unmodified IFN-α. Both forms of interferon were effective in protecting -------- cells from cytolysis induced by -------- infection. At lower concentrations, unmodified IFN-α had a slightly increased anti-viral effect as compared

to the PEG-conjugated material; however, at concentrations greater than --- ng/ml, the differences in anti-viral activity between the two IFN compounds were negligible. Using a newly developed assay to determine the anti-viral activity of interferons against HCV, ---------- ---------- were treated with either PEG-IFN or IFN-α, and the effects on viral replication were determined. Both interferon compounds showed inhibitory effects on HCV replication in this system, with complete replicon inhibition obtained after incubation of the transfected cells with 0.34 ng/ml IFN-α or 19.6 ng/ml PEG-IFN. PEGASYS® was approximately 200-fold less potent than the unmodified, parent compound in this assay, with an IC_{50} value of 2.7 ng/ml for PEG-IFN and an IC_{50} value of 0.018 ng/ml for IFN-α.

Anti-proliferative activity of PEG-IFN against several ---------- cell lines was assayed as compared to the unmodified, IFN-α after incubation for two to four days in the presence of either compound. The antiproliferative activity of PEG-IFN varied depending on the cell line being tested, but in all cases PEG-IFN was less active when compared on a total protein weight basis to the unmodified, parent compound. These results are summarized in Table I, below:

Table I – *In vitro* Anti-proliferative Activity of IFN-a and PEG-IFN against ---------- Cell Lines

--------- Cell Line	Type	IC_{50} (ng/ml)		Ratio[a]
		IFN-a	PEG-IFN	
--------	----------	0.012	0.748	0.016
-------	----------	0.14	17.2	0.008
--------	----------	0.06	11.6	0.005
---------	----------	0.95	11.5	0.08
---------	----------	992	36,819	0.03
----------	-------	7.8	209.4	0.04
---------	----------	2.2	204.1	0.01
---------	----------	1.2	330.6	0.004
----------	----------	7.4	522.8	0.01
----------	----------	92.4	3729	0.02

[a] ratio of IC_{50} of unmodified, IFN-α to the IC_{50} of PEG-IFN

In summary, PEG-IFN was demonstrated to effectively bind the type I interferon receptor, induce both signal transduction and gene expression associated with interferon-α, and have both *in vitro* anti-viral and anti-proliferative activities typical of interferon-a, although at lower potency than the unmodified, parent compound ROFERON®-A.

In Vivo Pharmacology of PEGASYS®

The *in vivo* pharmacologic and anti-tumor effects of PEGASYS® were evaluated in ---------- mice bearing established, ----------. In all three tumor models, PEG-IFN injected s/c in the contralateral flank to the tumor at a dose of 300 μg protein/week resulted in marked inhibition of growth and tumor regression after 4 to 6 weeks of treatment, while injection of the unmodified, parent IFN-α at 100 μg protein, three times weekly for the same duration resulted only in a slight to moderate inhibition

of tumor growth. It should be noted that in the *in vitro* assay systems described above, PEG-IFN was approximately 300 to 2000-fold less active than IFN-α in the inhibition of proliferation of these three tumor cell lines.

Safety Pharmacology of PEGASYS®

Safety pharmacology studies were conducted in rats and mice to evaluate the effects of PEGASYS® on the cardiovascular, renal, gastrointestinal, and central nervous systems. Saline-pretreated rats injected s/c with 6, 60, or 600 µg/kg PEG-IFN had significantly decreased urine volumes in the 0-3 hour interval after injection of PEGASYS®, as compared to animals dosed with either vehicle control or ---------- ($p \leq 0.05$, ANOVA). However, there was no dose-response in relation to urine output, and no changes in urinalysis profiles were noted in PEG-IFN treated rats as compared to control, with the exception of a slight, although statistically significant decrease in urine potassium levels in the mid-dose group at the 6-24 h interval after dosing ($p \leq 0.05$, ANOVA). It should be noted that these effects are of questionable biologic relevance, since PEG-IFN was previously demonstrated to be pharmacologically inactive in rodent species.

Cardiovascular and respiratory safety pharmacology studies were performed in conscious mice or ---------- monkeys. Mice were injected s/c with 6, 60, or 600 µg/kg PEG-IFN, and monkeys were injected intravenously with the same doses. In the mouse, PEG-IFN at all doses tested had no effects on either respiratory rate or core body temperature, as compared to vehicle control. By contrast, s/c injection of 10 mg/kg ---------- resulted in significant decreases in both parameters, as compared to vehicle control treated animals. In monkeys, i/v injection of 6, 60, or 600 µg/kg PEGASYS® had no remarkable effects on respiratory rate; however, increased heart rate(s) and a reduction in the Q-T interval on ECG evaluation were present in the group treated with 6 µg/kg PEG-IFN. These effects were statistically significant as compared to the vehicle control. However, since no dose-response relationship was present and the changes minor in magnitude, they were not considered biologically relevant. It should be noted that neither the heart rate increases or the decrease in Q-T intervals were observed in single or repeat-dose toxicology studies in cynomolgus monkeys, with doses of PEGASYS® of up to 6000 µg/kg.

The effects of PEG-IFN on general activity and behavior were studied in mice according to the methods of Irwin[1] following a single, s/c injection of 6, 60, or 600 µg/kg PEG-IFN. No evidence of mortality, clinical signs of toxicity, nor effects on general activity or behavioral changes were observed in any of the mice on study. There were no changes in duration of ----------induced sleeping time, or detectable pro- or anti-convulsant effects of PEG-IFN in mice at these same doses. In rats injected with 6, 60, or 600 µg/kg PEGASYS® s/c, there were no remarkable effects on locomotor, ambulatory, or rearing activity, and no anti- or pro-nociceptive effects in the tail flick test. PEGASYS® at dose of 6, 60, or 600 µg/kg had no effect on either gastric emptying or gastrointestinal transport in rats after s/c injection. Using isolated, guinea pig ileum, inclusion of up to 8.1 mg/ml PEG-IFN in the incubation bath had no effect on either basal smooth muscle tone or acetylcholine, histamine, or barium chloride-induced contractility. At the lowest dose of 0.081 mg/ml, a small increase in response to 5-hydroxytryptamine was noted; however, this effect was not present at the higher concentrations of PEG-IFN tested and was considered unrelated to PEGASYS®.

[1] Irwin, S. 1964. Drug screening and evaluation of new drugs in animals. *In*: Animal and Clinical Pharmacologic Techniques in Drug Evaluation, J.M. Nodine and P.I. Siegler, eds., Year Book Medical Publishers, Inc., Chicago, IL; pp. 36-64.

In summary, there were no remarkable behavioral changes, central nervous, cardiovascular, respiratory, or renal system effects, nor clinical toxicities noted in mice, rats, or conscious ---------- monkeys after a single, s/c administration of 6, 60, or 600 µg/kg PEG-IFN. It should be noted that the negative findings in the rodent models are not unexpected, given the species-specificity of the interferon present in the PEG-IFN conjugate.

Single Dose Pharmacokinetics of PEGASYS® in Rats

The pharmacokinetics of PEGASYS® were initially evaluated in female, ---------- rats after a single s/c or i/v injection. Three rats per group were treated with 850 µg/kg PEG-IFN by s/c injection, or 560 µg/kg PEG-IFN, i/v. An additional 3 rats per group were treated with either 60 µg/kg unconjugated ROFERON®-A, s/c or 40 µg/kg IFN-α, i/v as a comparative control. Blood samples were collected at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 h after s/c injection of PEG-IFN or ROFERON®-A, and at 5, 15, and 30 minutes, and 1, 3, 5, 12, and 24 h after i/v treatment. Serum samples were analyzed for interferon activity by inhibition of the cytopathic effects of ---------- on cultured, ---------- cells ----------. In this assay system, one unit of interferon activity is defined as the amount to inhibit the cytopathic effect by 50%, as compared to cultures of ----- cells exposed to ----- in the absence of IFN-α. Pharmacokinetic parameters were calculated from the serum IFN concentration *vs.* time profiles for all groups, using nonparametric, model-independent analyses.

After a single, i/v administration of PEG-IFN, high levels of (>100,000 U/ml) interferon activity could be detected in the rats up to 24 hours after dosing. Peak concentrations were observed at the first sampling time point of 5 min, then declined in a biphasic manner. These data are represented in Figure 1, below. Terminal elimination half-life of PEGASYS® after i/v injection was determined to be approximately 15 h by extrapolation of the curve over time, as compared to 2.1 hours for unconjugated, IFN-α. However, it should be noted that the apparent terminal half-life of PEG-IFN after i/v injection may be underestimated from these data, since the final time point of 24 h after injection was still too early to accurately define the elimination phase.

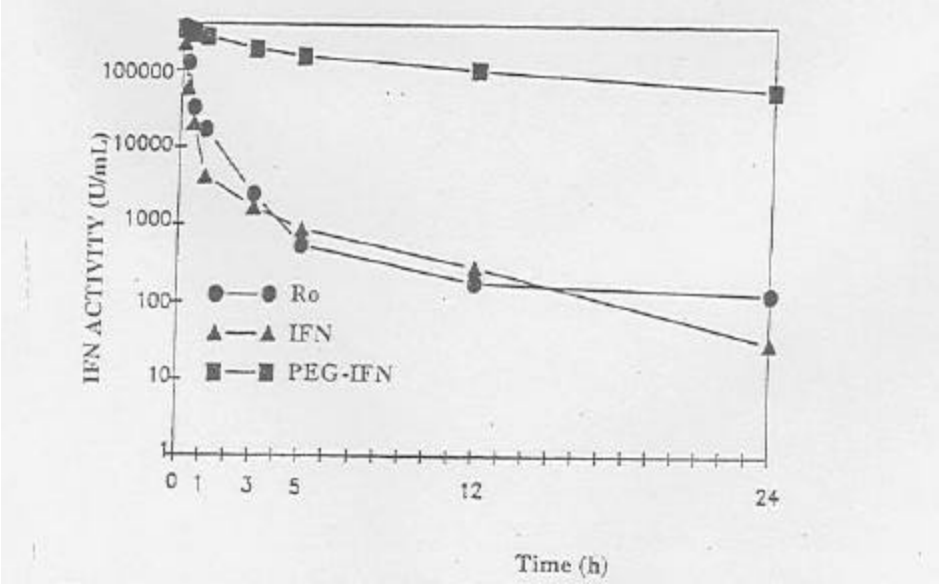

Figure 1 – Pharmacokinetic profile of PEG-IFN and IFN-a after i/v injection in rats.

Following s/c injection of rats with ROFERON®-A, serum concentrations of interferon activity peaked at 1 h after dosing, and were undetectable by 10 h (Figure 2). A different pattern of exposure was observed after s/c injection of PEG-IFN. In general, serum interferon activity increased slowly over time, T_{max} was not observed until 24 h after dosing in rats treated with 850 µg/kg PEGASYS®, and levels remained elevated out to 48 h after treatment (Figure 2). Because serum interferon activity remained elevated at the final time point (48 h after dosing), it was difficult to accurately estimate the terminal half-life of PEG-IFN after s/c injection. However, mean residence time was estimated to be 80 hours, as compared to 1.6 h for unconjugated, IFN-α. Peak serum interferon concentrations were approximately 20-fold lower after s/c injection than following i/v injection of a lower dose (560 µg/kg) of PEG-IFN. Bioavailability of PEG-IFN could not be determined from the present studies.

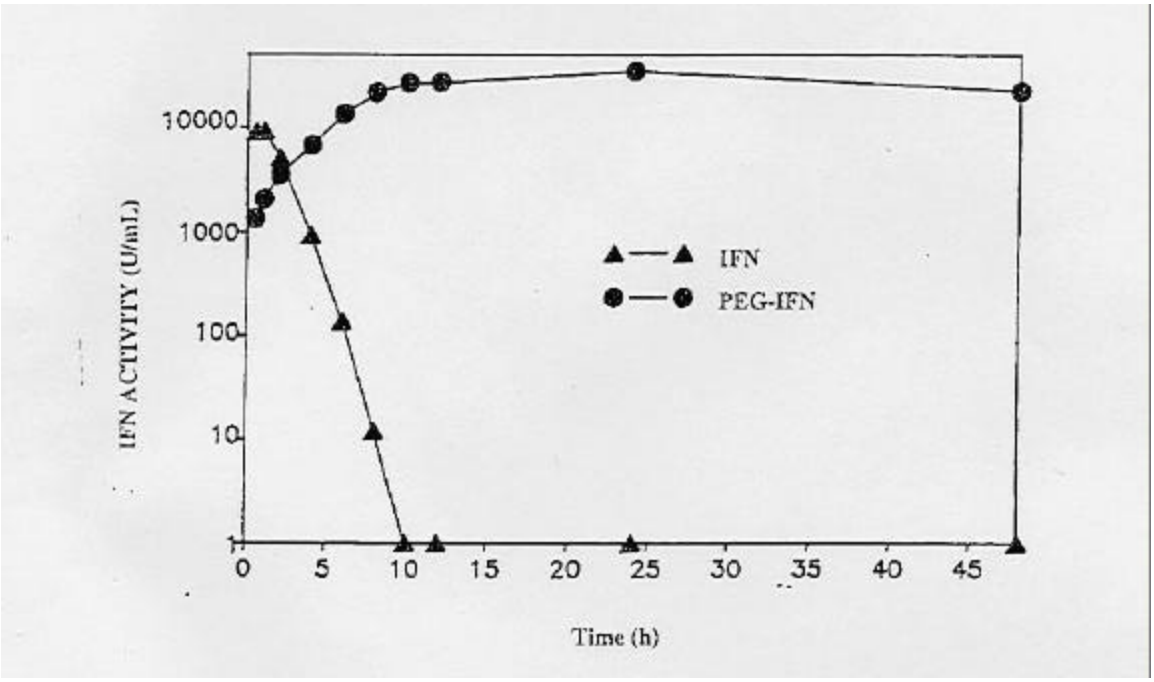

Figure 2 – Pharmacokinetic profile of PEG-IFN and IFN-a after s/c injection in rats.

In summary, treatment of female ---------- rats with PEGASYS® resulted in a more prolonged exposure to interferon activity than that observed following injection of unconjugated, IFN-α (ROFERON®-A). Terminal half-lives after s/c or i/v administration could not be accurately determined from the data due to high serum interferon activity at the final time points for each study. However, an estimated elimination half-life of 15 h for PEG-IFN in the rat was calculated by extrapolation of the data after i/v injection, and mean residence time after s/c injection was estimated to be 80 h for PEG-IFN, as compared to 1.6 h for unconjugated, IFN-α.

Single Dose Pharmacokinetics of PEGASYS® in Non-Human Primates

The pharmacokinetics, pharmacodynamic effects, and dose proportionality of PEGASYS® were determined in female ---------- monkeys, following a single s/c administration. Three monkeys per group were treated with 300 or 2250 µg/kg PEG-IFN by s/c injection. An additional three monkeys were treated 2250 µg/kg unconjugated IFN-α, as a comparative control.

Peripheral blood samples were obtained from each monkey at 0 (pre-dose), 4, 8, 12, 24, 30, 48, 72, 96, 120, 144, 168, and 192 h after injection of PEG-IFN. Serum samples from ROFERON®-A treated animals were obtained at 0 (pre-dose), 1, 2, 3, 4, 6, 12, 24, 30, 48, 72, 96, 120, and 140 h after injection. At the completion of the study, all monkeys were returned to the research colony.

Serum samples from PEGASYS® treated monkeys were analyzed for interferon activity using a ---------- and quantitated against a standard curve of PEG-IFN diluted in ----------. Serum concentrations of unconjugated IFN-α were determined by ------, and quantitated against a standard curve of ROFERON®-A diluted in ----------. To confirm that PEG-IFN retains the biologic activity of interferon-α *in vivo*, serum samples were also analyzed for 2', 5'-oligoadenylate synthetase (OAS) activity by ----------. Pharmacokinetic parameters were calculated from the serum IFN concentration *vs.* time profiles for all groups, using nonparametric, model-independent analyses.

All monkeys survived the dosing and observation periods with no apparent clinical signs of toxicity. After a single, s/c administration of PEG-IFN, interferon activity could be detected in the monkeys up to 192 hours after dosing. Peak concentrations were observed approximately 23 and 28 h after dosing with either 300 or 2250 µg/kg PEGASYS®, respectively, then declined gradually over the duration of the study. The values for C_{max} were approximately linear in relationship to dose, with mean values of 2574 ng/ml and 30,696 ng/ml achieved after a single dose of 300 or 2250 µg PEG-IFN/kg, respectively. Terminal elimination half-life after s/c injection of PEG-IFN was approximately 143 h and 186 h after injection of 300 and 2250 µg/kg PEGASYS®, respectively. By contrast, peak serum interferon activity after s/c injection of 2250 µg/kg unconjugated, ROFERON®-A was achieved by 4.4 h after injection, and the final time for detectable interferon serum levels was 30 h after dosing. The value for C_{max} of unconjugated, IFN-α after s/c injection was 1022 ng/ml, with an elimination half-life of 9.3 hours. These data are represented graphically in Figure 3, below.

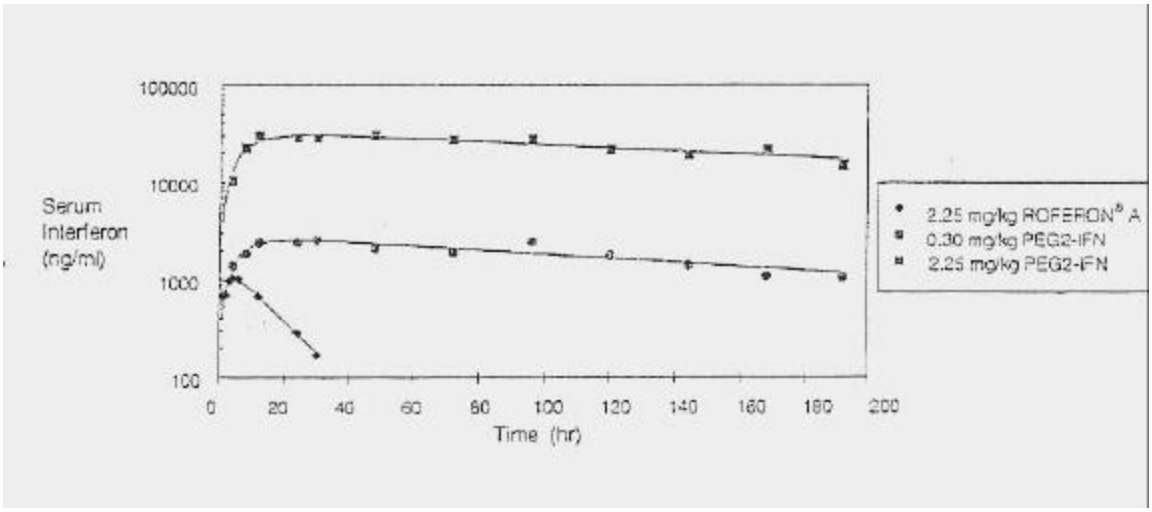

Figure 3 – Pharmacokinetic profile of PEG-IFN and IFN-a in ---------- monkeys after s/c injection.

Pharmacodynamic evaluation of 2', 5'-OAS levels after exposure to PEGASYS® showed increased levels of enzyme activity detectable as early as 8 h after injection of either PEG-IFN or IFN-α. Serum activity prior to dosing was approximately 0 pM/h, and increased to 1000 to 2500 pM/h by 24-30 h after treatment. For the unconjugated parent IFN-α compound, these levels were maintained out to at least 144 h after injection. High serum 2',5'-OAS activity remained detectable at the final time point (192 h) in both groups of animals treated with PEG-IFN, although there was no apparent relationship of the levels achieved to the dose of PEGASYS® administered.

In summary, s/c injection of female, cynomolgus monkeys with either 300 or 2250 µg/kg PEG-IFN resulted in both an increased, as well as a more prolonged exposure to interferon activity than that observed following injection of ROFERON®-A. Dose-related increases in both serum interferon concentrations and total exposure, as determined by AUC were observed for monkeys treated with PEGASYS®, and the elimination half-life was estimated to be 6 to 8 days, as compared to 9.3 hours for unconjugated, IFN-α. PEGASYS® was confirmed to be biologically active by induction of 2', 5'-OAS enzyme activity, which is a hallmark of interferon-α pharmacologic activity in both non-human primates and man.

Multiple Dose Toxicokinetics of PEGASYS® in Non-Human Primates

The toxicokinetic profiles of PEGASYS® following multiple dose adminstration were determined in conjunction with two 4-week, and one 13-week, repeat-dose, GLP toxicity studies in ---------- monkeys (please see PRECLINICAL TOXICOLOGY section, below). Antibody development (both total and anti-interferon neutralizing activity) was also investigated in each of these studies.

Blood samples for interferon activity (as determined by ----------) were obtained prior to dosing, and on study days 15 and 26 following twice weekly, s/c dosing of monkeys with 0, 15, 187.5, or 562.5 µg PEG-IFN/kg/dose for 4 weeks. Additional blood samples were obtained 8, 24, 48, and 72 h following the first and final doses of PEGASYS®, to better characterize the exposure. Serum samples for determination of anti-interferon antibody activity were prepared from blood collected prior to the first dose of PEG-IFN, and at 72 h after the fourth dose (study d 15), and final dose (study d 29). Toxicokinetic parameters were calculated from the serum concentration *vs.* time profiles, and the AUC values were determined using the linear trapezoidal rule.

Evaluation of the toxicokinetic profiles revealed that on study d 1, both the exposure and the maximal serum concentrations of interferon increased greater than expected in proportion to dose. A 12.5-fold increase in dose from 15 µg/kg/dose to 187.5 µg/kg/dose resulted in 21 to 22-fold increases in both C_{max} and AUC_{0-72h}, and an additional 3-fold increase in dose to 562.5 mg/kg resulted in an overall increase in AUC_{0-72h} of 65-fold and a 67-fold increase in C_{max}, as compared to the values obtained for the 15 µg/kg/dose group.

By d 26 on study, the mean value for C_{max} for the lowest dose group had increased by approximately 2-fold, and the mean AUC_{0-72h} had increased 63% from study d 1. This finding may be attributed to higher serum interferon concentrations in one male and one female monkey each in this dose group. However, both C_{max} and AUC_{0-72h} values for animals treated with 187.5 or 562.5 µg PEG-IFN/kg/dose had decreased by 10 to 15-fold. High neutralizing antibody titers were detected in all monkeys in these dose groups at 72 h following the final dose of

PEGASYS®, suggesting that the apparent decreases in serum levels were related to increased, antibody-mediated clearance of PEG-IFN. The toxicokinetic data are represented in the table, below:

Table II – Toxicokinetic Profile in ---------- Monkeys Following Twice Weekly Subcutaneous Dosing with PEG-IFN

Dose (mg/kg)	Day 1				Day 26			
	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose
15	24	98.2	6000	400	48	184.4	9770	652
187.5	72	2051	130000	694	0	222	13200	70
562.5	24	6565	392000	696	0	408	22400	40

In summary, the overall exposure to PEG-IFN on study d 26 in monkeys treated with 187.5 or 562.5 µg/kg/dose decreased by approximately 90%, as compared to AUC0-72h obtained on study d 1. These findings were accompanied by an increase in both total binding antibody and anti-interferon neutralizing antibody activity, suggesting that a dose- and time-dependent induction of immunogenicity was related to the increased clearance of PEGASYS®.

The toxicokinetics and immunogenicity of PEGASYS® after repeated, daily exposure were also determined as part of a 4-week, GLP toxicity study (please see below). Male and female monkeys (3/sex/group) were injected daily for 28 d with 0, 15, 100, or 600 µg/kg/dose PEG-IFN. Blood samples for evaluation of serum interferon activity by ---------- were collected from all monkeys prior to initiation of dosing, and at 4, 8, and 24 h after dosing on study days 1 and 8, prior to dosing on days 4, 11, 16, 18, 23, 25, and 24 h following the final dose on study d 29. Toxicokinetic profiles for PEGASYS® after daily exposure were determined using non-parametric analysis of the serum concentration *vs.* time curves, and the AUC_{0-24h} was calculated using the linear trapezoidal rule. Serum samples for anti-interferon antibody analysis were obtained from monkeys in all dose groups prior to dosing, prior to dosing on study d 16, and 24 h after the last dose. Serum samples for antibody and interferon levels were also collected from monkeys held for the 4-week, treatment-free recovery period on recovery days 16 and prior to sacrifice.

On study d 1, values for both the C_{max} and AUC_{0-24h} increased approximately linearly in proportion to the dose of PEGASYS® administered. By study d 8, the dose-proportionality was still approximately linear for both parameters, however, the absolute mean values for both C_{max} and AUC_{0-24h} had increased by 7 to 12-fold, suggesting that significant accumulation of PEG-IFN was occurring. The data are summarized in the table, below:

Table III – Toxicokinetic Profile in ---------- Monkeys Following Daily Subcutaneous Dosing with PEG-IFN

Dose (mg/kg)	Day 1				Day 8			
	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose
15	24	133	1970	131	24	1070	23700	1580
100	24	1250	18800	188	8	9240	209000	2090
600	24	6670	102000	170	8	48900	1120000	1870

The values for $AUC_{0\text{-}24h}$ and C_{max} for PEGASYS® could not be reliably determined for the final two weeks of the study, due to the development of significant, anti-interferon antibody and neutralizing activity. However, a plot of the concentration *vs.* time data for these time points clearly demonstrates that serum PEG-IFN levels clearly decreased to undetectable levels over the final two weeks of the study (Figure 4, below).

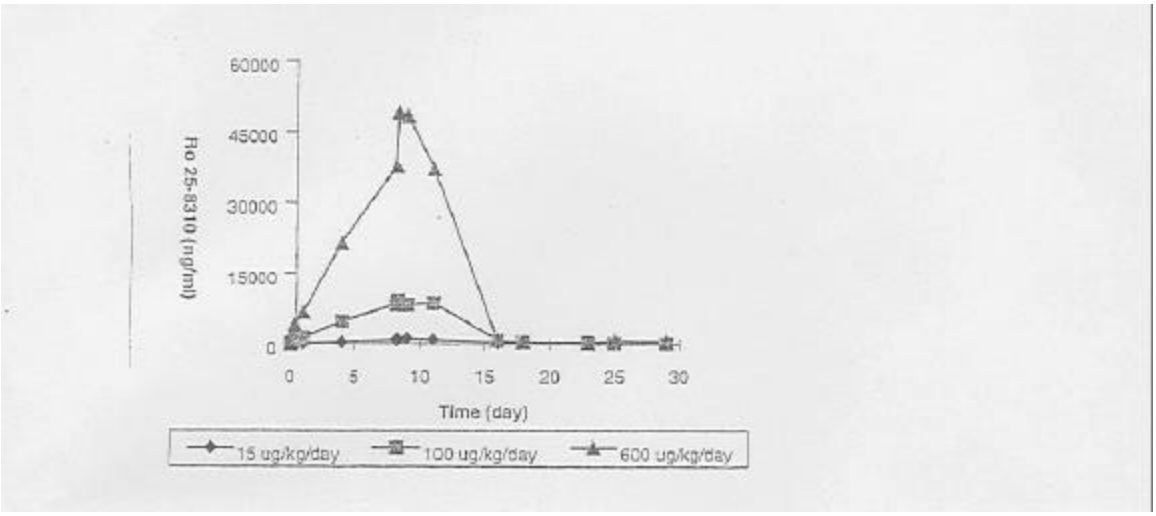

Figure 4 – Mean serum concentration *vs*. time profile of PEGASYS® in ---------- monkeys after s/c injection.

In summary, linear increases in both C_{max} and $AUC_{0\text{-}24h}$ were observed in ---------- monkeys following a single, s/c injection of PEGASYS® at dose levels of 15, 100, or 600 µg/kg. Repeated, daily administration of these same dose levels led to increases in both toxicokinetic parameters of 7 to 12-fold on study d 8 as compared to study d 1, suggesting that significant bioaccumulation of the conjugated IFN-α was occurring. Serum PEG-IFN concentrations were decreased after 8 days, due to development of significant anti-interferon total and neutralizing antibody.

Toxicokinetic evaluation of PEGASYS® was conducted as part of a 13-week, repeat dose GLP toxicology study of PEG-IFN in cynomolgus monkeys. Five monkeys per sex per dose group were injected twice weekly with PEGASYS® at dose levels of 0, 15, 50, or 150 µg/kg/dose. Blood samples for toxicokinetic profiles of PEG-IFN were collected from all monkeys prior to dosing, and at 8, 24, 48, and 96 h after the first, fifth, ninth, seventeenth, and 25th doses. Serum samples for antibody determination were collected prior to initiation of dosing, prior to doses 10 and 18, and approximately 72 h after the final dose of PEG-IFN. Serum concentrations of PEGASYS® were determined by the ---------- and the toxicokinetic profiles evaluated using non-parametric analysis and the linear trapezoidal rule for determination of $AUC_{0\text{-}96h}$. A summary of the data is provided in the table, below:

Table IV – Toxicokinetic Profile of PEG-IFN in ---------- Monkeys Following Twice Weekly, Subcutaneous Dosing for 13 Weeks

Dose (mg/kg)	Day 0			Day 14			Day 28		
	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose	C_{max} (ng/ml)	AUC_{0-t} ng*h/ml	AUC / Dose
15	161	11837	789	110	3679	245	86	7642	510
50	515	40643	813	425	23508	470	114	9819	196
150	1445	117626	784	423	36424	243	228	17798	119

Toxicokinetic parameters for animals treated with PEGASYS® could not be determined for the later time points on study, due to an ----------. The high antibody titers in these samples were found to interfere with the detection of PEG-IFN in the ----------, leading to values which were increased over those expected for these time points (please see immunogenicity data, above).

In summary, repeated dosing of ---------- monkeys with PEGASYS® for 13 weeks was associated with detectable serum levels of PEG-IFN for at least 4 weeks on study, after which time the development of significant, anti-interferon total antibody and neutralizing activity did not permit accurate quantitation of exposure.

PRECLINICAL TOXICOLOGY

Because IFN-α pharmacologic activity and interaction with its receptor is species-specific, the preclinical toxicology evaluation of PEGASYS® was conducted primarily in the ---------- monkey, which has previously been demonstrated to be pharmacologically responsive to the unmodified, parent compound ROFERON®-A. The duration of the studies was also limited by development of significant, anti-interferon binding and neutralizing antibody activity in the serum of treated animals, leading to its rapid inactivation and removal from the circulation and thereby preventing toxicity studies of greater than 4 weeks duration. The maximal duration of treatment of ---------- monkeys with PEG-IFN in the toxicology program was 13 weeks; however, no new or unexpected toxicities beyond what had previously been observed in the 4 week toxicity studies were detected in this study. Since traditional, segment II reproductive toxicities with unconjugated IFN-α have demonstrated significant abortifacient effects, PEG-IFN was assumed to have similar properties and was therefore not evaluated for its developmental toxicologic effects. However, serum sex steroid hormone levels and menstrual cycle durations in sexually mature, female monkeys were evaluated after PEG-IFN or IFN-α treatment, as a surrogate for reproductive function. The results of the toxicology program for PEG-IFN are summarized, below.

Acute Toxicology Studies in Non-Human Primates

PEG-IFN was well tolerated in ---------- monkeys following single, high-dose injections of 6750 µg/kg, s/c or up to 300 µg/kg, i/v. Toxicities after s/c injection included piloerection and unsteady gait, transient decreases in total leukocyte and neutrophil counts, transient anemia, and slight (1.7 to 2-fold) increases in serum ALT over baseline levels at study day 14. The hematologic findings were present at days 2 and 7 after treatment, but had resolved to baseline by d 14. Clinical chemistry findings also included reduction in total protein and serum albumin

levels at study days 7 and 14 as compared to baseline. Mild subcutaneous hemorrhage at the injection site was present in both monkeys treated by s/c injection, at one injection site each throughout the duration of the observation period, and was confirmed histologically in the female animal at terminal sacrifice. At necropsy, histologic evaluation revealed foci of subacute, inflammatory cell infiltrates in the liver of both monkeys, and mild, sub-mucosal edema in the gall bladder of the male animal, which were considered unrelated to PEGASYS® treatment.

Following i/v injection of ---------- monkeys with 15, 70, or 300 µg/kg PEG-IFN, there were no remarkable clinical signs of toxicity or changes in hematology profiles, with the exception of bruising at the injection site during the first week after dosing. One female monkey in the group treated with 300 mg/kg PEG-IFN developed a slight (1.6-fold) increase in serum ALT on study days 2 and 7 as compared to baseline, which had returned to pre-dose value by d 14. Other serum transaminases (ALT, GGT) and alkaline phosphatase were not affected by PEG-IFN treatment. There were no remarkable gross pathologic lesions, or effects on both absolute or relative organ weights, and no interferon-associated histologic findings at terminal sacrifice.

In summary, acute treatment of ---------- monkeys with 6750 mg/kg PEG-IFN by s/c injection was associated with transient anemia, decreases in leukocyte counts, total protein, and serum albumin, and slight increases in serum ALT in the absence of changes in other liver enzyme profiles. Similar effects on serum ALT were observed in one female monkey after 300 mg/kg PEG-IFN, i/v. In general, PEG-IFN exposure in a pharmacologically active species was well tolerated, at doses of up to 2250-fold higher than those planned for licensure.

Repeat-Dose Toxicology Studies in Non-Human Primates

A series of two 4-week, repeat dose toxicity studies of PEG-IFN and one 13-week study were conducted in cynomolgus monkeys to determine the cumulative toxicities after repeated exposure. A summary table of the comparative exposure of the AUC and Cmax values in humans and animals exposed to PEG-IFN in the phase 3 clinical trial and in these toxicity studies, respectively, is presented below:

Table V – Comparative Toxicokinetic Exposure of PEG-IFN in ---------- Monkeys Following Subcutaneous Dosing for 4 or 13 Weeks

Species	Dose	C_{max} (ng/ml)	AUC_{0-24h} (ng*hr/ml)	AUC_{0-168h} (ng*hr/ml)
Human	180 µg/week[a]	29	n.d.	4,129
Monkey (4-week)	15 µg/kg/day	1,070	23,700	165,900[b]
Monkey (4-week)	100 µg/kg/day	9,240	209,000	1,463,000[b]
Monkey (4-week)	600 µg/kg/day	48,900	1,120,000	7,840,000[b]
Monkey (13-week)	15 µg/kg/dose (twice weekly)	110	3,679[c]	6,438[d]
Monkey (13-week)	50 µg/kg/dose (twice weekly)	425	23,508[c]	41,139[d]
Monkey (13-week)	150 µg/kg/dose (twice weekly)	423	36,424[c]	63,472[d]

[a] last dose at week 48
[b] $AUC_{0\text{-}24h}$ x 7 to compare to weekly exposure in humans
[c] $AUC_{0\text{-}96h}$
[d] $AUC_{0\text{-}96h}$ x 1.75 to compare to weekly exposure in humans

The first study evaluated toxicity, toxicokinetics, and immunogenicity of PEG-IFN after twice weekly dosing with 0, 15, 187.5, or 562.5 µg/kg/dose PEG-IFN. Ophthalmic and electrocardiogram evaluations were included at pre-study and at various time points after treatment, for evaluation of product safety. No overt toxicities, clinical signs, or mortality were observed over the treatment period. Hematologic changes were limited to animals in the highest dose group, and included transient decreases in platelet counts and marked decreases in both neutrophil and total white cell counts in one male and one female monkey, during week 3 on study. Mean serum protein, albumin, globulin, and calcium levels were lower in monkeys treated with either 187.5 or 562.5 µg/kg/dose prior to the fifth injection. Similarly, serum ALT was increased in 2/3 male monkeys in the mid-dose group and in 1 male and one female monkey each treated with 562.5 µg/kg/dose at this same time point. However, these findings were no longer present at the end of the 4-week treatment period. At necropsy, statistically significant increases in mean absolute and relative liver and spleen weights were observed for monkeys treated with the highest dose of PEG-IFN, as compared to the vehicle control group. Mean absolute and relative thymus weights were also increased in all groups of PEG-IFN treated monkeys, without a clear dose-relationship in either incidence or severity. Histologically, there were no correlative findings in any of the three organs. Microscopic evidence of minimal to mild macrophage, neutrophil, and/or mononuclear cell infiltration, inflammation, and subcutaneous fibrosis was present at the injection site(s) of animals in all PEG-IFN treated dose groups, with no apparent relationship to the dose injected.

Toxicokinetic evaluation of serum samples from PEG-IFN treated monkeys revealed an initial, dose-related increase in serum IFN-α levels on d 1 after dosing, which was markedly decreased at 24 h following the final dose of PEG-IFN at study d 26. The decreases in serum IFN-α levels were associated with development of significant anti-interferon antibody activity. On study d 15, total antibodies to IFN-α were detected in 1/3 female monkeys in the 15 µg/kg/dose group, 1 male and 2 female monkeys in the 187.5 µg/kg/dose group, and in all six monkeys treated with 562.5 µg/kg/dose PEG-IFN. At 72 hours following the final dose of PEG-IFN, high neutralizing antibody titers were detected in all monkeys treated with PEG-IFN, with an apparent relationship to the dose level. These data, together with the transient hematologic and clinical chemistry findings suggest that PEG-IFN is immunogenic *in vivo* in ---------- monkeys, thereby limiting the toxicologic effects of PEG-IFN after repeated exposure.

In conclusion, twice weekly injection of PEG-IFN was well tolerated in ---------- monkeys at doses of up to 562.5 µg/kg/injection, or at levels of up to 375-fold greater than the maximum, human weekly exposure of 180 µg/dose proposed for licensure (as scaled for a 60 kg human).

A second, 4-week toxicology, toxicokinetics, and immunogenicity study of PEG-IFN was conducted in ---------- monkeys to determine the safety profile of the biologic following daily, s/c injections. Three monkeys per sex received daily injections with vehicle control, 15, 100, or 600 µg/kg/dose PEG-IFN. The reversibility of any toxicities was assessed after a 4-week, treatment-free recovery period. Again, ophthalmic and electrocardiogram examinations were performed as part of the safety evaluation, and were unremarkable for treatment-related effects. No effects of PEG-IFN treatment on body weights, body weight gains, or body temperature were

observed. One female monkey treated with 600 μg/kg/d PEG-IFN developed slight ataxia and listlessness, uncoordination, subdued behavior, and cold extremities on study d 12. Clinical pathology for this animal revealed elevations in serum ALT, AST, and alkaline phosphatase, cholesterol, triglycerides, and bilirubin levels, decreased serum calcium, total protein, BUN and marked decreases in total leukocytes and platelet counts. Interruption of dosing of this animal for ten days resulted in reversal of the clinical signs, which did not recur on re-administration of PEG-IFN, and the changes in clinical pathology had resolved to approximately baseline values by the end of the treatment period.

There were no overt, clinical signs of toxicity in any of the other animals on study. Transient, dose-related decreases in platelet, total leukocyte, and neutrophil counts were present in animals of all PEG-IFN dose groups as compared to the vehicle control at study d 8, and to a lesser degree on study d 15. Only the decrease in platelet counts was statistically significant ($p \leq 0.05$, ANOVA) in the male monkeys in all dose groups, as compared to control. Minimal decreases in erythrocyte parameters (hematocrit, hemoglobin concentrations, and red cell counts) were present in male monkeys treated with 600 μg/kg/d PEG-IFN at most of the time points on study; however, these values were not outside of the historical range for this species and were of questionable biologic significance. Decreases in fibrinogen were observed in male monkeys in the 100 and 600 μg/kg/d PEG-IFN dose groups at study d 8, as compared to either baseline or vehicle control animals. A concomitant increase in APTT was noted for male monkeys in the 600 μg/kg/d dose group, and to a lesser extent in some male and female monkeys in the 15 and 100 μg/kg/d PEG-IFN dose groups at study days 8 and 15. No treatment-related changes were noted, however, on microscopic evaluation of bone marrow smears from PEG-IFN treated monkeys, as compared to animals treated with the vehicle.

Dose-related decreases in serum total protein and albumin levels were noted in the 100 and 600 μg/kg/d dose groups at study days 8 and 15. Fractionation electrophoresis revealed that the decrease in total protein was due to lower levels of α- and β-globulins as well as the decrease in albumin. Transient decreases in serum calcium and BUN levels were noted in the female monkeys treated at the highest dose level on study d 8 only, and 1.4- and 3.6-fold increases in serum ALT and AST, respectively, were noted in one female monkey at this same time point in the 100 μg/kg/d dose group. These findings were all reversible by the end of the treatment period.

Subcutaneous reddening and edema at the injection site, which correlated histologically with the presence of subcutaneous fibrosis, hemorrhage, and inflammatory infiltrates were present in animals in all dose groups, including the control at terminal sacrifice. The degree of severity was mild to moderate, was unrelated to the dose of PEG-IFN, and was expected for repeated, s/c injection of a non-irritant compound. There were no other PEG-IFN related gross or microscopic pathologic findings, nor changes in absolute or relative organ weights noted in any group of PEG-IFN treated monkeys, as compared to the vehicle control group.

Mean serum toxicokinetic levels of PEG-IFN generally increased with time through study days 8-11 for animals in all dose groups of PEG-IFN. At these time points, both C_{max} and AUC_{0-24h} were increased in a dose-related fashion, and were approximately linear in relationship to dose administered. At time points after study d 11, AUC values could not be calculated due to development of anti-PEG-IFN antibody activity. On study d 16, total antibody against IFN-α was detectable in sera from 1/10, 6/10, and 6/9 monkeys treated with 15, 100, or 600 μg/kg/d PEG-IFN. By the end of the treatment period on study d 29, anti-interferon antibody was detectable in all PEG-IFN treated monkeys, with the exception of one animal each in the 15 and 100 μg/kg/d dose groups. All serum samples obtained from PEG-IFN treated monkeys on study days 16 and

24 contained detectable levels of "free" IFN-α, and could not be tested for neutralizing antibody activity. However, on recovery d 16, significant anti-interferon neutralizing activity was present in samples from all animals in the low-, mid-, or high-dose recovery groups. No anti-interferon antibody (either total or neutralizing) activity was present in serum samples from vehicle control-injected monkeys at any time point on study.

In summary, daily injection of PEG-IFN in cynomolgus monkeys at doses of up to 1400-fold greater than the anticipated, licensed dose of 180 µg/week was generally well-tolerated, with transient hematologic effects and elevations in serum hepatic transaminase levels. Development of anti-PEG-IFN total and neutralizing antibody activity accounted for the loss of toxicologic effects in this species. The NOAEL for PEG-IFN in this study was 15 µg/kg/d, or approximately 35 times higher than the dose of 180 µg/week proposed for licensure.

The cumulative toxicity of PEG-IFN was assessed in a 13-week toxicology, toxicokinetics, and immunogenicity study in ---------- monkeys. Animals were injected s/c twice weekly with PEG-IFN at doses of 0, 15, 50, or 150 µg/kg for 13 weeks, followed by a 4-week, treatment-free recovery period. Monkeys were assessed over the duration of the study for clinical signs of toxicity, hematologic and clinical chemistry profiles, serum levels of PEG-IFN and anti-interferon antibody activity, and for reversibility of these effects during the recovery period.

There were no mortalities on study, and clinical signs of toxicity were limited to occasional incidences of redness and/or edema at the injection site(s). No remarkable effects of PEG-IFN treatment on ophthalmologic parameters, ECG or urinalysis profiles, body weight, or behavioral changes were noted. Transient, mild decreases in platelet counts were noted for monkeys treated with either 50 or 150 µg/kg/dose PEG-IFN at study d 8, and in male monkeys treated with 150 µg/kg/dose at study d 29, as compared to the vehicle control group ($p \leq 0.05$, ANOVA). On study d 8, mean counts for both total leukocytes and neutrophils were decreased in the PEG-IFN treated groups as compared to the vehicle control; however, these findings were only statistically significant for female monkeys in all groups and for male monkeys treated with either 50 or 150 µg/kg/dose PEG-IFN ($p \leq 0.05$, ANOVA). In the highest dose group, mean prothrombin times were slightly decreased and mean APTT values were slightly increased at study d 15. Mean fibrinogen levels were also transiently increased in male monkeys in the 50 or 150 µg/kg/dose groups at study d 15. However, these values had decreased to the levels observed in the vehicle control group by study d 28 and throughout the remainder of the treatment period. There were no remarkable findings on histologic evaluation of bone marrow smears from any of the PEG-IFN treated monkeys.

Slight decreases in mean total protein values as compared to either baseline or to the control group were noted for all groups of PEG-IFN treated monkeys at study d 15, and were statistically significantly different from control for male animals in the 15 and 50 µg/kg/dose groups, and for animals of both sexes in the 150 µg/kg/dose group ($p \leq 0.05$, ANOVA). These changes were transient, and were due primarily to lower mean albumin levels in all PEG-IFN treated groups, as compared to the vehicle control. One male monkey and one female monkey each in the group treated with 150 mg PEG-IFN/kg/dose had slight (2- and 1.5-fold increases, respectively) elevations in serum ALT over baseline at study d 8. The ALT value for the male monkey remained elevated (approximately 1.3-fold over baseline) at study d 29, but was similar to values for the control group for the remainder of the treatment period.

At terminal sacrifice, gross pathologic findings were limited to presence of dark areas at the injection site(s) in all groups of animals, including the controls. Microscopically, these were

correlated with areas of subcutaneous hemorrhage and inflammation in both the vehicle control and PEG-IFN treated animals. After the 4-week recovery period, no macroscopic or microscopic evidence of inflammation or hemorrhage were present in monkeys from any group. Minimal inflammatory cell infiltrates were observed sporadically at the injection site(s) in some animals, and were felt to be of minimal biologic significance. There were no additional, treatment-related macroscopic or histologic findings observed in any of the PEG-IFN treated animals, and no changes in either absolute or relative organ weights as compared to the vehicle control.

Toxicokinetic evaluation of PEG-IFN serum levels at study days 0 and 14 revealed a dose-related increase in both C_{max} and $AUC_{0\text{-}96h}$. At study d 28, there was an apparent decrease in exposure (as determined by $AUC_{0\text{-}96h}$) in both male and female monkeys at all dose levels of PEG-IFN as compared to study d 0, which then increased again to values similar to d 0 by study d 56, and increased an additional 2 to 3-fold by study d 84. Subsequent investigation of the analytical method revealed that the apparent increase was due to an analytical artifact, and were not representative of true increases in PEG-IFN exposure. Total antibody activity, as determined by ELISA was increased beginning on study d 33 in all PEG-IFN treated monkeys with the exception of one female in the 15 µg/kg/d dose group. This animal had high levels of "free" interferon activity, as determined in the anti-viral assay and failed to develop measurable anti-IFN-α antibody levels over the duration of the study. Titers of neutralizing antibody activity were negative in all of the pre-treatment serum samples, increased to detectable levels by study d 33, and continued to increase as high as 1:12,800 in two monkeys in the mid-dose group and one monkey treated with 150 µg PEG-IFN/kg/dose by study d 61. Measurable serum anti-interferon neutralizing activity was also present in all samples obtained from PEG-IFN treated monkeys during the 4-week recovery period. These findings were not unexpected, given the previous findings with both the unconjugated IFN-α and the 4-week toxicity studies with PEG-IFN.

In summary, treatment of ---------- monkeys by s/c injection with PEG-IFN was associated with transient anemia, decreased platelets and increases in APTT and fibrinogen, decreased serum levels of total protein, albumin, and/or globulins, and a transient elevation of serum hepatic transaminases in the absence of any overt or histologic evidence of liver damage. All three studies showed minimal to mild irritation, edema, and inflammation at the injection site, which were partially to completely resolved at the end of a 4-week, treatment-free recovery period. The NOAEL for PEG-IFN given by s/c injection to ---------- monkeys is therefore 15 µg/kg/dose for either daily or twice weekly injections for 4 weeks, or twice weekly injections for 13 weeks. This dose is approximately 35-fold higher than the human dose of 180 µg/week proposed for licensure.

Reproductive/Developmental Toxicity Studies of PEGASYS®

Standard reproductive and developmental toxicity studies of PEG-IFN were not performed. Extensive information is available regarding the abortifacient effects of the type I interferons in non-human primates, including --------- and ---------- monkeys; however, there is no evidence of teratogenic activity of IFN-α in the surviving fetuses at any dose level tested (up to 25×10^6 IU/kg/d). Additionally, no adverse effects on male fertility were observed following treatment of male ------- monkeys at doses of unconjugated IFN-α up to 25×10^6 IU/kg/d for 5 months. However, irregularities in menstrual cycles and cyclic serum sex steroid hormone levels have been associated with type I interferon treatment in both ------ monkeys and humans, and were therefore used as a surrogate to evaluate the effects of PEG-IFN on reproductive function.

The effects of PEGASYS® treatment on the serum levels of sex steroid hormones were evaluated in two studies in sexually mature, female ---------- monkeys. Animals were treated three times weekly by s/c injection with doses of 100, 300, or 600 µg protein PEG-IFN/kg/day, or 25 x 10^6 IU/kg/day ROFERON®-A, i/m as a positive control, for the duration of one menstrual cycle. At 300 or 600 µg PEG-IFN/kg/dose, prolonged menstrual cycles and/or amenorrhea were observed in one of two female monkeys at each dose level in the pilot study, and in 5/5 monkeys treated with 600 µg/kg/dose in the confirmatory studies. Treatment with IFN-α at a dose of 25 x 10^6 IU/kg/d resulted in prolonged menstrual cycles in 2/2 and 4/5 female monkeys in the pilot and confirmatory studies, respectively. Mean cycle durations in the confirmatory study were 27.8, 41.0, and 61.8 days for monkeys treated with vehicle control, 100, or 600 µg/kg/dose PEG-IFN, respectively, and 39.2 days for the group of monkeys treated with ROFERON®-A as a positive control. The prolonged or absent menstrual cycles in these animals were accompanied by decreases in serum 17β-estradiol and progesterone levels, as well as delays to peak serum sex steroid hormone concentrations as compared to monkeys injected with the vehicle control. Menstrual cycle duration returned to normal during the recovery period for all monkeys in the confirmatory study, with the exception of one animal each in the IFN-α treated and 100 µg/kg/dose PEG-IFN treated groups. Toxicokinetic studies revealed that serum concentrations of PEG-IFN in monkeys receiving 100 or 600 µg/kg/dose in the confirmatory study were detectable throughout the duration of the treatment period, indicating that the monkeys were receiving continuous exposure to PEG-IFN. Exposure (as determined by $AUC_{0\text{-}48h}$) was increased approximately 3-fold on day 7 in both dose groups as compared to their respective values on d 1 of treatment. However, the $AUC_{0\text{-}48h}$ decreased significantly in both PEG-IFN dose groups on study d 14, due to the development of antibody activity against IFN-α. Neutralizing antibody activity was present at both 96 hours and 20 days following the last dose of PEG-IFN, as expected from the previous toxicology and toxicokinetic studies.

In summary, treatment of sexually mature, female ---------- monkeys with PEG-IFN for the duration of one menstrual cycle resulted in menstrual cycle irregularities, delays to peak serum 17β-estradiol and progesterone levels, and decreases in peak values for sex steroid hormone levels in these animals. The NOAEL for PEG-IFN effects on female reproductive function in the ---------- monkey was 100 µg/kg/dose (300 µg/kg/week), or approximately 200-fold higher than the dose of 180 µg/week proposed for licensing (scaled for a 60 kg woman).

Mutagenicity Studies

PEGASYS® was tested *in vitro* for its ability to induce genetic mutations using the standard,---------- assay in ----------, and for its potential to induce structural and chromosomal aberrations in ---------- ----------, in the presence and absence of exogenous metabolic activation by ---------- Concentrations of PEGASYS® tested in these assays ranged from ---------- µg protein/------ in the ------ assay, and from ----------µg/ml protein in the ---------- ---------- test. These concentrations of PEG-IFN were tested in pilot studies and found to have no cytotoxic effects on either test system, respectively. The sensitivity and specificity of these assays were demonstrated using the appropriate, positive control agents for each test system.

PEGASYS® did not increase the number of ---------- when tested in the ------- assay using the ---------- in the presence or absence of metabolic activation by ----------,

microsomes. Culture of ---------- in PEG-IFN for --------- hours did not increase the either the incidence of chromosomal aberrations, or the frequency of structural changes in the chromatids, in the presence or absence of ----------. In both test systems, the positive control agents produced the expected mutagenic responses, confirming the sensitivity of the test system and the activity of the metabolic activation.

In conclusion, PEGASYS® was not mutagenic or clastogenic under the conditions of these two assays.

SUMMARY AND CONCLUSION

The safety, biochemical, and pharmacokinetic activities of PEG-IFN were evaluated in ---- mice, ---------- rats, and ---------- monkeys *in vivo.* Pharmacokinetic studies in rats and ---------- monkeys demonstrated similar absorption and elimination profiles of PEG-IFN between the two species after s/c injection, with an approximate $t_{½elim}$ of 26 to 186 h. PEGASYS® has pharmacologic and toxicologic profiles similar to other type I interferons. Major findings in ---------- monkeys after repeated, every other day or daily, s/c dosing with PEG-IFN at doses of up to 600 µg/kg/dose for 4 to 13 weeks included slight to moderate decreases in erythrocyte parameters, platelet and leukocyte counts, transient elevation of hepatic transaminases (ALT), and transient decreases in serum total protein and albumin levels. The decrease in platelets and leukocytes observed in cynomolgus monkeys were related to the dose of PEG-IFN administered, and were only evident during the second and third weeks of treatment. All changes were reversible by the end of the treatment period, and were correlated with development of anti-interferon neutralizing activity in the serum. Mild to moderate, local irritation and/or inflammation at the site of injection were noted in all groups of PEG-IFN treated monkeys, including the vehicle control. Histologically, the most consistent finding was evidence of subcutaneous hemorrhage at the injection site, with minimal to mild acute, inflammatory infiltrates and/or subcutaneous fibrosis after single or repeated injections of up to 600 µg/kg/dose PEG-IFN. A loss of detectable IFN activity in the serum and development of neutralizing antibody activity was noted at the end of treatment period in repeat-dose studies in ---------- monkeys, with no apparent dose-relationship in either incidence or titer of antibody development induced. PEG-IFN exhibited no evidence of mutagenic potential in ----- tester strains of ----------, using the standard---------- tests. No evidence of clastogenic activity of PEG-IFN was detected in *in vitro* assays using ----------. Treatment of non-pregnant, female ---------- monkeys with 100, 300, or 600 µg/kg/dose PEG-IFN every other day for one menstrual cycle inhibited ovarian function in 2/2 animals treated with 300 µg/kg/dose, and in 7/7 monkeys at the highest dose level, as evidenced by lengthening of menstrual cycle duration during the treatment period, irregularities in cycle duration following cessation of treatment, and dose-related decreases in serum 17β-estradiol, and progesterone levels.

In conclusion, the pharmacologic and toxicologic profiles of PEG-IFN in rats and ---------- monkeys are similar to those observed for the unmodified parent compound, ROFERON®-A. Toxicities noted in repeat administration of PEG-IFN include transient decreases platelet, total leukocyte, and neutrophil counts, elevations in serum ALT levels in the absence of either overt or microscopic evidence of liver pathology, increase in menstrual cycle duration, and alterations in serum reproductive hormone levels in female monkeys. Other findings included reversible decreases in erythrocyte parameters, inflammation and/or irritation at the injection site, and

development of serum neutralizing activity after repeat administration. Based on the hematologic toxicities noted, the NOAEL for PEG-IFN in ---------- monkeys after repeat, daily or every other day injections for 28 days, or every other day injections for 13 weeks was 15 µg/kg/dose. The NOAEL for effects of PEG-IFN on reproductive hormone status and menstrual cycle duration in cynomolgus monkeys was 100 µg/kg/dose, or approximately 33-fold greater than the recommended human weekly dose of 180 µg (as scaled for a 60 kg female).

REGULATORY CONCLUSION

The preclinical pharmacology, pharmacokinetic, and toxicology data are adequate to support the safety of PEGASYS® pegylated interferon alpha-2a for the treatment of chronic hepatitis C infection, and support the proposed labeling. Minor changes to the PRECAUTIONS section, specifically *Impairment of Fertility* and *Pregnancy* sections are recommended, and have been communicated to the sponsor.

Key Words: chronic hepatitis C infection; interferon; PEG-interferon; toxicity

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