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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

BLA 103949/5002

Pharmacology Review(s)

TOXICOLOGIST'S REVIEW

BLA #: RMS BLA#103949/5002/0

SPONSOR: SCHERING-PLOUGH RESEARCH INSTITUTE

PRODUCT: pegylated interferon- α 2b (PEG-IFN, PEG-INTRON™, Schering-Plough) and ribavirin (REBETOL®, Schering-Plough)

FORMULATION: [

]

RELATED DOCUMENTS: [] PLA #83-415 (initial approval of INTRON®-A for hairy cell leukemia); ELA #83-414; PLA #89-0381 (approval of INTRON®-A for hepatitis C); BLA #99-1488 (RMS BLA#103949; PEG-IFN monotherapy for chronic hepatitis C infection)

PROPOSED INDICATION: treatment of chronic hepatitis C infection

ABBREVIATIONS: IFN, recombinant, human interferon- α 2b, derived from *Escherichia coli* (INTRON®-A); PEG, methoxypolyethylene glycol, average molecular weight 12,000 daltons; MIU, 1×10^6 anti-viral units of activity (by cytopathic effect bioassay); CHC, chronic hepatitis C virus; PBL, peripheral blood leukocytes; $t_{1/2elim}$, elimination half-life; AUC, area under the serum concentrations vs. time curve; C_{max} , maximal serum or plasma concentration after dosing; T_{max} , time to reach maximal plasma or serum concentration after dosing; kD, kilodaltons; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NOAEL, no observable adverse effect level; PT, prothrombin time; APTT, activated partial thromboplastin time; GGT, γ -glutamyl transpeptidase; PBS, phosphate buffered saline, pH 7.0; LDH, lactic dehydrogenase; BUN, blood urea nitrogen; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; ECL, electrochemiluminescent assay; LC/MS, liquid chromatography/mass spectrophotometry assay; CPE, cytopathic effect

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ABSTRACT:

The toxicity, effects on neutrophil function, and toxicokinetics of SCH 54031 PEG-IFN after every other day, s/c injections, alone or in combination with oral dosing with SCH 18908 ribavirin were evaluated in cynomolgus monkeys after a one month treatment period. Male and female cynomolgus monkeys were treated every other day with vehicle control or PEG-IFN. Monkeys received daily, oral dosing with either 50 or 75 mg/kg ribavirin by gavage. Treatment with PEG-IFN, alone or in combination with ribavirin was associated with inappetence in the absence of significant weight loss, dose-related anemia, mild to moderate leukopenia, lymphopenia, and decreases in platelets, moderate to severe neutropenia, and transient decreases in total serum protein, albumin, globulin, and A:G ratios. In the first study, mortalities were observed in 5 animals, and were associated with a decreased host response to opportunistic infections. The second study did not repeat the findings of the first, specifically, there was only one death on study and it was due to aspiration pneumonia. Pathologic findings related to PEG-IFN treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site, atrophy of the thymus, and minimal to mild changes in bone marrow cellularity. These findings were transient in nature, and had fully resolved by the end of the 4 week recovery period. Animals treated with either ribavirin alone, or in combination with PEG-IFN had persistent, dose-related anemia and evidence of bone marrow hyperplastic responses in the red cell lineage. Toxicokinetic evaluations of serum PEG-IFN and ribavirin levels confirmed that exposure was continuous throughout the duration of the study; however, after 4 weeks of treatment, detection of serum IFN levels was impeded by the development of anti-PEG-IFN and anti-interferon- α antibody titers. Reversal of toxicity was associated with development of neutralizing antibodies in all of the groups treated with PEG-IFN. Although no NOAEL for PEG-IFN alone or in combination with ribavirin could be determined for either study, the doses of PEG-IFN used in these two studies are approximately 20 to 300-fold greater than the dose of 1.5 μ g/kg/week used in the phase 3 clinical trial. The two doses of ribavirin used in the animal studies are approximately 6 and 8-fold higher than the clinical dose of 800 mg/d given in combination with the high-dose of PEG-IFN in the phase 3 clinical trial. Taken together, these data support the safety and expected toxicity profiles of SCH 54031 PEG-IFN in combination with ribavirin for licensure in chronic hepatitis C infection.

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.

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 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 2b (PEG-INTRONTM, PEG-IFN) is a semi-synthetic conjugate of recombinant, *Escherichia coli*-derived interferon- α 2b (INTRON[®]-A) and polyethylene glycol with an average molecular weight of 12,000 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 2/3 pivotal trial.

The intended clinical use of PEG-IFN is for the treatment of patients with chronic HCV infection. In the pivotal trial, 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 (INTRON[®]-A, Schering). Efficacy in the present study was evaluated by measurement of serum ALT levels over time, as well as quantitation of HCV RNA levels by PCR 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 24% and 29% for patients treated with 0.5 or 1.0 $\mu\text{g}/\text{kg}/\text{week}$ PEG-IFN, respectively, as compared to 11% complete responders in the group treated with INTRON[®]-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 (18% and 25 % of patients had undetectable RNA levels when treated with 0.5 or 1.0 $\mu\text{g}/\text{kg}/\text{week}$ PEG-IFN, as compared to 12% virologic responders in patients treated with 3 MIU INTRON[®]-A, t.i.w.). Combined response rates for both normalization of ALT and negative HCV RNA responses were 17%, 24%, and 12%, for patients treated with 0.5 or 1.0 $\mu\text{g}/\text{kg}/\text{week}$ PEG-IFN, or INTRON[®]-A, respectively.

The dose and schedule of PEG-INTRON™ intended for administration to chronically-infected HCV patients is 1.5 µg/kg, once weekly for 48 weeks. PEG-INTRON™ is formulated with dibasic sodium phosphate, monobasic sodium phosphate, sucrose, and polysorbate 80, and provided as a lyophilized powder for reconstitution with Sterile Water for Injection, U.S.P. The PEG-IFN used for all preclinical pharmacology, pharmacokinetics, and toxicology studies was produced at commercial scale, was greater than 99% pure, was formulated according to clinical procedures and was either of clinical grade, or representative of that used in the clinic.

PRECLINICAL PHARMACOLOGY AND PHARMACOKINETICS:

Pharmacology Study Summary and Review:

No preclinical pharmacology studies were included in the present BLA supplement. For a full description of the pharmacologic activity, pharmacodynamic profiles, and safety pharmacology of PEG-IFN in rats and cynomolgus monkeys, please refer to the original review for BLA #99-1488 (RMS BLA #103949).

Pharmacokinetics Study Summary and Review:

No preclinical pharmacokinetic studies were included in the present BLA supplement. Toxicokinetic evaluations of serum levels of PEG-IFN and ribavirin were incorporated as part of two one-month, repeat-dose toxicity studies of PEG-IFN and ribavirin in cynomolgus monkeys (Study #97060 and Study #97261), and are reported below. A complete review of the preclinical pharmacokinetics of PEG-IFN monotherapy in rats and cynomolgus monkeys may be found in BLA #99-1488 (RMS BLA #103949).

PRECLINICAL TOXICOLOGY:

Mutagenicity Study Summary and Review:

No mutagenicity studies of the combination of PEG-IFN and ribavirin were performed. In the previous review of SCH 54031 monotherapy (BLA #99-1488/RMS BLA #103949), PEG-IFN exhibited no evidence of mutagenic potential in five tester strains of *Salmonella typhimurium*, and in *E. coli* strain WP2uvrA, using the standard Ames microbial mutagenicity plate incorporation tests. PEG-IFN showed no evidence of clastogenic potential using an *in vitro* human peripheral blood lymphocyte chromosomal aberration assay at up to the highest feasible concentration (35 µg/ml) which could be evaluated. At doses of up to 30 mg/kg/d, i/p in mice, PEG-IFN did not induce any statistically significant changes in the incidence of micronucleated bone marrow cells, suggesting that it is not clastogenic after *in vivo* exposure.

Toxicology Study Summary:

1. One-month subcutaneous toxicity study of PEG₁₂₀₀₀-IFN-α 2b (SCH 54031) in combination with oral (gavage) dosing of ribavirin (SCH 18908) in cynomolgus monkeys. Study

#97060 (Schering Report #P-6671). *Macaca fascicularis* (weight range 2.2-4.5 kg, ♂; 2.0-3.2 kg, ♀), 3-5/sex/group; vehicle control (IFN placebo, lot #37-IQZ-101), 5494 $\mu\text{g}/\text{m}^2/\text{dose}$ SCH54031, lot #7-IQC-101, s/c, q.o.d. for 15 doses; with or without 50, 75 mg/kg/d SCH 18908, lot #36438-027, p/o, daily for 29 d; GLP; 4/15/97 – 6/3/99; Schering-Plough Research Institute, Lafayette, NJ. **Volume 3, pp. 1-397 and Volume 4, pp. 398-844.**

2. One-month subcutaneous toxicity and neutrophil function study of PEG₁₂₀₀₀-IFN- α 2b (SCH 54031) in combination with fixed oral (gavage) dosing of ribavirin (SCH 18908) in cynomolgus monkeys. Study #97261 (Schering Report #P-6752). *Macaca fascicularis* (weight range 2.2-3.7 kg, ♂; 2.0-3.0 kg, ♀), 3/sex/group; vehicle control (IFN placebo, lot #37-IQZ-101), 350, 1400, 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ SCH54031, lot #38101-016, s/c, q.o.d. for 13 doses; with or without 75 mg/kg/d SCH 18908, lot #36438-027, p/o, daily for 29-31d; GLP; 9/9/97 – 8/3/99; Schering-Plough Research Institute, Lafayette, NJ. **Volume 5, pp. 1-357 and Volume 6, pp. 358-844.**

Toxicology Review:

Study #97060 (report #P-6671). One-month subcutaneous toxicity study of PEG₁₂₀₀₀-IFN (SCH54031) in combination with oral (gavage) dosing of ribavirin (SCH 18908) in cynomolgus monkeys.

The toxicity and toxicokinetics of SCH 54031 PEG-IFN after every other day, s/c injections, alone or in combination with oral dosing with SCH 18908 ribavirin were evaluated in cynomolgus monkeys after a one month treatment period. Male and female cynomolgus monkeys (3/sex/group) were treated every other day with vehicle control (PEG-IFN placebo) or PEG-IFN at doses of 5494 $\mu\text{g}/\text{m}^2/\text{injection}$. Monkeys received daily, oral dosing with either 50 or 75 mg/kg ribavirin or vehicle (Sterile Water for Irrigation, U.S.P.) by gavage for 29 days. Two additional animals/sex were treated in the vehicle control and PEG-IFN plus high-dose ribavirin groups, and retained for a 4 week treatment-free recovery period.

Clinical observations for signs of morbidity or overt toxicities, as well as measurement of food consumption were performed daily, and body weights were determined weekly. Fasting peripheral blood samples for hematologic and serum biochemistry profiles were obtained twice during the pre-test period for determination of baseline values, then after one and four weeks of treatment with PEG-IFN, ribavirin, or controls, and at week 4 of recovery in the appropriate dose groups. Additional blood samples for hematologic evaluation only were obtained prior to dosing on study days 15 and 22 (weeks 2 and 3 on study, respectively), and in the recovery animals at week 2 after completion of treatment. Urinalysis and urine chemistries, physiologic parameters (ECG, respiratory and heart rates, blood pressure, body temperatures) were conducted twice during the pre-test period and at weeks 4 on treatment and twice during week 4 of recovery. General veterinary examinations, as well as ophthalmologic examinations were performed once during the baseline period, then at week 4 on study and at week 8 in the recovery animals. A full necropsy and gross pathologic evaluation was performed on each animal at terminal sacrifice (weeks 5 or 9 on study for the end-of-treatment and recovery groups, respectively), with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation.

Peripheral blood samples (approximately 3 ml/time point) were also obtained from SCH 54031 and ribavirin-treated monkeys for companion toxicokinetic and antibody development assays.

Serum and plasma samples were collected on days 1 and 5 of study prior to treatment and at 8 h after the injection of PEG-IFN and/or ribavirin dosing, and during week 2 of treatment on days which SCH 54031 was administered. Plasma and serum were also collected for toxicokinetic determination of PEG-IFN and ribavirin levels 24 h following the final dose of SCH 54031, and at terminal sacrifice following the 4-week recovery period. Serum interferon levels were measured using an electrochemiluminescence assay, and plasma ribavirin levels were determined by combination liquid chromatography/mass spectrophotometry. Antibody activity (both total Ig and neutralizing antibody) against IFN- α was determined using a biosensor assay (BIACORE []), which tests the binding activity of serum samples to immobilized PEG-IFN as well as unconjugated IFN- α . Positive samples from the BIACORE [] assay were analyzed further for neutralization of the anti-viral activity of IFN- α using a cytopathic effect (CPE) bioassay, as previously described (for a description of the CPE, please see review for BLA #98-1488).

Five animals were sacrificed moribund during the treatment period. One male monkey (animal #12M) in the group treated with 5495 $\mu\text{g}/\text{m}^2$ PEG-IFN alone was sacrificed on study d 18. Prior to sacrifice, this animal exhibited clinical signs of toxicity, including coolness to touch, abnormal gait and posture, and no feces. Hematologic profiles at terminal sacrifice showed decreases in total leukocyte, lymphocyte, and neutrophil counts with a degenerative left shift in polymorphonuclear neutrophil morphology indicative of release of immature precursor cells from the bone marrow, presumably in response to infection. Evidence of erythrocyte phagocytosis by neutrophils, suggesting low-grade extravascular hemolysis was also present. Moderate decreases in platelet counts (139,000/ μl at terminal sacrifice, as compared to 219,000/ μl at treatment initiation) were also observed, along with a concomitant left shift and nuclear dysplasia in the megakaryocyte lineage of this animal's bone marrow. Clinical pathology profiles for this monkey revealed a severe decrease in total serum protein, albumin, and total globulin, suggestive of vascular leakage. Five to seven-fold elevations in both ALT and AST were observed at terminal sacrifice for this animal, as compared to the two baseline values. At necropsy, the carcass of monkey #12M appeared thin, with cutaneous erosions and/or ulcerations present around and below both nostrils, and a mucopurulent discharge was present in and around both nares. Histologically, only part of the lesion was included in the sample, and the findings were consistent with the gross observation of ulceration. The routine skin section had no remarkable microscopic findings. Gross evaluation of the injection sites revealed several, approximately 5 mm diameter focal areas of discoloration, which on microscopic examination were found to consist of mild areas of subcutaneous hemorrhage accompanied by nonsuppurative perivascularitis, and infiltrating, foam-filled macrophages. The thymus gland in this animal was noted to be small at necropsy; histologically, evidence of thymic atrophy was present, as well as atrophied, ectopic thymic tissue present in the thyroid gland.

On gross pathologic evaluation, there were focal areas of discoloration in the left caudal and right middle lobes of the lungs, and the trachea contained a foamy material with no histologic evidence of inflammation. Microscopic evaluation of the lungs of monkey #12M revealed focal areas of mild to moderate, subacute pneumonia. Other histologic findings in this animal possibly related to treatment with SCH 54031 included minimal to mild areas of congestion and lymphadenitis in the mandibular lymph nodes, focal areas of hepatocellular necrosis, mild lymphoid depletion in the spleen, and mild to moderate hypocellularity and serous atrophy in the bone marrow. Atrophy of pericardial fat, as well as diffuse hyperplasia and decreased cytoplasmic vacuoles in the adrenal glands were also present in this animal, and are consistent with response of the host animal to severe stress. Although no bacterial colonies were found on histologic evaluation of samples from this animal, it is presumed that the changes are associated

with a subclinical infection, as evidenced by the shift in neutrophil morphology to a less mature phenotype, in the presence of a neutropenia.

One female monkey (#37F) was sacrificed moribund on d 25, after receiving approximately 11 doses of 5494 $\mu\text{g}/\text{m}^2$ SCH 54031 and 50 mg/kg/d ribavirin, p/o. During the fourth week on study, this animal exhibited signs of inappetence and decreased food consumption, resulting in no feces and a 0.3 kg loss of body weight from baseline by week 3 on study. Other clinical signs included abnormal posture, coolness to the touch, tremors and dehydration, beginning during the third week of treatment. Monkey #37F also developed several suppurative lesions at the injection sites for PEG-IFN, which on histologic evaluation were associated with moderately severe, subacute fibrogranulomas, with suppurative inflammation in the subcutis tissue.

Red cell counts, hematocrit, and hemoglobin levels in this monkey were decreased from the two baseline measurements beginning at the 2 week time point, suggesting a slight, treatment-related anemic effect. The decreases in erythrocyte parameters were also observed in the other two female monkeys, as well as in all male monkeys in this dose group at weeks 2 through 4 on study as compared to baseline, and were more severe in the two surviving female monkeys in this group than in animal #37F. Platelet counts were severely decreased in this animal, from a baseline count of 412,000/ μl at week -4, to 81,000/ μl at terminal sacrifice. Total leukocyte counts decreased by over 80% as compared to baseline for monkey #37F at week 4 on study, with a value of $1.7 \times 10^3/\text{mm}^2$ at this time point, as compared to 8.1 and $9.0 \times 10^3/\text{mm}^2$ at weeks -4 and -2, respectively, prior to treatment. Similar 70-95% decreases in both neutrophil and lymphocyte numbers from baseline time point were also observed in this animal at the week 4 time point.

Comment: Total leukocyte, lymphocyte, and neutrophil counts for this monkey were not remarkably different from baseline up until week 3 on study. However, the remaining two female monkeys in this dose group showed progressive decreases in all parameters, suggesting that the effects of the combined PEG-IFN and low-dose ribavirin on the hematologic profiles and marrow function are cumulative. One possible explanation for the relative increase in leukocyte parameters in monkey #37F is the presence of infection, as evidenced by positive cultures for opportunistic pathogens (see below).

The serum biochemistry samples taken from monkey #37F at terminal sacrifice revealed approximately two-fold elevations of both ALT and AST, as compared to the weeks -4 and -2 baseline values, and to week 1 on study. Increases in serum glucose to 301 g/dL from baseline values of 77 and 79 mg/dL, and 30-50% decreases in serum calcium, cholesterol, total protein, albumin, and globulin levels as compared to baseline were also observed in this monkey at the terminal sacrifice. There was a three-fold elevation in blood urea nitrogen at terminal sacrifice as compared to baseline values with no apparent increase in creatinine, which is consistent with the findings of dehydration in this animal.

Gross pathologic findings at necropsy of monkey #37F included effusions in the abdominal and thoracic cavities and pericardium, respectively. Samples of these fluids, as well as cerebrospinal fluid, blood from the ascending aorta, and swabs from the tracheal lumen and throat were collected for bacterial culture. There was no correlating pathology in the heart; however, microscopic findings of bacterial colonization in the stomach and kidney were reported. Culture specimens from monkey #37F were positive for *Staphylococcus aureus*, and the throat culture from this animal was also positive for *Streptococcus equisimilis*. At necropsy, a small thymus

was noted, which on histologic evaluation correlated with severe atrophy of the lymphoid tissue. Erosions and/or ulcerations of the skin were present at necropsy on both corners of the mouth, both eyebrows, the palm of the left hand, the lower abdomen, and both popliteal areas. Histologically, ulcerative dermatitis of mild to moderate severity was present on the mucosal surface of the lip, the eyebrows, and the popliteal surfaces, and a suppurative dermatitis of moderate severity was present in the lesions in the chin. The popliteal lesion contained bacterial colonies in the deep subcutaneous tissue. Gross evaluation of the lungs showed focal areas of dark red discoloration in the right cranial and left middle lobes, which on microscopic evaluation were associated with mild, focal areas of hemorrhage and nonsuppurative pleuritis. Focal areas of alveolar macrophage accumulation, of minimal severity were also present in the lungs of this animal. Other histopathologic findings included mild hypocellularity and serous atrophy of the fatty tissue in the bone marrow, focal areas of single cell hepatocyte necrosis in the liver and mucosal necrosis in the stomach of moderate severity, and atrophy of the epicardial and peripancreatic fat stores. The adrenal glands showed changes consistent with a response to severe stress in this animal, including diffuse hyperplasia and decreased cytoplasmic vacuolization in the zona fasciculata of the adrenal cortex, and focal areas of mineralization at the corticomedullary junction, both of mild severity. Skeletal muscle samples showed focal areas of granulomatous myositis with an accumulation of epithelioid cells, suggestive of muscle damage and repair secondary to infection. Together, all of these findings were considered by the sponsor to be an extension of the marrow suppressive effects of interferon- α , resulting in anemia, decreased total protein synthesis by the liver, and decreased leukocyte responses to infection.

Three monkeys in the group treated with 5494 $\mu\text{g}/\text{m}^2$ PEG-IFN and 75 mg/kg/d ribavirin, p/o were sacrificed moribund during weeks 2 or 3 on study. Male monkey #23M was sacrificed on study day 14, during week 2 on study. This animal was reported as having no observable clinical abnormalities on four occasions that week, and sacrificed moribund at the next observation time point. Two female monkeys in this group (animals #29F and #32F) were sacrificed during week 3 (days 19 and 18, respectively) after exhibiting clinical signs of scant or no feces, dehydration, abnormal posture, and coolness to touch for approximately one week prior to termination. At terminal sacrifice, all three animals had minimal to mild decreases in erythrocyte parameters as compared to baseline values, including red cell numbers, hemoglobin, and hematocrit levels. All three monkeys had evidence of hemolysis, as determined by presence of erythroid fragments within the cytoplasm of phagocytic neutrophils. However, male monkey #23M had no evidence of a bone marrow response to the mild anemia, as evidenced both by no increase in peripheral blood reticulocyte counts and a concomitant increase in circulating, nucleated red cells. Total leukocyte counts in this animal were moderately decreased from baseline values of 11.9 and 9.3 $\times 10^3/\text{mm}^3$ to 2.1 $\times 10^3/\text{mm}^3$ at terminal sacrifice. Lymphocytes were less severely affected in this animal than were neutrophils; an apparent, 2-fold decrease in lymphocyte counts was observed at terminal sacrifice as compared to baseline values. However, neutrophils were severely decreased in monkey #23M, from baseline values of 7.5 and 5.7 $\times 10^3/\text{mm}^3$ to 100/ mm^3 (Grade 4 neutropenia) at sacrifice, with a degenerative left shift in the hematologic profile (as defined by an increase in the number of immature PMN and band cells, in the presence of a severe neutropenia). Evidence of bacterial infection, as confirmed by cytoplasmic basophilia and intracellular bacteria within the neutrophils, as well as the presence of Gram-positive cocci in peripheral blood were prominent in this monkey.

Severe decreases in both total white cell and neutrophil counts, with a concomitant degenerative left shift in neutrophil morphology were also observed for female monkey #29F, sacrificed during week 3 on study. Neutrophil counts at sacrifice had dropped to 400/ μl (Grade 4

neutropenia), from baseline values of 2800 and 2300/ μl . Less severe decreases in total leukocyte, lymphocyte, and neutrophil counts were observed in monkey #32F; total leukocyte counts for this animal at terminal sacrifice were $5.4 \times 10^3/\text{mm}^3$, as compared to 8.3 and $14.5 \times 10^3/\text{mm}^3$ at baseline. Similarly, neutrophil counts for this monkey were only mildly decreased as compared to the other two animals sacrificed moribund in this group. At sacrifice, peripheral blood neutrophil counts were 2600/ μl , as compared to 2.9 and $8.6 \times 10^3/\text{mm}^3$ at baseline. However, all three monkeys sacrificed early in this dose group exhibited bone marrow hypoplasia, which was moderate in male monkey #23M and mild in severity in female animals #29F and #32F. Increased M:E ratios, consistent with granulocytic hyperplasia and erythrocyte hypoplasia were observed in animals #23M and #32M, while female monkey #29F had evidence of granulocyte hypoplasia. All three animals had minimal to mild decreases in peripheral blood platelet counts, with corresponding decreases in megakaryocyte counts and increased dysplasia of megakaryocytes in the bone marrow; however, female monkey #32F had no evidence of decreased peripheral blood platelet counts at terminal sacrifice, as compared to baseline values.

Clinical chemistry values for these three monkeys treated with $5495 \mu\text{g}/\text{m}^2$ SCH 54031 and 75 mg/kg/d ribavirin revealed hyperglycemia (263 mg/dL), mild elevations in AST, alkaline phosphatase, and urea nitrogen in female monkey #29F at terminal sacrifice as compared to baseline. No apparent changes from baseline were noted for these parameters in female monkey #32F, while male monkey #23M had a 2-fold increase from baseline values in both blood urea nitrogen and creatinine, a 3-fold increase in ALT, and a 9-fold increase in AST at terminal sacrifice. This animal also had a 5-fold elevation in serum triglycerides with no concomitant increase in cholesterol, a finding that was not present in the two female monkeys in this dose group. All three monkeys sacrificed moribund from this dose group had 25 to 66% decreases in total serum protein, albumin, globulin, and A:G ratios, consistent with leakage of plasma proteins into inflammatory sites. The decreases in serum protein values were most severe in male monkey #23M, sacrificed on study d 14.

On gross pathologic evaluation, all three animals had evidence of erosions and/or ulcerations of the skin, particularly around the mouth and nose. Microscopic evaluation revealed minimal to moderate ulcerative dermatitis, with bacterial colonies present in the lesions from animal #23M. Injection sites in these three animals all appeared discolored at necropsy, with histologic findings of mild to moderate subcutaneous hemorrhage, subacute inflammation, and perivascularitis. Monkeys #23M and #29F had serosanguinous effusions present in the thoracic cavity and fibrinous material in the pericardium, which histologically were correlated with moderate to severe fibrinopurulent myo- and epicarditis, suppurative pericarditis, coagulative myocardial necrosis and lymphocytic infiltrates, and evidence of bacterial colonization of the myocardium and epicardium. All three animals had evidence of focal parietal and/or mucosal cell necrosis, lymphocytic inflammation, and areas of hemorrhage in the stomach, and thymic atrophy, which was severe in female monkey #32F. Histologic findings in the adrenal glands included decreased cytoplasmic vacuolization in the zona fasciculata of all 3 animals, consistent with a response to stress. Additionally, male monkey #23M had microscopic evidence of intravascular bacterial colonies in the adrenal glands, accompanied by mild bacterial peritonitis in the surrounding abdominal area. Female monkey #29F had a moderately severe, suppurative peritonitis in the abdominal cavity and surrounding the stomach, which at necropsy was reported as a thickening of the omentum, with a minimal presence of cloudy fluid.

Histologic evaluation of bone marrow samples from these three animals treated with PEG-IFN and 75 mg/kg/d ribavirin revealed increased hypocellularity as compared to control monkeys,

which was minimal in severity in male monkey #23M, and mild in both female monkeys in this dose group. Taken together, these changes are suggestive sepsis in animal #23 M, and of decreased host resistance to infection in all three monkeys in this dose group, probably secondary to the marrow suppression associated with PEG-IFN treatment in these animals.

There were no mortalities noted, and no remarkable changes in body weight, food consumption, body weight gains, or clinical chemistry profiles in monkeys treated with 50 mg/kg/d ribavirin alone, when compared to either baseline values for each individual animals, or to the mean values for the vehicle control group.

During the treatment period, the remainder of the animals treated with the high dose of 75 mg/kg/d ribavirin in combination with 5495 $\mu\text{g}/\text{m}^2$ /dose SCH 54031 showed clinical signs of toxicity. Inappetence, dehydration, scant feces, and poor food consumption were noted in beginning at week 2 and increasing in severity at week 3 in 4/5 female monkeys in the highest dose group (animals #29F, #31F, #32F and #28F), and in 3/5 male monkeys (animals #4M, #23M, and #27M) at 2 weeks and one additional male (animal #25M) at 3 weeks on study. Three of the female monkeys (animals #28F, #29F, and #32F) were also dehydrated and cool to the touch, while animal #4M also developed diarrhea. However, beginning in week 3, normal food consumption was resumed in all of these animals, and by study termination, 3/4 surviving male animals, and 1/3 of the surviving female monkeys in this dose group had returned to baseline weight values. Mean body weights and body weight gains in this group were not statistically significantly different from either mean baseline values, or control group values for either sex at terminal sacrifice. Animals held for the 4-week recovery period continued to gain weight over the duration of the study, suggesting that the inappetence caused by PEG-IFN and/or ribavirin treatment was reversible following cessation of dosing.

Monkeys in the mid-dose group also displayed overt signs of toxicity; however, the severity of the effects was generally less than those observed in the high-dose group. All monkeys treated with 5494 $\mu\text{g}/\text{m}^2$ PEG-IFN and 50 mg/kg/d ribavirin, with the exception of female monkey #37F survived for the duration of the study. Slight inappetence and/or weight loss were observed in 2/3 male animals in this dose group, between weeks 2 and 3 on study but had returned to baseline values in all except animal #35M by the end of the study. The female monkeys had slight (0.1 – 0.2 kg) losses in body weight, however the severity was less than 10% decrease from initial body weight. Scant feces were noted on two occasions in all three male monkeys during weeks 2 and 3 on study, and diarrhea was observed on one occasion during week 4 in monkey #39M. Female monkey #36 F had two occasions of scant feces at week 2 on study, and animal #37F was sacrificed moribund during study week 3 (please see description, above). There were no other clinical abnormalities reported for animals in this dose group.

There were no overt clinical signs of toxicity seen in any of the vehicle control group animals, nor in the animals treated with 50 mg/kg/d ribavirin alone or 5494 $\mu\text{g}/\text{m}^2$ SCH 54031 PEG-IFN as a positive control, with the exception of male monkey #12M, who was sacrificed moribund at study d 18 (please see description, above). Although slight decreases in mean and individual animal body weights, food consumption, and body weight gains were observed for animals of both sexes in this treatment group, they were not statistically significant when compared either to baseline values or to the vehicle control group mean values.

There were no statistically significant, treatment-related changes in rectal body temperature, heart rate, blood pressure, respiratory rates, or ECG profiles in monkeys treated for four weeks

with either SCH 54031 PEG-IFN or ribavirin alone, or in combination as compared to the either the vehicle control group or to baseline values. Several animals in the high-dose ribavirin and PEG-IFN group had irregular respiratory rates during the week 4 EKG reading; however, this finding was not present in the monkeys treated with either PEG-IFN or 50 mg/kg/d ribavirin, and the biologic relevance of this finding is unknown. Ophthalmologic exams were normal in all monkeys at all time points on study, and there were no remarkable findings on urinalysis profiles.

Hematologic profiles showed evidence of anemia, decreased total leukocyte counts, and decreased platelets in all groups of monkeys treated with either ribavirin or SCH 54031 PEG-IFN, beginning at the week 1 time point. Dose-related, 10 to 45% decreases in mean red cell counts, hematocrit, and hemoglobin concentration were observed in both male and female monkeys treated with PEG-IFN and either dose of ribavirin, or with 50 mg/kg/d ribavirin alone, as compared to either baseline values or to the vehicle control group. These changes were less severe in the animals treated with SCH 54031 alone, but were still decreased approximately 15 to 20% from baseline at terminal sacrifice. The decreases in erythrocyte parameters were more severe in animals treated with PEG-IFN and the 75 mg/kg/d ribavirin dose level than in either the low-dose ribavirin alone or in combination with SCH 54031 groups. These changes persisted at the 4 week time point, and were only partially resolved after a 4-week treatment-free recovery period. Peripheral blood reticulocyte counts were also elevated over baseline values in all groups of monkeys treated with PEG-IFN with or without ribavirin, as well as in the group of monkeys treated with ribavirin alone. After a 2 week recovery period, monkeys treated with PEG-IFN and 75 mg/kg/d ribavirin demonstrated 6 to 10-fold increases in reticulocyte counts which then decreased to approximately baseline at the 4 week recovery time point, suggesting that the marrow suppressive effects of the pegylated interferon- α are related to its extended half-life.

At all time points on study, mean platelet counts were not significantly decreased from baseline, nor different from the vehicle control group in all groups of male monkeys treated with PEG-IFN, with or without ribavirin. By contrast, female monkeys treated with SCH 54031 alone or in combination with either dose of ribavirin had decreases in platelet counts of approximately 30 to 45% from baseline, beginning at week 1 and continuing through week 3 on study. No changes in platelet count were noted for female monkeys treated with ribavirin alone. By week 4, platelet counts had recovered to baseline values in almost all affected groups with the exception of the female monkeys treated with PEG-IFN and 75 mg/kg/d ribavirin, who remained approximately 10% below baseline values. However, platelet numbers continued to increase in this group to approximately 2-fold over baseline during the treatment-free recovery phase.

Total leukocyte counts were initially decreased by greater than 50% as compared to either baseline values or the placebo control in all groups of interferon-treated monkeys after one week on study ($p \leq 0.01$, ANOVA), with no apparent relationship in either the incidence or severity of the decreases to the dose of PEG-IFN. Recovery of total leukocyte counts was observed in all groups of animals at the week 4 time point and at the end of the recovery period; although not all mean values had reached baseline by that time, there were no statistically significant differences between the groups. Of interest, there was an increase in the mean white cell counts above baseline values in the PEG-IFN alone dose group at 4 weeks on study, suggesting an apparent rebound effect from the initial decrease at week 1. These data are presented in Table I, below.

Table I – Total Peripheral Blood Leukocyte Counts Following PEG-IFN ± Ribavirin

Dose of PEG-IFN	Mean Total Leukocyte Counts (x 1000/mm ³) + S.D.			
	Baseline	Week 1	Week 4	Recovery
Vehicle	11.9 ± 2.0	10.5 ± 3.1	10.9 ± 1.9	10.6 ± 2.9
PEG-IFN alone	9.6 ± 2.5	4.6 ± 1.0 ^{a,b}	12.1 ± 4.2	n.d. ^c
Ribavirin alone	11.3 ± 2.8	10.0 ± 2.3	10.1 ± 3.5	n.d. ^c
PEG-IFN + low	10.2 ± 2.7	5.5 ± 1.5 ^{a,b}	8.7 ± 4.5	n.d. ^c
PEG-IFN + high	11.5 ± 2.8	4.7 ± 0.9 ^{a,b}	8.1 ± 4.3	13.8 ± 7.1

^a significantly different from vehicle control ($p < 0.01$, ANOVA)

^b significantly different from baseline ($p \leq 0.005$, ANOVA)

^c n.d. = not done

Similar 30 to >75% decreases in both absolute neutrophil counts and lymphocyte counts were observed in interferon-treated monkeys, with or without ribavirin treatment at the week 1 time point, as compared to either vehicle control or to baseline values. Once again, there was no relationship to either the severity or the incidence of the changes to the dose of ribavirin administered to the monkeys. Recovery of both neutrophil and total lymphocyte counts to approximately baseline values was observed in all groups of animals treated with PEG-IFN either alone or in combination with ribavirin by the end of week 4 on study. These data are presented in Tables II and III, below.

Table II – Peripheral Blood Neutrophil Counts Following PEG-IFN ± Ribavirin Treatment

Dose of PEG-IFN	Mean Neutrophil Counts (x 1000/mm ³) + S.D.			
	Baseline	Week 1	Week 4	Recovery
Vehicle	7.3 ± 3.5	6.1 ± 3.7	6.6 ± 3.7	4.0 ± 4.1
PEG-IFN alone	6.0 ± 1.6	1.0 ± 0.4 ^{a,b}	6.9 ± 4.2	n.d. ^c
Ribavirin alone	5.9 ± 1.7	3.9 ± 1.3 ^b	4.8 ± 2.4	n.d. ^c
PEG-IFN + low	5.2 ± 2.3	1.6 ± 0.4 ^{a,b}	4.9 ± 3.0	n.d. ^c
PEG-IFN + high	5.8 ± 2.8	1.2 ± 0.5 ^{a,b}	3.4 ± 2.9	7.5 ± 7.6

^a significantly different from vehicle control ($p < 0.01$, ANOVA)

^b significantly different from baseline ($p \leq 0.05$, ANOVA)

^c n.d. = not done

Table III – Peripheral Blood Lymphocyte Counts Following PEG-IFN ± Ribavirin Treatment

Dose of PEG-IFN	Mean Lymphocyte Counts (x 1000/mm ³) + S.D.			
	Baseline	Week 2	Week 4	Recovery
Vehicle	6.0 ± 1.4	4.5 ± 1.6	5.1 ± 1.9	6.2 ± 1.4
PEG-IFN alone	5.4 ± 0.9	3.2 ± 0.9 ^a	4.1 ± 1.0	n.d. ^c
Ribavirin alone	4.7 ± 1.1	3.1 ± 1.0 ^a	5.0 ± 1.8	n.d. ^c
PEG-IFN + low	5.8 ± 2.4	3.6 ± 1.1 ^a	4.9 ± 2.1	n.d. ^c
PEG-IFN + high	6.0 ± 1.7	2.3 ± 0.8 ^{a,b}	5.0 ± 1.7	5.8 ± 2.2

^a significantly different from baseline ($p \leq 0.05$, ANOVA)

^b significantly different from placebo control ($p \leq 0.01$, ANOVA)

^c n.d. = not done

There were no remarkable changes in eosinophil, basophil, atypical lymphocytes, or immature (band) neutrophils in the surviving animals treated PEG-IFN with or without ribavirin, or in the group treated with ribavirin alone, as compared to either the vehicle control group or to baseline values. Beginning at week 1 and continuing through week 4, monocyte counts were elevated by approximately 1.3 to 2-fold as compared to baseline in all groups of monkeys treated with PEG-IFN, either alone or in combination with ribavirin. By week 4, monocyte counts had returned to baseline in male monkeys treated with either SCH 54031 alone or PEG-IFN and 50 mg/kg/d ribavirin, however, monocyte counts in the group of male monkeys treated with PEG-IFN and 75 mg/kg/d ribavirin remained elevated 1.5 to 2-fold over baseline from week 4 on study to the end of the recovery period. By contrast, the elevated monocyte counts had returned to baseline values by week 4 on study in all groups of female monkeys treated with PEG-IFN and either dose of ribavirin; however, mean monocyte counts remained elevated at week 4 by approximately 2-fold over baseline in the female monkeys treated with SCH 54031 PEG-IFN alone. There were no other remarkable changes in eosinophil, basophil, or atypical lymphocyte counts in any of the other dose groups.

There were no definitive, treatment-related changes in clinical chemistry profiles for the surviving animals treated with SCH 54031 at either 1 or 4 weeks, as compared to either the vehicle control group, or to baseline values. Slight, although statistically not significant elevations in total bilirubin, as compared to either baseline values or to the placebo control group were noted at week 1 on study in the male monkeys treated with PEG-IFN alone; however, these findings had resolved to baseline by the end of the 4-week treatment period. Decreases in total protein, globulin, and serum albumin levels, with concomitant 20 to 25% decreases in A:G ratios were noted in all groups of interferon-treated monkeys without an apparent relationship to the dose of ribavirin, beginning at week 2 and continuing until study termination. These changes had resolved to baseline at the end of the 4-week recovery period in the male and female monkeys treated with both PEG-IFN and 75 mg/kg/d ribavirin.

At necropsy, there were no adverse gross pathologic findings related to treatment in the control, or ribavirin only treated groups, with the exception of discoloration at the injection site (redness, bruising). This effect was observed in monkeys in all other groups as well, controls, without a dose-relationship in either incidence or severity. Small thymus glands were observed on gross evaluation in female monkey #15F and male monkeys #35M and #39M in the group treated with 5494 $\mu\text{g}/\text{m}^2/\text{dose}$ SCH 54031 PEG-IFN alone, and in 2/2 female monkeys (animals #36F and #38F) in the PEG-IFN plus 50 mg/kg/d ribavirin dose, and in female monkey #28F and male monkeys #4M and #27M in the group receiving PEG-IFN and 75 mg/kg/d ribavirin. Histologically, these effects were correlated with mild to moderate thymic atrophy, which is a known effect of interferon- α treatment. Monkeys #39M and #36F in the PEG-IFN and low-dose ribavirin group, and animals #4M and #28F in the group treated with PEG-IFN and 75 mg/kg/d had thin carcasses on gross evaluation, as compared to either the vehicle or ribavirin only control animals. This finding was likely related to inappetence induced by the PEG-IFN in combination with ribavirin. Histologically, atrophy of the epicardial and abdominal fat stores, as well as atrophy of the serous fat in the bone marrow were present in these animals. Ulcerations or erosions, particularly on the lips or skin surrounding the mouth and/or nose were present in all three low-dose ribavirin male monkeys, and in monkeys #4M and #28 F in the group treated with 75 mg/kg/d ribavirin in combination with SCH 54031 PEG-IFN. All of these findings had resolved by sacrifice at week 4 of recovery in the PEG-IFN and high-dose ribavirin monkeys.

Other, incidental findings in the animals surviving to terminal sacrifice included nodules or abnormal contents in the colon of several animals, small testes in one male monkey (#41M) in the vehicle control group (suggesting a sexually immature animal), and adhesions and areas of discoloration in the lungs from one vehicle control male monkey (#5M), one female monkey in the ribavirin only group (animal #22F), and one male monkey (#4M) in the high-dose ribavirin and PEG-IFN groups. Focal discoloration and reddish areas of the stomach mucosa in either the fundus or the pylorus were reported for animals #10F and #11M in the vehicle control and 5494 $\mu\text{g}/\text{m}^2$ PEG-IFN treated groups, respectively. After the 4 week, treatment-free recovery period, there were no gross pathologic findings noted in either the vehicle control or the PEG-IFN and 75 mg/kg/d ribavirin treated monkeys.

Histologic findings related to treatment were limited to the injection site, and included subcutaneous hemorrhage, inflammation, perivascularitis and endarteritis, and fibrosis. These findings occurred with approximately equal incidence and severity in all of the treatment groups, including the controls. The three monkeys showing macroscopic changes in the lungs had findings on histologic evaluation that correlated with the gross pathology, including focal areas of mild, pleural fibrosis in control monkey #5M, ribavirin control monkey #22F, and monkey #4M in the group treated with PEG-IFN and high-dose ribavirin. Other microscopic findings included minimal decreases in cellularity in the bone marrow in animals of both sexes in all PEG-IFN treatment groups at terminal sacrifice, mononuclear cell infiltrates, mineral deposition, and tubular pigmentation in the kidneys, mononuclear cell infiltrates in the lacrimal glands, increased pigment and lymphoid hyperplasia in the mandibular lymph nodes, and focal areas of hepatocellular vacuolization, necrosis, and mononuclear cell infiltrates in the liver. These findings occurred at approximately equal incidence and severity across all treatment groups, and were considered by the reviewing pathologist to be incidental to treatment with the test articles.

The toxicokinetic profiles of both PEG-IFN and ribavirin were evaluated as part of this repeat-dose toxicity study. Samples of peripheral blood for determination of PEG-IFN levels were obtained from all monkeys at baseline (immediately prior to dosing on day 1), and at 8 h after the first dose of PEG-IFN or ribavirin. Peripheral blood samples for cumulative exposure were also obtained at 8 h post-dosing on study days 11 and 29, and at terminal sacrifice on d 30 or after the 4 week recovery period.

Serum interferon levels were measured both by an electrochemiluminescence (ECL) assay, with a lower limit of quantitation of \sim pg/ml. This assay involved the formation of an immune complex between the PEG-IFN in the sample, and α antibody. The ECL signal produced is proportional to the concentration of both SCH 54031 and any free interferon- α present in the sample, and concentrations in the serum samples were determined against a standard curve of SCH 54031 PEG-IFN. Ribavirin levels were determined from plasma samples, using a validated liquid chromatography/mass spectrophotometer with ^{13}C -ribavirin as the internal standard. The lower limit of detection of this assay is \sim ng/ml ribavirin. The results from samples obtained 8 h after dosing at each time point on study, as determined by each assay are presented in Tables IV and V below:

Table IV – Toxicokinetic Evaluation of PEG-IFN in Cynomolgus Monkeys**ECL Assay for IFN levels**

Week on Study	Mean Serum IFN Value (ng/ml) ± % CV		
	PEG-IFN + Ribavirin Dose Level		
	Vehicle Control	50 mg/kg/day	75 mg/kg/d
-4	0 ± 0	0 ± 0	0 ± 0 ^c
1	873 ± 18	1260 ± 36	1140 ± 20 ^c
2	1120 ± 59	1260 ± 32 ^b	1200 ± 47 ^c
5	137 ± 38 ^a	143 ± 66 ^b	589 ± 103 ^d

^a n = 3 animals^b n = 5 animals^c n = 10 animals^d n = 7 animals**Table V – Toxicokinetic Evaluation of Ribavirin in Cynomolgus Monkeys****LC/MS Assay for Ribavirin Levels**

Week on Study	Mean Plasma Ribavirin Value (ng/ml) ± % CV		
	PEG-IFN + Ribavirin Dose Level		
	Vehicle Control	50 mg/kg/day	75 mg/kg/d
-4	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^c
1	923 ± 42 ^a	572 ± 35 ^a	788 ± 33 ^c
2	1210 ± 68 ^a	1330 ± 52 ^a	2070 ± 27 ^c
5	1740 ± 42 ^a	1850 ± 41 ^b	2640 ± 22 ^d

^a n = 6 animals^b n = 5 animals^c n = 10 animals^d n = 7 animals

No interferon activity was detected by the ECL assay in serum samples from either the vehicle control (SCH 54031 placebo) or ribavirin alone-treated monkeys at any time point on study. Similarly, no ribavirin was detected in plasma samples from either vehicle control or SCH 54031-treated monkeys at any time point on study. The decreases in PEG-IFN serum levels seen in all groups at week 5 on study are suggestive of antibody generation against the biologic; this effect was confirmed both by the BIACORE assay for anti-PEG-IFN antibodies, as well as by the loss of pharmacodynamic and toxic effects associated with PEG-IFN at week 5 on study. Because only a single time point after test article administration was sampled for PEG-IFN and ribavirin levels, it is not possible to calculate standard pharmacokinetic parameters (*i.e.* AUC, C_{max}, and T_{1/2elim}). However, in all groups treated with ribavirin, the mean plasma values obtained at weeks 2 and 5 on study were increases by approximately 1.5 to 2-fold, as compared to the values obtained at week 1. These data suggest that bioaccumulation or ribavirin is occurring, either by saturation of the clearance mechanism for the drug, or by a possible synergistic effect with PEG-IFN.

Antibody activity (both total Ig and neutralizing antibody) was determined using a biosensor assay, which tests the binding of serum samples to immobilized PEG-IFN or unconjugated interferon- α . Samples positive for total anti-IFN antibody activity were further analyzed for

neutralizing activity of the anti-viral effects of IFN- α by the CPE bioassay, respectively. The results of the BIACORE assay are presented in Table VI, below.

Table VI – Anti-Interferon Total Antibody Activity in PEG-IFN Treated Cynomolgus Monkeys

BIACORE Assay for Total Antibody Titers

Week on Study	Relative Serum Anti-PEG-IFN Ab + % CV			
	PEG-IFN + Ribavirin Dose Level			
	Vehicle Control	50 mg/kg/day	75 mg/kg/d	
Pre-Test	0 + 0	0 + 0	0 + 0	
5	1685 + 56	23961 + 99	540 + 118	
Week on Study	Relative Serum Anti-IFN- α Ab + % CV			
	Pre-Test	0 + 0	0 + 0 ^a	0 + 0 ^c
	5	1648 + 61	3488 + 71	576 + 120

All serum samples from either vehicle or ribavirin control animals were negative for both anti-PEG-IFN and anti-interferon- α total antibody at all time points tested.

Comment: The protocol and the final, audited study report state that any positive samples for anti-interferon- α antibody activity by the BIACORE assay will be analyzed by the CPE assay for evidence of anti-interferon neutralizing activity. However, nowhere in the final report is there any indication that these studies were conducted, and there are no amendments to the protocol that provide an explanation for the lack of data.

In summary, treatment of both male and female cynomolgus monkeys with PEG-IFN at a dose of 5494 $\mu\text{g}/\text{m}^2$, either alone or in combination with 50 or 75 mg/kg/d ribavirin, p/o for one month was associated with inappetence in the absence of significant weight loss, dose-related anemia, mild to moderate leukopenia, lymphopenia, and decreases in platelets, moderate to severe neutropenia, and transient decreases in total serum protein, albumin, globulin, and A:G ratios. Mortalities were observed in 5 animals, one each in the PEG-IFN and PEG-IFN plus 50 mg/kg/d ribavirin dose groups, and in 3/10 animals in the group treated with PEG-IFN and 75 mg/kg/d ribavirin. The early deaths on study were associated with a decreased host response to opportunistic infections, as evidenced by mucopurulent lesions and/or abscesses on the skin, bacterial colonization in the heart, kidneys, adrenal glands, and blood of several animals, degenerative left shifts in neutrophil morphology in the peripheral blood, moderate to severe bone marrow hypoplasia, and presence of *Staphylococcus aureus* and *Streptococcus equinimus* in culture samples from one animal. Pathologic findings related to PEG-IFN treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site, atrophy of the thymus, and minimal to mild changes in bone marrow cellularity. These findings were transient in nature, and had fully resolved by the end of the 4 week recovery period. Toxicokinetic evaluations of serum PEG-IFN and ribavirin levels confirmed that exposure was continuous throughout the duration of the study; however, after 4 weeks of treatment, detection of serum IFN levels was impeded by the development of anti-PEG-IFN and anti-interferon- α antibody titers. Reversal of toxicity was associated with development of neutralizing antibodies in all of the groups treated with PEG-IFN. Although no NOAEL for PEG-IFN alone or in combination with ribavirin could be determined for this study, the dose of PEG-IFN used in this study is approximately 300-fold greater than the dose of 1.5 $\mu\text{g}/\text{kg}/\text{week}$

used in the phase 3 clinical trial. The two doses of ribavirin used in the animal study are approximately 6 and 8-fold higher than the clinical dose of 800 mg/d given in combination with the high-dose of PEG-IFN in the phase 3 clinical trial.

Study #97261 (report #P-6752). One-month subcutaneous toxicity and neutrophil function study of PEG₁₂₀₀₀-IFN (SCH54031) in combination with fixed oral (gavage) dosing of ribavirin (SCH 18908) in cynomolgus monkeys.

The toxicity, toxicokinetics, and effects on neutrophil function of SCH 54031, alone or in combination with ribavirin were evaluated in male and female cynomolgus monkeys after a one-month treatment period. A previous toxicity study of PEG-IFN and ribavirin (study #97060 [Schering report #P-6671]) had demonstrated significant mortalities in cynomolgus monkeys beginning in the second week of dosing due to opportunistic infections, bacteremia, and sepsis that were presumed secondary to the marrow suppressive effects of PEG-IFN. To investigate this hypothesis, the purpose of the present study was to determine the effects of increasing doses of PEG-IFN on mortality in monkeys, as well as on neutrophil functions associated with surveillance and prevention of infection (phagocytosis, chemotaxis, and intracellular killing).

Male and female cynomolgus monkeys (3/sex/group) were treated every other day with vehicle control (PEG-IFN placebo) or PEG-IFN at doses of 350, 1400, or 5500 $\mu\text{g}/\text{m}^2$ /injection for a total of 25 days (13 doses). Monkeys received daily, oral dosing with either 75 mg/kg SCH 18908 ribavirin or vehicle (Sterile Water for Irrigation, U.S.P.) by gavage for 29-31 days. Clinical observations for signs of morbidity or overt toxicities, as well as measurement of food consumption were performed daily, and body weights were determined weekly. General veterinary examinations, as well as ophthalmologic examinations were performed once during the baseline period, then at week 4 on study. A full necropsy and gross pathologic evaluation was performed on each animal at terminal sacrifice at week 5 on study, with organ weights recorded, and tissue samples taken and processed for histopathologic evaluation.

Fasting peripheral blood samples for hematologic and serum biochemistry profiles were obtained twice during the pre-test period for determination of baseline values, then after two and four weeks of treatment with PEG-IFN, ribavirin, or controls (study days 9 and 23, respectively). Additional blood samples for evaluation of coagulation parameters were obtained twice during pre-test, and prior to necropsy on study days 30, 31, or 32. Urinalysis and urine chemistries, physiologic parameters (ECG, respiratory and heart rates, blood pressure, body temperatures) were conducted twice during the pre-test period and once during weeks 2 and 4 on treatment (study days 9, 10, or 11 and days 24 or 35, respectively).

Samples of peripheral blood neutrophils were obtained from all monkeys in the vehicle control group and the groups treated with 75 mg/kg/d ribavirin alone, 5500 $\mu\text{g}/\text{m}^2$ /dose PEG-IFN, or the combination of the high-dose PEG-IFN and ribavirin only. Blood samples (at least 5 ml/animal) were collected once during the pre-test period, and once during weeks 2 or 3 on study, using EDTA as the anti-coagulant, transferred to [] tubes, and shipped on wet ice to the testing laboratories. Samples from 2 monkeys/sex/group in the 5500 $\mu\text{g}/\text{m}^2$ /dose PEG-IFN only, ribavirin only, and vehicle control groups and from all 6 animals treated with the combination of PEG-IFN and ribavirin were evaluated for chemotaxis at []

] Peripheral blood neutrophils isolated from the remaining animals were assayed the same day for phagocytic activity and intra-cellular

killing of *Staphylococcus aureus* and for phagocytosis of opsonized *Saccharomyces cerevisiae* at the Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ.

Comment: Although the samples shipped to the laboratory in [] were sent to arrive the same day, the final study report does not specify if the chemotactic activity of these cells was assayed the same day. Neutrophils have a low viability once isolated from peripheral blood, and should be evaluated immediately after isolation.

Peripheral blood samples (approximately 2-3 ml/time point) were also obtained from SCH 54031 and ribavirin-treated monkeys for companion toxicokinetic and antibody development assays. Serum and plasma samples were collected once during the pre-test period, and at 8 h after dosing on study d 1 and during weeks 3 and 5 on treatment. Plasma and serum were also collected for toxicokinetic determination of PEG-IFN and ribavirin levels 104 h following the final dose of SCH 54031. Blood samples were also collected from the vehicle control animals, but were not analyzed for either PEG-IFN or ribavirin levels.

Serum interferon levels were measured using the ECL assay previously described, and plasma ribavirin levels were determined by combination liquid chromatography/mass spectrophotometry. Antibody activity (both total Ig and neutralizing antibody) against IFN- α was determined using the BIACORE [] biosensor assay, which tests the binding activity of serum samples to immobilized PEG-IFN as well as unconjugated IFN- α . Positive samples from the BIACORE [] assay were analyzed further for neutralization of the anti-viral activity of IFN- α using the previously described CPE bioassay. These samples were also inadvertently assayed for IFN- α activity using the CPE bioassay; a summary of these data was provided in the final report.

One male monkey (animal #26M) in the group treated with 350 $\mu\text{g}/\text{m}^2$ PEG-IFN and 75 mg/kg/d ribavirin was sacrificed moribund on study d 25. Beginning week 2 on study, this animal decreased its food consumption by greater than 50%, and had several bouts of either loose stool or diarrhea. In the ten days prior to sacrifice, this animal exhibited clinical signs of toxicity, including coolness to touch, abnormal posture, dehydration and weakness, and soft, scant, or no feces. Hematologic profiles at terminal sacrifice showed 33 to > 50% decreases in total leukocyte, lymphocyte, and neutrophil counts from baseline. The decreases in total leukocyte and neutrophil counts were accompanied by a degenerative left shift in polymorphonuclear neutrophil morphology indicative of release of immature precursor cells from the bone marrow, presumably in response to infection. Moderate increases in platelet counts (945,000/ μl at terminal sacrifice, as compared to 544,000/ μl at treatment initiation) were also observed, along with a concomitant left shift and nuclear dysplasia (hyperlobularity) in the megakaryocyte lineage of this animal's bone marrow. Bone marrow findings also included a mild shift towards immaturity in the erythrocyte lineage, with an increase in the myeloid:erythroid ratio and no evidence of increased reticulocyte formation, either in the peripheral blood or in the marrow itself. These findings are consistent with the known, marrow-suppressive effects of PEG-IFN in both non-human primates and man.

Clinical pathology profiles (serum biochemistry) were not determined in this particular study. At necropsy, the carcass of monkey #26M appeared moderately thin, with evidence of depletion of body fat stores as well as an empty intestinal tract on gross pathologic evaluation. The injection site contained a small (approximately 5 mm in diameter), focal area of red discoloration, which histologically was characterized as grade 1 hemorrhage surrounding an old scar, and was considered unrelated to treatment with the test article. The thymus gland in this animal was

noted to be small at necropsy; histologically, evidence of thymic atrophy was present, as well as atrophied, ectopic thymic tissue present in the thyroid gland.

On gross pathologic evaluation, there was a moderate (10 x 10 mm) area of dark discoloration at the base of the right middle lobe of the lung. Microscopic evaluation of the lungs of monkey #26M revealed multi-focal areas of mild to moderate, subacute pneumonia, characterized by intra-alveolar and intra-bronchiolar infiltrates of neutrophils, edema, and foreign particulate matter including hair and plant material. These findings are consistent with aspiration pneumonia, which was ultimately determined to be the cause of this animal's demise.

Other histologic findings in this animal possibly related to treatment with SCH 54031 included Grade 2 hypocellularity and congestion in the femoral bone marrow, prominent, although mild in severity amyloidosis in the germinal centers of the mesenteric lymph nodes, and multi-focal areas of intra-trabecular hemorrhage and mild lymphoid hyperplasia in the spleen (both findings were Grade 2 in severity). Decreased cytoplasmic vacuolization, accompanied by diffuse hyperplasia in the zona fasciculata of the adrenal glands were also present in this animal, and are consistent with response of the host animal to severe stress. Other histologic and gross pathologic findings were considered consistent with the moribund condition of the animal and necropsy, and incidental to treatment with SCH 54031 PEG-IFN and ribavirin.

Although no bacterial colonies were reported on histologic evaluation of samples from this animal, it is presumed that the changes are associated with a subclinical infection, as evidenced by the shift in neutrophil morphology to a less mature phenotype, in the presence of a neutropenia. Samples of pericardial fluid and fluid from the right side of the thoracic cavity were obtained at necropsy for bacteriologic culture, which revealed the presence of *Streptococcus bovis* and *Shingobacterium multivorum* in the two samples, respectively. These findings appear to be consistent with opportunistic infection secondary to the development of aspiration pneumonia in this animal.

All remaining animals on study survived until scheduled sacrifice. There were no remarkable effects of either SCH54031 PEG-IFN or SCH18908 ribavirin treatment on ophthalmologic findings, electrocardiograms, or other physiologic parameters including heart and respiration rates, blood pressure, or body temperature, or urinalysis profiles as compared to either baseline values or to the vehicle control group.

There were no adverse clinical observations, and no remarkable changes in body weight, food consumption, or physiologic parameters in monkeys in the vehicle control group over the course of the study. Mean values for body weights for this group were not significantly increased over baseline at study week 4 although monkeys #1M and #3M both gained 0.1 kg over the study duration, while female monkey #5F lost 0.1 kg in this same time period. All monkeys in the vehicle control group demonstrated progressive, 10 to 30% decreases in hemoglobin, hematocrit, and red cell counts from weeks -7 and -2 baseline through week 4 on study. Concomitant increases in MCV, MCH, MCHC, and reticulocyte counts were also observed in the control group over the duration of the study. These findings are consistent with an appropriate marrow response to iatrogenic anemia, induced by the multiple blood draws required in the study design.

There were no mortalities or adverse clinical signs noted, and no remarkable changes in body weight, food consumption, body weight gains, or physiologic parameters (mean body temperatures, heart and respiratory rates, or blood pressure) in monkeys treated with 75 mg/kg/d ribavirin alone, when compared to either baseline values for each individual animals, or to the

mean values for the vehicle control group. Hematologic profiles demonstrated 10 to 30% decreases in erythrocyte parameters, including red cell counts, hemoglobin, and hematocrit, with concomitant increases in MCV, MCH, and reticulocyte counts at week 4 on study, as compared to both baseline values for the group, as well as to mean values for animals in the vehicle control group (see Table VII, below). Total leukocyte, neutrophil, monocyte, lymphocyte, and eosinophil counts, as well as platelet counts, PT and APTT were not significantly affected by daily treatment with ribavirin alone after 4 weeks on study, as compared to either vehicle control or baseline values for this group (please see Tables VIII and IX, below). Microscopically, evidence of bone marrow hypercellularity was observed in monkey #20M in this group, and was minimal (Grade 1) in severity. This animal also had evidence of poikilocytosis and anisocytosis on evaluation of bone marrow smears and peripheral blood differentials. These findings are consistent with the known, hematologic toxicity (hemolytic anemia) associated with ribavirin treatment, and have been reported previously both in test animal species and in humans.

No remarkable clinical signs or toxicities were noted in monkeys treated with either 350 or 5500 $\mu\text{g}/\text{m}^2$ SCH54031 alone, until week 3 on study. At this time point, one male monkey (animal #14M) in the group treated with 5500 $\mu\text{g}/\text{m}^2$ PEG-IFN had two incidences of soft feces, and one incidence of loose stool. These changes resolved spontaneously, and were no longer observed during week 4 of treatment. One male monkey (animal #7M) had a laceration at the tip of the tail 2 weeks prior to beginning treatment with 350 $\mu\text{g}/\text{m}^2$ SCH54031 PEG-IFN, which was determined to be a traumatic injury and unrelated to the study treatment.

Mean body weights for both male and female monkeys treated with either dose of PEG-IFN alone were decreased by approximately 0.1 kg from baseline at study week 1, but were not significantly different either from baseline values, or for the mean values obtained for animals treated with vehicle control. By weeks 2 and 3 on study, additional, 0.1 to 0.2 kg decreases in mean body weights were noted for these groups. Maximal weight loss was observed at study week 3 in male monkeys treated with 350 $\mu\text{g}/\text{m}^2$ PEG-IFN, and in both male and female animals treated with 5500 $\mu\text{g}/\text{m}^2$ PEG-IFN (mean loss of 0.2 kg from baseline in all three groups). Mean values for body weights in female monkeys treated with the 350 $\mu\text{g}/\text{m}^2$ dose of PEG-IFN did not further decrease over the duration of the study from the initial loss at week 1. No one animal showed consistently greater weight loss over the study duration than the others in these two groups. Food consumption was qualitatively lower than both baseline and vehicle control group at weeks 1 and 2 on study (decreased to approximately 51-75% of daily ration consumed), but had returned to 100% consumption by study week 3. At study week 4, all animals had begun to regain body weight, so that the final values for mean body weights were decreased by only 0.1 kg from baseline in monkeys of both sexes treated with either dose of SCH54031. The final mean body weight groups for monkeys treated with either dose of PEG-IFN alone were not statistically significantly different from either baseline, or the vehicle control group at study end.

By contrast, monkeys treated with either 350, 1400, or 5500 $\mu\text{g}/\text{m}^2$ PEG-IFN and 75 mg/kg/d ribavirin demonstrated gastrointestinal changes, including scant or no feces, loose stool and/or diarrhea, and dehydration and coolness to the touch beginning week 2 on study, and continuing through week 4. There was no apparent dose-relationship in either the incidence or number of animals affected to the dose of PEG-IFN administered. One female monkey (animal #30F, 350 $\mu\text{g}/\text{m}^2$ /dose SCH54031 plus 75 mg/kg/d ribavirin) remained slightly dehydrated during study weeks 4 and 5, while all other animals with the exception of male monkey #26M (sacrificed moribund, above) recovered by week 4. Mean body weight values for animals in these three groups were decreased by 0.1 to 0.2 kg from baseline over the duration of the study in all groups

of female monkeys treated with the combination of PEG-IFN and ribavirin, regardless of dose, and in the male monkeys treated with either 350 or 1400 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin. By contrast, mean body weight values in male monkeys treated with the combination of ribavirin and 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN were decreased approximately 0.4 kg from baseline, and 0.5 kg from their week one value by week 3 on study, and remained depressed at study week 4. Individual animals in this group lost between 11 and 20% of their initial body weight by study week 3, and did not recover it by study termination.

Food consumption was decreased to 51-75% of the daily ration beginning study week 1 in 2/3 male and 1/3 female monkeys in all three groups treated with the combination of PEG-IFN and ribavirin, and decreased to 26-50% of food consumed in one female (animal #40F) monkey treated with 5500 $\mu\text{g}/\text{m}^2$ PEG-IFN and 75 mg/kg/d ribavirin. By study week 2, food consumption was normal in all female monkeys in the groups receiving either 350 or 1400 $\mu\text{g}/\text{m}^2/\text{dose}$ SCH 54031 PEG-IFN and ribavirin, and remained at 100% of daily ration for all female monkeys in these two groups for the remainder of the study. Food consumption had decreased by 25 to 49% in 2/3 female monkeys treated with the high dose of PEG-IFN and 75 mg/kg/d ribavirin at study week 2; however, food intake returned to 100% of daily ration for all three females in this group by study week 3, and remained normal for the duration of the study.

By contrast, food intake in 2/3 male monkeys in each group treated with either 350 or 1400 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin was decreased to 26-50% of daily ration at study week 2, and remained decreased from normal (51-75% of ration consumed) in 1/3 male monkeys (animal #31M) in the mid-dose group at study week 3. All three male monkeys treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and 75 mg/kg/d ribavirin had severely decreased food intake at study week 2; monkey #37 M had consumed less than 25% of the daily ration during this time period, while monkeys #38M and #39M in this same group consumed between 26-50% of their daily food supply in this same time. By week 3, all three animals were consuming between 51 and 75% of their daily ration, and by week 4 had recovered fully to normal food intake.

Hematologic profiles showed evidence of anemia, decreased total leukocyte counts, and decreased platelets in all groups of monkeys treated with either ribavirin or SCH 54031 PEG-IFN, alone, or in combination, beginning at the week 1 time point. Dose-related, 25 to 52% decreases in mean red cell counts, hematocrit, and hemoglobin concentration were observed in both male and female monkeys treated with all dose levels of PEG-IFN and 75 mg/kg/d ribavirin, or with 75 mg/kg/d ribavirin alone, as compared to either baseline values or to the vehicle control group. These changes were less severe in the animals treated with SCH 54031 PEG-IFN alone, but were still decreased approximately 12 to 25% from baseline at terminal sacrifice. The decreases in erythrocyte parameters were more severe in animals treated with 1400 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and 75 mg/kg/d ribavirin dose level than in either the low- or high-dose SCH54031 PEG-IFN and ribavirin combination groups.

At the 4 week study time point, peripheral blood reticulocyte counts were elevated 1.25 to 5-fold over baseline values in either group of monkeys treated with PEG-IFN alone, all groups of monkeys treated with either 350 or 1400 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN in combination with ribavirin, and in both male and female treated with ribavirin alone. Female monkeys treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and 75 mg/kg/d ribavirin demonstrated 2.2 to 6-fold increases in reticulocyte counts from baseline at the 4 week time point as well. By contrast, male monkeys treated with this same dose of SCH54031 and ribavirin failed to increase reticulocytes in response to the ribavirin-induced hemolysis, and actually demonstrated a >50% decrease in

reticulocyte counts from baseline at study week 4, suggesting that the marrow suppressive effects of the pegylated interferon- α are dose-related, with a possible threshold effect on the ability of the marrow to recover.

At all time points on study, mean platelet counts were not significantly different from baseline, nor different from the vehicle control group in all groups of monkeys treated with PEG-IFN, with or without ribavirin. Although slight increases in PT and APTT were noted in all dose groups at study week 4 as compared to baseline values for each group, there were no statistically significant differences from either vehicle control or baseline in any dose group, and the findings are not considered to be biologically meaningful.

Total leukocyte counts were initially decreased by greater than 50% as compared to the vehicle control in all groups of interferon-treated monkeys, with or without ribavirin after two weeks on study ($p \leq 0.05$, Student's *t* test), with an apparent dose-relationship in either the both the incidence and severity of the decreases to the dose of PEG-IFN. At the two week time point, total leukocyte counts in the groups treated with 350 or 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin were also significantly decreased from their respective baseline values ($p \leq 0.05$, ANOVA). Recovery of total leukocyte counts was observed in all groups of animals at the week 4 time point and at the end of the recovery period; although not all mean values had reached baseline by that time, there were no statistically significant differences between the groups. Of interest, there was an increase in the mean white cell counts above baseline values in both of the PEG-IFN alone dose groups at 4 weeks on study, suggesting an apparent rebound effect from the initial decrease at week 2. This increase was not observed to as great an extent in all groups receiving ribavirin, regardless of whether they received PEG-IFN or not. These data are presented in Table VII, below.

Table VII – Total Peripheral Blood Leukocyte Counts Following PEG-IFN \pm Ribavirin

Dose of PEG-IFN	Mean Total Leukocyte Counts ($\times 1000/\text{mm}^3$) \pm S.D.			
	Week -7 Baseline	Week -2 Baseline	Week 2	Week 4
Vehicle	10.1 \pm 1.6	13.5 \pm 1.3	12.9 \pm 4.3	10.8 \pm 1.8
350 $\mu\text{g}/\text{m}^2$ PEG-IFN alone	9.3 \pm 2.3	15.5 \pm 3.6	8.0 \pm 1.9 ^a	14.1 \pm 3.5
5500 $\mu\text{g}/\text{m}^2$ PEG-IFN alone	6.7 \pm 2.4	12.1 \pm 2.9	5.1 \pm 1.5 ^a	14.3 \pm 4.3
75 mg/kg/d ribavirin alone	8.7 \pm 2.7	13.1 \pm 3.3	11.1 \pm 2.2	9.7 \pm 2.2
350 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin	7.9 \pm 2.1	10.5 \pm 2.4	5.6 \pm 1.0 ^{a,b}	9.8 \pm 4.8
1400 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin	8.5 \pm 1.9	14.1 \pm 3.0	6.7 \pm 1.9 ^a	11.5 \pm 2.9 ^{a,b}
5500 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin	9.4 \pm 3.5	12.6 \pm 6.3	5.5 \pm 2.4 ^{a,b}	9.8 \pm 2.8

^a significantly different from vehicle control ($p \leq 0.05$, Student's *t* test)

^b significantly different from baseline ($p \leq 0.05$, ANOVA)

Similar 20 to >65% decreases in both absolute neutrophil counts and lymphocyte counts were observed in PEG-IFN-treated monkeys, with or without ribavirin treatment at the week 2 time point, as compared to either vehicle control or to baseline values. Once again, there was no apparent relationship to either the severity or the incidence of the changes to the dose of SCH

54031 PEG-IFN administered to the monkeys. However, the decreases in absolute neutrophil counts were more severe in animals receiving any dose of PEG-IFN with concomitant ribavirin than those seen in the corresponding doses of PEG-IFN alone. Recovery of both neutrophil and total lymphocyte counts to approximately baseline values was observed in all groups of animals treated with PEG-IFN either alone or in combination with ribavirin by the end of week 4 on study. These data are presented in Tables VIII and IX, below.

Table VIII – Peripheral Blood Neutrophil Counts Following PEG-IFN ± Ribavirin Treatment

Dose of PEG-IFN	Mean Absolute Neutrophil Counts (x 1000/mm ³) ± S.D.			
	Week -7 Baseline	Week -2 Baseline	Week 2	Week 4
Vehicle	4.3 ± 1.3	4.2 ± 1.7	6.3 ± 2.9	4.9 ± 2.0
350 µg/m ² PEG-IFN alone	4.8 ± 2.7	6.6 ± 3.2	2.3 ± 1.2 ^a	6.6 ± 3.1
5500 µg/m ² PEG-IFN alone	3.0 ± 2.1	4.6 ± 2.0	1.9 ± 1.3 ^a	7.0 ± 4.2
75 mg/kg/d ribavirin alone	3.6 ± 1.1	5.9 ± 3.3	4.7 ± 1.2	4.1 ± 1.2
350 µg/m ² PEG-IFN + ribavirin	2.9 ± 1.5	3.4 ± 1.5	1.6 ± 0.6 ^a	3.4 ± 2.7
1400 µg/m ² PEG-IFN + ribavirin	3.2 ± 2.0	5.3 ± 2.7	1.6 ± 0.8 ^a	4.2 ± 1.6
5500 µg/m ² PEG-IFN + ribavirin	4.6 ± 2.9	6.0 ± 4.9	1.5 ± 0.9 ^{a,b}	4.0 ± 2.1

^a significantly different from vehicle control ($p \leq 0.01$, Student's *t* test)

^b significantly different from baseline ($p \leq 0.05$, ANOVA)

Table IX – Peripheral Blood Lymphocyte Counts Following PEG-IFN ± Ribavirin Treatment

Dose of PEG-IFN	Mean Absolute Lymphocyte Counts (x 1000/mm ³) ± S.D.			
	Week -7 Baseline	Week -2 Baseline	Week 2	Week 4
Vehicle	5.4 ± 1.9	7.6 ± 0.6	5.0 ± 1.6	4.7 ± 0.8
350 µg/m ² PEG-IFN alone	4.3 ± 1.0	7.2 ± 1.7	4.6 ± 0.9	6.2 ± 1.3 ^a
5500 µg/m ² PEG-IFN alone	3.5 ± 0.7	6.6 ± 1.6	2.3 ± 1.0 ^{a,b}	5.9 ± 2.1
75 mg/kg/d ribavirin alone	4.7 ± 2.0	6.1 ± 1.7	5.4 ± 1.8	4.7 ± 1.6
350 µg/m ² PEG-IFN + ribavirin	4.6 ± 0.8	5.9 ± 0.7 ^a	3.3 ± 0.5 ^{a,b}	5.5 ± 2.1
1400 µg/m ² PEG-IFN + ribavirin	5.1 ± 1.1	7.3 ± 2.6	4.1 ± 2.1	5.9 ± 1.8
5500 µg/m ² PEG-IFN + ribavirin	4.6 ± 1.9	5.7 ± 1.8	3.1 ± 1.5	4.6 ± 0.9

^a significantly different from vehicle control ($p \leq 0.05$, Student's *t* test)

^b significantly different from baseline ($p \leq 0.05$, ANOVA)

There were no remarkable changes in either absolute or differential eosinophil, basophil, or immature (band) neutrophil counts in the surviving animals treated PEG-IFN with or without ribavirin, or in the group treated with ribavirin alone, as compared to either the vehicle control group or to baseline values. Beginning at week -2 of baseline and continuing through week 4, monocyte counts were elevated by approximately 1.3 to 6.4-fold as compared to week -7 baseline in all groups of monkeys, including the vehicle control. By week 4, monocyte counts remained elevated 3.6 to 6.6-fold in male monkeys and 4.7 to 6-fold in female monkeys treated with either 350 or 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ of PEG-IFN alone, respectively. At study week 4, monocyte counts in the group of male monkeys treated with all three dose levels of PEG-IFN and 75 mg/kg/d ribavirin remained elevated 1.3 to 4-fold over baseline (week -7), but were reduced approximately 2-fold from the values obtained at week -2 baseline and week 2 on study. These effects were not observed in the female monkeys in these three dose groups.

At necropsy, there were no adverse gross pathologic findings related to treatment in the vehicle control, or ribavirin only treated groups, with the exception of discoloration at the injection site (redness, bruising) in animals in the control group. This effect was observed in monkeys in all of the PEG-IFN treatment groups as well, without an apparent dose-relationship in either incidence or severity. Small thymus glands were observed on gross evaluation in female monkey #5F in the vehicle control group, and in animals #11F and #46F in the group treated with 350 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN alone, and monkeys #13M, #17F, and #18F in the group treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ SCH 54031 PEG-IFN alone. Small thymus glands were also observed macroscopically in 2/3 female monkeys (animals #22F and #23F) in the 75 mg/kg/d ribavirin dose groups, in animals #28F and #30F and #26M (sacrificed moribund on study d 25) in the group receiving 350 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and 75 mg/kg/d ribavirin, in 2/3 male (animals #31M and #33M) and #36F in the mid-dose PEG-IFN and ribavirin group, and in all 6 monkeys treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin. Histologically, these effects were correlated with mild to moderate thymic atrophy, which is a known effect of interferon- α treatment. Monkeys #26M and #30F in the 350 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin group, animal #34F in the mid-dose group, and monkeys #37M and #40F in the group treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and 75 mg/kg/d had thin carcasses on gross evaluation, as compared to either the vehicle or ribavirin only control animals. These findings were likely related to inappetence induced by the PEG-IFN in combination with ribavirin. Histologically, atrophy of the epicardial and abdominal fat stores, as well as atrophy of the serous fat in the bone marrow were present in these animals.

Other, incidental findings in the animals surviving to terminal sacrifice included focal areas of discoloration, nodules or cysts in the liver of several animals, small testes in one male monkey (#19M) in the ribavirin control group (suggesting a sexually immature animal), and adhesions and areas of discoloration in the lungs from one vehicle control female monkey (#5F), one female monkey in the low dose PEG-IFN alone group (animal #46F), one male monkey #14M treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN alone, one male monkey (#26M, sacrificed moribund) and one female monkey (#28F) in the group treated with 350 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin. Additionally, adhesions of the left, caudal lobe of the lung to the chest wall were present in monkeys #31M and #34F in the mid-dose PEG-IFN and ribavirin group. Focal discoloration and reddish areas of the stomach mucosa in either the fundus or the pylorus were reported for animals #3M and #26M in the vehicle control and 350 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN and ribavirin treated groups, respectively.

Histologic findings related to treatment were limited to the injection site, and included subcutaneous hemorrhage, inflammation, perivasculitis and endarteritis, and fibrosis. These

findings occurred with approximately equal incidence and severity in all of the treatment groups, including the controls. The five monkeys showing macroscopic changes in the lungs had findings on histologic evaluation that correlated with the gross pathology, including focal areas of mild, pleural fibrosis, inflammatory infiltrates, and chronic interstitial pneumonia. Other microscopic findings included minimal to mild decreases in cellularity in the bone marrow in animals of both sexes in all PEG-IFN treatment groups at terminal sacrifice, mononuclear cell infiltrates, mineral deposition, and tubular pigmentation in the kidneys, mononuclear cell infiltrates in the lacrimal glands, increased pigment and lymphoid hyperplasia in the mandibular lymph nodes, and focal areas of hepatocellular vacuolization, necrosis, and mononuclear cell infiltrates in the liver. These findings occurred at approximately equal incidence and severity across all treatment groups, and were considered by the reviewing pathologist to be incidental to treatment with the test articles.

The toxicokinetic profiles of both PEG-IFN and ribavirin were evaluated as part of this repeat-dose toxicity study. Samples of peripheral blood for determination of PEG-IFN levels were obtained from all monkeys at baseline (2 weeks prior to dosing), and at 8 h after the first dose of PEG-IFN or ribavirin. Peripheral blood samples for cumulative exposure were also obtained at 8 h post-dosing on study days 15 and 29, and at terminal sacrifice on d 30 or d 31.

Serum interferon levels were measured both by an electrochemiluminescence (ECL) assay, with a lower limit of quantitation of $1 \mu\text{g/ml}$. This assay involved the formation of an immune complex between the PEG-IFN in the sample, and α antibody. The ECL signal produced is proportional to the concentration of both SCH 54031 and any free interferon- α present in the sample, and concentrations in the serum samples were determined against a standard curve of SCH 54031 PEG-IFN. Ribavirin levels were determined from plasma samples, using a validated liquid chromatography/mass spectrophotometer with ^{13}C -ribavirin as the internal standard. The lower limit of detection of this assay is 1 ng/ml ribavirin. The results from samples obtained 8 h after dosing at each time point on study, as determined by each assay are presented in Tables X and XI below:

Table X – Toxicokinetic Evaluation of PEG-IFN in Cynomolgus Monkeys

ECL Assay for IFN levels

Week on Study	Mean Serum IFN Value (ng/ml) \pm % CV				
	PEG-IFN Dose Level, +/- 75 mg/kg/d Ribavirin				
	350 $\mu\text{g/m}^2$ PEG-IFN alone	5500 $\mu\text{g/m}^2$ PEG-IFN alone	350 $\mu\text{g/m}^2$ PEG-IFN + ribavirin	1400 $\mu\text{g/m}^2$ PEG-IFN + ribavirin	5500 $\mu\text{g/m}^2$ PEG-IFN + ribavirin
-2	0 + NC ^{a, b}	0 + NC	0 + NC	0 + NC	0.01 \pm 245
1	36.7 \pm 37	992 \pm 23	37.8 \pm 19	189 \pm 21	1010 \pm 40
3	3.2 \pm 69	233 \pm 69	21.9 \pm 91	92.2 \pm 74	891 \pm 66
5	0 + NC	0 + NC	0.12 \pm 224 ^c	0 + NC	0.01 \pm 245

^aNC = not calculable

^bn = 6 animals in all groups except where specified

^cn = 5 animals

Table XI-- Toxicokinetic Evaluation of Ribavirin in Cynomolgus Monkeys**LC/MS Assay for Ribavirin Levels**

Week on Study	Mean Plasma Ribavirin Value (ng/ml) ± % CV			
	PEG-IFN Dose Level, +/- 75 mg/kg/d Ribavirin			
	75 mg/kg/d ribavirin alone	350 µg/m ² PEG-IFN + ribavirin	1400 µg/m ² PEG-IFN + ribavirin	5500 µg/m ² PEG-IFN + ribavirin
-2	0 ± NC ^{a, b}	0 ± NC	0 ± NC	0 ± NC
1	623 ± 44	651 ± 17	549 ± 19	642 ± 17
3	1960 ± 43	2120 ± 18	2010 ± 15	1920 ± 33
5	2180 ± 25	2380 ± 20 ^c	2580 ± 17	2580 ± 23

^aNC = not calculable^bn = 6 animals in all groups except where specified^cn = 5 animals

No serum samples were analyzed for interferon activity by the ECL assay from either the vehicle control (SCH 54031 placebo) or ribavirin alone-treated monkeys at any time point on study. Similarly, no plasma samples from these two groups were analyzed for ribavirin levels. The decreases in PEG-IFN serum levels seen in all groups at week 5 on study are suggestive of antibody generation against the biologic; this effect was confirmed both by the BIACORE assay for anti-PEG-IFN antibodies, as well as by the loss of pharmacodynamic and toxic effects associated with PEG-IFN at week 5 on study. Because only a single time point after test article administration was sampled for PEG-IFN and ribavirin levels, it is not possible to calculate standard pharmacokinetic parameters (*i.e.* AUC, C_{max}, and T_{1/2elim}). However, in all groups treated with ribavirin, the mean plasma values obtained at weeks 3 and 5 on study were increased by approximately 3 to 4-fold, as compared to the values obtained at week 1. These data suggest that bioaccumulation of ribavirin is occurring, either by saturation of the clearance mechanism for the drug, or by a possible synergistic effect with PEG-IFN.

Antibody activity (both total Ig and neutralizing antibody) was determined using a biosensor assay, which tests the binding of serum samples to immobilized PEG-IFN or unconjugated interferon-α. Samples positive for total anti-IFN antibody activity were further analyzed for neutralizing activity of the anti-viral effects of IFN-α by the CPE bioassay, respectively. The results of the BIACORE assay are presented in Table XII, below.

Table XII – Anti-Interferon Total Antibody Activity in PEG-IFN Treated Cynomolgus Monkeys

BIACORE Assay	Relative Serum Anti-PEG-IFN Ab + % CV				
	PEG-IFN Dose Level, +/- 75 mg/kg/d Ribavirin				
Week on Study	350 $\mu\text{g}/\text{m}^2$ PEG-IFN alone	5500 $\mu\text{g}/\text{m}^2$ PEG-IFN alone	350 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin	1400 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin	5500 $\mu\text{g}/\text{m}^2$ PEG-IFN + ribavirin
-2	negative	negative	negative	negative	negative
5	86 + 49 ^a	340 + 57	50 + 78 ^b	70 + 74	117 + 82
Bioassay Results					
-2	negative	Negative	Negative	negative	negative
5	507 + 42	640 + 0	320 + 71 ^c	244 + 102 ^d	328 + 88 ^d

^an = 6 animals in all groups except where specified

^bn = 5 animals

^cn = 4 animals

^done animal in this group had a negative titer for anti-IFN antibody at the week 5 timepoint

The ability of isolated neutrophils from SCH54031 PEG-IFN or ribavirin-treated monkeys to phagocytize foreign particles and kill bacteria, and to migrate in response to a chemotactic stimulus was assessed using *ex vivo* assays of phagocyte function. Neutrophils were isolated from EDTA-anti-coagulated blood by centrifugation over discontinuous Percoll gradients after sedimentation through 4% dextran in saline solution. The resulting pellet was washed twice in sterile PBS, and held prior to assay in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum, 200 mM L-glutamine, and 1% penicillin-streptomycin solution. Neutrophils obtained by this method were > 95 % viable by trypan blue dye exclusion, and were $\geq 95\%$ pure, with eosinophils as the major cell contaminant.

Comment: The final report does not specify at what time during treatment these samples were obtained and assayed for their phagocytic capacity, although the protocol states that samples will be collected pre-test, and during weeks 2 and 3 on study.

Phagocytic activity of isolated neutrophils was assayed using monkey serum opsonized, heat-killed *Saccharomyces cerevisiae*. Cells were incubated in a shaking water bath at 37°C for one hour with opsonized yeast, and stained following centrifugation with combination trypan blue/eosin Y dyes. This method allows microscopic determination of uningested, viable yeast particle (unstained) as opposed to phagocytosed, dead yeast (stained purple). Thirty phagocytes per sample were counted for the number of ingested yeast particles, and the percentage of phagocytes was determined in a total of 200 cells in each preparation. The phagocytic index was determined by calculating the mean number of yeast particles per cell, times the percent phagocytes per sample.

Neutrophils from vehicle control-treated monkeys were capable of phagocytosing a mean of 3.1 ± 0.1 yeast particles per cell (n = 2). This value was decreased, although not statistically so, in animals treated with either 5500 $\mu\text{g}/\text{m}^2$ /dose PEG-IFN or 75 mg/kg/d ribavirin (2.3 ± 0.4 and 2.8 ± 0.1 particles/cell, respectively). Phagocytic indices in these two groups were also depressed

relative to neutrophils isolated from control monkeys; values were 264 ± 25 , 177 ± 44 , and 188 ± 28 for the vehicle control, PEG-IFN, and ribavirin treated groups, respectively ($n = 2/\text{group}$).

Comment: This method is a very crude estimation of phagocytic capability, and is highly dependent on how the cells are treated prior to conducting the assay. It has been this reviewer's experience that the inclusion of fetal calf serum in the test medium can actually interfere with uptake of opsonized yeast particles, and the assay provides only qualitative results at best, since microscopic evaluation is subject to investigator error and bias. A simpler, more quantitative method to determine phagocytic activity is to use both opsonized and non-opsonized, ^{51}Cr -labeled sheep red blood cells. This method would also permit analysis of samples from all 36 animals on study, rather than two per group, and allow replicate samples to be performed, permitting statistical evaluation of the results.

Phagocytosis and intra-cellular killing of opsonized *Staphylococcus aureus* were also evaluated on peripheral blood neutrophil samples from these three dose groups. Isolated neutrophils were incubated in a shaking water bath at 37°C for 2 hours with approximately 20,000 bacteria resuspended in a 1:10 dilution of guinea pig complement and normal monkey serum (not heat-inactivated). Following incubation, the cells were recovered by centrifugation, stained with acridine orange, washed, and evaluated by fluorescence microscopy for phagocytosis and killing of the opsonized bacteria. Using this method, live bacteria stain green and dead bacteria stain red under fluorescent illumination. Two separate fields of 100 cells per field were evaluated for each monkey, and samples were evaluated in triplicate. Neutrophils were scored as positive for bacterial phagocytosis and killing if they contained at least 5 red bacteria per cell. The percentage of phagocytic cells was then calculated for each animal, and the mean, \pm standard error for each group was calculated.

The mean percentage of phagocytic neutrophils from vehicle control-treated animals was $87.1 \pm 5.1\%$, while phagocytosis was decreased to 58.7 ± 8.5 and $79.6 \pm 2.8\%$ of the cells from animals treated with PEG-IFN or ribavirin alone, respectively. There was no significant difference in the percent of neutrophils capable of phagocytosing and killing opsonized *Staphylococcus aureus* between the vehicle control and PEG-IFN or ribavirin-treated groups. However, a trend towards decreased function in PEG-IFN treated animals was observed when compared to the value for the vehicle control group ($p \leq 0.07$, Student's paired t test)

The chemotactic ability of neutrophils isolated from PEG-IFN or ribavirin treated monkeys was evaluated using the modified Boyden chamber technique. In this assay, vehicle control or a stimulus is placed in the lower well of the chamber, and a nitrocellulose filter containing pores of a specific size is set over the top of the fluid. The tested stimuli included buffer control, 1% zymosan-activated, monkey serum (ZAMS; a source of the complement fragment C5a), 10 nM N-formyl-methionyl-leucine-phenylalanine (fMLP), and 5 ng/ml leukotriene B4 (LTB4). Isolated neutrophils are then added to the top portion of the chamber, which is then incubated at 37°C . Following incubation, the filters are removed, non-migrated cells on the top of the filter are removed by gentle scraping, the filters are fixed in 100% methanol, stained with Wright's-Giemsa stain, and evaluated microscopically under oil immersion for the number of migrating cells per well. Typically, cells are counted in 10 high-power (1000 X magnification) fields per well, and the farthest distance the cells have migrated into the filter (in microns) is determined for each group.

Comment: There was no protocol provided for the conduct of this assay, so no information is provided about the composition of the buffer, the duration of the incubation, or the methodology used to evaluate cell migration. However, this reviewer has extensive experience conducting these assays, and the methods described above are what is typically employed by the field.

Comment: The nitrocellulose filters allow the determination of how far cells have migrated into the filter in response to the chemotactic stimulus. Using this method, a "leading front," or distance from the top surface of the filter is calculated, using a special optic that allows the evaluator to score the distance each cell has migrated into the filter in microns. While effective, this method is very tedious and requires floating the chemotactic filters in 100% xylene during the evaluation, to clear the nitrocellulose. A simpler method, using polycarbonate filters with a 5 micron pore size through which neutrophils can easily migrate has been in use for at least 20 years, and provides a quantitative index of neutrophil (or other leukocyte) migration in response to a chemotactic stimulus.

Neutrophils were isolated from a total of 18 monkeys, in the vehicle control (n=4), 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN alone (n=4), 75 mg/kg/d ribavirin alone (n=4), and 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN plus ribavirin groups (n=6), both prior to initiating treatment, and after 2 weeks on study. Baseline (prior to treatment) chemotactic responses to ZAMS, fMLP, and LTB₄ were similar in all of the test groups, and the mean chemotactic indices (distance migrated in response to stimulus, minus distance migrated in response to buffer control, divided by distance migrated in the buffer control group) ranged from 0.54 to 1.7 regardless of chemotactic stimulus or *in vivo* test treatment. Following treatment with test article for two weeks, chemotactic indices to all stimuli were increased 2 to 3-fold over baseline values for neutrophils isolated from animals in the vehicle control group. An approximate 50% increase in chemotactic index as compared to baseline was observed in samples from monkeys treated with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN. However, the values obtained for this group were approximately 50% lower than those obtained for the same chemotactic stimuli in the vehicle control animals. Neutrophils from animals treated with 75 mg/kg/d ribavirin alone also displayed a 30 to >80% increase in chemotactic activity to all three stimuli at two weeks as compared to baseline, with an approximate 30% decrease in activity as compared to the vehicle control group at this same time point. Although a similar, 2 to 3-fold increase in chemotaxis to ZAMS, fMLP, and LTB₄ was observed in neutrophils from PEG-IFN and ribavirin treated animals at 2 weeks on study as compared to their baseline control, there was no remarkable difference in the chemotactic response of these cells as compared to the vehicle control.

Taken together, these data suggest that treatment of cynomolgus monkeys with PEG-IFN can result in an inhibition of neutrophil function as compared to the vehicle control group, although statistical significance could not be determined due to the small sample numbers.

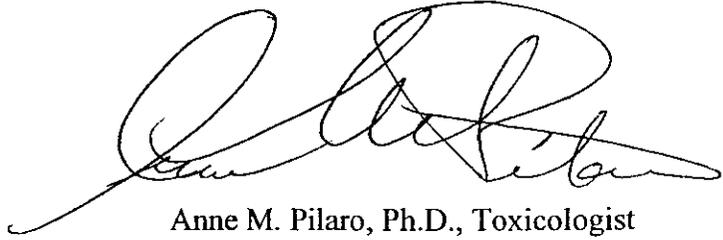
In summary, treatment of both male and female cynomolgus monkeys every other day by s/c injection with PEG-IFN at doses of 350, 1400, or 5500 $\mu\text{g}/\text{m}^2/\text{dose}$, either alone or in combination with 75 mg/kg/d ribavirin, p/o for one month was associated with inappetence in the absence of significant weight loss, dose-related anemia, leukopenia, lymphopenia, and mild to moderate neutropenia. One male monkey (#26M) did not survive until study termination, due to an aspiration pneumonia; however, there were no other mortalities observed in this study, as compared to Study #97060 (above). Pathologic findings related to PEG-IFN treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site, atrophy of the thymus, and minimal to mild changes in bone marrow cellularity.

Toxicokinetic evaluations of serum PEG-IFN and ribavirin levels confirmed that exposure was continuous throughout the duration of the study; however, after 4 weeks of treatment, detection of serum IFN levels was abrogated by the development of anti-PEG-IFN and anti-interferon- α antibody titers. Neutrophil phagocytosis, bacterial cell killing, and chemotactic response to complement fragment C5a, fMLP, and LTB₄ were depressed, although not significantly, in monkeys treated for 2 weeks with 5500 $\mu\text{g}/\text{m}^2/\text{dose}$ PEG-IFN as compared to the vehicle control group. Although no NOAEL for PEG-IFN alone or in combination with ribavirin could be determined for this study, the doses of PEG-IFN used in this study are approximately 20 to 300-fold greater than the dose of 1.5 $\mu\text{g}/\text{kg}/\text{week}$ used in the phase 3 clinical trial. The dose of ribavirin used in the present study is approximately 8-fold higher than the clinical dose of 800 mg/d given in combination with the high-dose of PEG-IFN in the phase 3 clinical trial.

SUMMARY AND CONCLUSION:

In summary, PEG-IFN treatment of cynomolgus monkeys for one month either alone or in combination with 50 or 75 mg/kg/d ribavirin leads to transient inappetence in the absence of significant weight loss, dose-related anemia, mild to moderate leukopenia, lymphopenia, and decreases in platelets, moderate to severe neutropenia, and transient decreases in total serum protein, albumin, globulin, and A:G ratios. These findings are consistent with the known toxicities of interferon- α , and were reversible by the end of a 4 week, treatment-free recovery period. Pathologic findings related to PEG-IFN treatment included macroscopic and microscopic evidence of hemorrhage, inflammation, and fibrosis at the injection site, atrophy of the thymus, and minimal to mild changes in bone marrow cellularity. These findings were transient in nature, and had fully resolved by the end of the 4 week recovery period. Toxicokinetic evaluations of serum PEG-IFN and ribavirin levels confirmed that exposure was continuous throughout the duration of the study; however, after 4 weeks of treatment, detection of serum IFN levels was abrogated by the development of anti-PEG-IFN and anti-interferon- α antibody titers. Reversal of toxicity was associated with development of neutralizing antibodies in all of the groups treated with PEG-IFN.

In conclusion, treatment of cynomolgus monkeys for one month with PEG-IFN alone or in combination with ribavirin was associated with transient, reversible toxicities consistent with those of other type I interferons. Hemolytic anemia associated with ribavirin treatment was also observed, and is a known toxicity of ribavirin in humans as well. Although no NOAEL for PEG-IFN alone or in combination with ribavirin could be determined for either of these two studies, the doses of PEG-IFN used are approximately 20 to 300-fold greater than the dose of 1.5 $\mu\text{g}/\text{kg}/\text{week}$ used in the phase 3 clinical trial. The two doses of ribavirin used in the animal studies are approximately 6 and 8-fold higher than the clinical dose of 800 mg/d given in combination with the high-dose of PEG-IFN in the phase 3 clinical trial. Taken together, these data support the safety and toxicity profiles of PEG-IFN in combination with ribavirin for licensure as treatment for chronic hepatitis C infection.



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

concurrences: *Merck Serenby for MD6*
OTRR/C, CP-T/MGreen

cc:
OTRR/C, CP-T/MGreen
OTRR/C, IID/WSchwieterman