

lymphomas of thymic origin in positive control (MNU-treated) mice validates the use of the p53(+/-) model for oncogenicity evaluation.

- **Evaluation of tumor findings:** Ribavirin did not induce neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks.

Carcinogenicity summary: Ribavirin did not result in neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks. The predicted early development of malignant lymphomas of thymic origin in positive control (MNU-treated) mice validates the use of the p53(+/-) model for oncogenicity evaluation.

Carcinogenicity conclusions:

Ribavirin did not result in neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks.

Executive CAC Recommendations and Conclusions:

The Committee agreed that the 6-month carcinogenicity study in p53 (+/-) C57BL/6 mice (p53 knock-out mice) with ribavirin was adequate (10, 50, or 100 mg/kg/day for 26-weeks, orally by gavage). The committee noticed that the protocol and doses were approved by Exec CAC and the predicted early malignant lymphoma of thymic origin in positive controls (90 mg/kg methylnitrosourea for a single oral dose, by gavage) were developed, which validated the assay.

Two male mice in the 50 mg/kg/day ribavirin group had malignant lymphomas. The sponsor stated that this was within the historical control range, but did not include historical control data in this report. The committee stated that the pharmacologist reviewer could add the historical control data to the file, when the historical data are available.

The committee concluded that ribavirin was not positive for carcinogenicity when administered to P53 (+/-) mice by gavage at dose levels up to 100 mg/kg/day for 26 weeks.

Recommendations for further analysis: A 2-year oral gavage study is presently ongoing in Crl:BR rats to determine the oncogenic potential of ribavirin (Ro 20-9963) in rats. Dose levels of 0, 10, 30 and 60 mg/kg/day are being used for this study and are supported by results from the 13-week and 6-month toxicity studies in rats. In the 2-year carcinogenicity study, ribavirin is being administered to 60 rats/sex/group; two control groups are receiving sterile water. In-life observations/determinations consist of clinical observations of toxicity, and body weight and food consumption determinations. Additionally, selected clinical pathology parameters will be evaluated at two timepoints during the course of the 2-year study, including study termination. Terminal endpoints will include full necropsy, organ weights, and comprehensive histopathology. On Days 0 (after the first dose), 91 and 182, blood will be collected from toxicokinetic animals for evaluation of serum and whole blood levels of ribavirin. Concentrations of ribavirin in RBCs will be calculated based, in part, on serum and whole blood levels of ribavirin. The dosing phase of this study will be completed in ——— The final study report will be submitted in ——— as a Phase IV commitment.

Labeling Recommendations: Minor label revisions are recommended as follows:

Carcinogenesis

Mutagenesis

Ribavirin demonstrated mutagenic activity in the *in vitro* mouse lymphoma assay. No clastogenic activity was observed in an *in vivo* mouse micronucleus assay at doses up to 2000 mg/kg. However, results from studies published in the literature show clastogenic activity in the *in vivo* mouse micronucleus assay at oral doses up to 2000 mg/kg. A dominant lethal assay in rats was negative, indicating that if mutations occurred in rats they were not transmitted through male gametes. However, the potential of carcinogenic risk to humans cannot be excluded.

Addendum/appendix listing:

1. **Dose-ranging study reports:** (The following studies were reviewed and were included in the General Toxicology Section of this NDA review)

- Pharmacologist Review: Four-week oral gavage dose range finding and toxicokinetic study with Ro 20-9963/000 in C57BL/6 mice, Report No 1002209; Study No. 6131-296 and Study No. 07296
- Pharmacologist Review: 13-Week oral gavage toxicity and toxicokinetics study with Ro 20-9963 in Wistar rats, Report No 1003487)

2. **Sponsor's incidence of histopathology findings:**

Neoplastic:

Findings		Incidence of Tumors (Numeric)									
		Males					Females				
		Ribavirin (mg/kg/day)					Ribavirin (mg/kg/day)				
		0	10	50	100	90*	0	10	50	100	90*
Thymus	<i>M-lymphoma</i>	0/19	0/20	2/20	0/19	15/15	0/20	0/20	0/20	0/20	13/15
Lung	<i>M-lymphoma</i>	0/18	0/18	1/16	0/20	2/2	0/20	0/20	0/20	0/20	2/3
Spleen	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	8/8	0/20	0/20	0/20	0/20	6/6
Thyroid	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Liver	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	2/2	0/20	0/20	0/20	0/20	0/1
Kidney	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	2/2	0/20	0/20	0/20	0/20	1/2
Pancreas	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	1/1	0/20	0/19	0/20	0/20	0/2
Lymph Nodes	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	5/5	0/20	0/20	0/20	0/20	3/3
Harderian gland	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Skeletal muscle	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Sciatic nerve	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Prostate	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	—	—	—	—	—
Marrow (femur)	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Urinary bladder	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	—	0/20	0/20	0/20	0/20	—
Hemato neoplasia	<i>M-lymphoma</i>	0/20	0/20	1/20	0/20	15/15	0/20	0/20	0/20	0/20	15/15
Stomach	<i>M-leiomyosarcoma</i>	0/20	0/20	1/20	0/20	1/1	0/20	0/20	1/20	0/20	1/1
Subcutaneous tissue	<i>M-sarcoma**</i>	0/20	1/20	0/20	0/20	1/1	1/1	1/1	5/5	—	—

*:90 mg/kg methyl nitrosourea, once, on Day 1. —: not examined. ** related to biomedic implant.

3. Histopathology in 26-week gavage oncogenicity study in P53(+/-)C57BL/6 mice with ribavirin (Study No. 07402; NDA#21-511)

Tissue	Ribavirin (0 mg/kg)	Ribavirin (10 mg/kg)	Ribavirin (50 mg/kg)	Ribavirin (100 mg/kg)	MNU (90 mg/kg/day)
No animals	20/20 (M) 20/20 (F)	20/20 (M) 20/20 (F)	19/20 (M) 18/20 (F)	18/20 (M) 20/20 (F)	15/15 (M) 15/15 (F)
Adrenal	X	X	X	X	X
Aorta	X	X	X	X	X
Brain*	X	X	X	X	X
Bone, femur	X	X	X	X	X
Cecum	X	X	X	X	X
Colon	X	X	X	X	X
Cervix	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymides*	X	X	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Harderian gland	X	X	X	X	X
Heart*	X	X	X	X	X
Ileum	X	X	X	X	X
Jejunum	X	X	X	X	X
Kidney*	X	X	X	X	X
Liver	X	X	X	X	X
Lung	X	X	X	X	X
Lymph node(s)	X	X	X	X	X
Mediasinal	X	X	X	X	X
Axillary	X	X	X	X	X
Mandibular	X	X	X	X	X
Muscle, skeletal	X	X	X	X	X
Nerve, sciatic	X	X	X	X	X
Ovary*	X	X	X	X	X
Prostate	X	X	X	X	X
Pancreas	X	X	X	X	X
Parathyroid	X	X	X	X	X
Pituitary	X	X	X	X	X
Rectum	X	X	X	X	X
Salivary gland	X	X	X	X	X
Seminal vesicles	X	X	X	X	X
Skin	X	X	X	X	X
Spleen*	X	X	X	X	X
Spinal cord	X	X	X	X	X
Stomach	X	X	X	X	X
Testes*	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X	X	X	X	X
Vagina	X	X	X	X	X

*Organs of the toxicity portion rats were weighted. Paired organs were weighted together.

4. CAC-Exec Minutes, Pharmacologist Review:

Executive CAC Meeting Minutes (Twenty – six week gavage oncogenicity study with Ribavirin in p53(+/-) C57BL/6 Mice):

Date of Meeting: September 3, 2002

Committee: Joseph Contrera, Ph.D., HFD-900, Acting Chair

Abby Jacobs, Ph.D., HFD-540, Alternate Member
Alex Jordan, Ph.D., Alternate Member
James Farrelly, Ph.D., Team Leader
Hao Zhang, M.D., Presenting Reviewer

Author of Minutes: Hao Zhang

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: 21-511

Drug Name: COPEGUS™ (Ribavirin; Ro 20-9963/000)

Sponsor: Hoffmann-La Roche Inc. 340 Kingsland Street, Nutley, NJ 07110; 973-562-2930 (phone); 973-562-3700/3554 (fax)

P53 Mouse Carcinogenicity Study

Background Information:

To support NDA#21-511 for PEG-IFN alfa-2a/ribavirin, the sponsor submitted results from the carcinogenicity studies characterizing the potential carcinogenicity of ribavirin in p53 (+/-) C57BL/6 mice to CDER for review. Prior to the conduct of the 6-month carcinogenicity study, the CAC-EC reviewed and approved the dose levels and protocol design for this study (CAC-EC fax of July 18, 2000).

Executive CAC Recommendations and Conclusions:

The Committee agreed that the 6-month carcinogenicity study in p53 (+/-) C57BL/6 mice (p53 knock-out mice) with ribavirin was adequate (10, 50, or 100 mg/kg/day for 26-weeks, orally by gavage). The committee noticed that the protocol and doses were approved by Exec CAC and the predicted early malignant lymphoma of thymic origin in positive controls (90 mg/kg methylnitrosourea for a single oral dose, by gavage) were developed, which validated the assay.

Two male mice in the 50 mg/kg/day ribavirin group had malignant lymphomas. The sponsor stated that this was within the historical control range, but did not include historical control data in this report. The committee stated that the pharmacologist reviewer could add the historical control data to the file, when the historical data are available.

The committee concluded that ribavirin was not positive for carcinogenicity when administered to P53 (+/-) mice by gavage at dose levels up to 100 mg/kg/day for 26 weeks.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/JFarrelly, HFD-530
/HZhang, HFD-530
/DSullivan, HFD-530
/ASeifried, HFD-024

Executive CAC Minutes (2-Year rat carcinogenicity study protocol, and summary of toxicology data from the 13-Week Range-finding Study with Ribavirin in Wistar Rats)**February 27, 2001**

Committee: Joseph DeGeorge, Ph.D., Chair
Joseph Contrera, Ph.D., HFD-901, Member
Karen DavisBruno, Ph.D., HFD-510, Alternate Member
Frank Sistare, Ph. D., HFD-910, Member
David Morse, Ph.D., HFD-150, Member
James Farrelly, Ph.D., Team Leader
Hao Zhang, M.D., Presenting Reviewer

Author of Draft: Hao Zhang

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review. The Committee did not address the sponsor's statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately.

IND # 58,827**Drug Name:** Ribavirin; Ro 20-9963**Sponsor:** Hoffmann-LaRoche, Inc., 340 Kingsland Street, Nutley, New Jersey 07110**Sponsor contact name:** Jennifer A Dudinak, Pharm D., Program Manager, Drug Regulatory Affairs**Sponsor/Applicant telephone and fax number:** (973) 562-2930 (phone); (973) 562-3700 (fax)**BACKGROUND**

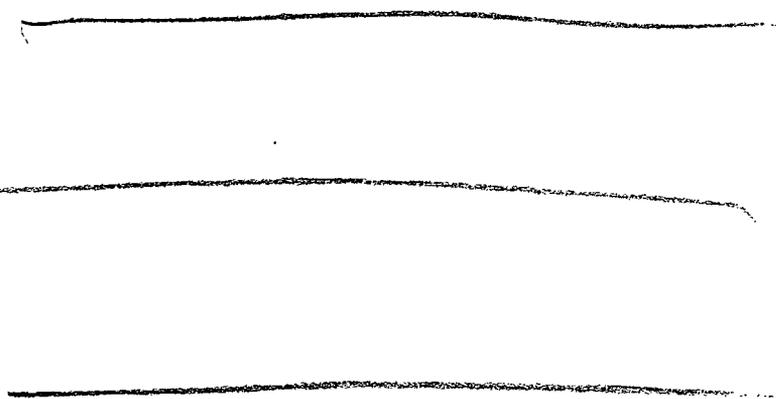
The Sponsor has filed a request for 'Special Carcinogenicity Protocol Assessment' for its ribavirin hepatitis C indication. The agency advised that the Sponsor conduct a 2-year rat carcinogenicity study with ribavirin at the time of the BLA/NDA submission to support the NDA filing for PEGASYS (Peg-interferon)/ribavirin (Re: meeting minutes; October 20, 1999 between CDER and CBER and Hoffmann-La Roche). This submission contains the draft protocol for the recommended 2-year oral carcinogenicity study in rats (Hoffmann-La Roche Study No. 07473).

In the 13-week oral rat dose range-finding study, Wistar rats were administered 0, 10, 40, 80 or 160 mg/kg/day ribavirin for 13-weeks by oral gavage. Toxicities seen at 160 mg/kg after 13 weeks administration in rats included mortality, body weight reductions (13% in males and 6% in females), body weight gain reductions (26% in males and 25% in females), food consumption and histopathological changes (bone marrow hypocellularity, splenic hematopoiesis, splenic lymphoid depletion, splenic hemorrhage, hepatocentrilobular necrosis and vacuolar degeneration, and thymic lymphocytic depletion). At 80 mg/kg, mortality (1/30 in the PK rats only), body weight reductions (11% in males and 4% in females), body weight gain reductions (19% in males and 20% in females), food consumption and histopathological changes (mesenteric lymph node hemorrhage, hepatocellular necrosis, and decreased thymic weights associated with thymic lymphocytic depletion) were seen. At 40 mg/kg/day, body weight reductions (3% in males and 2% in females) and body weight gain reductions (4% and 12% for males and females, respectively) were observed. At 10 mg/kg/day, overall body weight gains were comparable to controls for males but reduced by 10% for females. The Sponsor proposed a

high dose for the rat carcinogenicity study of 60 mg/kg/day, administered by oral intubation, once daily. The Sponsor stated that the 60 mg/kg dose should be considered the maximum tolerated dose (MTD).

Executive CAC Recommendations and Conclusions:

The Committee concurred with the sponsor's proposed doses of 10, 30, and 60 mg/kg.



Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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/Division File, HFD-530
/HZhang, HFD-530
/JFarrelly, HFD-530
/DSullivan, HFD-530
/ASeifried, HFD-024

C. ATTACHMENT 1- Background Information

Review and Evaluation of Pharmacology and Toxicology Data for CAC

Key Words: For the treatment of Chronic Hepatitis C
Reviewer: Hao Zhang, M.D.
Division: Antiviral Drug Products, HFD-530

Date: February 13, 2001

IND No: 58,827

Amendment No.	Date	Purpose
N058	1/26/01	Request for special protocol assessment: Dose level justification for the 2-year rat carcinogenicity study protocol

Information to Sponsor: Yes (x) No ()

Sponsor: Hoffmann-La Roche Inc.
340 Kingsland Street, Nutley, New Jersey 07110
Tel: 973-562-2930; Fax: 973-562-3700/3554

Drug: Ribavirin; Ro 20-9963

Chemical Name(s): 1-beta-D-ribofuranosyl -1,2,4-triazole-3-carboxamide

CAS Number: 36791-04-05

Molecular Formula: C₈H₁₂N₄O₅

Molecular Weight: 244.21

Melting Point: _____

Solubility: _____

Relevant IND and NDA Submissions:

INDs: _____

BB-IND 7823

NDAs: 18-266 (ICN); 18-859 (ICN); _____

20,903 (Schering Corp.)

Drug Class: Antiviral nucleoside analogue (IMPDH inhibitor)

Indication: Treatment of Hepatitis C

Route of Administration: Oral

Clinical Formulation: Tablet formulation (200 mg/tablet)

Proposed Clinical Protocol or Use: The maximum anticipated clinical oral dose of ribavirin (Hoffmann-La Roche Inc.) for the treatment of hepatitis C is _____ for 12 weeks (Study NV 15801).

BACKGROUND

Ribavirin is a purine nucleoside analog that has shown *in vitro* inhibitory activity against several DNA and RNA viruses. It was approved in the U.S. as an aerosol product for the treatment of severe low respiratory tract infections in children caused by respiratory syncytial virus (RSV) (VIRAZOLE®; NDA 18-859). On 18 December 2000, the Agency approved Schering Corporation's ribavirin (Rebetol)/Intron A (Interferon α-2b) combination therapy for the treatment of chronic hepatitis C in naïve patients and in patients who relapsed after previous α-interferon therapy. On the other hand, the Sponsor, Hoffmann-La Roche Inc., has initiated clinical trials with ribavirin (Roche Inc) administration (800 to 1200 mg/day, orally) in combination with PEG-interferon (PEG-interferon 2α-2b, 1.5 μg/kg, once a week, subcutaneously) for the treatment of chronic hepatitis C recently in Europe. The sponsor is planning to initiate clinical studies with ribavirin (Roche)/PEG-intron combination for the treatment of hepatitis C in the United States.

A proposed mechanism for the antiviral activity of ribavirin (Schering corp.) is through changes of the cellular purine metabolism via the inhibition of inosine 5'-monophosphate dehydrogenase (IMPDH) and the *de novo* synthesis of purine nucleotide.

Pre-clinical animal toxicity data and previous human experience indicate that ribavirin (Schering Corp)

induced anemia (due to direct hemolytic effect and suppression of the bone marrow), reticulocytosis, lymphoid atrophy following high dose, acute administration or low dose, repeat administration. The anemia is generally reversed within a few weeks following the cessation of ribavirin administration. Study data showed that ribavirin accumulated in tissues during repeat dosing procedures, but that it generally stabilizes 1-3 weeks following the start of dosing. The administration of ribavirin was associated with slight reductions in serum protein, albumin and ALT levels in dogs at 20 mg/kg/day and rats at 160 mg/kg/day (estimated human equivalent doses of 10.8 and 23 mg/kg/day, based on body surface area conversion). Ribavirin has significant adverse effects on rapidly proliferating tissues (lymphoid tissue, mucosa, testes, thymus and spleen) and tissues with high cellular metabolic rates (heart, liver and secretory cells of the intestinal mucosa). Of concern are potential inhibitions of protein synthetic and/or metabolic capacity of the liver, as demonstrated by changes in multiple serum proteins, albumin and globulins, and elongations of PT and APTT. Histological abnormalities evident in either liver or kidneys were not consistent with these changes.

Ribavirin (Schering Corp.) has demonstrated significant teratogenic and/or embryocidal potential in all animal species. Teratogenic effects have been seen after daily oral doses of 0.3 and 1.0 mg/kg in the rabbit and rat, and after single oral daily doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. The incidence and severity of the teratogenic effects generally increased with escalation of the drug dose. Viability of the fetuses and offspring is typically reduced. Ribavirin may produce significant dose and time dependent toxic responses in the testes in the CD-1 mouse, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis. However, fertility studies conducted in rats revealed no significant effects of ribavirin on reproductive behaviors or any indices of fertility when the drug was administered for 2-12 weeks prior to mating (females and males; high doses of 10 and 160 mg/kg/day). In a peri- and post- natal rat study, ribavirin administration at doses up to 1 mg/kg/day has no adverse effects on pregnant rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

The results of multiple pharmacokinetic and ADME studies in the mouse, rat and dog suggested that ribavirin (Schering Corp.) given orally is well absorbed with approximate bioavailability of 80% (in the human: 40-65%). The data from the three animal species suggest that ribavirin reached maximal levels in the plasma or serum within 1-2 hours of dosing, and decayed with an initial half-life of between 4-10 hours. Drug levels were comparable for male and female animals. The absorption of ribavirin from the gastrointestinal tract may be reduced at high doses, possibly due to saturation of a carrier transport.

Ribavirin (Schering Corp.) increased the incidence of cell transformations and mutations in mouse Balb/c 3T3 fibroblasts and L5178Y lymphoma cells at concentrations of 0.015 and 0.03-5 mg/ml, respectively, without metabolic activation. Increased mutation rates (3-4x) were seen at concentrations between 3.75-10 mg/ml in L5178Y cells *in vitro* in the presence of rat liver S9. Ribavirin was clastogenic at doses of 20 to 200 mg/kg (estimated human dose equivalent of 1.67-16.7 mg/kg, based on body surface area adjustment for a 60 kg person) in the *in vivo* mouse micronucleus assay.

Carcinogenicity studies with ribavirin (Schering Corp.) have been conducted. Unfortunately, these studies were considered inadequately designed (drug doses too low), were not conducted in accordance with the study protocol or were incomplete, and were inadequately reported/documented (Re: Summary of carcinogenicity and mutagenicity study findings: by CDER CAC -Executive session on 28 April 1998). In these studies, increased incidences of vascular lesions (microscopic hemorrhages) and retinal degeneration were seen in mice and rats, respectively. Additionally, a chronic feeding study with ribavirin in rats, at doses of 16 to 100 mg/kg/day suggested that ribavirin may induce benign mammary, pancreatic, pituitary and adrenal tumors.

It appears likely that Roche's ribavirin is more toxic than Schering's ribavirin in rats. A 2 to 5-fold increase in the mortality was seen in the 13-week oral rat dose-ranging study with Roche's ribavirin, when it is compared with that of the 13-week oral rat dose-ranging study with Schering's ribavirin. Note that different strains of animals were used in these studies.

Rebetol (Schering)				Ribavirine (Roche)			
Dose mg/kg/day	Rat/sex/group (Sprague-Dawley)	No of Death	Mortality (%)	Dose mg/kg/day	Rat/sex/group (Wistar)	No of Death	Mortality (%)
20	10	0	0	10	12	0	0
40	10	0	0	40	12	0	0
80	10	0	0	80	12 or 15*	1*	3.3
150	10	1 (F)	5	160	12	8; 8*	33; 26*
200	10	4 (1F,3M)	20	-	12	-	-

*In the 13-week oral rat TK study, 15 rats/sex/group was used.

Note that the sponsor did not submit any animal toxicity studies, except for the 13-week oral rat study data included in this submission. Therefore, the division has requested any additional animal toxicology data with ribavirin from the Sponsor. Towards this end, the agency advised that the Sponsor conduct a 2-year rat carcinogenicity study at the time of the BLA/NDA submission to support the NDA filing for PEGASYS (Peg-interferon)/ribavirin (Re: meeting minutes; October 20, 1999 between CDER and CBER and Hoffmann-La Roche).

This submission contains the following studies and protocols and is reviewed as follows:

1. Draft Protocol for the 2-Year oral (Intubation) Carcinogenicity Study in Wistar Rats With Ribavirin (Hoffmann-La Roche Study No. 07473)
2. Summary of toxicology data from the 13-Week Range-finding Study with Ribavirin in Wistar Rats (Hoffmann-La Roche Study No. 07320; IND 58827-S058: Appendix B-M)

The Sponsor has filed a request for 'Special Carcinogenicity Protocol Assessment' for its ribavirin hepatitis C indication. This submission contains the draft protocol for the recommended 2-year oral carcinogenicity study in rats (Hoffmann-La Roche Study No. 07473). The draft protocol for the oral rat carcinogenicity study was concurrently filed to IND 58,827 (S-058) in the Division of Antiviral Drug Products. The background information and briefing documents for ExecCAC presentations are attached to this review, in compliance with the Guidance for Industry on Carcinogenic Study Protocol Submissions (45-day review).

1. Summary of toxicology data from the 13-Week Range-finding Study with Ribavirin in Wistar Rats (Hoffmann-La Roche Study No. 07320)
2. Draft Protocol for the 2-Year oral (Intubation) Carcinogenicity Study in Wistar Rats With Ribavirin (Hoffmann-La Roche Study No. 07473)

Summary of toxicology data from the 13-Week Range-finding Study with Ribavirin in Wistar Rats (Hoffmann-La Roche Study No. 07320)

Vol. No.: 1 and 2; Conducting Laboratory: — Sponsor: Hoffmann-La Roche Inc., Nutley, NJ 07110; Date of Initiation: March, 2000 GLP Compliance: Yes (X) Drug Lot: 990543 Formulation: ribavirin was dissolved in deionized water

Methods

Five groups of Crl:WI(Glx/BRL/Han) (12/sex/group; 6-8 weeks of age; body weight: 181-216g for males and 137-156 g for females) were administered by oral gavage 0 (water), 10, 40, 80, or 160 mg/kg/day ribavirin for 13 weeks. Animals were observed for clinical signs and food consumption daily throughout the dosing and recovery periods. Body weights were recorded at baseline, prior to each dose administration, and at study termination. Food consumption was recorded weekly. Ophthalmoscopic examinations were conducted prior to dose administration and again during Week 12. Hematology, coagulation parameters, and clinical chemistry were evaluated on weeks 2, 4, 8, and 13. All animals were subjected to a gross necropsy, and external body features and internal organs were carefully examined and any alterations or gross lesions were recorded. Wet tissue weights were obtained from the following organs: adrenal glands, brain, epididymides, heart, kidney, liver, lung, thymus, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroid, and uterus. Tissues were processed and examined microscopically by a pathologist (Appendix 1).

Results

- Mortality:** Eight of 24 (33%) animals at 160 mg/kg/day were found dead or sacrificed moribund during the study. In addition, 8 of 30 (26%) toxicokinetic animals at 160 mg/kg/day and 1 of 30 rats in the toxicokinetic group at 80 mg/kg/day were sacrificed moribund.
- Clinical signs:** Clinical signs were seen at 80 mg/kg/day or greater, including sores, scabs, hunched appearance, hypoactivity, and labored respiration.
- Body weights:** Mean absolute body weights (Week 1-13) reduced by 13% and 6% for males and females, respectively, at 160 mg/kg/day. Mean absolute body weights (Week 1-13) reduced by 11% and 4% for males and females, respectively, at 80 mg/kg/day. Mean absolute body weights (Week 1-13) reduced by 3% and 2% for males and females, respectively, at 40 mg/kg/day. Mean body weight gains (Week 1-13) were significantly reduced in rats at 80 mg/kg/day (19-20%↓) and 160 mg/kg/day (25-26%↓). Body weight gains reduced by 4% and 12% for males and females, respectively, at 40 mg/kg/day. At 10 mg/kg/day, overall body weight gains were comparable to controls for males but reduced by 10% for females (Tables 1 and 2).
- Food consumption:** Food consumption was reduced in rats at 80 mg/kg/day or greater (Tables 3).
- Ophthalmology:** No treatment-related changes were seen.
- Hematology:** RBC, hemoglobin, and hematocrit were moderate-to-markedly reduced in rats at 160 mg/kg/day during the 13-week rat study. Mild decreases in these parameters were seen in rats at 80 mg/kg/day. Increases in reticulocyte counts and decreases in lymphocyte counts were seen in rats at 160 mg/kg/day. Increases in platelet counts were seen in rats at 80 mg/kg/day or greater. Slight decreases in hemoglobin and hematocrit were seen in rats at 10 mg/kg/day and 40 mg/kg/day (Table 4).
- Clinical Chemistry:** Decreased creatinine, total protein, albumin, sodium, globulin, cholesterol, and triglycerides were seen in rats at 160 mg/kg/day. Decreased cholesterol was seen in females at 80 mg/kg/day (Table 5).

Histopathology:

Increased heart, lung, and splenic weights were seen in rats at 160 mg/kg/day. Decreased thymic weights were seen in rats at 40 mg/kg/day or greater, which were generally correlated with microscopic observations of thymic lymphoid depletion. Increased lung weights were noted in rats at 80 mg/kg/day or greater, which were correlated with an increase in the incidence and/or severity of alveolar and interstitial macrophage, or foamy alveolar macrophage infiltration in the lungs (Table 6). Hepatocellular necrosis was seen in rats at 80 mg/kg/day. Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day. Increases in splenic weights were seen in rats at 160 mg/kg/day, which were associated with splenic extramedullary hematopoiesis, lymphocytic depletion, and hemorrhage. Additionally, depletion of thymic-dependent areas of lymph nodes and decreased numbers of erythroid and myeloid precursors in the bone marrow were noted in rats at 160 mg/kg/day. Skin lesions (ulceration, dermis and epithelial sclerosis) were also noted microscopically in one female rat at 40 mg/kg/day, and in both sexes at 80 mg/kg/day or greater. Toxicology summary tables from the 13-week range-finding toxicity study in Wistar rats are included in Appendixes 1 and 2.

Table 1. Summary of mean absolute body weights from the 13-week range-finding study with ribavirin in Wistar Rats

Dose (mg/kg)	Mean Absolute Body Weight (g) (Male)					Mean Absolute Body Weight (g) (Female)				
	Week 1	Week 3	Week 6	Week 9	Week 13	Week 1	Week 3	Week 6	Week 9	Week 13
0	195±18	270±23	333±31	373±37	412±42	148±9	181±12	209±14	221±16	223±19
10	196±19	267±17	334±22	373±27	411±30	146±9	176±10	198±12	210±16	222±17
40	196±18	265±20	326±29	363±33	400±29	147±10	174±13	196±15	208±16	219±17
80	195±18	254±21	308±30	339±33	367±37*	147±9	169±11*	191±12*	203±11*	215±12*
160	198±19	238±28*	270±41*	319±41*	355±41*	147±8	168±9*	185±13*	201±10*	211±13*

*P<0.05

Table 2. Summary of body weight gains from the 13-week range-finding study with ribavirin in Wistar Rats

Dose mg/kg	Mean Body Weight Changes (g) (Male)						Mean Body Weight Changes (g) (Female)					
	1-2	4-5	6-7	13-14	1-14	(↓%)	1-2	4-5	6-7	13-14	1-14	(↓%)
0	42±4	19±23	16±3	3±4	220±30	—	21±4	7±5	6±4	2±4	86±16	—
10	42±4	20±17	17±5	5±2	221±27	—	17±4	6±5	9±5	2±3	77±13	10
40	40±8	19±20	16±2	6±2	209±29	4	17±5	4±5	7±3	3±3	75±11	12
80	35±6	24±21	15±3	5±3	177±31*	19	12±5*	5±5	7±2	1±2	69±11*	20
160	24±7*	1±22*	19±10	4±5	162±41*	26	13±3*	-3±9*	7±10	2±2	64±16*	25

*P<0.05

Table 3. Summary of mean food consumption data from the 13-week range-finding study with ribavirin in Wistar Rats

Dose mg/kg	Mean Food Consumption (g) (Male)					Mean Food Consumption (g) (Female)				
	Week 1	Week 3	Week 6	Week 9	Week 13	Week 1	Week 3	Week 6	Week 9	Week 13
0	167±15	177±16	177±19	169±18	169±17	125±10	129±12	127±10	123±13	122±14
10	159±12	170±17	166±11	169±14	167±11	117±7	121±7	125±11	121±14	116±12
40	158±15	164±15	168±20	161±19	160±19	115±8	118±10	119±11	115±12	114±9
80	152±13*	155±15*	157±14*	152±14*	146±15*	111±9	115±8	119±7	115±8	114±8
160	140±12*	144±27*	165±14	153±11	155±14	108±12	114±11	129±18	124±16	119±21

*P<0.05

Table 4a. Summary of clinical hematology data from the 13-week rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 2) (Male)							Hematological Parameters (Week 2) (Female)						
	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$
0	7.6	14.9	44	946	216	2.9	7.6	7.9	15.3	45	899	160	2.0	6.4
10	7.4	14.6	43	933	177	2.4	7.3	7.8	14.7*	44	950	130	1.7	6.7
40	7.4	14.6	43	880	191	2.6	6.8	7.7	14.4*	43*	876	138	1.8	6.0
80	7.5	14.4*	43	972	172	2.4	6.6	7.3*	14.1*	42*	845	145	2.0	5.7
160	6.5*	13.1*	38*	1135*	122	1.9	5.9	6.9*	13.4*	40*	934	140	2.0	6.0

*P<0.05

Table 4b. Summary of clinical hematology data from the 13-week rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 4) (Male)							Hematological Parameters (Week 4) (Female)						
	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$
0	8.6	16.1	47	977	92	1.1	8.0	8.2	15.5	44	903	115	1.4	6.8
10	8.2	15.4	45	955	94	1.1	7.9	8.2	15.0*	44	1031	142	1.7	6.7
40	8.2	15.1	44*	951	147	1.8*	7.3	8.0	14.3*	42*	986	113.8	1.4	7.1
80	8.0*	14.6*	43*	1055	128	1.6	6.9	7.6*	13.9*	41*	958	152	2.0	5.6
160	6.8*	13.6*	38*	1281*	142	2.1	5.9*	6.6*	12.3*	35*	1273	114	1.7	6.7

*P<0.05

Table 4c. Summary of clinical hematology data from the 13-week rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 8) (Male)							Hematological Parameters (Week 8) (Female)						
	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$
0	8.9	16.3	47	924	113	1.3	7.0	8.6	16.0	46	862	70	0.8	5.8
10	8.7	15.4*	45*	970	127	1.5	7.8	8.5	15.0*	44*	965	94	1.1	5.8
40	8.6	15.0*	44*	922	104	1.2	6.7	8.4	14.2*	42*	990	106	1.3	5.6
80	8.4	15.0*	43*	1102*	138	1.6	7.0	7.9*	13.9*	41*	1065*	109	1.4	5.1
160	5.3*	11.5*	32*	1120*	153	2.9	4.3*	5.1*	10.8*	29*	1376*	138	2.8*	4.8

*P<0.05

Table 4d. Summary of clinical hematology data from the 13-week dose-rage finding study with ribavirin in Wistar rats

Dose (mg/kg)	Hematological Parameters (Week 14) (Male)							Hematological Parameters (Week 14) (Female)						
	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$	RBC $10^6/\mu\text{l}$	HGB g/dL	Hct %	Platele $10^3/\mu\text{l}$	Retic $10^3/\mu\text{l}$	Retic %	WBC $10^3/\mu\text{l}$
0	9.1	15.7	46	870	146	1.6	4.0	8.5	15.2	45	819	149	1.7	3.0
10	8.8	14.7*	44*	938	162	1.8	4.8	8.6	14.3*	43*	973	162	1.9	3.0
40	8.7	14.3*	43*	899	178	2.1	4.4	8.3	13.6*	41*	999	148	1.8	3.1
80	8.3*	14.2*	42*	1078*	152	1.8	4.0	7.4*	13.0*	39*	989	167	2.3	2.7
160	5.7*	12.4*	35*	1055*	221	4.0	3.1	4.0*	8.7*	23*	1348*	202	5.1*	2.3

*P<0.05

and females of all treated groups after a single (Day 1) and multiple (Days 28 and 91) doses. Peak serum ribavirin levels were reached by approximately 1 hour after dosing in both males and females at 10 mg/kg/day. However, peak serum ribavirin levels were reached by approximately 2-8 hours after dosing in both males and females at 40, 80 or 160 mg/kg/day. Serum Ribavirin concentrations were linearly proportional to dose in both sexes and at both Days 1, 29, and 91. Males had 1.4 -fold higher mean serum ribavirin levels than females. Dose-related systemic exposures (AUC_{0-24h}) were demonstrated in all treatment groups (Table 7). Serum ribavirin concentrations were 2.5 to 3-fold higher after 13-weeks of dosing compared with levels after the first dose, suggesting a ribavirin accumulation in tissues and in the serum. Systemic exposure to ribavirin was greater in males than in females on Day 1, but not on Days 28 and 91.

Table 7. Pharmacokinetics of ribavirin in rats after 13-week oral doses of ribavirin (Ro 20-9963/000)

Ribavirin	RIBAVIRIN (PHAMACOKINETIC PARAMETERS)																	
	Day 1						Day 28						Day 91					
Dose (mg/kg)	C _{max} (ng/ml)		AUC (ng•hr/ml)		T _{max} (hr)		C _{max} (ng/ml)		AUC (ng•hr/ml)		T _{max} (hr)		C _{max} (ng/ml)		AUC (ng•hr/ml)		T _{max} (hr)	
10	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
40	133	91	1114	973	1	1	157	133	2116	1821	1	2	122	142	1780	1941	1	0.5
80	434	292	6665	4585	1	4	538	539	9258	8103	8	8	465	610	8281	9622	4	8
160	811	589	11382	8478	4	4	857	859	15290	11877	2	2	961	933	16738	16946	8	8
	1346	1058	18887	15038	4	4	2070	1973	40400	31415	4	2	2927	2690	46633	46067	2	2

M: male; F: female

Comment:

Based on the MTD (less than 80 mg/kg) identified in the 13-week oral toxicity study in Wistar rats, systemic exposure in rats in the oral carcinogenicity study are expected to be 1.6-fold lower than the anticipated clinical oral dose for hepatitis in humans (Ribavirin, 600 mg once daily for 14 weeks; multiple doses; AUC_{0-12hr}: 25467 ng•hr/mL; Study No. NV 15801)

Comments

No ADME data are available on Roche's ribavirin. However, Ribavirin (Schering Corp.) has two pathways of metabolism in rats and humans: (i) a reversible phosphorylation pathway in nucleated cells; and (ii) a degradative pathway involving deribosylation and amide hydrolysis to yield a triazole carboxylic acid metabolite. Ribavirin and its triazole carboxamide and triazole carboxylic acid metabolites are excreted via the kidney. After oral administration of 600 mg of ¹⁴C-ribavirin in the human, approximately 61% and 12% of the radioactivity was eliminated in the urine and feces, respectively, in 336 hours. Unchanged ribavirin accounted for 17 % of the administered dose. Results of *in vitro* studies using both rat and human liver micrososome preparations indicated little or no cytochrome P450 enzyme mediated metabolism of ribavirin, with minimal potential for P450 enzyme-based drug interaction.

Draft Protocol: A Two Year Oral Ribavirin Carcinogenicity Study in Wistar Rats (Study No. 07473)

IND 58827 (S-058); Sponsor: Hoffmann-La Roche Inc.; Testing Facility: Hoffmann-La Roche Inc., Nutley, NJ 07110
 GLP Compliance: Yes

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Histopathology in 13-week oral dose ranging study in Wistar rats with ribavirin

Tissue	Ribavirin (0 mg/kg)	Ribavirin (10 mg/kg)	Ribavirin (40 mg/kg)	Ribavirin (80 mg/kg)	Ribavirin (160 mg/kg)
No lesions	11/12 (M); 12/12(F)	10/12 (M); 10/12(F)	7/12 (M); 9/12(F)	6/12 (M); 9/12(F)	5/12 (M); 1/12(F)
Adrenal	X	X	X	X	X
Aorta	X	X	X	X	X
Brain*	X	X	X	X	X
Bone, femur	X	X	X	X	X
Cecum	X	X	X	X	X
Colon	X	X	X	X	X
Cervix	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymides*	X	X	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Harderian gland	X	X	X	X	X
Heart*	X	X	X	X	X
Ileum	X	X	X	X	X
Jejunum	X	X	X	X	X
Kidney*	X	X	X	X	X
Liver	X	X	X	X	X
Lung	X	X	X	X	X
Lymph node(s)	X	X	X	X	X
Mediasinal	X	X	X	X	X
Axillary	X	X	X	X	X
Mandibular	X	X	X	X	X
Muscle, skeletal	X	X	X	X	X
Nerve, sciatic	X	X	X	X	X
Ovary*	X	X	X	X	X
Prostate	X	X	X	X	X
Pancreas	X	X	X	X	X
Parathyroid	X	X	X	X	X
Pituitary	X	X	X	X	X
Rectum	X	X	X	X	X
Salivary gland	X	X	X	X	X
Seminal vesicles	X	X	X	X	X
Skin	X	X	X	X	X
Spleen*	X	X	X	X	X
Spinal cord	X	X	X	X	X
Stomach	X	X	X	X	X
Testes*	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X	X	X	X	X
Vagina	X	X	X	X	X

*Organs of the toxicity portion rats were weighted. Paired organs were weighted together.

Incidence of microscopic observation in 13-week oral dose ranging study in Wistar rats with ribavirin

Tissue	Ribavirin (0 mg/kg)	Ribavirin (10 mg/kg)	Ribavirin (40 mg/kg)	Ribavirin (80 mg/kg)	Ribavirin (160 mg/kg)
Bone	X	N.E	N.E	N.E.	X
<i>Femur, sternum</i>					
Marrow- Femur:					
<i>Myeloid hypocellularity</i>	X	X	X	X	4/12, M; 2/12, F
<i>sternum-Myeloid hypocellularity</i>	X	X	X	X	3/12, M; 2/12, F
Eye	X	N.E.	N.E.	N.E.	X
Brain	X	N.E.	N.E.	N.E.	X
Spinal cord	X	N.E.	N.E.	N.E.	X
Muscle, skeletal	X	N.E.	N.E.	N.E.	X
Nerve, sciatic	X	N.E.	N.E.	N.E.	X
Lung					
<i>-infiltrate, macrophage, alveolar</i>	3/12, M	9/12, M; 3/12, F	12/12, M; 2/12, F	12/12, M; 6/12, F	5/12, M
<i>-infiltrate, macrophage, interstitial</i>	X	2/12, M; 8/12, F	5/12, M; 3/12, F	8/12, M; 3/12, F	2/12, M
<i>-infiltrate, macrophage, foamy, alveolar</i>	2/12; M, F	2/12, M	2/12, M; 1/12, F	2/12, M; 1/12, F	5/12, M; 10/12, F
Esophagus	X	N.E.	N.E.	N.E.	X
Trachea	X	X	X	X	X
Kidney	X	X	X	X	X
Heart	X	X	X	X	X
<i>-cardiomyopathy</i>	X	X	2/12(M)	X	2/12(M)
Tongue	X	N.E.	N.E.	N.E.	X
Liver					
<i>-cyst, mineralized</i>	X	X	X	X	X
<i>-infiltrate, lymphohistiocytic</i>	1/12, M;	1/12, F	1/12, M	1/12, M; 1/12, F	1/12, M; 1/12, F
<i>-stasis, bile</i>	4/12	X	X	X	2/12, M; 2/12, F
<i>-degeneration/necrosis, vacuolar</i>	X	X	X	X	1/12, M
<i>-cytomegaly, hepatocellular</i>	X	X	X	X	1/12, M
<i>-necrosis, centrilobular and bridging</i>	X	X	X	X	3/12, F
<i>-necrosis, hepatocellular</i>	X	X	X	1/12, F	X
Spleen					
<i>-hematopoiesis, extramedullary</i>	X	X	X	X	4/12, M; 5/12, F
<i>-depletion, lymphocytic, periarterial</i>	X	X	X	X	4/12, M; 10/12, F
<i>-hemorrhage</i>	X	X	X	X	1/12, M; 3/12, F
Lymph nodes, mesenteric					
<i>-infiltrate, macrophage, pigmented</i>	X	X	X	X	1/12, M
<i>-depletion, lymphocytic, paracortical</i>	X	X	X	X	4/12, M; 6/12, F
<i>-hemorrhage</i>	1/12, F	1/12, F	X	1/12, F	X
Thymus					
<i>-depletion, lymphocytic</i>	X	X	3/12, M	11/12, M; 8/12, F	12/12, M; 12/12, F
<i>-hyperplasia, lymphoreticular</i>	X	X	X	X	X
<i>-hemorrhage</i>	1/12, F	X	4/12, M; 1/12, F	1/12, F	X
Thyroid					
<i>-cyst, ultimobranchial</i>	2/1, F	X	X	1/12, F	2/12, F
Parathyroid	X	N.E.	N.E.	N.E.	X
Adrenal	X	N.E.	N.E.	N.E.	X
Aorta	X	N.E.	N.E.	N.E.	X
Pituitary	X	N.E.	N.E.	N.E.	X
Pancreas-atrophy, acinar	2/12, F	N.E.	N.E.	N.E.	1/12, M
Stomach					
<i>-edema</i>	X	X	X	X	2/12, M
<i>-erosion</i>	1/12, F	X	X	X	1/12, M
Duodenum-paucity, epithelial cell (hypertrophy, squamous metaplasia)	X	X	X	X	X
Jejunum-paucity, epithelial cell (hypertrophy, squamous metaplasia)	X	X	X	X	3/12, M
Ileum-paucity, epithelial cell (hypertrophy, squamous metaplasia)	X	X	X	X	3/12, M
Cecum -paucity, epithelial cell	X	X	X	X	2/12, M
Colon- paucity, epithelial cell	X	X	X	X	2/12, M

Rectum- paucity, epithelial cell	X	X	X	X	2/12, M
Salivary gland	X	N.E.	N.E.	N.E.	X
Harderian gland	X	N.E.	N.E.	N.E.	X
Skin					
-inflammation, chronic active					
-ulceration	X	X	1/12,F	2/12, F	3/12, M; 9/12, F
-sclerosis, dermis	X	X	1/12, F	2/12, F	3/12, M; 9/12, F
-sclerosis, epithelium	X	X	1/12, F	2/12, F	3/12, M; 8/12, F
-atrophy, adnexal	X	X	X	X	1/12, M
-vesicle	X	X	X	X	1/12, M; 2/12, F
-superficial crust	X	X	X	1/12, F	1/12, M
	X	X	1/12,F	1/12,M; 2/12, F	3/12,M; 9/12,F
Mammary, Female					
Urinary bladder	X	X	X	X	X
Uterus-dilatation	X	N.E.	N.E.	N.E.	X
Cervix	2/12,F	1/12, F	1/12, F	X	1/12,F
Vagina	X	N.E.	N.E.	N.E.	X
Ovary	X	N.E.	N.E.	N.E.	X
	X	N.E.	N.E.	N.E.	X
Prostate					
-infiltrate, lymphohistiocytic					
-inflammation, acute	3/12, M	N.E.	N.E.	N.E.	1/12, F
Seminal vesicles	1/12, M	N.E.	N.E.	N.E.	X
	X	N.E.	N.E.	N.E.	X
Epididymides					
-infiltrate, lymphohistiocytic	1/12, M	N.E.	N.E.	N.E.	X
-inflammation, acute	X	N.E.	1/1,F	N.E.	X
Testes-atrophy/degeneration	X	N.E.	N.E.	N.E.	N.E.

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VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

To support an NDA/BLA filing for PEG-IFN alfa-2a/ribavirin, the sponsor submitted results from the non-clinical studies, and the literature data characterizing the potential teratogenicity of ribavirin in rodent and non-rodent species to the division for review.

List Reproductive Toxicology Studies:

1. An Oral Gavage Study for Effects of Ro 20-9963/000 (ribavirin) on Fertility and Early Embryonic Development to Implantation in Sprague-Dawley Rats (Roche Study No. 07392; Study No. 6131-316).

Reproductive Toxicology Study Reviews:

Summary of Reproductive Toxicity Study Findings in the Published Literature:

Extensive data in the published literature regarding the teratogenic potential of ribavirin is included in the submission to support the NDA application. Based on the open literature reports, it is concluded that ribavirin is a teratogen and reproductive toxicant in animals.

Hamster:

In the pregnant golden hamster, administration of a single dose of ribavirin on gestation day 8 (2.5 or 5.0 mg/kg, i.p.) resulted in a broad spectrum of anomalies in several different tissues/organ systems including, the central nervous system, eye, limbs and axial skeleton. A dose of 5 mg/kg of ribavirin also induced an increase in resorptions, indicating that the most severely malformed embryos that likely died *in utero* and were resorbed. Similar teratogenic findings were noted in hamsters administered ribavirin via the intravenous and oral routes (single injection of 2.5, 3.75 or 5 mg/kg on gestation day 8), with the oral route of exposure resulting in a greater degree of teratogenicity compared to the parenteral routes. Repeat-dose intraperitoneal administration of ribavirin (25 mg/kg) to pregnant hamsters at later stages of gestation was associated with cerebellar hypoplasia, tooth germ defects, epidermal defects, and retinal defects.

Mice:

A study with ribavirin (10-200 mg/kg) in pregnant ICR mice (single i.p. injection on gestation day 10, 11, 12 or 13) showed teratogenicity at all ribavirin doses above 25 mg/kg. Depending on the dose and gestational day of treatment, virtually all parts of the skeleton, including the craniofacial and limb bones, were affected.

Rats:

Administration of a single oral or intraperitoneal dose of ribavirin to CD-1 strain rats on gestation day 9 also resulted in teratogenic effects. Doses approximately 10-fold higher than those associated with teratogenic effects in hamsters were required to elicit teratogenic effects in rats, with the effects generally restricted to the head region. In a Segment II reproductive toxicity study in SD rats, ribavirin (p.o. administration at dose levels of 0.1, 1, or 10 mg/kg from gestation days 6 through 15) showed central nervous system malformations at the high dose of 10 mg/kg in the absence of definitive maternal toxicity. In a peri- and post-natal exposure study, ribavirin administration at doses up to 1.0 mg/kg/day did not provoke significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning.

Rabbits:

An increased incidence of early and late resorptions was seen in rabbits at 1 mg/kg. However, the incidences were reported to be in the historical control range and occurred in mainly one litter. No adverse effects were reported following oral administration of ribavirin (60 or 120 mg/kg) to pregnant baboons during critical periods of organogenesis. Notably, these non-rodent studies included only a small number of animals per group, low dose levels and/or minimal dosing periods. Thus, a definitive assessment of the teratogenic and embryotoxic potential of ribavirin in these non-rodent species cannot be made from these studies.

1. An Oral Gavage Study for Effects of Ro 20-9963/000 (ribavirin) on Fertility and Early Embryonic Development to Implantation in Sprague-Dawley Rats (Roche Study No 07392; Study No. 6131-316)

Sponsor: Hoffmann-La Roche, Nutley, NJ; Roche Study No.: 07392; Testing Facility: _____
 Study No.:6131-316; Study Initiation Date: 11 April 2000; GLP: Yes (X); Drug and Drug Lot Numbers: Ro 20-9963/000 Lot No.:990543; Purity: 100.2%; Formulation: Ro 20-9963/000 in sterile water for injection, USP

The effects of ribavirin on fertility and early embryonic development were assessed in male and female rats in the study. The reversibility of an adverse effect on the fertility was also assessed in the treated animals. The rats received oral doses of 0, 10, 30, or 100 mg/kg/day of ribavirin.

Key study findings

- Results obtained from the mating of treated females with untreated males showed an increased pre-implantation loss and the resorption rate in the mid- and high dose groups.
- Results obtained from the mating of treated males with untreated females showed slightly reduced sperm counts in the high dose males.
- Exposures (AUC_{0-24hr}) of 2.0 (both sexes), 6.8 (males) and 4.9 (females) and 18.3 (both sexes µg•hr /ml were obtained on Day 27 (females) or Day 62 (males) following administration of doses of 10, 30 and 100 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 µg•hr /ml/ml.

Methods

Species: Crl:CD®(SD)IGS BR rats ;
 Age,: The breeder males - 6 weeks old; the breeder females – 8-15 weeks old; body weight: unknown

Dosage and group designation:

Part I					
Group	Dosage mg/kg/day	Concentration mg/ml	Number of animals		
			Breeder Males	Females	TK females ^a
1 (Control)	0	0	20	40	-
2 (Low)	10	1	20	40	11
3 (Mid)	30	3	20	40	11
4 (High)	100	10	20	40	11

Note: Females were treated, breeder males were not treated
^aTwo additional rats/group were included as possible replacements

Part II					
Group	Dosage mg/kg/day	Concentration mg/ml	Number of animals		
			Breeder Females	Males	TK Males ^a
1 (Control)	0	0	20	40	-
2 (Low)	10	1	20	40	11
3 (Mid)	30	3	20	40	11
4 (High)	100	10	20	40	11

Note: Males were treated, breeder females were not treated
^aTwo additional rats/group were included as possible replacements

Dosing regimen	
First breeding treated males	Dosed for 9 weeks prior to mating and throughout the mating period (2 weeks) for a total of 11 weeks
SECOND BREEDING TREATED MALES	Dosed for 11 weeks and then held off treatment for 9 weeks prior to mating
Treated males; no breeding	Dosed for 11 weeks and then held off treatment until sacrifice
First breeding treated females	Dosed for 4 weeks prior to mating, throughout the mating period, and until gestation Day 7
SECOND BREEDING TREATED FEMALES	Dosed for 4 weeks and then held off treatment for 6 weeks prior to mating
TK males	Dosed for 9 weeks and held off treatment for 9 weeks
TK females	Dosed for 4 weeks

Route: oral, by gavage

Drug: Ro 20-9963/000 (Lot No. 990543) in Sterile Water for Injection, USP

Clinical signs: once daily

Body weight: twice weekly; on GD 0, 3, 7, 10, and 13 for the breeding females

Food consumption: weekly during the pre-mating treatment period for the treated males and females only

Mating period: daily examinations were performed to detect the presence of sperm (vaginal lavage) or retained copulatory plug (GD 0 of gestation); the mating period was 2 weeks

Estrous cycle: daily vaginal smears for stage of estrus during the mating period of the first breeding of the treated females, until confirmation of mating or until the end of the mating period

Hematology: red blood cell counts and hematocrit for toxicokinetic animals only

Clinical chemistry: not determined

Urinalysis: not determined

Gross pathology: at sacrifice (selected organs were weighed, Appendix Table 1). Macroscopic lesions and the thymus from positive control animals were examined microscopically.

Histopathology: All females (treated and breeder) were sacrificed and cesarean-sectioned on gestation day 13, while males were sacrificed following the mating period. At necropsy (gestation day 13 for females), the uterus from each gravid female was examined for the number and placement of implantation sites, live and dead fetuses, and early and late resorptions. Additionally, the ovaries were examined for the number of corpora lutea. For males, organ weights (testes/epididymides, seminal vesicles, prostate) were determined and then the tissues (with the exception of the right testis/epididymis) were preserved for histopathological examination. The right testis/epididymis was processed for reproductive capacity (sperm motility, count, morphology)

Toxicokinetics: Blood samples for the determination of serum concentrations of ribavirin were collected from toxicokinetic animals predose and at approximately 30 minutes and 1, 2, 3, 4, 8, 12 and 24 hours after oral dosing on Day 1 (first day of dosing) and immediately prior to breeding (Day 27/28 for females and Day 62/63 for males). Ribavirin concentrations in whole blood were evaluated 24 hours post-dose on Day 27/28 (females) or Day 62/63 (males). Male rats were then maintained without treatment for 9 weeks. On Day 125, six males/group were bled for analyses of serum and whole blood concentrations of ribavirin. Erythrocyte levels of ribavirin were calculated, in part, from serum and whole blood ribavirin concentrations.

Results**Mortality:**

There were no treatment-related mortalities seen in the study.

Clinical signs:

There were no treatment-related clinical signs.

Body weight:

No treatment-related changes in mean body weights or overall body weight gains were seen in females treated for 4 weeks with ribavirin and then immediately mated with breeder (untreated) males (Weeks 1-4). However, mean weekly body weights and overall body weight gains ($\downarrow 20\%$) were reduced for treated females at 100 mg/kg/day and then held off treatment for 6 weeks, which was reversed by the end of the recovery period. Decreased mean weekly body weights and overall (Weeks 1-11) body weight gains were seen in males at 30 or 100 mg/kg/day and then immediately mated with breeder (untreated) females. Overall body weight gain was reduced by 9% and 15% for 30 and 100 mg/kg/day males, respectively, after 11 weeks of treatment, as compared to controls. Mean weekly body weights and overall (Weeks 1-11) body weight gains were also reduced for treated males at 100 mg/kg/day and then held off treatment for 9 weeks. Overall body weight gain was reduced by 20% for 100 mg/kg/day males after 11 weeks of treatment, as compared to controls. Mean body weights for these males in the 100 mg/kg/day group were still reduced at the end of the recovery period.

Food consumption:

There was a slight decrease in mean food consumption during the first week of gestation for females that had been treated with 100 mg/kg/day of ribavirin and then immediately mated with untreated males. Mean food consumption was reduced during the pre-mating treatment period for females treated with 100 mg/kg/day of ribavirin and then withheld treatment for 6 weeks prior to mating. Males at 100 mg/kg/day and then immediately mated with untreated females, and males at 100 mg/kg/day of ribavirin and then withheld treatment for 9 weeks prior to mating, also showed a slight reduction in mean food consumption during the pre-mating treatment period.

Estrous cycle evaluations:

No treatment-related changes in the estrous cycle were seen in females treated with ribavirin and then immediately mated with untreated males.

Reproductive performance:

No effects on male or female reproductive performance were noted in this study at any dose level for any of the matings, as indicated by comparable copulation and pregnancy rates across all groups.

Cesarean-section data:

No effects on the mean number of implants or mean number of corpora lutea were noted in this study for any of the matings. A slight increase in preimplantation loss was noted at 30 and 100 mg/kg/day for treated females which were immediately mated with untreated males (i.e., no recovery period for females). Additionally, there was an increase in early resorptions for these females in the 30 and 100 mg/kg/day groups, with 6 animals in the 100 mg/kg/day group exhibiting total litter resorption. Correspondingly, the mean live litter size was reduced in the 100 mg/kg/day group. All effects had reversed when females were treated for 4 weeks and then held off treatment for 6 weeks prior to mating with untreated males. Cesarean-section data obtained from the mating of treated males (with and without a recovery period) with untreated females was comparable for all groups.

Organ weights:

No direct treatment-related effects on organ weights (i.e., testes/epididymides, prostate, seminal vesicles) were observed for treated males (either with or without a recovery period).

- Sperm evaluations:** No effects on sperm motility or morphology were observed for treated males (either with or without a recovery period). The sperm count was slightly reduced for males treated with 100 mg/kg/day of ribavirin for 11 weeks. This slight effect on sperm count had reversed when males were treated for 11 weeks with 100 mg/kg/day of ribavirin and then subjected to a 9-week recovery period prior to sacrifice.
- Gross findings:** Soft and small testes were noted for one male in the 30 mg/kg/day group and one male in the 100 mg/kg/day group which were sacrificed with no recovery period. Similar findings were not noted in males treated with ribavirin and then sacrificed following a 9-week recovery period.
- Histopathology:** No treatment-related effects on the testes (the only organ evaluated microscopically) were observed for treated males (either with or without a recovery period).
- Toxicokinetics:** Exposure to ribavirin increased as the dose level increased from 10 to 100 mg/kg/day. In general, the increases in C_{max} were not consistently dose proportional to the increase in the dose level. The increases in AUC_{0-24hr} in males and females were approximately dose proportional to the increase in the dose level on both sample collection days. In general, males had higher C_{max} but similar AUC values at the different dose levels on Day 1 compared to females. The AUC values on Day 27/28 for females and on Day 62/63 for males were generally similar to those on Day 1 indicating no ribavirin accumulation in serum. Exposures (AUC_{0-24hr}) of 2.0 (both sexes), 6.8 (males) and 4.9 (females) and 18.3 (both sexes $\mu g \cdot hr / ml$) were obtained on Day 27 (females) or Day 62 (males) following administration of doses of 10, 30 and 100 mg/kg/day, respectively, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu g \cdot hr / ml / ml$. Ribavirin whole blood and calculated erythrocyte concentrations of ribavirin generally increased with the increase in dose level. However, it should be noted that no anticoagulant was added to the samples processed for the determination of ribavirin in whole blood. Because bioanalytical analyses of whole blood samples requires the addition of anticoagulant in order to prevent the blood from clotting, the whole blood and erythrocyte results are considered unreliable for the accurate determination of whole blood and erythrocyte levels of ribavirin.

Summary of individual study findings:

- Increased pre-implantation loss and resorption rates were observed in the litters of females treated with 30 and 100 mg/kg/day of ribavirin. The increased resorption rate is consistent with ribavirin's known inhibitory effect on rapidly proliferating cells and confirms that ribavirin is embryotoxic. These effects reversed following a 6-week recovery period.
- The mean sperm count in the males treated with 100 mg/kg/day was marginally reduced, an expected finding based on ribavirin's mode of action and the published literature. No adverse effects on sperm count or any other parameters of male reproductive performance were evident following a 9-week recovery period.

Reproductive and developmental toxicology (Segment I Study) summary:

- The results of the sponsor-conducted segment I rat study, suggest that oral gavage administration of ribavirin once daily to male Crl:BR rats for at least 9 weeks and to female rats for a pre-mating treatment period of 4 weeks did not affect the fertility of the treated animals. However, ribavirin produce increased preimplantation loss and resorption in the litters of females at 30 and 100 mg/kg/day. The increased resorption rate is consistent with ribavirin's known inhibitory effect on

rapidly proliferating cells and confirms that ribavirin is embryotoxic. These effects reversed following a 6-week recovery period.

- In the present study, the mean sperm count in the males treated with 100 mg/kg/day was marginally reduced, an expected finding based on ribavirin's mode of action and the published literature. No adverse effects on sperm count or any other parameters of male reproductive performance were evident following a 9-week recovery period. However, in a segment I study in the CD-1 mouse (published literature data), ribavirin produced significant dose and time dependent toxic responses in the testes, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis.
- Ribavirin (Rebetol) has demonstrated significant teratogenic and/or embryocidal potential in the mice, rat, rabbits and hamster. Teratogenic effects have been seen after daily oral doses of 0.3 to 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. In general, the incidence and severity of the teratogenic effects increased with increases of the drug dose. Viability of the fetuses and offspring is also reduced.
- In a peri- and postnatal exposure study with Rebetol by another sponsor, treatment at doses up to 1 mg/kg/day of ribavirin was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning. Note that no ribavirin-related toxicities were seen in the study in either parent generation or offspring, suggesting the 1 mg/kg dose might be too low.

Reproductive and developmental toxicology conclusions:

- Oral gavage administration of ribavirin once daily to male Crl:BR rats for at least 9 weeks and to female rats for a pre-mating treatment period of 4 weeks did not affect the fertility of the treated animals. However, increased pre-implantation loss and resorption rates were observed in the litters of females treated with 30 and 100 mg/kg/day of ribavirin. These effects reversed following a 6-week recovery period. The mean sperm count in the males treated with 100 mg/kg/day was marginally reduced, without adverse effects on sperm count or any other parameters of male reproductive performance following a 9-week recovery period. In contrast, in a segment I study in the CD-1 mouse (published literature data), ribavirin produced significant dose and time dependent toxic responses in the testes, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis.
- Ribavirin (Rebetol) has demonstrated significant teratogenic and/or embryocidal potential in the mice, rat, rabbits and hamster. Teratogenic effects have been seen after daily oral doses of 0.3 to 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. In general, the incidence and severity of the teratogenic effects increased with increases of the drug dose. Viability of the fetuses and offspring is also reduced.
- In a peri- and postnatal exposure study with Rebetol by another sponsor, treatment at doses up to 1 mg/kg of ribavirin was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning. Note that no ribavirin-related toxicities were seen in either parent generation or offspring rats at 0.1 mg/kg, indicating that the study may be inadequate because of an inappropriately low dose of treatment in the study.

Labeling Recommendations:

Impairment of Fertility

In a fertility study in rats, ribavirin showed a marginal reduction in sperm counts at the dose of 100 mg/kg/day with no effect on fertility ([redacted]). Upon cessation of treatment, total recovery occurred after 1 spermatogenesis cycle.

[redacted] . Upon cessation of treatment, total recovery from ribavirin-induced testicular toxicity was apparent within 1 or 2 spermatogenic cycles.

No reproductive toxicology studies have been performed using [redacted] in combination with [redacted] . However, peginterferon alfa-2a and ribavirin when administered [redacted] each has adverse effects on reproduction. It should be assumed that the effects produced by either agent alone will also be caused by the combination of the two agents.

Animal Toxicology Long-term study in the mouse and rat (18-24 months; dose 20-75 and 10-40 mg/kg/day, respectively (r

[redacted] have demonstrated a relationship between chronic ribavirin exposure and increased incidences of vascular lesions (microscopic hemorrhages) in mice. In rats, retinal degeneration occurred in controls, but the incidence was increased in ribavirin-treated rats.

Pregnancy Category X (see CONTRAINDICATIONS)

[redacted] produced significant embryocidal and/or teratogenic effects in all animal species in which adequate studies have been conducted. Observed no effect dose levels were well below those intended for clinical use (0.3 mg/kg/day for both the rat and rabbit; approximately 0.06 x the recommended human 24-hour dose of ribavirin). Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were noted. The incidence and severity of teratogenic effects increased with escalation of the drug dose. Survival of fetuses and offspring was reduced. No maternal toxicity or effects on offsprings were observed in a peri- and postnatal study in rats dosed orally at up to 1 mg/kg/day.

approximately 0.01 x the maximum recommended human daily dose of ribavirin).
Ribavirin is known to accumulate intracellular components from where it is cleared very slowly. It is not known whether ribavirin: _____ will exert a potential teratogenic effect upon fertilization of the ova. In a study in rats, it was concluded that dominant lethality was not induced by ribavirin at doses up to 200 mg/kg/day for 5 days (_____; up to 1.7 x the maximum recommended human daily dose of ribavirin).

COPEGUS should not be used in pregnant women or by men whose female partners are pregnant. _____ of childbearing potential: _____ are using effective contraception (two reliable forms) during the therapy _____

VIII. SPECIAL TOXICOLOGY STUDIES: None

**APPEARS THIS WAY
ON ORIGINAL**

**APPEARS THIS WAY
ON ORIGINAL**

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Ribavirin was first innovated by the ICN Pharmaceuticals and was licensed to Schering-Plough Research Institute in 1995 for clinical development. It has been approved as an oral product (Rebetol, NDA 20-903), in combination with interferon alpha (Intron®A, and related interferons: Roferon®, Wellferon®, Infergen®, Peg-intron™), for use in the treatment of chronic active hepatitis C infection in patients since 1998.

The sponsor has submitted an original BLA and original NDA (NDA 21-511) for the use of Pegasys (peginterferon alfa-2a, Ro 25-8310) in combination with Ribavirin (Copegus, R0 20-9963) for the treatment of patients with hepatitis C without cirrhosis or with cirrhosis with compensated liver disease who have not been previously treated with interferon alpha and are at least 18 years of age. The proposed duration of treatment is 6 months.

General Toxicology Issues:

Nonclinical toxicology studies performed with ribavirin included: 4-week oral gavage range-finding toxicity and toxicokinetic study in C57BL/6 mice; 13-week oral gavage toxicity and toxicokinetic study in Crl:WI(G1x/BRL/Han) IGS BR rats; 26-week oral gavage toxicity and toxicokinetic in Crl:WI(G1x/BRL/Han) IGS BR rats with a 6-week recovery period; 26-week oral gavage toxicity and toxicokinetic study in dogs with a 6-week recovery period. Repeat-dose toxicity studies in mice, rats or dogs are summarized as follows.

Summary of repeat-dose toxicity studies in mice, rats, dogs or monkeys

Species	Dose mg/kg/day	C _{max} µg/ml	AUC _{0-24 h} ^a µg·hr/mL	NOAEL ^c mg/kg/day	HED ^f mg/kg/day	Ratio of Animals to Human AUC Following Ribavirin Administration ^g	Toxicity
Human NV 15801	16 ^b 1200mg/day	2.781 ^e	25.5 ^d 51 ^e	—	—	—	
Mouse 4-week, p.o. Roche Study No 07296	30 100 200 400	1.148 3.394 4.585 19.6	11.6 35.5 71.9 142.5	<30	<2.5	0.23 0.70 1.41 2.79	↓RBC, ↓Hb, ↓Hct, Splenic hematopoiesis: ≥30 mg/kg Mortality: ≥200 mg/kg
Rat 13-week, p.o. Roche Study No 07320	10 40 80 160	0.13 0.54 0.95 2.8	1.9 9.0 16.8 46.3	10	1.6	0.04 0.18 0.33 0.91	↓RBC, ↓Hb, ↓Hct, erythroid hypoplasia, splenic hematopoiesis, ↓ thymic weights, thymic lymphoid depletion: ≥10 mg/kg; Mortality: 160 mg/kg
Rat 26-week, p.o. Roche Study No 07321	10 35 70	0.20 0.54 1.14	2.3 7.9 19.1	10	1.6	0.05 0.15 0.37	Mild ↓RBC, ↓Hb, ↓Hct: ≥10 mg/kg/day. Skin ulceration, erythroid hypoplasia, splenic hematopoiesis, ↓ thymic weights, thymic lymphoid depletion: ≥35 mg/kg Mortality: 70 mg/kg

Dog	5	1.60	10.4			0.20	↓RBC, ↓Hb, ↓Hct and ↑reticulocyte counts: ≥20 mg/kg/day. ↓Body weights and food consumption, ↑intestinal crypt dilatation/necrosis
26-week, p.o.	10	3.70	23.1	10	5.4	0.45	
Roche Study No 07322	20	6.14	40.1			0.79	

^a Obtained 24 hours after last dose of the study; ^b Based on a 75 kg body weight for the patient; ^c C_{max} and AUC_{0-12h} values were calculated from data obtained after the morning dose only (i.e., 600 mg/patient); ^d AUC_{0-12h}; ^e NOAEL: no-observed adverse effect level; ^f HED: human equivalent dose; ^g Margin of Safety: based on multiple dose following administration of ribavirin, i.e., AUC_{0-12h} (25.5µg•hr/mL), multiplied by 2 to obtain exposure for 24 hours

General Toxicology issues identified in these studies are as follows.

Hematological toxicity: Preclinical toxicology data indicate that the hematological tissue is one of the major target sites of ribavirin. Ribavirin induces a significant degree of anemia, reticulocytosis, and lymphoid atrophy in rats and dogs following a repeat-dose administration up to 6 months. In general, the anemia is reversed within 6-weeks following the cessation of ribavirin in rats and dogs. In a 13-week oral-rat study, effects on the hematological parameters (↓RBC, ↓Hb, ↓Hct, ↑platelet counts, and ↓lymphocyte counts) were seen at 160 mg/kg/day. Bone marrow smears revealed elevated myeloid-to-erythroid ratios and erythroid hypoplasia at the same dose level. Decreased thymic weights were seen in rats at 40 mg/kg/day or greater, which were generally correlated with microscopic observations of thymic lymphoid depletion. Increases in splenic weights were seen in rats at 160 mg/kg/day, which were associated with splenic extramedullary hematopoiesis, lymphocytic depletion, and hemorrhage. In a 26-week oral toxicity study in rats with a 6-week recovery period, mild hematological toxicity was present in rats at ≥10 mg/kg/day. Thymic lymphoid depletion was seen at all dose levels, with a dose-dependent increase in the incidence and severity of this finding from 10 to 70 mg/kg/day. Hypercellularity of the femur marrow was seen in both sexes at 70 mg/kg/day and in females at 35 mg/kg/day. Increased splenic extramedullary hematopoiesis and hepatic pigment deposition were seen in females at 70 mg/kg/day. In general these hematological effects and microscopic findings were reversed at the end of the recovery period. In a 26-week oral study in dog with a 6-week recovery period, ↓RBC, ↓Hb, ↓Hct, and ↓lymphocyte counts, and ↑reticulocyte counts were seen in dogs at 20 mg/kg/day. Mean lymphocyte counts remained decreased for animals in all treated groups at recovery (Week 33) as did WBC counts for females at 20 mg/kg/day. In general, erythrocyte parameters at recovery (Week 33) were comparable to those of control dogs, indicating the reversibility of changes in RBC parameters.

Gastrointestinal toxicity: In the four-week toxicity study in mice, crypt cell necrosis and regenerative hyperplasia in the small and large intestine were seen in mice died at ≥200 mg/kg/day. In the 26-week oral gavage toxicity and toxicokinetic dog study with a 6-week recovery period, increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Ribavirin treatment-related gastrointestinal toxicity in rats and dogs was similar to that reported in the literature for cats. The clinical relevance of this finding is unknown in the human following administration of ribavirin.

Skin: In the 13-week oral gavage toxicity study in rats, skin lesions (ulceration, dermis and epithelial sclerosis) were also noted microscopically in one female rat at 40 mg/kg/day, and in both sexes at 80 mg/kg/day or greater. In the 26-week oral gavage toxicity and toxicokinetic dog study with a 6-week recovery period, skin ulceration was seen at ≥35 mg/kg/day. The skin lesions were reversed within 6-weeks following the cessation of ribavirin in rats and dogs.

Liver toxicity: Liver centrilobular necrosis and vacuolar degeneration was seen in rats at 160 mg/kg/day in the 13-week oral toxicity study. Decreased cholesterol levels in males at 70 mg/kg/day, increased inorganic phosphorus levels in females at 70 mg/kg/day, and slightly decreased ALT levels in males at 35 mg/kg/day or greater were seen in the 26-week oral toxicity study. These changes had reversed following the 6-week recovery period.

Testes and epididymis: In a 4-week oral dose range finding study in mice, a statistically significant decrease in relative (to body weight) testes/epididymis weights was observed for surviving male mice at 200 mg/kg/day, accompanied by the treatment-related histopathologic changes in the epididymis (hypospermia).

Other issues: Results from studies published in the literature show a relationship between chronic ribavirin exposure and increased incidences of vascular lesions (microscopic hemorrhages) in mice at 20-75 or 10-40 mg/kg/day, respectively, for 18 to 24 months (estimated human equivalent doses of 1.7-6.3 and 1.4-5.7 mg/kg/day, respectively, based on body surface area adjustment for a 60 kg adult; approximately 0.1-0.4 × the maximum human daily dose of ribavirin).

Toxicology Summary:

Ribavirin: Summary of Non-clinical Pharmacology

Ribavirin is a nucleoside drug with antiviral activity, both *in vitro* and *in vivo*, against a wide range of RNA and DNA viruses, including HCV. The anti-HCV mechanism of action of ribavirin is likely to involve multiple mechanisms. Ribavirin inhibits HCV RNA replication *in vitro* in human hepatoma cells. Inhibition of HCV RNA-dependent RNA polymerase (NS5B) by ribavirin triphosphate could contribute to the inhibition by ribavirin in the HCV replicon assay. Ribavirin increases Type 1 (Th1) cytokine and decreases Type 2 (Th2) cytokine secretion from stimulated human T cells *in vitro*, in addition to inhibiting T cell proliferation. Ribavirin inhibits cellular IMP dehydrogenase (IMPDH) and results in a depletion of intracellular GTP pools, which could contribute to the anti-proliferation effect of ribavirin.

Ribavirin: Summary of Safety Pharmacology

No safety pharmacology studies conducted with ribavirin alone were included in this NDA submission. Repeat-dose toxicity studies with ribavirin in mice, rats and dogs do not reveal any potential for CNS, renal or cardiovascular effects. However, repeat-dose toxicity studies with ribavirin in mice, rats and dogs reveal ribavirin-related gastrointestinal effects. Intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were evident in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. At the end of recovery period, intestinal sections from recovery animals at 20 mg/kg/day were comparable to controls.

Ribavirin: Summary of General Toxicology

Preclinical toxicology data indicate that the hematological system and intestines, and skin are the target sites of ribavirin. Ribavirin induces a significant degree of anemia, reticulocytosis, and lymphoid atrophy in rats and dogs following up to 6-month, repeat-dose oral administration. The anemia is generally reversed within 6-weeks following the cessation of ribavirin. Increased intestinal crypt dilatation/necrosis in the duodenum and erosion in the ileum were seen in dogs at 20 mg/kg/day, with increased inflammation in all sections of the small intestine. Skin ulceration was seen in rats at ≥ 35 mg/kg/day. In addition, ribavirin causes clinical chemistry changes in rats at 70 mg/kg/day, which included decreased cholesterol levels in males, increased inorganic phosphorus levels in females, and slightly decreased ALT levels in males. These changes had reversed following the 6-week recovery period. The results of the sponsor-conducted studies (mice, rats, and dogs) suggested that ribavirin has significant adverse effects on rapidly proliferating tissues with high metabolic rate (lymphoid, mucosa, bone marrow, testes, spleen, liver and skin). Following 6-month administration of 10, 35 and 70 mg/kg/day of ribavirin, respectively, exposures (AUC_{0-24hr}) at Day 182 in rats were 2.3, 7.9 and 19.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$, as compared to the therapeutic AUC_{0-12hr} of 26.4 $\mu\text{g}\cdot\text{hr}/\text{ml}$. The NOAEL in rats for ribavirin could not be established in the 6-month study. The NOAEL for ribavirin was considered to be less than 10 mg/kg/day in rats following a 6-month repeat-dose administration. Exposures (AUC_{0-24hr}) of 10.4, 23.1 and 40.1 $\mu\text{g}\cdot\text{hr}/\text{ml}$ were achieved at treatment termination following administration of dose levels of 5, 10 and 20 mg/kg/day, respectively, as

compared to the therapeutic AUC_{0-12hr} of 26.4 µg•hr/ml in the human. The no-effect level (NOAEL) for ribavirin in dogs after 26 weeks of daily administration is considered to be 10 mg/kg/day.

Ribavirin: Summary of Reproductive Toxicology

Oral gavage administration of ribavirin once daily to male Crl:BR rats for at least 9 weeks and to female rats for a pre-mating treatment period of 4 weeks did not affect the fertility of the treated animals. However, increased pre-implantation loss and resorption rates were observed in the litters of females treated with 30 and 100 mg/kg/day of ribavirin. These effects reversed following a 6-week recovery period. The mean sperm count in the males treated with 100 mg/kg/day was marginally reduced, an expected finding based on ribavirin's mode of action and the published literature. No adverse effects on sperm count or any other parameters of male reproductive performance were evident following a 9-week recovery period. However, in a segment I study in the CD-1 mouse (published literature data), ribavirin produced significant dose and time dependent toxic responses in the testes, including decreases in spermatid concentration, increases in abnormal sperm morphology, and germinal epithelia necrosis.

Ribavirin's teratogenic and/or embryocidal effects in animals have been reported. Teratogenic effects have been seen after daily oral doses of 0.3 to 1.0 mg/kg in the rabbit and rat, and after single oral doses of 2.5 mg/kg or greater in the hamster. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and gastrointestinal tract were evident. In general, the incidence and severity of the teratogenic effects increased with increases of the drug dose. Viability of the fetuses and offspring is also reduced.

In a peri- and postnatal exposure study with Rebetol by another sponsor, treatment at doses up to 1 mg/kg of ribavirin was without significant adverse effects on pregnant SD rats or their offspring when exposure began after the period of organogenesis and continued through weaning. Clinical studies with ribavirin administration to pregnant women have not been conducted. It should be assumed that ribavirin may cause fetal harm in humans.

Ribavirin: Summary of Genetic Toxicology

Ribavirin (Ro 20-9963/000) was evaluated as negative for inducing reverse mutations in the *Salmonella-Escherichia coli* reverse mutation assay conducted with four tester strains of *S. typhimurium* (TA1535, TA1537, TA98 and TA100) and *E. coli* WP2uvrA, using nonactivation and activation conditions. However, ribavirin was evaluated as positive for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells using non-activation and activation conditions, with a clearly less positive response in the presence of S9 metabolic activation. In a confirmatory non-activation assay at concentrations from 7.85 to 2500 µg/ml, concentrations from 125 µg/ml and higher induced a 3 to 4-fold increase in mutant frequencies. In contrast, under activation conditions, the positive response was clearly weaker than under non-activation conditions. Seven of these 8 concentrations (1000, 1500, 2000, 2100, 2200, 2300, 2400, and 2500 µg/ml) induced a 2.2 to 2.7-fold increase in mutant frequencies indicative of a weak positive response.

The sponsor-conducted *in vivo* micronucleus assay in CD-1 mice showed that ribavirin did not induce clastogenic or spindle-damaging effects in mouse bone marrow cells at any of the dose levels tested (from 500 to 2000 mg/kg/day x 3 days; the estimated human dose equivalent: 42 – 168 mg/kg, based on body surface area adjustment for a 60 kg adult). Although published literature data show CD-1 mice to be an adequate strain for detecting micronucleated PCEs following administration of other purine nucleoside analogues in the micronucleus assay, two non-GLP studies (data from published literature) conducted with the same or a different dosing regimen of ribavirin, using different strain of mice (Swiss albino mice or B6C3F1 mice), demonstrated that ribavirin was genotoxic. Thus, mouse strain differences in the susceptibility to induce micronucleated polychromatic erythrocytes (PCEs) by clastogenic nucleosides should also be considered.

Ribavirin: Summary of Carcinogenicity

Ribavirin did not result in neoplastic lesions when administered to p53(+/-) mice by gavage at dose levels of 10, 50, or 100 mg/kg/day for 26 weeks. The lack of treatment-related neoplastic lesions in this study is consistent with the negative results obtained in the sponsor conducted mouse micronucleus assay. However, these data are inconsistent with the data from the mouse lymphoma assay. The predicted early development of malignant lymphomas of thymic origin in positive control (MNU-treated) mice validates the use of the p53(+/-) model for oncogenicity evaluation.

A 2-year oral gavage study is presently ongoing in Crl:BR rats to determine the oncogenic potential of ribavirin (Ro 20-9963) in rats. Dose levels of 0, 10, 30 and 60 mg/kg/day are being used for this study and are supported by results from the 13-week and 6-month toxicity studies in rats. The dosing phase of this study will be completed in _____. The final study report will be submitted in _____ as Phase IV commitment.

Ribavirin: Summary of Non-clinical Pharmacokinetics and ADME Studies

The results of pharmacokinetic/toxicokinetic studies conducted by the sponsor, and the published data on ADME and PK data in the mouse, rat and dogs, suggest that ribavirin is readily absorbed and slowly eliminated in mice, rats and dogs with an approximate bioavailability of 80%. The absorption of ribavirin from the gastrointestinal route was reduced at high doses, suggesting a dose-dependent saturation of the carrier transport. In addition, published literature data for the mouse, rat and dog suggest that ribavirin achieved maximal levels in the serum or plasma within 1-2 hours of dosing, and delayed with an initial half-life of between 4 to 10 hours.

The uptake of ribavirin into RBCs is via a nucleoside transporter (es-transporter) which been identified in other human cell types. The es-transporters maintain intra and extracellular ribavirin concentrations at equilibrium in all cells. Following administration of [¹⁴C] ribavirin to monkeys and humans 20% to 30% of the total dose was retained in body tissue after 72 hours. After 2 hours, the concentration of radioactivity in the RBCs exceeded that in the plasma and continued to increase rapidly. Accumulation of ribavirin in serum was seen in mice, rats and cynomolgus monkeys after repeat oral administration, except for dogs. In contrast, accumulation of ribavirin in RBCs was observed in all tested species (dogs, mice, rats and cynomolgus monkeys) after repeat oral administration. These results suggest that RBCs may serve as a drug (or the triazole carboxamide metabolites, and the phosphorylated drug metabolites) reservoir with delayed release following drug withdrawal. The accumulation of ribavirin nucleotide metabolites within the RBCs may contribute to the observed anemia in patients administered ribavirin in high doses or for prolonged periods of time. Compared to the human and monkey, the rat does not accumulate radioactivity in RBCs to the same extent as the human and monkey. In monkeys and humans the amount of parent drug in blood cells increases through 48 hours and remains stable for 72 hours, whereas in rats, the concentration of ribavirin decreases at a rate similar to the plasma disappearance curve. Serum drug levels were comparable for male and female animals.

Ribavirin has two pathways of metabolism in rats and humans: (i) a reversible phosphorylation pathway in nucleated cells; and (ii) a degradative pathway involving deribosylation and amide hydrolysis to yield a triazole carboxylic acid metabolite. The enzymes responsible for the deribosylation and/or amide hydrolysis have not been definitively identified; however, production of these metabolites has been reported in the literature to be associated with liver cytosolic fractions. In human and monkey erythrocytes cells, ribavirin is phosphorylated to mono- di and triphosphate nucleotides, with the major metabolite being the triphosphate nucleotide. Adenine kinase is the rate-limiting enzyme for the initial phosphorylation of ribavirin. RBCs are able to concentrate ribavirin triphosphate nucleotide due to the lack of dephosphorylation enzymes. In contrast, nucleated cells have been shown to dephosphorylate ribavirin. Ribavirin has minimal effect on P450s in either animals or humans. Results of *in vitro* studies

using both rat and human liver micromosome preparations indicated little or no cytochrome P450 enzyme mediated metabolism of ribavirin, with minimal potential for P450 enzyme-based drug interaction.

The principal route of elimination is renal for both ribavirin and its triazole carboxamide and triazole carboxylic acid metabolites, with the majority of the drug being eliminated as metabolites rather than the parent compound. It has been reported that 50 to 100% of the administered radioactivity were eliminated in the urine in mice, rats, dogs, and monkeys, within 24 to 48 hours of dosing, and approximately 5 to 20% of the administered radioactivity were recovered in the feces after dosing (depending on the species). After oral administration of 600 mg of ^{14}C -ribavirin in the human, approximately 61% and 12% of the radioactivity was eliminated in the urine and feces, respectively, in 336 hours. Unchanged ribavirin accounted for 17 % of the administered dose. Published literature data showed that the majority of ribavirin in plasma is available as free drug and not bound to plasma proteins.

Ribavirin elicits significant toxicity on RBCs. Results of the pharmacokinetic and ADME studies of ribavirin suggested that the affected tissue is also the primary site of drug (and the phosphorylated metabolites of ribavirin) deposition after oral dosing. These results suggest that RBCs may serve as a drug (or the triazole carboxamide metabolites, and the phosphorylated drug metabolites) reservoir with delayed release following drug withdrawal. The accumulation of ribavirin nucleotide metabolites within RBCs may contribute to the observed anemia in patients administered ribavirin in high doses or for prolonged periods of time. Unfortunately, there is no validated method for the measurement of these phosphorylated metabolites of ribavirin in RBCs, as well as in the rapid proliferating tissues.

Recommendations:

As part of a Phase 4 Agreement, the following non-clinical studies are recommended for the drug product in the pharmacology and toxicology perspective.

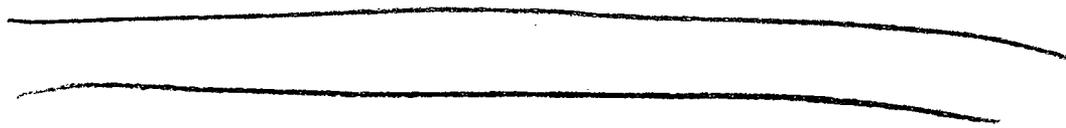
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- As part of a Phase 4 Post-marketing Agreement, it is understood that the sponsor should be required to submit the currently on-going 2-year rat carcinogenicity study report to the division for review and concurred by the CDER-CAC, when the study is completed.

Labeling with basis for findings: see Appendix 1

Labeling Review (NDA): see Appendix 1

**APPEARS THIS WAY
ON ORIGINAL**

4 pages redacted from this section of
the approval package consisted of draft labeling



X. APPENDIX/ATTACHMENTS:

Attachment 1. Pharmacologist's review: IND 58827, SN159

IND 58,827 Serial No: 159 (GC)
Date Submitted: 4/1/02
Date Assigned: 4/15/02
Date review completed: 5/22/02
Assigned reviewer: Hao Zhang

SPONSOR Hoffmann-La Roche Inc.; 340 Kingsland Street, Nutley, New Jersey 07110-1199; Telephone: 973-562-2930

DRUG Peginterferon alfa-2a: (PEGSYS®; PEG-INF α-2a: PEG-INF α-2a is synthesized by

Ribavirin: 1-β-D-ribofurabosyl-1H-1, 2, 4-triazole-3-carboxamide; Ro 20-9963; COPEGUS™; C₈H₁₂N₄O₅; Molecular Weight: 244.21; White crystalline powder that is freely soluble in water (155.3 mg/mL) and is slightly soluble in ethanol (2.8 mg/mL)

INFORMATION TO SPONSOR No

FORMULATION The [redacted] contains [redacted] 4 PEGSYS®
180 µg/vial, [redacted]

ROUTE OF ADMINISTRATION PEGSYS®: Subcutaneous injection; COPEGUS™; oral administration

RELATED INDs PEGASYS BB-IND 7823 (S-238); [redacted]
IND 58,827 (S-125)

INDICATION (S) Treatment of hepatitis C and hepatitis B

INTRODUCTION

SUMMARY

The Sponsor submitted an initial request for a Pre-BLA/NDA meeting on June 5, 2001 (S-072 to IND 58,827; S-164 to BB-IND 7823) to discuss the clinical portion of the BLA/NDA. The meeting request

was denied because comparability between the PEGSYS clinical and commercial materials was not established. Based on the demonstration of comparability in study NP16569 (S-262 to BB-IND 7823 and S-143 to IND 58,827), CBER informed the sponsor that a Clinical Pre-BLA/NDA meeting would be granted. The sponsor submitted a second request on March 13, 2001 (S-270 BB-IND 7823 and S150 to IND 58,827). The sponsor submitted the briefing package for the Clinical Pre-BLA/NDA meeting and justification of maintaining fast-track designation and receiving priority review.

The meeting objectives included:

- To present and discuss data from the pivotal trials, NV15801 (*A phase III randomized, multicenter efficacy and safety study comparing the combination of Peglated interferon alfa-2a and ribavirin to Rebertron in the treatment of patients with chronic HCV infection*) and NV15942 (*A phase III randomized, multicenter, efficacy and safety study examining the Effects of the Duration of Treatment and the daily dose of Ribavirin in patients with chronic hepatitis C virus infection treated with the combination of Peglated interferon alfa-2a and ribavirin*), which support the BLA/NDA filing
- To obtain concurrence that the overall benefit-risk profile and scope of the data in the phase III pivotal trial justify fast-track designation and priority review for the BLA/NDA.

In the Attachment 6 of the briefing package, the sponsor provided a summary of agreements between the agency and the sponsor (Roche). The sponsor listed the recommendations that the agency made on October 20, 1999 to the sponsor:

- Complete genotoxicity testing
- Conduct a two-year carcinogenicity study in rats
- A six-month carcinogenicity study in P53 knockout mice
- Segment 1 fertility study with a recovery endpoint design (to evaluate whether or not fertility function recovers after treatment is stopped)

The sponsor informed the division that all studies have been completed, and the results will be included in the BLA/NDA submission except for the 2-year carcinogenicity study in rats, which is ongoing.

Comments

The sponsor submitted its pre-clinical toxicology plan, dated August 9, 1999, for a BLA/NDA for the PEGSYS/ribavirin combination for use in the treatment of patients with chronic hepatitis C infection. On October 20, 1999, the agency (both CBER and CDER Pharmacology and Toxicology reviewers: Drs. Anne Pilaro, David Morse, James Farrelly) and the sponsor discussed the suitability of these toxicology plans. Note that the agency's concurrence to begin the following studies (Re: IND58,827, Record of Industry Meeting; Meeting Date: October 20, 1999):

- To conduct three mutagenicity studies according to the ICH guidelines
- Segment 1 reproductive toxicology studies addressing the time following ribavirin discontinuation before which pregnancy may safely be initiated. Both the safe initiation of pregnancy in females and the recovery of reproductive function in males after termination of ribavirin therapy should be demonstrated (The Segment I open reference listed in the submission is not acceptable, as the study was audited and found to be deficient).
- Conduct two-year carcinogenicity study in either the rat or mouse (Specifically, completion of the 2-year bioassay in either the rat or mouse would be acceptable as a Phase 4 commitment. If the short-term study is in mice, the 2-year carcinogenicity study should be in rats)
- A six-month carcinogenicity study in P53 knockout mice

After reviewing the submission (GC), the reviewer found no new pharmacology/toxicology issues raised in this submission. The reviewer agreed that the above studies were designed to address the issues

regarding the pre-clinical toxicology plan for a BLA/NDA for the PEGSYS/ribavirin combination for use in the treatment of patients with chronic hepatitis C infection, raised by the pharmacology/toxicology review team on October 20, 1999. Note that the sponsor will submit the pre-clinical studies and the table of contents for NDA submission in which ribavirin application as a full NDA/BLA under section 505 (b) (2) of the Federal Food, Drug, and Cosmetic Act.

Conclusion

There is no Pharmacology/Toxicology issue that will preclude the pending BLA/NDA filings at this time. No regulatory action is associated with this review at this time.

Hao Zhang, M.D.
Pharmacologist

Concurrence:

HFD-530/JFarrelly
HFD-530/HZhang

cc:

HFD-530/IND
HFD-530/DSullivan
HFD-530/RFleischer
HFD-530/RKambhampati
HFD-530/NBattula

Disk:

HFD-530/JFarrelly

Attachment 2 Pharmacologist's review: IND 58827, SN046

END 58,827 Serial No: 046 (YY)
 Date Submitted: 11/16/2000
 Date Assigned: 12/24/2000
 Date review completed: 12/20/2000
 Assigned reviewer: Hao Zhang

SPONSOR Hoffmann-La Roche Inc.; 340 Kingsland Street, Nutley, New Jersey 07110-1199; Telephone: 973-562-2930

DRUG Peginterferon alfa-2a (PEG-INF α -2a: PEG-INF α -2a is synthesized by

Ribavirin: 1- β -D-ribofurabosyl-1H-1, 2, 4-triazole-3-carboxamide; Ro 20-9963; $C_8H_{12}N_4O_5$; Molecular Weight: 244.21; White crystalline powder that is freely soluble in water (— mg/mL) and is slightly soluble in ethanol (2.8 mg/mL)

INFORMATION TO SPONSOR No

FORMULATION An injectable solution containing benzyl alcohol, sodium chloride, sodium acetate trihydrate, acetic acid, polysorbate 80, and — For studies in hepatitis C, concentrations of — are used, packaged as — unit doses in — glass vials.

ROUTE OF ADMINISTRATION Subcutaneous

RELATED INDs PEGASYS BB-IND 7823

INDICATION (S) Treatment of hepatitis C and hepatitis B

INTRODUCTION

The recombinant INF α is a product of a cloned human leukocyte interferon gene expressed in *E. coli*. It has — amino acids and a molecular mass of — daltons.

Ribavirin has been combined with Interferon α in the treatment of chronic hepatitis C. It is a guanosine analogue that inhibits the *in vitro* replication of a variety of RNA

and DNA viruses. Combination of ribavirin with interferon α -2b (Intron A, IFN- α -2b) resulted in a 2-fold increase in sustained virological response in naïve patients compared with interferon α -2b monotherapy.

SUMMARY

The Sponsor submitted an annual report for a review by the division. In the Investigator's Brochure, the Sponsor included the preliminary results of clinical studies of PEG-IFN α -2a in combination with ribavirin. Note that two new indications, _____ were added in this Investigator's Brochure. The cutoff for the ribavirin combination data is April 15, 2000. All deaths and all unexpected serious adverse events for both the monotherapy and combination therapy programs that occurred up to October 31, 2000 were included.

A phase I study showed that the addition of a branched PEG moiety to interferon α -2 α (PEG-IFN α -2a) results in sustained absorption and reduced clearance of PEG-IFN α -2a compared with IFN α -2a, which allow it to be given once a week. In phase II and III studies involving 1600 patients with hepatitis C, nine patients died in the IFN α -2a monotherapy studies at doses of 45 to 180 μ g daily (NV15489, NV15495, NV15496, NV15497). Two died of cerebral hemorrhage or pneumonitis, which occurred at 135 and 180 μ g daily, respectively. These deaths were considered to be related to treatment. Additionally, the incidence of dermatitis and injection site inflammation was higher in the PEG-IFN α -2a compared with IFN α -2a.

Additionally, the Sponsor included nonclinical information on ribavirin that is current to November 1999 (Appendix 1). Note that the remaining nonclinical information was not updated. Note that Dr. David Morse, the previous pharm/tox reviewer in the division for this IND had reviewed these nonclinical pharmacology/toxicology studies. These studies were summarized in Table 1. After reviewing the Investigator's Brochure, the reviewer found no new pharmacology/toxicology issues raised in the annual report when compared with the previously submitted reports. Therefore, with respect to pharmacology/toxicology no actions are indicated at this time.

Conclusion

No regulatory action is associated with this review.

Hao Zhang, M.D.
Pharmacologist

Attachment 3 **Other relevant materials:** The sponsor provided published literature data to support the NDA submission;

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Appendix F Any compliance issues: not indicated