

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-903

MICROBIOLOGY REVIEW(S)

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA #: 20-903

REVIEWER :N. Battula
CORRESPONDENCE DATE :12-03-97
CDER RECEIPT DATE :12-03-97
REVIEW COMPLETE DATE :04-27-98

SPONSOR: Schering Corporation
2000 Galloping Hill Road
Kenilworth, NJ 07033

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-HCV

INDICATION: Treatment of chronic hepatitis C infection

DOSAGE FORM: Intron A as lyophilized powder and Ribavirin as capsules

PRODUCT NAMES:

A. PROPRIETARY: (1) Intron A and (2) Rebetol

B. NONPROPRIETARY: (1) Intron alfa-2b and (2) Ribavirin

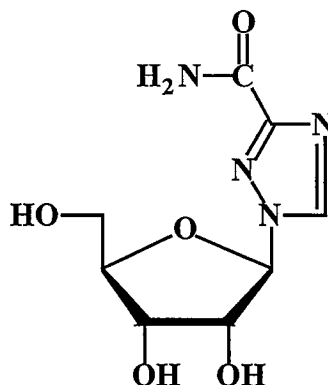
C. CHEMICAL:

(b) (4)



(2) 1-β-D-1*H*-ribofuranosyl-1,2,4-triazole-3-carboxamide

STRUCTURAL FORMIULA: (2)



SUPPORTING DOCUMENTS: IND 33,988 and IND 49,923

BACKGROUND: Schering Corporation submitted this NDA in support of their application for use of a two drug combination consisting of Intron A (Interferon-alfa-2b, recombinant) plus Ribavirin vs. Intron A alone, for the treatment of chronic hepatitis C in individuals who have relapsed following a course of interferon-therapy. Intron A was previously approved for the treatment of hepatitis C infection and Ribavirin was approved for respiratory syncytial virus infections. In November, 1994, Ribavirin, however, was not approvable for monotherapy of chronic hepatitis C based on lack of clinical benefit (NDA # 20-442). Ribavirin was subsequently licensed to Schering Corporation for further development in the treatment of HCV infection.

The sponsor, Schering Corporation, requested a priority review of this application. The clinical portion of this application was submitted as a computer assisted new drug application. The requested indication is based on changes in virologic (HCV RNA), hepatohistopathologic (Knodell Score) and biochemical (alanine aminotransferase) surrogate markers scored as end-of-treatment response at 24 weeks of therapy and sustained response at 12 and 24 weeks of off-therapy follow-up.

In the microbiology portion of the submission the sponsor has not provided information on the mechanism of hepatitis C virus inhibition by interferon and/or ribavirin or the method for the determination of HCV RNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). However, after repeated oral and written requests, the sponsor submitted scant information related to the RT-PCR. The microbiology review here deals with 1. Virology of hepatitis C virus, 2. An analysis of the experimental RT-PCR for determination of HCV RNA, 3. Postulated mechanism of action of interferons and ribavirin and 4. HCV RNA determination in clinical studies.

1. HCV VIROLOGY: Hepatitis C virus has a short history. Until 1975 only two hepatitis viruses, hepatitis A virus and hepatitis B virus were known. A proposal for the potential existence of non-A and non-B hepatitis viruses was put forth in 1975 when it was discovered that some hepatitis patients lacked serological reactivity against hepatitis A and B viruses. The presence of non-A and non-B virus i.e., HCV, was confirmed in 1989 when the cDNA of HCV genome was cloned, characterized and a diagnostic test developed. Lack of permissive cell culture systems for in vitro cultivation of hepatitis C virus hampered studies on the replication and pathology of the virus. However, the use of molecular biology and biotechnology lead to a tremendous progress in HCV virology including its diagnosis. To date six hepatitis viruses, Hepatitis A, B, C, D, E, and G have been recognized and characterized.

HCV structure and genotypes: Hepatitis C viruses are a family of small (50nm) spherical enveloped viruses with single stranded linear RNA genome of positive polarity and hence the viral RNA can serve as a message for synthesis of viral proteins. The size of HCV genome is approximately 9,600 nucleotides (nt) consisting of a relatively long (332 to 341 nt) 5'-NTR (non-translated region), a large open reading frame encoding a polyprotein of >3,000 amino acids (~9033 nt) and a relatively short (45 nt.) 3'-NTR followed by homopolymeric tail of poly A or U residues.

The precursor polyprotein of HCV is proteolytically processed by the combined action of viral and cellular proteases to generate ten mature virion proteins. Three structural proteins; C, M and E (core, matrix and structural protein, respectively), localized at the 5'-end are involved in forming the viral particles and the seven nonstructural proteins NS1, NS2a, NS2b, NS3, NS4, NS4b and NS5, localized to the 3'-end are thought to be involved in the replication of the viral genome. The 5'-NTR of HCV is relatively the most conserved portion among all HCV isolates and as such it is often used for determination of HCV RNA by RT-PCR.

HCV being an RNA virus is highly mutable and accordingly, in infected individuals the HCV complex consists of a continuum of genetic variants. The extent of genetic variation in HCV-infected individuals is suggested by recent kinetic studies¹ which indicate a half-life of 8 hours for HCV with a turnover rate of $\geq 3.7 \times 10^{11}$ virions /day to maintain a serum virion concentration of 10^7 virions/ml. The high rate of replication and turnover of HCV at $\geq 3.7 \times 10^{11}$ virions/day far exceeds that of the HIV turnover rate of 10^8 to 10^9 virions/day. This extraordinarily large number of replication cycles in vivo results in evolution of highly divergent quasispecies of HCV and thus could challenge even the most promising antiviral agent(s).

Based on phylogenetic analysis of the coding regions, HCV has been classified into at least six distinct genotypes and the number of genotypes and their sub-types has been expanding as more isolates from different parts of the world are discovered and sequenced. HCV genotypes 1, 2 and 3 show a relatively broad geographic distribution, and 4, 5 and 6 are more restricted. Recent studies suggest that genotypes influence the efficiency of transmission and response to therapy. These studies were mostly based on assay procedures that detect and quantify HCV RNA genotypes with varying efficiencies² and lack of genotype specific quantitation makes the results unreliable.

Detection and diagnosis of HCV: To date there are no in vitro models to support the growth of HCV. Molecular biology techniques lead to the discovery of HCV in 1989 and biotechnology lead to our current ability to detect, diagnose and prevent HCV infection. There are two general categories of tests that define HCV infection: (1) Serological assays that can detect antibodies to HCV and (2) Molecular tests that claim to detect, quantify and characterize HCV RNA genomes.

Serological tests: Table 1 shows the progressive development of three generations of anti-HCV enzyme immunoassays (EIA) for the detection of HCV infection, their relative sensitivities and their positive predictive value. The first serological screening assay for detecting anti-HCV contained a single HCV antigen produced by recombinant DNA techniques. Although this assay was very useful in identifying individuals with serological evidence of HCV infection, it lacked optimal sensitivity and specificity. The EIA-1 test was subsequently replaced (Table 1) with EIA-2 and EIA-3 that contained additional viral epitopes which lead to substantial improvements in sensitivity and specificity of HCV antibody detection relative to the EIA-1. In addition, the latter EIA tests shortened the average 'window period' i.e., the time interval between primary HCV infection to seroconver-

¹ Lam, N. et al., Hepatology (1977) 26: 226-231

² Hawkins. A. et al., J Clin Microbiol (1977) 35: 187-192

sion, so that the mean time to detection was reduced to 10 weeks with EIA-2 compared to 16 weeks with EIA-1.

Table 1. Sensitivity and positive predictive value of EIA for anti-HCA*

Year ¹	Assay	Sensitivity	Positive Predictive Value (%)	
			Low prevalence	High prevalence
1990	EIA-1	70-80	30-50	70-85
1992	EIA-2	92-95	50-61	88-95
1997	EIA-3	97	25	Not done

* Most contents of the table are from ³

¹ Year of assay availability

The anti-HCV EIA tests were primarily designed to optimize the sensitivity of detection, thereby preventing transmission of HCV infection from blood and blood products and as such EIA tests were not optimized for exquisite specificity. Often these tests also showed false positivity in some individuals who had no other laboratory or clinical evidence of infection. To resolve false positivity of EIA tests, other supplementary confirmatory tests such as the recombinant immunoblot assays (RIBA), which were technically more demanding but conferring greater specificity compared to EIA, were developed and these additional tests have been used in some cases to aid in the diagnosis of HCV infections. Immunodetection methods including RIBA test are not always indicative of ongoing HCV infection because HCV recovered patients may remain HCV antibody positive for many years. Therefore, molecular tests such as RT-PCR diagnostic of ongoing HCV infection need to be used for confirmation of infection.

Molecular tests: Molecular tests directly detect HCV infection and are thus independent of seroconversion. These tests in addition to being confirmatory of HCV infection also detect infection early in the 'window period'. Available molecular assays for HCV can be divided into two types: 1. qualitative tests that detect the presence or absence of HCV RNA in test samples and 2. quantitative tests or viral load tests that determine quantity of HCV RNA in test samples. The molecular tests are based on two approaches: A. signal amplification based methods such as the Chiron b-DNA assay and B. sequence amplification methods such as the RT-PCR. Thus far none of the molecular tests have received approval by the FDA.

³ Gretch, D. R. Hepatology (1977) 26: 43S-47S

Qualitative assays: In the published literature many "home-brew" RT-PCR based qualitative assays have been described. These tests reported different degrees of sensitivity for the detection of HCV with the most sensitive tests claiming a capability of detecting 100 copies /ml. In general, the RT-PCR assays have played an important role in confirmation of HCV diagnosis and also in assessing antiviral response to therapies such as interferons. However, several attempts to standardize these assays showed lack of uniformity. For example, in a large international collaborative study⁴, 86 labs submitted 136 data forms on a panel of coded sera. In these data sets only 16% of the labs scored perfectly on the comprehensive standardized test panel, 55% reported false positive and/or false negative results and 29% missed weak positive sample. Thus, lack of rigorous quality control and proficiency testing of these "home-brew" assays render them often unreliable for use in clinical testing.

Quantitative tests: Two different types of techniques namely, RT-PCR, a sequence amplification method, and/or b-DNA, a signal amplification procedure⁵, have been used to determine HCV RNA concentration in infected individuals. Many labs have described their "home-brew" quantitative RT-PCR tests. Roche Monitor assay⁶ is perhaps the only quantitative (not FDA approved) RT-PCR assay available in a kit format. In general quantitative RT-PCR methods have high analytical sensitivity and the Monitor assay is claimed to measure HCV RNA at levels of 1000 copies/ml. The RT-PCR assays for the determination of HCV RNA have high variability and limited dynamic range (1 million copies/ml) and there is limited published experience with these methods. In the clinical studies conducted for this NDA the sponsor used the services of a testing laboratory for determination of HCV RNA in patient sera by an in-house RT-PCR procedure.

In the b-DNA signal amplification procedure HCV RNA in plasma or serum is captured in microtiter wells by hybridization to oligonucleotide probes complementary to the conserved 5'-NTR and the 5' third of the core region of the HCV genome. The amount of RNA captured is quantified by chemiluminescent signal amplification and the quantity is expressed as genome Eq/ml. This method is standardized and there is published experience in clinical studies. However, the hybridization efficiencies varies² among HCV genotypes and refinements on oligonucleotide probe became necessary to increase the sensitivity and specificity of the HCV RNA in clinical specimens. Moreover, the b-DNA-1 assay and the b-DNA-2 assay have low analytical sensitivity with a lower limit of quantification of 350,000 and 200,000 RNA equivalents/ml, respectively, and therefore RT-PCR is necessary on b-DNA negative specimens to rule out low-level of viremia.

⁴ Damen, M. et al., J. Virol. Meth. (1996) 58: 175-185

⁵ Detmer, J. et al., J. Clin. Microbiol. (1996) 34: 901-907

⁶ Nolte, F. S. J. Clin. Microbiol. (1995) 33: 1775-1778

In the clinical studies of this NDA the sponsor determined HCV RNA copy number by RT-PCR. In the estimation of HCV-RNA load the RNA copy number used as a reference standard was calculated by the b-DNA assay which expresses RNA as equivalents/ml. The sponsor pooled plasma samples from HCV positive individuals and the viral RNA equivalents/ml of plasma, as determined by b-DNA assay, was used as reference standard for HCV RNA copy number. HCV RNA in clinical trial patient sera was determined against the plasma control to calculate HCV RNA. A variety of inconsistent criteria renders this unapproved b-DNA assay inadequate as gold standard for calculating RNA copy number. At best this assay may represent a qualitative procedure for estimating HCV RNA in patient serum samples.

HCV genotyping: Published evidence suggests that HCV genotype may be an independent predictor of response to therapy and therefore genotyping of HCV has become an important aspect of clinical trials. Tests for HCV genotyping include: A. Restriction length polymorphism analysis⁷ of the conserved 5'-noncoding region of HCV; B. Line probe assay (LIPA)⁸ which is a dot blot hybridization analysis of 5'-noncoding region sequences; and C. Nested PCR analysis of the HCV core gene using genotype-specific probes⁹. These screening tests can characterize HCV genotypes and their subtypes. The sponsor in this NDA used the LIPA dot blot hybridization assay for genotyping of HCV.

2. DETERMINATION OF HCV RNA BY RT-PCR: The sponsor of this NDA, Schering Corporation, employed a testing service laboratory (b) (4) for the determination of HCV RNA in serum samples. HCV RNA was determined by coupled reverse transcription and polymerase chain reaction (RT-PCR). The RT-PCR is an experimental assay developed in-house by (b) (4) and was exclusively used by (b) (4) at their facilities. From the initial submission of the IND in 1996, FDA requested the sponsor (both orally and in writing) to provide details of the assay protocols and the performance characteristics of their assay for FDA evaluation. Written requests transmitted to the sponsor are attached to this review as Appendix 1 and Appendix 2. In July, 1997, FDA submitted a list of specific questions (see Appendix 1) regarding the sponsor's RT-PCR, and in response the sponsor in October, 1997, provided very general information on their assay without addressing the questions by data. They made summary statements of their protocols, what they did and the conclusions drawn without providing raw data. The sponsor's RT-PCR procedure essentially involves the following steps:

⁷ Simmonds, P. et al., J. Gen. Virol. (1993) 74: 661-668

⁸ Stuyver, L. et. al., J. Gen. Virol. (1993) 74: 1093-1102

⁹ Okamoto, H. et al., J. Gen. Virol. (1993) 73: 673-679

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Review comments on the RT-PCR protocol: There are numerous deficiencies in this experimental RT-PCR procedure and for brevity the comments are limited to a few aspects.

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B)

¹⁰ Lemon, S. M. and Honda, M. Seminars in Virol. (1997) 8: 274-288

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C)

The summary comment on this experimental RT-PCR assay is that it is a qualitative, in house assay with an undefinable number for the lower limit of detection of HCV RNA copies in serum samples.

3. MECHANISM OF ACTION: Neither ribavirin nor Intron A have well recognized targets on HCV to effect inhibition of the virus replication. However, these substances have been in use for other indications and multiple hypotheses have been extended to explain their effects. A brief summary of the proposed modes of action is presented.

Ribavirin: Ribavirin is a 'guanosine nucleoside analogue' which undergoes biotransformation into its mono, di and triphosphate forms in the body. These metabolic forms interfere either directly or indirectly with the nucleic acid biosynthesis of body cells as well as with infectious agents that infect or invade cells. Ribavirin monophosphate, for example, is a well recognized inhibitor of inosine monophosphate (IMP) dehydrogenase which is required for the synthesis of guanylate. The primary effect of IMP dehydrogenase is a reduction in intracellular guanylate nucleotide pools including GTP and dGTP, precursors in nucleic acid biosynthesis. Reduced supply of these precursors in the presence of ribavirin limits the rate of nucleic acid biosynthesis, a consequence that affects both cells and the infecting viruses. Furthermore, dGTP and/or GTP play critical roles in essential cellular signaling and alterations in levels in these molecules may adversely affect cellular metabolic pathways.

An additional recognized effect of ribavirin is its interference with mRNA 'cap' structure formation which is essential for maximal translational efficiency of both cellular and viral mRNAs. The mRNA capping involves the addition of guanosine phosphate on the 5'-ends of mRNAs and subsequent methylation of the guanylated site. Ribavirin inhibits the capping of mRNA either by decreasing guanylate pools or by direct interference with methyl transferases required for the capping process. The positive stranded HCV RNA which

naturally lacks 5'-cap structure is translationally competent and initiates protein synthesis by an alternate mechanism, through an internal ribosomal entry site. Therefore, ribavirin may not inhibit HCV by this mode of action i.e., through 'cap structure' mediated translation process. It is interesting to note that several clinical studies^{11,12} showed a lack of inhibitory effect of ribavirin on HCV viral load or improvements in hepatic histopathology. On the contrary ribavirin decreases levels of ALT and hemoglobin which are translated from capped mRNAs.

The precise molecular mechanism of action of ribavirin on cells and infectious agents is unknown. It is believed that the drug acts in a complex, multi-site fashion¹³. Unlike classical nucleoside analogues which inhibit nucleic acid biosynthesis by their incorporation and chain termination, ribavirin does not appear to incorporate into nucleic acids and thus its mechanism of action appears to be different from that of the classical nucleoside analogues.

Interferon alfa-2b, recombinant: Interferon alfa-2b is a water soluble protein of 165 amino acids with a molecular mass of 19,271 daltons. The protein is recombinant DNA product expressed in *E.coli* from a plasmid bearing cloned interferon alfa-2b DNA sequence derived from human leukocytes.

Interferons are biological response modifiers. They exhibit redundant functions with overlapping activities on a range of responding cells with their effects varying depending on the tissue. Interferons both up and down regulate gene expression, exhibit both beneficial and detrimental effects and play roles in both physiology and pathology. Genetic and biochemical evidence suggests that the diverse functions of interferons include antiproliferative, antiviral, immunomodulatory and central nervous system effects¹⁴. Current evidence supports that interferons exert their cellular activities by binding to specific membrane receptors on the cell surface. Upon binding to the cell membrane, interferons initiate a complex sequence of intracellular events through signal transduction pathways¹⁵ that lead to the expression of numerous interferon induced gene products. However, the specific mechanism by which interferon exerts anti-HCV activity is unknown.

¹¹ Di Bisceglie, A. M. Ann. Intern. Med. (1995) 123: 897-903

¹² Dusheiko, G. J. Hepatol. (1996) 25: 591-598

¹³ Patterson, J. L. and Fernandez-Larsson, R. Rev. Infect. Dis. (1990) 20: 1139-1146

¹⁴ Gutterman, J. A. Proc. Natl. Acad. Sci. (1994) 91: 1198-1205

¹⁵ Ransohoff, R. M. N. Engl. J. Med. (1998) 338: 616-618

The specific mechanism of inhibition by ribavirin or Intron A and/or enhancement of inhibition by the combination use of ribavirin and Interferon alfa-2b, recombinant is unknown.

4. HCV RNA DETERMINATION IN CLINICAL STUDIES: The sponsor conducted two similar Phase 3 clinical studies: 1. C95-144, a U.S. trial (n=153), and 2. C95-145, an international trial (n=192). These studies were double blind, placebo-controlled, randomized, parallel-group, multi-center trials, comparing interferon monotherapy with interferon plus ribavirin combination therapy, in patients with chronic hepatitis C who had relapsed following a previous response to a course of interferon. In the two studies HCV-infected individuals were equally randomized to receive either: Interferon 3MU TIW for 24 weeks or Interferon 3MU TIW + Ribavirin, 1000-1200 mg/day (depending on weight) for 24 weeks followed by 24 weeks of off-therapy follow-up.

Virologic criteria for enrollment into these studies was serum HCV RNA positivity as determined by an experimental, in-house, RT-PCR assay. With regard to virology the enrolled patients were stratified by two variables: 1. baseline RNA level, into those bearing $\leq 2 \times 10^6$ RNA copies/ml or $> 2 \times 10^6$ RNA copies/ml and 2. HCV genotype, into those bearing the genotype-1 (which included genotype-1 plus mixed genotype) and those bearing other than the genotype-1.

Serum HCV RNA was evaluated at weeks 4, 12, 24 during therapy and at weeks 12 and 24 following the end of therapy. One of the efficacy endpoints was sustained virological response. A virologic responder was defined as a subject whose HCV RNA was below the assay limit of detection at the time point tested. A sustained virologic responder was defined by the sponsor as a subject whose HCV RNA falls below the assay limit of detection at end of 24 weeks of follow-up. FDA modified the sponsor's definition of sustained responders to include those that responded at the end of treatment period of 24 weeks, and at 12 and 24 weeks of post-therapy follow-up. If HCV RNA was detectable by the experimental RT-PCR at any of the 3 time points, then the subject was considered as a non-responder. In these studies the sponsor also evaluated the response rates on the basis of genotypes and viral load.

In the determination of HCV RNA in patient sera an experimental in-house RT-PCR assay was used. The sponsor indicated that their RT-PCR is a dichotomous assay in which the assay was qualitative when the RNA copy number was below 10^4 RNA copies/ml of serum and quantitative assay at and above 10^4 RNA copies/ml of serum. The sponsor claimed that the lower limit of detection of the qualitative assay was 100 copies of RNA /ml and the linear range for the quantitative assay was 10^4 to 10^6 copies of RNA/ ml of serum. FDA

evaluation of their assay showed that the procedure in its entirety was qualitative by design and that there were numerous deficiencies in the protocol to support a claim that this was a quantitative method. Right from the submission of the IND in 1996 till now the sponsor was requested to provide details of the assay protocol and its performance characteristics. The scant information that the sponsor recently provided was inadequate to suggest that it is a quantitative procedure.

With regard to the assay's lower limit of detection it has been difficult to project a specific number because of multiple deficiencies such as the uncertainty of reference copy number control, lack of other controls to evaluate the efficiency of RNA extraction, reverse transcription, DNA amplification and DNA detection. Therefore, for the purpose of this analysis it is simply stated as "lower limit of detection of the assay". The lower limit of detection was further complicated due to the use of different matrices; **plasma** for HCV RNA as copy number as reference control and **serum** as test sample to determine HCV RNA in patients. The RNA equivalents/ml estimated from b-DNA assay was considered synonymous with the RNA copy number/ml estimated by the experimental RT-PCR assay. Additionally published evidence suggests that plasma is a better matrix for preserving RNA viruses than serum¹⁶ and the RNA copy number in plasma by RT-PCR score at higher levels than in serum¹⁷.

Genotyping of HCV was carried out by dot blot hybridization analysis of 5'-nontranslated region of the HCV RNA by Line Probe Assay (LIPA). The assay has been reported to detect all of the six HCV genotypes and their subtypes. The method is more of a screening procedure and is generally considered acceptable for genotyping. However, the gold standard for genotyping is sequence determination of the HCV RNA. The sequencing procedure for genotyping at this time is not practical for multiple samples and as such it was not used in these studies.

Prior clinical studies suggested that both HCV genotype and baseline viral load were useful in predicting a sustained treatment response to interferon monotherapy¹⁸. To assess this notion the sponsor stratified their clinical study population into genotype 1 and non-genotype 1. Based on the analysis of genotype response to therapy the sponsor suggested that the non-genotype 1 may respond better than the genotype 1 (see clinical and statistical reviews). With regard to response rates as a function of virus load, subjects with lower levels of HCV RNA appear to respond slightly better than those with higher levels of HCV

¹⁶ Ginocchio, C. C. J. Clin. Microbiol. (1997) 35: 2886-2893

¹⁷ Griffith, B. P. J. Clin. Microbiol. (1997) 35: 3288-3291

¹⁸ Martinot-Peignoux, M. et al., Hepatology (1995) 22: 1050-1056

RNA (see clinical and statistical reviews). However, a similar study¹⁹ comparing the effect of interferon alone with interferon plus ribavirin in previously untreated patients reported that sustained response rates in the interferon and ribavirin combination was independent of virus load.

Determination of HCV RNA in clinical samples by an experimental RT-PCR protocol adopted and conducted by the sponsor exclusively at their facilities by design (and in effect) was a qualitative procedure capable of scoring HCV positive samples from HCV negative samples. It was not possible to determine the assay's lower limit of detection of HCV RNA copies with the meager data supplied. Furthermore, the extent of false positivity and false negativity of the assay was unknown as the sponsor had not addressed this aspect of the assay. Thus, while the experimental RT-PCR procedure used in the clinical studies was capable of detecting HCV RNA, it was incapable of defining a lower limit for detection of HCV RNA copy number. Therefore, in the label where appropriate the HCV RNA response was indicated as being below the limit of detection using a research based RT-PCR assay without specifying a number for the HCV RNA copies.

Draft Microbiology label: *Mechanism of action: Interferon alfa-2b, recombinant/ribavirin.* The mechanism of inhibition of hepatitis C virus (HCV) RNA by combination therapy with INTRON A and REBETOL has not been established.

RECOMMENDATION: The microbiology portion of the draft label as currently written is acceptable. With respect to microbiology this NDA is approved.

(b) (6)

Narayana Battula, Ph.D.
Microbiologist

¹⁹ Reichard, O. et al., Lancet (1998) 351: 83-87

CONCURRENCES:

HFD-530/Deputy Dir

HFD-530/SMicro

CC:

HFD-530/Original IND

HFD-530/Division File

HFD-530/MO

HFD-530/Pharm

HFD-530/Chem

HFD-530/SMicro

HFD-530/Review Micro

HFD-530/CSO, Crescenzi, T.

(b) (6)

Signature 6/10/98 DateSignature 6/1/98 Date

APPENDIX 1

Micro comments conveyed to Schering on July 22, 1997

HCV RNA quantification by PCR

One of Schering-Plough's proposed primary endpoint in evaluating ribavirin and interferon combination treatment effects in HCV infected individuals is a fall of HCV RNA below detectable level (100 copies/ml of serum) as determined by an experimental quantitative PCR for HCV. The sponsor, however, has not provided any procedural details on the quantification of HCV RNA copies in plasma samples. Therefore, the sponsor is requested to HCV RNA provide in a step-wise fashion full details of the reagents, methods and procedures used to amplify, identify and quantify HCV RNA in the test subject's plasma samples. Below is a partial list of general methodology information requests for evaluating the specificity and selectivity of HCV RNA assay by PCR. Additional assay specific details may be requested after the initial assay information is received. Please insure to include complete assay details, all aspects of its performance characteristics and copies of all cited references for independent evaluation of your experimental procedure by the FDA.

1. Please indicate the manner of sample collection, transport storage and stability of HCV RNA during the course of these events
2. Please define the target sequence(s) selected for amplification and their suitability for quantifying different HCV genotypes
3. Please define the efficiency of conversion of HCV RNA into cDNA. Please indicate the primers and enzyme systems used
4. Please indicate the design and choice of the various components selected for DNA amplification such as the primers, probes, amplicons and their sizes, DNA polymerases, thermal cycling conditions and specific steps taken to prevent carryover contamination
5. Please submit information on the methods for identification, quantification of the amplicons, their reliability and reproducibility

6. Please define the amplification and quantification standards included as internal and parallel controls in each of the reaction
7. Please define the limits of sensitivity dynamic range (linearity), and reproducibility of the assay
8. Please evaluate the biological variability (intra and interindividual) and assay specific variability (intra and interassay)
9. Please indicate if and how this assay is amenable for "batch" testing
10. Please comment on the ability of your method to detect potential HCV variants and genotypes that may emerge due to inherent and intervention effects
11. Please indicate the method used to ensure that the sample tracking system traces the sample back to the original donation
12. Please indicate the manner of archival of plasma samples for future verification and reevaluation of HCV RNA copy number
13. Please indicate the qualifications of all of the reagents used in the assays and the assay performance characteristics
14. Please show the observed loss of HCV-specific information in each step of the assay from sample collection through HCV RNA quantification

APPENDIX 2

Micro comments conveyed to Schering on March 26, 1998

The procedure used to determine HCV RNA in plasma samples is dichotomous, consisting of a qualitative assay in which the HCV RNA in plasma was reported to be detected at 100 copies/ml and a quantitative assay in which the HCV RNA sequences in lab solutions were reported to be quantified in the range of 10^4 - 10^6 copies/ml. We previously requested performance characteristics of the assay(s) used in your NDA studies to be provided in sufficient detail to permit an independent review of the assay parameters. However, the data provided consisted primarily of a list of protocols and conclusions. Data relevant to the determination of the upper and lower limits of detection, precision, reproducibility, and linearity of the assay for a standardized virus preparation of known HCV RNA copies/ml were not provided. In addition, data relevant to several controls incorporated into the assay were not provided. Therefore, please provide the data for the specifically identified parameters as described below.

1. It is stated in amendment N237 (IC), attachment 7, page 143, paragraph three under *Materials* that, "the titer of this pool (HCV by b-DNA) was confirmed by several independent analyses and PCR analysis of samples at limiting dilutions." Please define these other methods and provide the data obtained relevant to determining the HCV RNA copy number for this pool.
2. Please include a detailed step-by-step procedure for the qualitative assay, starting with plasma (including volumes used) through the detection of DNAs. Please submit a few representative immunoblots (not cut-outs) that show the test samples, pAW and other in-process internal and external controls. Please specify the amplification conditions such as, cycling temperatures, cycling times, and probes used to identify different controls. (Please note that previous submissions contained inconsistent cycling times, temperatures, and probes). Please limit your response to the characterization of the assay(s) used for your NDA studies.
3. As in item 2, please submit the same information for the quantitative procedure.
4. In your previous submission, the pAW copy number reported varied between assays. In addition, a description of the amplification and detection for pAW RNA and DNA

was not defined. Please define the input copy number and amplification and detection conditions for the positive controls, pAW RNA and DNA.

5. Please provide data that characterize the false positive and false negative rates reported for the assay. A sufficient number of sample analyses should be reported to permit a statistical evaluation of the results.
6. Please provide a summary table of the raw data used to construct the standard curve(s) for the assay's reported ability to detect HCV RNA in the range of 10^2 - 5×10^6 copies/ml. The table should show the dilution tested, the number of samples assayed at each dilution, the % detectable, mean $\pm$ standard deviation, %CV, and predicted value based upon dilution of your standard virus preparation. A graphical representation of each standard curve should be provided.