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PEGYLATED INTERFERON +/- RIBAVIRIN FOR CHILDREN WITH HCV (PEDS-C)

PROTOCOL

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A. SPECIFIC AIMS

The over-all goals of this research project are:

I. To assess the safety and efficacy of peginterferon alfa-2a (PEG-2a) in combination with ribavirin (RV) and PEG-2a alone for the treatment of chronic hepatitis C virus (CHC) infection in children.

II. To determine whether PEG-2a in combination with RV or PEG-2a alone will result in a higher sustained virologic response rate in children with CHC.

III. To examine the effects of PEG-2a (with or without concomitant RV) treatment on body mass index, body composition, and linear growth in children infected with hepatitis C.

IV. To characterize short- and long-term outcomes, including health-related QOL, cognitive and developmental and psychological functioning, and behavior in children treated with PEG-2a (with or without concomitant RV).

To accomplish these aims, we propose to do the following:

1. Establish a Multi-Center Group to Carry Out a Randomized, Placebo-Controlled Trial of PEG-2a in Combination with RV or PEG-2a Alone

Eleven pediatric liver centers with experience and expertise in caring for children with CHC will participate in this trial. Each center will provide a physician investigator and a study coordinator. These individuals will identify clinic sites within their institution and will follow established procedures for collecting, preparing, and shipping of all samples, for monitoring patient clinical and laboratory data, and for reporting adverse events. In addition, they will identify and coordinate the participation of an investigational pharmacy to store and distribute the research drugs..

2. Establish a Collaborative Infra-Structure with Hoffmann-La Roche, Inc (hereafter referred to as ROCHE, Drug Sponsor, the Clinical Research Organization (CRO) and Data Coordinating Center (DCC) for the Study, and NIDDK Through a U01 Cooperative Grant

Roche has agreed to supply the PEG-2a and RV, the cross-reference with their Investigational New Drug (IND) for these drugs, and the clinical chemistry and virology laboratory costs. Roche will also provide sufficient funding so that data management and study monitoring can be carried out. We have identified Maryland Medical Research Institute (MMRI) as the CRO and DCC for this study. MMRI will be responsible for over-all study co-ordination, for monitoring of adverse events, for developing a communication system for monitoring and reporting laboratory and clinical data, and for working with the Data and Safety Monitoring Board. The NIDDK will provide scientific oversite of the study and will participate regularly in the investigator meetings and monthly teleconferences.

3. Identify 112 Children with CHC Who Meet the Inclusion and Exclusion Criteria and Enroll Them in the Study

Each center will enroll at least 5 and no more than 15 children, up to 112 total for the group of centers. (However, this policy will be reconsidered after six months of enrollment. If some sites are having difficulty in meeting the enrollment target and others are able to enroll more than 15 patients, the limitation of 15 patients per site will be removed.) Children 5-18 years of age (screening must be complete prior to 18th birthday) will be considered eligible for enrollment if they have CHC as defined by detectable HCV RNA by any test on 2 determinations at least 6 months apart, if they are able to swallow the RV capsule, if they are RV and interferon-naïve and if they meet the other inclusion and exclusion criteria detailed in the protocol. In addition, children must have compensated liver disease and histologic findings consistent with CHC on a liver biopsy obtained within 24 months prior to study screening as assessed by a qualified local pathologist. Signed informed consent will be obtained.

4. Randomize the Participating Children to Treatment with PEG-2a + RV or PEG-2a + Placebo

The children will be randomized to one of the two groups in a 1:1 ratio (PEG-2a +RV vs PEG-2a + placebo), according to a computer generated random code. Children in the PEG-2a + RV group and the PEG-2a + placebo group will be assessed at 24 weeks of therapy. If children in the PEG-2a + RV group do not exhibit viral disappearance (by the Roche AMPLICOR™ HCV PCR assay) at 24 weeks, PEG-2a and RV will be discontinued and the children will be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long -term follow-up period. If at 24 weeks they exhibit viral disappearance, they will be treated for an additional 24 weeks for a total of 48 weeks. These children will be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long -term follow-up period.

Children randomized to PEG–2a plus placebo who fail to exhibit viral disappearance at week 24 will be considered monotherapy treatment failures and will be moved to a compassionate "monotherapy/combination" arm with PEG-2a plus RV. This group will receive their first dose of RV at week 28. These children will be assessed after 24 weeks of combination therapy (week 52). If they do not exhibit viral disappearance after 24 weeks of combination therapy, PEG-2a and RV will be discontinued and the children will be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long -term follow-up period. If after 24 weeks of combination therapy they exhibit viral disappearance, they will be treated for an additional 24 weeks for a total of 48 weeks. They will then be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long -term follow-up period. Children will be treated with PEG-2a 180 mcg/1.73m^2 sq. weekly with placebo or with RV 15 mg/kg/day divided b.i.d. p.o.(maximum dose of 1200 mg/day if $\geq$ or equal to 75 kg and 1000 mg/day if <75 kg).

All children, regardless of treatment group or treatment outcome, will have two annual visits during the long-term follow-up period at one and two years after completion of treatment.

5. Examine Body Mass Index and Body Composition Changes in Children Treated with PEG-2a for Chronic Hepatitis C

All children will have serial measurements of body weight, height and body composition (DXA scans). In addition, changes in diet and physical activity during and after treatment will be assessed by a standardized questionnaire. Specific assessments will characterize changes in body mass index (BMI) and rate of weight gain, changes in body composition (absolute and relative lean body and fat mass) using dual-energy x-ray absorptiometry (DXA) and bioelectric impedance analysis (BIA), changes in total energy intake and physical activity and changes in linear growth velocity in all children treated with PEG-2a with or without concomitant RV.

6. Assess Health Related Quality of Life (QOL), Cognitive and Emotional Outcomes in Children Treated with PEG-2a and RV

Short- and long-term outcomes will be characterized including health-related QOL, and cognitive, developmental and psychological functioning. Assessments will be conducted at baseline (pre-randomization), at 24 weeks (before crossover, if applicable), at 48 weeks, and at annual intervals for 2 years post-treatment.

7. Analyze the Data

For specific aims I and II, the primary analysis will be on an intent-to-treat basis in children originally randomized to either therapy. The primary endpoint will be Sustained Viral Response (SVR), non-detectable plasma HCV-RNA (by the AMPLICOR™ HCV PCR assay) at the end of the 24 week post treatment period (72 weeks after baseline visit).The secondary endpoint is sustained biochemical response defined as two consecutive normal serum ALT assessments taken at weeks 60 and 72. Safety endpoints to be assessed include vital signs, laboratory tests and clinical adverse events (AE's). Virological responses at intervals during and at the end of

treatment will also be assessed. The secondary analysis will be on the basis of ultimate assignment of treatment. Three groups are possible: PEG-2a monotherapy; PEG-2a RV, and compassionate monotherapy/combination.

The primary outcome variables for specific aim III are change in BMI, weight for age Z score, linear growth velocity and lean body mass evaluated at 48 weeks. A specific objective will be to determine the relationship between the primary endpoints and demographic, clinical, and biochemical variables including age, hepatitis disease activity, dietary intake, exercise, and treatment regimen.

The primary outcome variables for specific aim IV are changes in QOL, cognitive and developmental functioning and psychosocial and/or behavioral functioning as evaluated at 48 weeks.

The significance of this multi-center trial is that it would be the first large-scale randomized, placebo-controlled trial of antiviral therapy in children with CHC. This carefully executed study should thus provide essential data for rational and safe product development for the ~ 150,000 children in the United States with CHC.

B. BACKGROUND AND SIGNIFICANCE

Hepatitis C virus (HCV) infection is a global health problem. The importance of HCV infection lies in its propensity to cause insidiously progressive liver damage; chronic hepatitis develops in about three-quarters of those infected. In the United States alone, there are estimated to be nearly 4 million cases of chronic HCV infection, at least 20% of whom are expected to develop cirrhosis of the liver (1). Of these cases, approximately 25% will die from hepatic failure or require liver transplantation (2). Hepatocellular carcinoma has also been linked to chronic HCV infection (3).

The incidence and prevalence of HCV infection in children is not well defined. In the NHANES III survey of random US populations, the estimated prevalence of anti-HCV positive children was 0.2 (6 -12 years of age) to 0.4% in children 12 - 19 years of age = 132,000 (95%CI 51,000 – 355,000) (4). Given that chronic infection rates tend to be lower (~50%) among persons infected as children (5) compared to rates for adults, one could estimate that ~66,000 of the anti-body positive children were HCV RNA positive (95%CI 25,500 – 177,500). For children < 6 years of age, the prevalence could be estimated as follows: 4,000,000 births/year x 0.01 (prevalence of anti-HCV in pregnant women) = 40,000 anti-HCV positive women x 0.02 (weighted incidence from anti-HCV positive mothers) = 800 infants infected through peri-natal transmission x 0.5 (chronicity rate) = 400 chronic infections per year x 6 years = 2400 HCV-infected children < 6 years of age (6). Thus the prevalence of HCV infection in US children is less than 200,000 even using the 95%CI rates from NHANES III; children with HCV therefore represent an appropriate target for orphan drug development.

Although less is known about the natural history and pathobiology of HCV infection in pediatric patients, HCV has been shown to be an important viral pathogen in this population (7). HCV infection leads to chronic liver disease in a proportion of children. It has been suggested that HCV infection may run a more benign course in young patients than in adults (8). However, significant histologic liver disease, including cirrhosis has been observed in a proportion of children (9). In our centers, HCV infection has led to chronic liver failure requiring orthotopic liver transplantation in several children.

Recombinant interferons have been shown to normalize serum alanine aminotransferase (ALT) and reduce serum HCV RNA below detectable levels in up to 40-50% of adults by the end of treatment. However, the majority of these patients “relapse”, as manifested by elevations in serum ALT and reappearance of serum HCV RNA within 6 months after discontinuation of therapy. Thus sustained, long-term responses are achieved in only 8-35% of adults (10).

The use of different treatment regimens in published small, uncontrolled, clinical trials of interferon for chronic hepatitis C in children makes direct comparisons to adults difficult. However, reported sustained responses are better in children (30-60%) than adults (8-35%) (11). We have recently reviewed the English literature of interferon mono-therapy of children with chronic hepatitis C virus infection (CHC) and observed that published response rates in children may be 2-3-fold higher than those in adults (12). This is biologically plausible because, in general, children have a combination of characteristics known to predict a higher likelihood of response to interferon in adults: short duration of infection, low viral load, and pre-cirrhotic liver disease (13). Attempts to improve the virologic response have included the addition of RV to interferon and development of a type of long-acting interferon, Peg-2a, administration of which is associated with sustained blood levels for a week, thus permitting weekly administration of the drug.

RV is a guanosine analogue that inhibits the in vitro replication of a wide range of RNA and DNA viruses (14). As detailed in the Investigator Brochure in the Manual of Procedures, Roche has performed detailed pharmacokinetics of RV in adults. The drug was absorbed rapidly following oral administration of a single dose (mean Tmax = 1.5 hours), followed by rapid distribution and prolonged elimination phases. Absorption was extensive with approximately 10% of the radio-labeled dose excreted in the feces. Upon multiple-dose administration, RV accumulated extensively in plasma. A steady state was reached by approximately 4 weeks. Upon discontinuation of dose administration, the half-life was approximately 300 hours, probably reflecting slow elimination from non-plasma compartments. The bio-availability of a single oral 600mg dose of RV was

increased by co-administration of a high-fat meal. Thus in order to achieve optimal RV plasma concentrations, it is recommended that RV be taken with food (15).

Combination of RV with interferon increased sustained response rates in adults to 30-40%(16), and this combination therapy is now FDA-approved for adults and children 3 years and older. In 1993, Connor et al (17) reported on the safety, tolerance and pharmacokinetics of orally administered RV in children with human immunodeficiency virus infection. Peak concentrations in plasma were reached at 90 min after single oral doses. The mean systemic availability of oral RV was 43.5%. The drug was well tolerated. Christensson et al (18) administered interferon-alpha (5 x 106 U thrice weekly in combination with oral RV (15 mg/kg/day) to 11 children with malignancy in remission. Seven of the 11(64%) patients had sustained virological responses 6 and 12 months after therapy. Lackner et al (19) had similar results in a comparable population. Treatment-induced toxicity was moderate and reversible and included influenza-like symptoms and a decrease in blood counts in all 12 patients; hemolysis was noted in 4 and weight loss of >10% in 2 of the 12. The authors concluded that treatment with IFN-alpha and RV also seemed to be an effective and safe therapeutic option for children and adolescents with CHC after malignancy. In contrast, Suoglu et al (20) performed a similar study, in 12 patients and showed that although combination therapy was associated with improved end-of-treatment responses, SR rates were 30% in the combination group compared to 42% in the monotherapy group. These authors concluded that large-scale, controlled trials were necessary to determine whether or not combination therapy will be necessary for optimum treatment for children with CHC.

A recent pediatric trial deserves detailed commentary because of the larger numbers of children studied. Kelly et al (21) presented a preliminary report at the American Association for the Study of Liver Disease (AASLD) in October, 2001, on an uncontrolled study of RV 8, 12, or 15 mg/kg/day in combination with interferon-alpha 3MIU/m^2 (TIW) for 48 weeks in 61 children with CHC 5-16 years of age who were interferon-naïve or relapsers after interferon therapy. Only 2 children discontinued therapy because of adverse side effects. The most frequent reason for dose modification was neutropenia (5/61) followed by depression (4/61). No life threatening, adverse effects were noted. Children experienced a lower maximum hemoglobin drop in the first 8 weeks of therapy (-1.8 g/dL) vs. adults (-2.6g/dL). Maximum mean neutrophil drop during the same time frame was -1.5×10^9/L to -1.8×10^9/L. Sustained viral response rates 24 weeks post cessation of therapy were 33% for 8mg/kg/day, 35% for 12 mg/kg/day, and 45% for 15 mg/kg/day RV. The authors concluded that the combination of RV and thrice weekly interferon was safe and effective for children.

The results of the larger (n = 70) phase III trial of interferon-alpha plus RV which evolved out of the above study was presented at the AASLD in Boston in November 2002 by one of the investigators (RGP) in our proposed PEG-2a +/- RV study. Using interferon-alpha 2b 3MU/m^2 (TIW) plus RV (15 mg/kg/d div bid), the main results were as follows:

Based on intent-to-treat, 35/70 (49%) of all treated children had a sustained virologic response. A sustained virologic response occurred in 29/43 (67%) of children who received 80% of the interferon-alfa2b and RV doses and completed at least 38 weeks of treatment and in 5/27 (19%) who did not ($p<0.001$). A sustained virologic response was more common in children $\leq$ 12 years old (57% vs 30% for > 12 years, $p = 0.04$) and in those infected with HCV type 2/3 (82% vs 38% for HCV type 1, $p = 0.002$). HCV RNA levels $\leq$ 2 million copies/mL were associated with improved sustained virologic response rates in children infected with HCV type 1 (53% vs. 18% for HCV RNA > 2 million copies/mL, $p = 0.02$) but not in those infected with HCV type 2/3. The rate of sustained virologic response was not associated with ethnicity, gender, mode of acquisition, estimated duration of infection, baseline ALT or RV formulation. Adverse events included fever (n = 48), headache (n = 45), fatigue (n = 36), influenza-like symptoms (n = 9), anorexia (n = 38), weight loss (n = 22) and depression (n = 9). During treatment, anemia occurred in 10/70 (14%), neutropenia (ANC < 1000 cells/mm^3), in 19/70 (27%) and thrombocytopenia (platelet count <100K/mm^3) in 1/70 (1%). Adverse events led to dose modification in 14/70 (20%) and discontinuation in 5/70 (7%) of the treated children (22).

Roche has synthesized a pegylated version of interferon (IFN) alpha-2a by chemically conjugating one branched 40 kilodalton polyethylene glycol molecule to IFN alpha-2a (23). Compared with IFN alpha-2a, PEG-2a exhibits sustained absorption, decreased systemic clearance, and an approximately tenfold increase in serum half-life. This superior pharmacokinetic and pharmacodynamic profile, as detailed at length in the Investigator's

Brochure in the Manual of Procedures, allows weekly administration of the drug. As noted in the Brochure, the safety and efficacy of weekly PEG-2a has now been compared with thrice weekly IFN alpha-2a. The Investigator Brochure details the four large multi-center trials of the Roche PEG-2a in adults with CHC, involving a total of 1600 individuals [1] Study NV15495 and N-181406 - CHC with cirrhosis or transition to cirrhosis, n = 271, 30 centers); [2] Study NV15496 and N-181408 CHC with or without cirrhosis or transition to cirrhosis, n = 639, 52 centers [3] Study NV15497 and N-1811410 – CHC with or without cirrhosis or transition to cirrhosis, n = 531, 36 centers and [4] Study NV15489/N-181405. – CHC without cirrhosis, n = 159, 11 US centers. In general PEG-2a administration to adults with chronic HCV has resulted in improved virologic sustained response rates (30-40%) compared to IFN monotherapy (24). Patients with chronic HCV who are treated with PEG-2a who exhibit viral disappearance by week 12 have approximately a 60% chance of achieving a sustained viral response (Roche Study NV 15942).

The major safety results from the four monotherapy studies are as follows: the incidences and types of adverse events were similar among patients treated with PEG-2a and IFN alpha-2a. The most frequently reported adverse events were those often observed for interferon, including headache, fatigue, myalgia, pyrexia, rigors, arthralgia, nausea, alopecia, insomnia, diarrhea, abdominal pain, depression, and injection site reaction. Most of these events were of mild to moderate intensity. The most common serious adverse events were infections, psychiatric disorders, and gastrointestinal disorders and were seen with equal frequency in the PEG-2a (9%) and IFN (7%) groups. For any given treatment-related serious adverse event, the incidence was <1% in any group. Nine patients died and only two of the nine deaths were considered to be possibly or probably related to the study drug. Approximately 20% of both groups needed dose modification during treatment, most frequently due to neutropenia. Treatment was discontinued prematurely in 10% of the patients in both groups. Depression and fatigue were the two most common adverse events and thrombocytopenia and elevation in serum ALT concentration were the two most common laboratory abnormalities leading to premature treatment discontinuation. Most treatment-related serious adverse events resolved without sequelae.

The highest efficacy rates in adults with CHC have been achieved with the combination of PEG + RV, up to 56% sustained virologic clearance 6 months after cessation of therapy (25). As detailed in the Investigator Brochure, Roche has performed safety studies regarding PEG-2a and RV in a phase III, multicenter, randomized, controlled study comparing the efficacy and safety of PEG-2a plus RV combination therapy with that of IFN alpha-2b plus RV combination therapy and PEG-2a monotherapy. The studies were done in a general population of non-cirrhotic and cirrhotic adult patients with CHC. A total of 1149 patients were enrolled. The main safety findings were as follows: Most patients from all three treatment groups experienced adverse events during the study, most commonly events that were consistent with IFN monotherapy. The incidence of certain IFN-associated events (depression, psychiatric disorders, and some “flu-like” symptoms) was lower in the two PEG-2a treatment arms than in the combined IFN alpha-2b and RV treatment arm. Patients in the two PEG-2a treatment groups experienced slightly more frequent serious adverse events (12% for both groups) compared to the IFN alpha 2b plus RV group (9%). Three patients died – none of the deaths were considered secondary to the treatments. 27-32% of the PEG groups had dosage modification compared to 18% of the IFN alpha-2b combination therapy group. As a result of adverse events or laboratory abnormalities, study drug treatment was discontinued prematurely in 10-11% of the 2 combination therapy groups compared with 7% of the PEG-2a monotherapy group, most commonly due to psychiatric disorders. Median neutrophil counts, platelet counts, and hemoglobin concentrations rapidly declined in the first few weeks of treatment and returned gradually toward their pretreatment baselines after the end of the 48 week treatment period. For most body systems, the incidence of serious adverse events was <1%.

PEG- 2a + RV was recently approved by the FDA for use in adults 18 years and older. Although the above experience in adults suggests that PEG plus RV is of greatest efficacy and results in children may well be similar, it is reasonable to predict that response rates of children to the pegylated form of interferon alone may also be superior to that observed in adults. Thus a major purpose of the present proposal is to perform a prospective trial to investigate whether or not the addition of RV to PEG-2a is necessary to achieve the highest efficacy in young subjects.

The importance of this aim is that a major potential problem in the treatment of subjects with CHC under the age of 18 years of age is that RV is a teratogen (26-28) and embyrotoxin (29). Thus great caution should be used

when treating women of childbearing age with this drug. If our proposed studies demonstrate that combination therapy yields the best response rates, then this type of therapy would be predicted to yield the greatest public health benefits by preventing the consequences of chronic CHC. On the other hand, if efficacy rates are comparable, then it would of great public health benefit to avoid the use of this teratogenic drug in young women of childbearing age. Since the combination has already been released to the public under FDA approval, without this trial, it is highly likely that there will be indiscriminant uncontrolled use of this combination therapy in young subjects.

Additionally, concern about potential side effects of IFN-alpha in children has hampered the process of studying this agent as a treatment for HCV in this population. It has been noted that weight loss, slow weight gain, and changes in linear growth occur in children treated with IFN-alpha for either chronic hepatitis B or HCV (30-31). Some reversibility of these effects has been documented after cessation of treatment. However, few details are known about this side effect. In particular, whether the changes in weight represent changes in body composition, with preferential loss of lean body mass or fat, has never been studied.

Preservation and/or augmentation of lean body mass has been linked with improved health outcomes in a number of settings including recovery from protein-energy malnutrition, HIV infection, cystic fibrosis, and a variety of other chronic illnesses (32-35). Known determinants of lean body and fat mass include age, dietary intake (energy, macronutrients, micronutrients), exercise, medication use, neurohormonal factors, and likely genetic factors. The possible effect of IFN on changes in weight, height, and body composition is an important factor to consider when considering the relative benefits and risks of this type of therapy in children.

Changes in body weight, growth velocity, and body composition of the children who will be receiving treatment will be characterized. This prospective cohort study will allow the identification of significant correlates of altered body composition among children with HCV receiving PEG-2a, thereby suggesting possible dietary, medical or lifestyle interventions aimed at preserving or augmenting lean body mass.

Concern about potential side effects of IFN in children has hampered the process of studying this agent as a treatment for HCV in this population. In adults, IFN-based regimens result in significant rates of psychological disorders, including depression (36-38). While the addition of RV to IFN has been associated with enhanced rates of virologic response, this improved outcome has been associated with higher rates of irritability and depression compared to IFN alone (39). The onset of affective symptoms is associated with poor compliance and the need to decrease or stop antiviral therapy (36). In addition, there is growing empirical evidence that HCV in adults is strongly associated with decrements in health-related QOL and cognitive functioning, and the relative impact of IFN on these parameters is unknown (40-42).

Despite the potential effectiveness of IFN and RV therapies, the impact of these treatments on children's health-related QOL, cognitive and developmental functioning, and psychological functioning is not currently known. Furthermore, there has been no systematic attempt to integrate these outcomes into controlled clinical trials involving children with HCV. In the only published study examining QOL in children with HCV, Iorio et al. found that while tolerability was not a limiting factor in IFN treatment in children, health related QOL deteriorated significantly during treatment and returned to baseline within 3 months of stopping IFN (43). These investigators noted a 3 to 4 fold increases in irritability and all the psychosocial dimensions of the Sickness Impact Profiles (e.g. Social Interaction, Emotional Behavior, and Alertness Behavior) during treatment. These observations suggest that very significant morbidity may accompany IFN treatment in children as in adults.

The inclusion of QOL, cognitive, and psychological assessments in clinical trials involving children with HCV is important for several reasons. First, the impact of both the disease process and drug therapies on these parameters in children is not presently known and warrants careful investigation. Second, it is important to examine the differential effects on these variables of the different therapies that are available to treat HCV in children. Third, identifying the QOL, cognitive, and psychological sequelae associated with both the disease and therapies will allow health professionals to better inform patients and their guardians about the range of possible side-effects. Fourth, it is critically important that changes in any of these variables be carefully evaluated and monitored to appropriately characterize the child's academic and psychological needs throughout therapy. Finally, identifying the complete range of morbidity associated with HCV and IFN and RV therapies may facilitate the

development and implementation of possible psychotherapeutic or psychopharmacological interventions aimed at attenuating morbidity in these children.

HCV is a costly disease for children and their families, both in terms of financial costs as well as human suffering and social stigmatization. The Children's Liver Council of the American Liver Foundation ishas published a Pediatric Liver Research Agenda, in which the costs over the next decade for all US children with HCV infection are estimated (44). We projected that $160 million would be spent for anti-viral treatment and $6 million, for liver transplantation. Thus identification of safe and effective treatments for children suffering from the medical, psychological and social issues related to this infection is of great public health importance. Since children with HCV do not number in the millions, it has been difficult to identify resources to support large scale, randomized controlled clinical trials and most studies have involved only a small number of patients, often without a comparison group. Thus the proposed trial will provide essential data for product development for children with CHC and for pediatric labeling.

C. PRELIMINARY STUDIES/PROGRESS REPORT

The published experience of Roche with PEG-2a and PEG-2a + RV in adults have been detailed in the Background section. Thus Section C will concentrate on four additional issues:

1) Preliminary studies and the experience of the group of investigators and study pathologist in anti-viral trials, and in the area of growth and body composition.

2) Our analysis of the literature in English on interferon monotherapy of children with CHC to demonstrate that response rates are superior to those in adults with CHC.

3) Our pilot pharmacokinetic/pharmacodynamic(PKPD) study of PEG-2a in 14 children 2-8 years of age.

4) A summary of children with CHC in each of the 11 centers who meet inclusion and exclusion criteria for the study.

1) Preliminary studies and the experience of the group of investigators and study pathologist in anti-viral trials, and in the area of growth and body composition.

Each of the investigators invited to participate in this trial has demonstrated experience in the management of children with liver disease, and, in particular, in the management of children with CHC. Dr. Jonas of Harvard University was the Principal Investigator in a large-scale multi-center trial of lamivudine for children with chronic hepatitis B virus infection and several investigators in the proposed trial, including the P.I., participated (45). Dr. Rosenthal of UCSF and several other investigators participated in the first large-scale multi-national trial of alpha-interferon for children with chronic hepatitis B virus infection (46). Dr. Schwarz of Johns Hopkins University (PI of the proposed study) was the PI of the first FDA-approved trial of alpha-IFN for children with CHC and inherited disorders of coagulation (47).

Many of the investigators are involved in large scale studies of CHC in children, including Dr. Schwarz, the PI, who is PI of an NIH RO1 grant entitled: "Viral Hepatitis in Children of Injection Drug Users" in which she is doing HCV epidemiology in ~900 children 2-18 years of age in Baltimore City who are living in homeless shelters or transitional housing. Dr. Jonas has studied the epidemiology and natural history (48-49) in children with CHC. Dr. Mohan has been involved in a bloodbank lookback study. Dr. Smith is doing a large-scale study of maternal-fetal transmission of HCV. Drs Narkewicz, Molleston, Mohan, and Schwarz are currently involved with a PKPD study of PEG-2a in children 2-8 years of age. Dr. Gonzalez-Peralta has studied the significance of HCV infection in infants with neonatal cholestasis and is the leading investigator in the largest trial of interferon plus RV in children with CHC, results of this study have been summarized in the Background and Significance section (22). Dr. Murray has recently completed a study entitled "Prevalence of Hepatitis C Infection and Risk Factors in an Incarcerated Juvenile Population: A Pilot Study" (50).

Dr. Goodman, Chief of Hepatic Pathology of the Armed Forces Institute of Pathology, is an internationally recognized expert in hepatic pathology in general and in the various techniques to quantify hepatic histopathology and liver injury in hepatitis C. He is currently assessing hepatic pathology in the HALT-C trial, a multi-center prospective trial which is studying the effect on several interventions, including PEG-2a, on liver injury in several thousand adults with CHC followed over several years. He is thus eminently suited to assess the hepatic histopathology of children with CHC in the present study.

Decreases in weight and height z-scores have been demonstrated in children who received standard interferon and RV for the treatment of chronic hepatitis C (51). There have been no reports of analysis of body composition in this population. Dr. Jonas has treated children and adolescents with standard interferon-alfa as part of several clinical trials. Between 1993 and 1998, interferon monotherapy was used. Between 1999 and 2000, combination therapy (interferon and RV) was used. During those years, a total of 31 patients ages 5-19 years had data available for assessment of baseline weight, height and BMI as well as weight, height and BMI after 6 months of treatment. Fifteen of 31 subjects (48.4%) lost weight, and 22 of 31 (71%) had a decrease in BMI percentile. The mean (SD)

decrease in BMI was 0.70 (1.38) ($p < .01$), and the mean (SD) decrease in BMI percentile was 5.74 (11.93) ($p < .05$). These data suggest significant weight loss in a large number of children treated with interferon. This has not been further characterized, indicating the need for a prospective, inclusive trial in children.

Dr. Shepherd joined the Osteoporosis and Arthritis Research Group (OARG) a research group in the Department of Radiology at UCSF, in January of 1999 from Hologic where he was a Principal Scientist and had the management role of Science Support Coordinator. At Hologic, he played a key role in the company's development of bone density, body composition, and imaging products. He did the theoretical work used on how Hologic fan beam densitometers measure body mass and is the principal inventor in the associated patent. While at Hologic, he was instrumental in the design, construction, and validation of Hologic's Whole Body Phantom. He has been at UCSF for 4 years and is responsible for DXA data quality including the following studies: NHANES IV (22,000 subject-visits), NICHD BMD in Children Study (BMDCS) (1500 pediatric subject-visits), Glaser Pediatric Research Network's Alendrodate in Children Study (400 subject-visits). Professor Shepherd is now the Associate Technical Director of Osteoporosis and Arthritis Research Group (OARG), providing oversight and management for all technical aspects of the research, equipment testing and quality assurance work performed by the OARG.

2) An Analysis of Published Trials of Interferon Monotherapy in Children with Chronic Hepatitis C
Jacobson K, Murray K, Zellos A and Schwarz KB. (12).

BACKGROUND: There have been a number of pediatric interferon alpha (IFN-alfa) trials. The purpose of our study was to perform a critical review of these trials so as to determine: 1) end-of-treatment (ETR) and sustained response (SR) rates 2) predictors of response to therapy and 3) safety of and tolerance to IFN-alfa in children with CHC.

METHODS: Relevant studies in the English-language medical literature and abstracts (1/90-11/00) were identified by searching for manuscripts that contained the key words "children", "Hepatitis C", and "interferon." Trials were considered eligible for inclusion in this analysis if criteria for treatment included positive serum polymerase chain reaction for HCV RNA (HCV PCR).

RESULTS: Twenty published manuscripts of the use of IFN-alfa in children with CHC were found, of which twelve met our inclusion criteria. Twenty-two abstracts, of which seven met our inclusion criteria, were identified. In the 19 included trials, 366 treated and 105 untreated children were followed; five countries were represented. Average ETR was 54% (0-91%) and average SR was 36% (0-73%). SR in children with genotype 1 was 27% vs. 70% for non-genotype 1 ($p=0.001$). Five of 105 (5%) untreated controls exhibited spontaneous viral clearance.

CONCLUSIONS: To date, there is no published large-scale, multi-center, prospective, placebo-controlled randomized trial of the use of IFN-alfa in children with CHC. The data in this review suggest that IFN-alfa in children with CHC does have reasonable efficacy and safety. This review highlights the need for more systematic design of future pediatric CHC trials. Ideally, such trials would be large scale, prospective and controlled, and would include HCV genotype and viral load, histology, quality of life measures, systematic recording of adverse events and effects of therapy on growth and development.

The relevant data is shown in Tables 1 and 2:

Table 1. Clinical Data in Children with Chronic Hepatitis C Treated with Interferon-alpha

MANUSCRIPTS	IFN/ CONTROL	EXCLUDED	AGE RANGE (years)	% MALE	IFN DURATION (weeks)	FOLLOW UP (weeks)
Bortollotti, F (1995)	14/13	4/0	2-14	50	52	52
Cesaro, S (1994)	6/0	3/0	11-17	67	52	52
Clemente, M (1994)	51/14	0/0	6-21	67	26+39 for responders	156
Fujisawa, T (1995)	18/0	1/0	Mean=10.1	78	24	52
Iorio, R (1996)	11/10	3/0	2.5-13	63	52	78
Jonas, M (1998)	23/0	2/0	2-17	52	26+26 for responders	52
Komatsu, H (1996)	13/0	1/0	5-17	92	24	78
Marcellini, M (1997)	10/0	0/0	5-18	N/A	26	26
Matsuoka, S (1997)	22/0	0/0	6-18	59	28	26 or 52
Pensati, P (1999)	15/0	0/0	2.8-14.5	59	52	76
Sawada, A (1998)	26/0	2/0	3-18	71	24	104
Zwiener, R (1996)	5/0	0/0	2-15	100	24	52
Manuscript Totals	**214/37**	**16/0**	**2 to 21**			
ABSTRACTS						
Fujisawa, T (2000)	37/68	0/0	3-20	73	24	156
Jara, P (1997)	35/0	5/0	4-19	N/A	52	26
Karyda, S (1997)	11/0	0/0	10-15	64	52	52
Ko, J (2000)	17/0	0/0	N/A	N/A	24	188.2
Schwarz, K (1997)	13/0	0/0	N/A	N/A	26	26
Sluzewski, W (2000)	16/0	0/0	2.5-15	N/A	24	96
Marcellini, M (2000)	23/0	0/0	6-18	48%	24	96
Abstract Totals	**152/68**	**5/0**	**N/A**			
Manuscript + Abstract Totals	**366/105**	**21/0**				

[a]Excluded in IFN treatment/control as inclusion criteria were not met

Table 2. Response to Interferon - alpha in Children with Chronic Hepatitis C

	IFN TREATMENT			CONTROL		
MANUSCRIPTS	**N**	**ETR[1] # (%)**	**SR[2] # (%)**	**N**	**ETR# (%)**	**SR#(%)**
Bortolotti, F (1995)	14	9 (64)	5 (36)	13	0 (0)	0 (0)
Cesaro, S (1993)	6	0 (0)	0 (0)	0	0 (0)	0 (0)
Clemente, M (1994)	51	21 (41)	19 (37)	14	0 (0)	0 (0)
Fujisawa, T (1995)	18	10 (56)	10 (56)	0	0 (0)	0 (0)
Iorio, R (1996)	11	5 (45)	5 (45)	11	1 (10)	1 (10)
Jonas, M (1998)	23	7 (30)	7 (30)	0	0 (0)	0 (0)
Komatsu, H (1996)	13	10 (77)	5 (38)	0	0 (0)	0 (0)
Marcellini, M (1997)	10	4 (40)	4 (40)	0	0 (0)	0 (0)
Matsuoka, S (1997)	22	15 (68)	8 (36)	0	0 (0)	0 (0)
Pensati, P (1999)	15	7 (47)	3 (20)	0	0 (0)	0 (0)
Sawada, A (1998)	26	18 (69)	12 (46)	0	0 (0)	0 (0)
Zwiener, R (1996)	5	2 (40)	0 (0)	0	0 (0)	0 (0)
Means/totals	**214**	**108 (50.5)**	**78 (36.4)**	**37**	**1 (2.7)**	**1 (2.7)**
ABSTRACTS						
Fujisawa, T (2000)	37	33 (89)	17 (46)	68	N/A	4 (6)
Jara, P (1997)	35	13 (37)	10 (29)	0	0 (0)	0 (0)
Karyda, S (1997)	11	10 (91)	8 (73)	0	0 (0)	0 (0)
Ko, J (2000)	17	9 (53)	7 (41)	0	0 (0)	0 (0)
Schwarz, K (1997)	13	6 (50)	1 (8)	0	0 (0)	0 (0)
Sluzewski, W (2000)	16	8 (50)	5 (31)	0	0	0
Marcellini, M (2000)	23	10 (43)	4 (17)	0	0	0
Means/totals	**152**	**89 (58.6)**	**52 (34.2)**	**68**	**N/A**	**4 (6)**

[1]ETR = end-of-treatment
[2]SR = sustained response 6 months or more after cessation of treatment

3) The Safety, Viral Kinetics and Pharmacokinetics of Pegasys™ after Multiples Doses in Young Children with Chronic Hepatitis C Infection. Roche Protocol Number NR16141. P.I. Kathleen B. Schwarz, M.D. (Johns Hopkins University); Co-investigators – M Narkewicz (University of Colorado), A Mohan (George Washington University), and J Molleston (Indiana University).

Our aim was to do a pilot study of the safety, viral kinetics and pharmacokinetics of PEG-2a in young children (2-8 years of age) with chronic HCV. This trial was developed in the spring of 2000, was approved by the Institutional Review Boards at all centers, and enrollment closed January 2002. 14 children were enrolled. The children enrolled in the trial are 2-8 years of age, have serologic evidence of chronic hepatitis C infection by an anti-HCV antibody test, serum HCV-RNA quantifiable at $\geq$ 600 IU/mL (by the Roche AMPLICOR HCV MONITOR™ Test), have chronic liver disease, without evidence of cirrhosis, consistent with chronic hepatitis C infection on a liver biopsy obtained within 24 months of screening as judged by a qualified pathologist, and have compensated liver disease (Child-Pugh Grade A clinical classification). Signed informed consent was obtained from legal guardian. (a complete copy of the protocol is provided in the Manual of Procedures).

Exclusion criteria were as follows:

Interferon or RV therapy at any previous time, any investigational drug $\leq$ 6 weeks prior to the first dose of study drug, any systemic antiviral therapy $\leq$ 6 weeks prior to the first dose of study drug (with the exception of patients who have taken or were expected to require acyclovir for herpetic lesions), positive test at screening for anti-HAV IgM Ab, HBsAg, anti-HBc IgMAb, or anti-HIV Ab, history or other evidence of a medical condition associated with chronic liver disease other than HCV (abnormal ceruloplasmin, alph-1-antitrypsin, ANA > 1:160, SMA > 1:80), history or other evidence of bleeding from esophageal varices, decompensated liver disease (e.g. conjugated bilirubin > 1.5 mg/dl, jaundice, ascites, varices, Child-Pugh Grade B or C clinical classification), history of immunologically mediated disease (e.g. inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, scleroderma, severe psoriasis, clinical evidence of rheumatoid arthritis), neutrophil count <1500 cells/mm^3, Hgb <10 g/dL, WBC >17.5 x 109/L, or platelet count <90,000 cells/mm^3, serum creatinine level >1.5 times the upper limit of normal or advanced renal disease at screening, major depression according to the DSM III, or a history of severe psychiatric disorder, such as major psychoses, suicidal ideation and/or suicidal attempt, history or other evidence of chronic pulmonary disease associated with functional limitation, current evidence of severe cardiac disease, history of thyroid disease poorly controlled on prescribed medications. Patients with elevated thyroid stimulating hormone (TSH) concentrations with elevation of antibodies to thyroid peroxidase and any clinical manifestations of thyroid disease were excluded. Other exclusion criteria include poorly controlled diabetes, autoimmune disease, evidence of severe retinopathy, history of solid organ transplantation with an existing functional graft, history of bone marrow transplantation, coagulopathy (PT >14.5 sec), evidence of an active or suspected cancer or a history of malignancy where the risk of recurrence is >20% within 2 years, and history of other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study.

The main outcome measures were as follows:

Viral kinetics – HCV-RNA levels
Pharmacokinetics – apparent total body clearance, volume of distribution and absorption rates
Safety-tolerability – vital signs, laboratory values, clinical adverse events and premature withdrawals for safety reasons; periodic review of data by Safety Review Board

PROCEDURES:

A screening period (time from the first screening assessment to the first administration of study drug) of up to 35 days preceded the treatment portion of the trial. RV and interferon naïve patients meeting all eligibility criteria were enrolled. Patients received subcutaneous injections of Pegasys™ (180 mcg/1.73m^2) once a week for 48 weeks. Vital signs, laboratory assessments, and clinical adverse events were monitored throughout the study. Blood samples were collected for pharmacokinetic analysis of serum concentrations of Pegasys™. Serial blood samples were collected at baseline, 24, 96, and 168 hours after the first dose of Pegasys™ administration and after

multiple doses of Pegasys™ administration in week 24 of the treatment period. In addition, pre-dose samples were collected at weeks 4, 8, 12, 40, 48, and 60. The exact date and time of dosing and collection of blood samples was recorded in the source document and Case Report Form. Population analysis was applied to assess pharmacokinetic parameters.

All patients completing the 48 weeks of treatment were assessed for an additional 24 weeks following treatment cessation for safety evaluations and to evaluate sustained response rates. Safety data were shared with a Safety Review board consisting of three experienced hepatologists who are advising the sponsor as to the safety and tolerability of Pegasys™ . The PK PD and safety data were analyzed when the study was completed and serves as a basis for the final PEG-2a dose for the multicenter trial.

Results to date which were presented at DDW May 2003 are summarized in the following abstract:

THE SAFETY, EFFICACY, AND PHARMACOKINETICS OF PEGINTERFERON ALFA-2a (40KD) IN CHILDREN WITH CHRONIC HEPATITIS C

Authors: Kathleen B. Schwarz, Johns Hopkins University School of Medicine, Baltimore, MD; Parvathi Mohan, Children's National Medical Center, Washington, DC; Michael Narkewicz, University of Colorado School of Medicine, Department of Pediatrics, Denver, CO; Jean Pappas Molleston, James Whitcomb Riley Hospital for Children, Indianapolis, IN; Helen S. Te, University of Chicago, Chicago, IL; Sylvia Hu, Susan Sheridan, Matthew W Lamb, Stephen C. Pappas, George Harb, Roche Laboratories, Inc., Nutley, NJ.

The role of antiviral therapy for chronic hepatitis C (CHC) in children remains unclear. Some studies have shown the safety and efficacy of standard interferon (IFN), with or without RV in children with CHC, but no data is available regarding the use of pegylated IFN in this population**.**
Objective: To investigate the safety, efficacy, and pharmacokinetics (PK) of peginterferon alfa-2a (40KD) in children with CHC.
Methods: This is a multicenter, open-label study of treatment-naïve children with established CHC. Children received peginterferon alfa-2a (40KD) once weekly for 48 weeks with a 24-week post treatment follow-up. The dose was normalized for patient body surface area (BSA) [(180 µg/1.73 m^2) X Patient BSA). Multiple blood samples were obtained to determine single (SD) and multiple dose (MD) PK. Adverse events (AEs) were assessed by clinical exam. HCV RNA was measured at weeks 12, 24, 48 and 72 using AMPLICOR MONITOR™ HCV Test v.2.0.
Results: Fourteen patients, 8 males, 6 females, aged 2-8 (median = 3.5) years were enrolled; majority (13/14) were Caucasian and genotype 1(12/13). CHC was acquired by vertical transmission in 11 patients. After administration of the first peginterferon alfa 2a (40KD) dose, there was rapid and sustained absorption with mean concentrations of 22.3 ± 8.2 ng/ml and 19.0 ± 8.4 ng/ml at 24 and 96 hours post dose. Mean steady state trough concentrations were 24.3 ± 13.7 ng/ml. Peginterferon alfa-2a (40KD) concentrations increased 1.5- 2.5- fold before reaching steady-state by week 12. At weeks 24, 48, and 72, 57% (8/14), 43% (6/14) and 38% (5/13) of patients were HCV RNA negative, respectively. Most frequently reported AEs were pyrexia, headache, vomiting, anorexia and abdominal pain; no serious AEs were observed. The majority of these AEs were mild in intensity. Four patients were withdrawn from therapy (1 due to lack of viral response at week 24, 2 due to elevated transaminases, 1 due to exacerbation of baseline hypertriglyceridemia). Five patients required dose reductions due to neutropenia.

Conclusions: Peginterferon alfa-2a (40KD) was well tolerated. Exposure to peginterferon alfa-2a (40KD) in pediatrics is slightly higher than in adults following a fixed 180 µg dose. The efficacy of peginterferon alfa-2a (40KD) monotherapy in children appears higher than that reported for adults and approaches that for standard IFN+RBV in children. These results support further study of peginterferon alfa-2a (40KD), with and without RBV, in children with CHC.

4) A summary of children with CHC in each of the 11 centers who meet inclusion and exclusion criteria for the study.

PI	Center	Number of Eligible patients
Kathleen Schwarz	Johns Hopkins University	21
Philip Rosenthal	University of California, San Francisco	49
Michael Narkewicz	The Children's HospitalUniversity of Colorado	28
Maureen Jonas	Children's Hospital BostonHarvard University	29
Jean Molleston	Indiana University	25
Parvathi Mohan	George Washington University	10
Karen Murray	University of Washington	25
Regino Gonzalez-Peralta	University of Florida	18
Barbara Haber	Children's Hospital of PhiladelphiaUniversity of Pennsylvania	39
Lesley Smith	Columbia University	8*
William Balistreri	University of Cincinnati	16
Total		**268**

D. RESEARCH DESIGN AND METHODS

This section details:

1. Objectives
2. Study Design
3. Schedule of Assessments and Procedures
4. Investigational Products
5. Safety Issues
6. Statistical Considerations and Analytical Plan
7. Data Coordinating Center Role in Training of Study Personnel
8. Receipt and Processing of Study Forms at the Data Coordinating Center
9. HIPAA Compliance
10. Monitoring Study Progress And Data Quality
11. Randomization and Study Drug Labeling
12. Study Documentation, CRFs And Record Keeping
13. Collaboration In Study Publications
14. Publications Committee

1. Objectives of the Study

a. Primary objective:

To assess the safety and efficacy of peginterferon alfa-2a (Pegasys™, PEG-2a) in combination with RV and PEG-2a alone for the treatment of chronic HCV in children

b. Secondary objectives:

i. To determine whether PEG-2a in combination with RV or PEG-2a alone will result in a higher sustained virologic response rate in children with CHC

ii. To compare the effects of PEG-2a (with or without concomitant RV) treatment on body mass index, body composition, and linear growth in children infected with hepatitis C

iii. To compare short- and long-term outcomes, including health-related QOL, cognitive and developmental and psychological functioning and behavior in children treated with PEG-2a (with or without concomitant RV)

2. Study Design

a. <u>Overview of Study Design and Dosing Regimen</u>

This study is a Phase III, multicenter, randomized, blinded (through the first 24 weeks of treatment), placebo-controlled trial to compare safety and efficacy of PEG-2a plus placebo vs. PEG-2a plus RV. It is a collaborative study by 11 university centers and the NIDDK. Roche is authorizing the FDA to cross reference their INDs BB-IND5102 PEGASYS™, BB-IND 7823 PEGASYS™ with RV, and IND 58,827 for RV. A total of 112 children 5-18 years of age will be enrolled in the study. Patients who have not previously been treated with RV or interferon and meeting all eligibility criteria will be enrolled after informed consent is obtained. Enrolled patients will receive a subcutaneous PEG-2a injection once a week given with placebo or RV tablet/s given orally once or twice daily, depending on the patient's weight. Patients will be randomized to PEG-2a plus placebo vs PEG-2a plus RV in a 1:1(50:50) ratio by a computer-generated randomization scheme. Randomization will be stratified by center and by HCV genotypes (I vs all others) so as to best balance the groups. Following the initiation of study drug, patients will return for evaluation at weeks 1,3,5, and 8, and then every 4 weeks thereafter, while receiving study drug therapy. The type of treatment for responders at week 24 will remain blinded until week 72 (the primary endpoint) at which time it will be unblinded to both families and investigators. For the non-responders at week 24 the type of treatment will be unblinded to both families and investigators at week 28.

Children randomized to PEG–2a plus placebo who exhibit viral disappearance at week 24 will be treated for a total of 48 weeks and followed closely for 24 weeks after discontinuation of therapy and all children will have 2 annual visits during the long-term follow-up period. Children randomized to PEG-2a plus placebo who fail to exhibit viral disappearance at week 24 will be considered monotherapy treatment failures and will be moved to a compassionate "monotherapy/combination" arm with PEG-2a plus RV. This group will receive their first dose of RV at week 28. These children will be assessed after 24 weeks of combination therapy (week 52). If they do not exhibit viral disappearance after 24 weeks of combination therapy, PEG-2a and RV will be discontinued and the children will be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long-term follow-up period. If after 24 weeks of combination therapy they exhibit viral disappearance, they will be treated for an additional 24 weeks (for a total of 48 weeks) and then followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long-term follow-up period. All children, regardless of treatment group or treatment outcome, will have two annual visits during the long-term follow-up period at one and two years after completion of treatment.

The rationale for adding RV is the experience in adults with HCV that treatment with the combination of PEG-2a plus RV is associated with the highest viral clearance rates. An additional rationale for adding RV is that the combination PEG-2a plus RV therapy is already available in the market place and it is well known in the HCV community that the highest viral clearance rates in adults have been achieved with combination therapy. Rebetol® (ribavirin, USP) Oral Solution to be used in combination with Intron A® has recently been FDA approved for the treatment of chronic hepatitis C among previously untreated pediatric patients at least three years of age and older. This group of pediatric investigators believes that recruitment will be impossible without the provision to add RV.

Children randomized to PEG-2a plus RV who fail to exhibit viral disappearance at week 24 will be considered combination therapy failures. PEG-2a and RV will be discontinued and the children will be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits during the long -term follow-up period. If at 24 weeks children receiving combination therapy they exhibit viral disappearance they will be treated for an additional 24 weeks, for a total of 48 weeks. They will then be followed closely for 24 weeks after discontinuation of medications and all children will have 2 annual visits for long-term follow-up.

Children will be treated with PEG-2a 180 mcg/1.73m^2 sq. weekly with placebo or with RV 15 mg/kg/day divided b.i.d. p.o.(maximum dose of 1200 mg/day if $\geq$ or equal to 75 kg and 1000 mg/day if <75 kg). Younger children may receive RV once daily depending on their weight. Participating patients will complete treatment, unless study drug is discontinued because of intolerance, investigator withdrawal of the patient or consent is withdrawn. Patients will continue to be evaluated closely for 24 weeks from the end of treatment and all children will have 2 annual visits during the long-term follow-up period. Patients who discontinue treatment will be monitored and followed up in the same schedule as those undergoing treatment. All subjects will have a follow-up study visit at 72 weeks to include interim history, physical exam, laboratory analysis, review of adverse events, depression assessment, growth and body composition measurements and QOL testing. Please see diagram of study design in this section. The long-term follow-up annual visits will include all clinical and laboratory assessments included in the week 72 visit except for thyroid function tests, serum pregnancy test and patient diary review.

Since ~ 60% of children will receive at least 48 weeks of PEG-2a, primary outcome observations for growth and body composition will be made at baseline, 24 weeks and after the 48 weeks. Some patients will have 76 weeks of PEG-2a treatment, and all will be followed for 24 weeks after the end of treatment. Observations will be recorded 24 weeks after the end of treatment for secondary analyses.

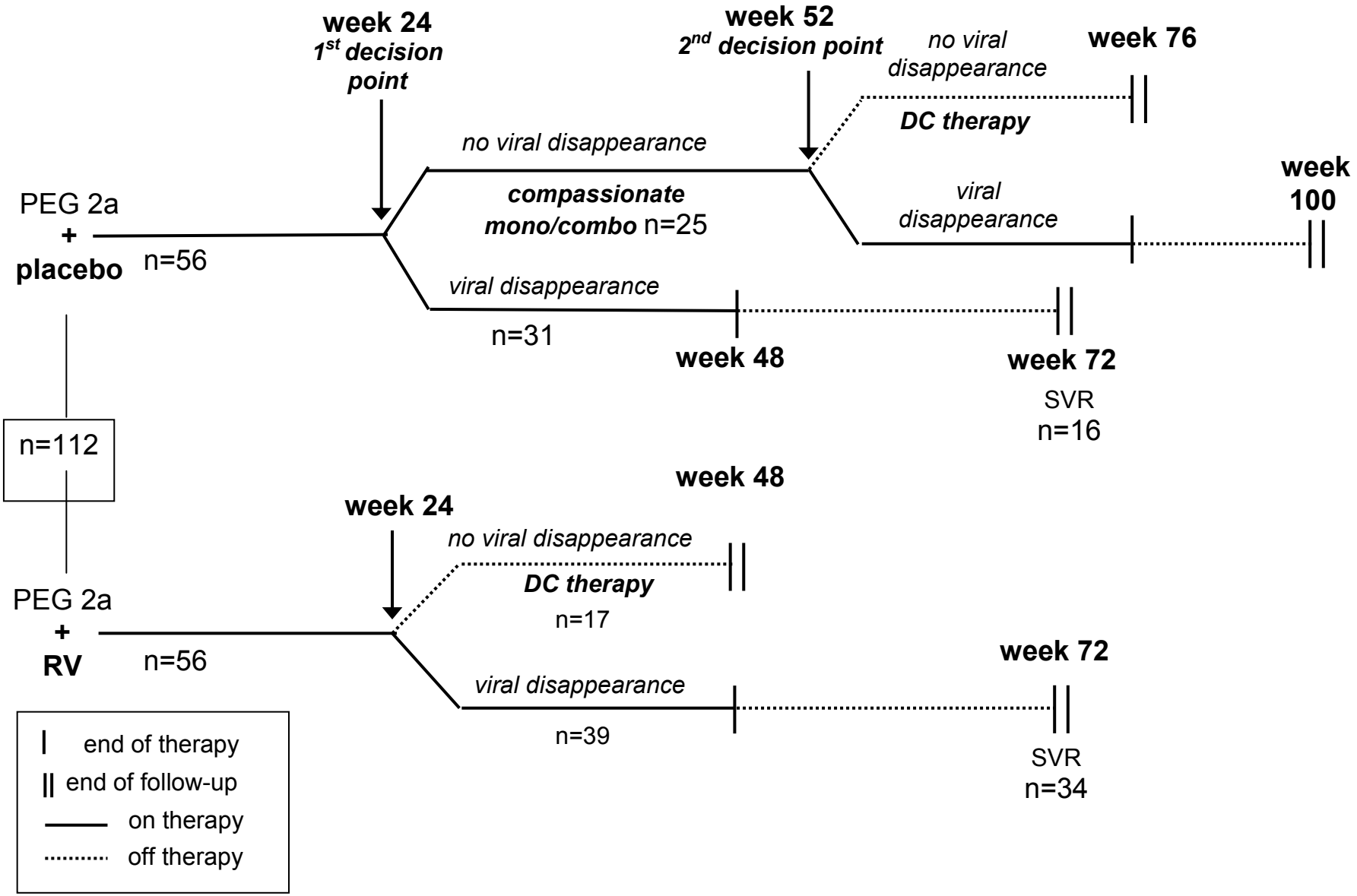
The health-related QOL, cognitive and developmental and psychological functioning in the 112 children with HCV will be examined. Various combinations of these outcomes will be assessed at baseline (pre-randomization), at 24 weeks (before crossover, if applicable), at 48 weeks, and at annual intervals for 2 years post-treatment.

The assessment protocol for this sub-project is designed to evaluate the child's health-related QOL, cognitive and developmental and psychological functioning. Some of the constructs will be measured from multiple sources (e.g., child and guardian) depending on child age. All assessment tools have been selected on the basis of their demonstrated reliability and validity.

Screening will begin within 35 days prior to the first dose of study drug. To be eligible, patients must demonstrate evidence of HCV viremia by any test on two occasions separated by at least 6 months and a liver biopsy within 24 months of screening consistent with CHC as assessed by a qualified local pathologist.

Patients must not have previously been treated with RV or interferon. Patients with other forms of liver disease, anemia, hemoglobinopathy, hemophilia, HIV infection, hepatocellular carcinoma, pre-existing severe depression or other psychiatric disease, active cardiac disease or advanced renal disease are excluded.

The overall study design is diagrammed on p. 20, using n = 56 for each of the two initial treatment groups (see Sample Size calculations, Sections 6a). The underlying assumptions are as follows: For the PEG monotherapy group, NR = 45%; SVR = 30%; and relapse = 25%. For the PEG + RV group, NR = 30%, SVR = 60%, and relapse = 10%.


week 24
1st decision point
week 52
2nd decision point
no viral disappearance
week 76
DC therapy
no viral disappearance
compassionate mono/combo n=25
viral disappearance
week 100
PEG 2a
+
placebo
n=56
viral disappearance
n=31
week 48
week 72
SVR
n=16
n=112
week 48
week 24
no viral disappearance
DC therapy
n=17
PEG 2a
+
RV
n=56
week 72
viral disappearance
n=39
SVR
n=34
end of therapy
end of follow-up
on therapy
off therapy

b. Assessments of Efficacy and Safety

Efficacy

Primary: Non-detectable plasma HCV-RNA (by the AMPLICOR™ qualitative HCV RNA assay) at the end of the 24 week post treatment period (72 weeks after baseline visit).

Secondary: Sustained biochemical responses (two consecutive normal serum ALT assessments at week 60 and week 72.

Safety

Vital signs, laboratory tests and clinical adverse events (AEs)

c. Study Duration and Early Termination

It is anticipated that in January 2005 the first patient will be enrolled in this trial. Recruitment time is scheduled for 12 months. Maximum treatment plus follow-up time will be 100 weeks; thus the last visit of the last patient is expected in November 2007 for the 3 year treatment protocol and November 2009 for Years 4 and 5 for the long-term follow-up visits.

If the study is discontinued prematurely for any reason, the reason will be documented and the date of final data collection will be announced (clinical cut-off). The study monitor will arrange to visit the investigators shortly after this cut-off date. The investigators will ensure that all Case Report Forms are completed with all data collected prior to the date of clinical cut-off and that a final assessment of each patient is performed before the monitor's visit. Data analysis will be performed and a Final Study Report will be prepared as soon as possible using all data collected before clinical cut-off.

d. Data Safety Monitoring Board (DSMB) Functions and Reports

DSMB Functions

The DSMB will monitor the study for the occurrence of adverse events (both serious and otherwise) in the two treatment groups. A significant increase in the rate of adverse events in one treatment group would be cause for concern for the safety of participants in the study. Information on adverse events will be presented in several ways: (1) listings of serious adverse events in the two treatment groups with accompanying narrative summary by the clinic PI or appropriate medical staff; (2) summaries of adverse events by body system and type of event. This information will be presented by blinded treatment group (i.e., Treatment A and Treatment B) to the DSMB.

The DSMB will also monitor the efficacy and the conduct of the study. Interim monitoring reports for the DSMB will include, but will not be limited to, such tabulations by blinded treatment group as:

1. Comparison of proportion of participants with non-detectable plasma HCV-RNA at the end of 24 week post treatment period (primary outcome);
2. Proportion of participants with sustained biochemical responses at weeks 60 and 72;
3. Comparison of vital signs, hematological and chemical determinations over the course of the study (weeks 1-72) with appropriate longitudinal models to identify any points of significant differences;
4. Comparison of mean depression (CDI) total scores and subscale scores at weeks 12, 24, 48
5. Comparison of urinalysis results at weeks 12, 24, 36, 48, and 72;
6. Baseline description of participants;
7. Recruitment (as appropriate) and follow-up rates by clinical site;
8. Data completion rates by clinical site; and
9. Adverse event tabulations and serious adverse event listings and tabulations.

The DSMB will meet at least twice per year and possibly more frequently given the expected speed of recruitment and study medication administration.

DSMB Members to be named by NIDDK.

A safety analysis will be performed after approximately 30 patients have received 4 weeks of treatment or, if enrollment begins slowly, 3 months after the first patient is enrolled. Safety data will be shared with the DSMB. If there are significant safety issues, the study will be unblinded to the DSMB who will re-analyze the data as per treatment group. There will be no further enrollment until the DSMB makes recommendations. An investigator in this study may not be a member of the DSMB. Members of the DSMB will receive safety data approximately every 2 to 3 months for review (schedule to be agreed upon between members of the DSMB and MMRI). Safety assessments will consist of vital signs, AEs, and laboratory evaluations. A cumulative listing of patient withdrawals, dose adjustments, and serious adverse events (SAEs) will also be reviewed. DSMB members will be notified of all SAEs reported expeditiously to regulatory authorities. A copy of the DSMB charter is provided in the Manual of Procedures.

e. Inclusion criteria

* Male or female patients who are 5-18 years of age
* HCV viremia (by any test) present on 2 tests separated by at least 6 months
* Chronic liver disease, as indicated by inflammation and/or fibrosis, consistent with chronic hepatitis C virus infection on a liver biopsy obtained within 24 months of screening, as assessed by a qualified local pathologist, not consistent with other known liver disease and not normal
* Compensated liver disease (Child-Pugh Grade A clinical classification)
* Signed informed consent from legal guardian and willingness of legal guardian to abide by the requirements of the study
* Hemoglobin values >11 g/dL for females;> 12 g/dL for males
* Normal TSH
* Able to swallow a 100 mg tablet
* Demonstration of ability to swallow a placebo tablet

f. Exclusion criteria

* Any prior treatment with Interferon or RV
* Receipt of any investigational drug <6 weeks prior to the first dose of study drug
* Any systemic antiviral therapy <6 weeks prior to the first dose of study drug. Exception: patients who have taken or are expected to require acyclovir for herpetic lesions
* Positive test at screening for anti-HAV IgM Ab, HBsAg, anti-HBc IgM Ab, or anti-HIV Ab
* History or other evidence of a medical condition associated with chronic liver disease other than HCV (abnormal ceruloplasmin, alpha-1-antitrypsin, ANA>1:160, SMA>1:80, anti LKM antibody > 60 units)
* History or other evidence of bleeding from esophageal varices
* Decompensated liver disease (e.g. conjugated bilirubin >1.5mg/dl, ascites, varices, Child-Pugh Grade B or C clinical classification)
* History of autoimmune or immunologically mediated disease (e.g. inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, scleroderma, severe psoriasis, clinical evidence of rheumatoid arthritis)
* Absolute neutrophil count <1500 cells/mm^3 , Hgb <11 g/dL for females and <12 g/dL for males, WBC>17.5 x 10^9/L, or platelet count <90,000/mm^3
* Serum creatinine level >1.5 times the upper limit of normal for age
* Major depression according to the American Psychiatric Association (see Table 7, Criteria for Major Depressive Episode) or a history of severe psychiatric disorder, such as major psychoses, suicidal ideation and/or suicidal attempt
* History or other evidence of chronic pulmonary or cardiac disease associated with functional limitation
* History of thyroid disease poorly controlled on prescribed medications. Patients with elevated thyroid stimulating hormone (TSH) concentrations with elevation of antibodies to thyroid peroxidase and any clinical manifestations of thyroid disease are excluded

* Poorly controlled diabetes as defined by hemoglobin A1C of > 8%
* History of solid organ or bone marrow transplantation
* Evidence of severe retinopathy
* Coagulopathy (INR>1.5)
* Evidence of an active or suspected cancer or a history of malignancy where the risk of recurrence is >20% within 2 years
* Hemoglobinopathy
* Hemophilia
* History of other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study
* Sexually active females of child-bearing potential (defined as age 10 years and older) and sexually active men who are not practicing two forms of effective contraception during treatment and during the 6 months after treatment has been concluded
* Females who have a positive serum pregnancy test within 7 days of initiation of treatment or who are breast-feeding
* Males whose female partners are pregnant
* Active substance abuse
* A sibling and/or any other child living in the same household (or sharing the same primary caregiver) enrolled in the study

g. Concomitant Medication and Treatment

All concomitant medications taken at anytime between screening and study termination will be recorded on the appropriate section of the Case Report Form. This includes any medication that is new, discontinued or changed in dose.

Systemic antiviral, anti-neoplastic and immunomodulatory treatments (including steroids and radiation) are not allowed during the study. Exception: patients who have taken or are expected to require acyclovir for herpetic lesions. Steroids given as physiologic replacement are permitted or as a short course (<7 days) for asthma management. Other investigational drugs and herbals are excluded as are other remedies being taken by the patient for possible or perceived effects against HCV.

The total daily dose of acetaminophen should not exceed 1 gram per day.

3. Schedule of Assessments and Procedures for Original Mono Therapy Groups with Viral Disappearance at Week 24 and for Original Combo Therapy Groups

Note that for original combo patients found to be HCV + at week 24, next assessment will be week 52
Note that BL refers to the Baseline (studies obtained prior to initiation of treatment on that day and week 1 refers to the beginning of the second week of therapy (i.e. 7 days after initiation of treatment) (Shaded area = assessment for all patients)

Table 3 Schedule of Assessments

Assessment	Screen Days	Study Treatment Period (weeks)															Untreated Follow-up (weeks)						Annual Visits⌂
	-35 to -1	BL	1	3	5	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72°	
Follow-up week #																	4	8	12	16	20	24	
Informed Consent	x																						
Complete Medical History	x																						
Vital Signs & Physical Exam	x															x						x	x
Vital Signs & Symptom Directed Physical		x	x	x	x	x	x	x	x	x		x		x			x	x	x				
Telephone Aassessment											x		x		x					x	x		
Immunology	x																						
Hematology	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
PT/PTT	x									x												x	x
Chemistry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
HCV-RNA Clinical[3]	x[1]	x[1]					x[2]			x[2]						x[2]			x[2]			x[2]	x[2]
HCV-RNA Research	x	x	x	x	x		x			x						x			x			x	
Thyroid Function Tests	x					x				x			x			x						x	
HCV Genotyping	x																						
Serum Bank	x	x					x			x				x		x			x			x	x
Pregnancy Test Serum**	x			x		x	x		x	x	x	x	x	x	x	x	x	x	x			x	
Pregnancy Ttest Uurine**		x						x												x	x		
Urinalysis	x						x			x			x			x						x	x
Ophthalmology Exam ***	x									x						x							
Adverse Events, Concomitant Medications, and Compliance	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Depression Screen	x	x					x			x						x						x	x
Growth/Body Composition +		x								x						x						x	x
QOL/Outcomes ++		x								x						x						x	x
Patient Diary Review		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x						

(See key page 27)

Table 3a Schedule of Assessments For Mono/Combo Group: no viral disappearance at week 24 and 52 (shaded area = all patients)

Assessment	Screen Days	Study Treatment Period (weeks) PEG 2a + placebo									Study Treatment Period (weeks) PEG 2a + RV									Untreated Follow-up						Annual Visits ⌂
Week #: a: Covance Kit Identifier	-35 to -1	BL	1	3	5	8	12	16	20	24	28 a	30 a	32 a	34 a	36 a	40 a	44 a	48 a	52 a	56 a	60 a	64 a	68 a	72 a	76E a	
Follow-up week #																				4	8	12	16	20	24	
Informed Consent	x																									
Complete Medical History	x																									
Vital Signs & Physical exam	x																		x						x	x
Vital Signs & Symptom Directed Physical		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x				
Telephone Assessment																							x	x		
Immunology	x																									
Hematology	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
PT/PTT	x									x									x						x	x
Chemistry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
HCV-RNA Clinical*	x^1	x^1					x^2			x^2						x^2			x^2			x^2			x^2	x^2
HCV-RNA Research	x	x	x	x	x					x																
Thyroid Function Tests	x					x				x					x				x						x	
HCV Genotyping	x																									
Serum Bank	x	x					x			x	x					x			x			x			x	x
Pregnancy Ttest Sserum **	x			x		x	x		x	x			x		x	x		x	x	x	x	x			x	
Pregnancy Test Urine **		x						x			x						x						x	x		
Urinalysis	x						x			x						x			x						x	x
Ophthalmology Eexam ***	x									x									x							
AE, Con Meds, and Compliance	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Depression Screen CDI	x	x					x			x									x						x	x
Growth/Body Comp +		x								x								x							x	x
QOL/Outcomes ++		x								x								x							x	x
Patient Diary Review		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							

See key page 27

Table 3b Schedule of Assessments For Mono/Combo Group: no viral disappearance at week 24; viral disappearance at week 52 (shaded area = all patients)

Assessment	Screen Days	Study Treatment Period (weeks) PEG 2a + placebo									Study Treatment Period (weeks) PEG 2a + RV															Untreated Follow-up (weeks)						Annual Visits ⌂
Week #: a / b: Covance Kit Identifier	-35 to -1	BL	1	3	5	8	12	16	20	24	28 a	30 a	32 a	34 a	36 a	40 a	44 a	48 a	52 a	56 b	60 b	64 b	68 b	72 b	76 b	80	84	88	92	96	100E	
Follow-up Week #																										4	8	12	16	20	24	
Informed Consent	x																															
Complete Medical History	x																															
Vital Signs & Physical Exam	x																		x						x						x	x
Vital Signs & Symptom Directed Physical		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x		x			x	x	x				
Telephone Aassessment																				x		x		x					x	x		
Immunology	x																															
Hematology	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
PT/PTT	x									x									x												x	x
Chemistry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x			x	x
HCV-RNA Clinical*	x[1]	x[1]					x[2]			x[2]						x[2]			x[2]						x[2]			x[2]			x[2]	x[2]
HCV-RNA Research	x	x	x	x	x					x																						
Thyroid Function Tests	x					x				x					x				x			x			x						x	
HCV Genotyping	x																															
Serum Bank	x	x					x			x	x					x			x				x		x			x			x	x
Pregnancy Ttest Sserum **	x			x		x	x		x	x			x		x	x		x	x	x	x	x	x	x	x	x	x	x			x	
Pregnancy Test Urine **		x						x			x						x												x	x		
Urinalysis	x						x			x						x			x			x			x						x	x
Ophthalmology Exam ***	x									x								x							x							
AE, Con Meds, and Compliance	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Depression Screen	x	x					x			x									x						x						x	x
Growth/Bbody Ccomp +		x								x								x							x						x	x
QOL/Ooutcomes ++		x								x								x							x						x	x
Patient Diary Reveiw		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x						

See key page 27

Note that for the compassionate "mono/combo" arm, the monitoring lab protocol will start with a new set of baseline labs the day the child is switched to combination therapy (week 28) and the laboratory studies will be carried out for the additional 48 weeks of combo therapy and 24 weeks off therapy (100 week total study period). If there is no viral disappearance at week 52 (second decision time point) begin schedule for untreated follow-up at week 56 (labs shown as untreated follow-up weeks 52-72 in schedule of assessments).

Note that for the combination group, if no viral disappearance at week 24, begin schedule for untreated follow-up at week 28 (labs shown as untreated follow-up weeks 52-72 in schedule of assessments).

Note there may be a variance of $\pm$ 7 days for monthly visits and $\pm$ 30 days for annual visits.

[1] Quantitative
[2] Qualitative

⌂ Annual visit: Visit #1 will be 6 months after completing follow-up week # 24. Annual visit # 2 will be 12 months after annual visit #1.

E Or upon discontinuation

* HCV RNA Clinical:
SC, BL, 1,3,5 of therapy, 12, 24, 48 of therapy; and week 60 and 72 of untreated follow-up. : A 3.0ml blood sample will be collected for HCV qualitative (COBAS AMPLICOR HCV Test, v2.0™) and quantitative (COBAS AMPLICOR HCV MONITOR Test, v2.™) tests. The quantitative test will be done at screening and pre-dose at baseline. Qualitative tests will be done at weeks 12, 24 and 48 of therapy and during weeks 60 and 72 of the treatment follow-up period.
HCV RNA Research:
3.0ml blood samples for HCV RNA will also be obtained at SC, BL, weeks 1, 3, 5, 12, 24, and 48 of therapy; and weeks 12 and 24 of untreated follow up. Plasma will be banked for performance of viral kinetic studies at a later date.

** Serum or urine pregnancy tests: As detailed above are to be performed in fertile or potentially fertile females only (age 10 years and older) within 24 hours prior to first tablet (RV/placebo) or at any time of a secondary amenorrhea of more than 1 week. Pregnancy test to be performed every 4 weeks during treatment and for the 6 months following completion of treatment.

*** Ophthalmologic exam: In addition, any patient who develops ocular symptoms should receive a prompt and complete eye examination. Study drug (s) should be discontinued in patients who develop severe retinopathy. Patients with severe retinopathy at screening are excluded from the trial.

+ Growth and Body Composition measurements: (with the exception of DXA scan) will be obtained by a dietitian at:

- Baseline
- 24 weeks
- After 48 weeks of PEG-2a treatment
- 24 weeks after the end of treatment (72 or 100 weeks)

At each of these points, subjects will undergo: (see Table 4 Growth and Body Composition Schedule of Assessments and Procedures)

1. Body composition analysis by DXA scan including bone density scans (performed by a technician
2. Bioelectrical impedance analysis (BIA)
3. Detailed anthropometry analysis
4. Diet and physical activity history

Table 4 Growth and Body Composition Schedule of Assessments and Procedures

Assessment	Baseline	Week 24	Week 48*	24 weeks after End of Treatment	2 annual visits (Long-term Follow-up)
				Week 72 or 100^	
DXA scan[#]	X	X	X	X	X
BIA	X	X	X	X	X
Anthropometry	X	X	X	X	X
Diet and physical activity assessment	X	X	X	X	X

*All subjects will be assessed at 48 weeks regardless of whether they are stopping treatment or require additional weeks of treatment

^For those subjects who fail PEG –2a monotherapy at 24 weeks and go on to another 48 weeks of combination therapy (76 weeks total treatment)

[#]DXA will include assessment of both body composition and bone density

DXA. Dual-energy x-ray absorptiometry is a radiographic technique that can determine the composition and density of different body compartments (fat, lean tissue, fat free mass, and bone mineral content) and their distribution in the body. All measurements will be made from head to toe at 2mm intervals while the child is lying supine. Data will be expressed as grams of fat, grams of lean tissue mass, percent lean body mass, and percent fat mass. Data will be expressed as age appropriate z scores. The scan will require 15 minutes to complete.

BIA. Bioelectrical impedance analysis will be measured with a four terminal portable impedance analyzer . Measurements will be made while the child is in the supine position. Current-injector electrodes will be placed just below the phalangeal-metacarpal joint in the middle of the dorsal side of the right hand and below the metatarsal arch on the superior side of the right foot. Detector electrodes will be placed on the posterior side of the right wrist, midline to the pisiform bone of the medial (fifth phalangeal) side with the wrist semiflexed.

Anthropometric assessment. Body weight will be measured by an electronic digital scale accurate to 0.1 kg. Standing height will be measured by stadiometer to the nearest 0.1 cm. Body mass index (BMI) will be calculated as kg/m^2. BMI provides a reasonable measure of overall fatness in children. Moderate to strong correlations have been observed between body mass index and percent body fat as measured by DXA or hydrodensitometry in youth populations (52-53). Triceps, biceps, iliac, and subscapular skinfold thickness will be measured to the nearest 0.2 mm using Lange skin calipers. Mid-arm circumference will be measured to the nearest 0.1 cm using a flexible non-stretchable plastic tape. All anthropometric measurements will be made by a single observer at each site in order to reduce inter-individual variation. Data will be expressed as age appropriate z scores.

Diet and physical activity assessment: A three-day food record and a physical activity assessment will be collected to obtain information on dietary intake and physical activity. This will take place at baseline and as noted in Table 4.. During those visits, a physical activity assessment will be completed. For the food record, the patient will be provided with detailed instructions and a handout on visualizing food portion sizes in order to more accurately complete the 3-day food record. They will have one week after the baseline visit to complete the 3-day food record (the 3-day record should include 2 week days and 1 weekend day). The food diary will be returned at the next study visit. The total intake of calories, protein, carbohydrate, fat, and micronutrients will be analyzed using the Food Processor (ESHA Research) diet analysis program (ESHA Research Professional Nutrition Analysis Software and Databases, PO Box 13028, Salem, OR 97309 USA).

++ The assessment protocol is designed to evaluate the child's health-related QOL, cognitive and developmental and psychological functioning. Some of the constructs will be measured from multiple sources (e.g., child and guardian) depending on child age. All assessment tools have been selected on the basis of their demonstrated reliability and validity. All measures will be completed at the time of scheduled clinic visits.

Health-related QOL. QOL will be measured using the Child Health Questionnaire (CHQ), which is one the best-validated measures of children's QOL and is completed by both children (at least 10 years old) and guardians. Information is gathered on several QOL domains, including physical functioning, role/social limitations, general health, bodily pain/discomfort, guardian impact, self-esteem, mental health, general behavior, and family impact.

Cognitive functioning. Cognitive functioning will be measured using the Behavior Rating Inventory of Executive Function (BRIEF) at all assessment points. There is a preschool version and a version normed for older children and adolescents. It contains 86 items in 8 non-overlapping clinical scales and 2 validity scales. These theoretically and statistically derived scales form two broader Indexes: Behavioral Regulation (3 scales) and Metacognition (5 scales), as well as a Global Executive Composite score. The BRIEF measures emotional and behavioral dysregulation, difficulties with response inhibition, working memory and the ability to quickly transition into new situations or tasks. It has demonstrated high internal consistency, test-retest reliability, and validity.

Psychosocial functioning. In addition to the CHQ, which has a mental health component, guardians will complete the Child Behavior Checklist (CBCL) at all assessment points. This well-validated measure permits an assessment of child adaptation across both internalizing (e.g., depression, anxiety) and externalizing (e.g., conduct problems, aggression) domains. If a participant reaches age 19 years of age, he will then be given the Adult Behavior Checklist (ABCL) (54).

In addition to the foregoing, the assessment protocol will include a measure of life stress and a measure of parental QOL. Specifically, guardians will complete the Life Events Checklist (LEC), which measures their perception of 46 life events and the degree to which they represent positive or negative stressors. Also, guardians will complete the MOS 36-Item Short-Form Health Survey (SF-36) to assess their physical and mental health status, including physical function, social function, pain, psychological well being, energy, impairment due to emotional problems, and general health perceptions. The results of cognitive, psychosocial and behavioral measures will be available to those interested within 4 weeks after discontinuation of treatment, and within 4 weeks after testing for Years 2 and 3.

Table 5: QOL/Health Outcomes Assessment Protocol and Timeline

Measure	Baseline		Week 24		Week 48		Years 2, 3	
	C	P	C	P	C	P	C	P
CHQ	X*	X	X*	X	X*	X	X*	X
BRIEF		X		X		X		X
CBCL/ABCL		X		X		X		X
SF-36		X		X		X		X
LEC		X		X		X		X

* Not completed by children <10 years of age
[C = Child completes or administered to child; P = Parent completes].
A sample of each assessment tool is provided in the Manual of Procedures.

a. Screening Examination and Eligibility Screening Form

Screening will begin within 35 days prior to the first dose of study drug after written informed consent is obtained. An Eligibility Screening Form (ESF) will be used as a checklist to document whether or not a patient fulfills the entry criteria. The ESF will be signed and dated by the investigator for all patients considered for enrollment in the study. A copy of the ESF should be kept with the patient's Case Report Form, or if the patient is excluded, the ESF should be kept as the patient's exclusion record. Patients must meet the entry criteria to be enrolled in the study.

b. Screening Assessments

The following screening assessments (Table 6) must be obtained within 35 days prior to the initiation of study drug.

Table 6 Screening Assessments

Medical History and Physical Exam	Body weight, height, vital signs, body temperature, complete medical history and concomitant medications, Depression screen
Immunology	Ceruloplasmin*, alpha1-antitrypsin*, anti-HBc IgM Ab*, anti-HAV IgM Ab*, HBsAg*, anti-HCV Ab*, anti-mitochondrial Abs*, anti-nuclear Abs*, anti-smooth muscle Abs*, anti-HIV Ab* anti-LKM antibody*
Hematology	Complete blood count (hemoglobin, hematocrit, WBC, Platelets), including differential, prothrombin time, partial thromboplastin time, INR
Hematology	Hemoglobin A1C
Chemistry	ALT, AST, total bilirubin, direct bilirubin, alkaline phosphatase, total protein, albumin, BUN, creatinine, creatinine phosphokinase, uric acid, calcium, phosphorus, cholesterol, triglycerides, glucose, sodium, chloride, and potassium
Virology	Clinical:HCV genotype, qualitative and quantitative HCV-RNA; research
Thyroid Function Tests	TSH, Free T4, T4 total and T3 uptake, Thyroid Peroxidase Antibody
Serum Bank	Samples will be stored in a serum bank in the event some tests need to be repeated or additional testing is warranted
Pregnancy test	Serum pregnancy test to be performed in fertile or potentially fertile females only
Urinalysis	Urine sample to be sent to central lab for analysis
Ophthalmology	Complete ophthalmologic exam with slit lamp exam and dilation (dilation recommended but not required)

Note:
1. Tests marked above with an asterisk (*) do not need to be repeated if patients do not receive study drug following initial screening but are subsequently re-screened for this protocol, unless dictated by a change in the patient's medical condition.
2. An extra screening visit may be necessary to complete the screening labs for small children. If this is necessary, use Covance Screening Form #2 and screening lab kit #2.

Baseline assessments, unless as otherwise noted in Tables 3, 3a and 3b should be obtained on the first day of study drug administration prior to the initiation of study drug therapy.

Additional blood samples will be collected at screening, baseline, week 12, 24, 40, 48, 60 and 72. The samples will be stored in a serum bank in the event some tests need to be repeated or additional testing is warranted.

If any future research of these samples other than delineated in this protocol is desired, the sera will not be released unless an IRB approves a protocol for this purpose. The PI (KBS) of the study will be responsible for these samples. They will be stored at the central lab for the duration of the study and then will be transferred to the NIDDK repository where they will be stored for an indefinite period of time. No genetic testing will be done on these samples, as they do not contain DNA.

For purposes of analyzing the relationships between study endpoints and initial hepatic histology the previously performed liver biopsies will be processed as follows. Sections of the liver biopsies will be stained with hematoxylin-eosin and Masson's trichrome for histologic evaluation by Dr. Zachary Goodman, study pathologist. Each biopsy will be evaluated for degree of hepatocellular injury, inflammation and fibrosis with the three scoring systems most widely used in hepatitis treatment studies: Knodell score(55), Ishak score(56) and Metavir score(57). Other histologic features, such as steatosis and dysplastic foci will be noted when present.

c. Laboratory Assessments

Clinical laboratory samples will be analyzed by:
Covance Central Laboratory Services Inc.
8211 SciCor Drive
Indianapolis, Indiana 46214-2985
Tel: (317) 271-1200

Laboratory testing specified in this protocol can be done locally, at the discretion of the investigator, if there are problems with the laboratory analyses sent to the central laboratory (Covance) at the scheduled visits (eg tube breakage, sample QNS) and/or if the laboratory analyses need to be repeated at unscheduled times for the purposes of safety monitoring outside the scheduled visits. For the purposes of the protocol, "local laboratory" refers to the laboratory at the investigator's site and/or a laboratory within the geographic proximity of the patient, provided the investigator verifies that the laboratory is CLIA-certified and confirms that a curriculum vitae of the laboratory director is available for review. The verification may be done verbally prior to the time the testing is performed at the local laboratory. However, the investigator will obtain both the written verification of the CLIA certification of the laboratory and a copy of the curriculum vitae of the laboratory director and will submit these to MMRI. The costs for the performance of the locally performed laboratory tests will be covered by the Patient Care Costs of the subcontract and will not be charged to the patient.

d. Total Blood Requirements

The screening assessments require about 30 mL of blood, and regular visits require between 2 mL and 16 mL of blood depending on the laboratory assessments for that visit.

During screening, about 30 mL of blood will be collected for screening assessments (see Screening Assessments Tables 3, 3a and 3b and Table 6)
During the study the following blood samples will be obtained:
Viral kinetic analysis (2mL)
Hematology analysis (2mL)
Chemistry/Pregnancy/Thyroid function analysis (2-3 mL)
Serum blood bank storage (2mL)

4. Investigational Products

a. Dose and Schedule of Study Drugs

Each patient will receive a weekly dose of PEG-2a (Peginterferon alfa-2a Ro 25-8310) administered subcutaneously, in the thigh or abdominal wall. The exact weekly dose in micrograms for each patient will be calculated as 180 ug/1.73 m^2 x BSA in m^2 of the child. (See Manual of Procedures for PEG-2a dosing guidelines)

Each patient randomized to receive RV (or who is switched from PEG-2a plus placebo to compassionate combination therapy) will receive twice daily doses of RV administered by mouth. The exact daily dose in milligrams for each patient will be calculated as 15 mg/kg/day divided bid with a maximum dose of 1200 mg/day if $\geq$ or equal to 75 kg and 1000 mg/day if <75 kg. (see Manual of Procedures for RV dosing guidelines). A 100 mg Roche RV tablet will be used in this study. A "look alike" tablet will be used for patients in the placebo group. Bioequivalence of a 100 mg Roche RV tablet and the currently marketed 200 mg RV capsule has been demonstrated in a fasted, single-dose crossover study in 40 subjects. The 100 mg tablet is the same dosage form as the 200 mg tablet and the active and inactive ingredients are proportionally similar between the two tablets. The tablets are not scored and they are not to be split. As delineated in the dose reduction portion of the protocol,

if doses are to be reduced below 200 mg, the dose regimen will be changed to 100 mg po qd. The long half-life of RV permits once daily dosing if necessary. All doses will be specified in the nearest 100 mg units.

All patients should receive RV treatment with food. By definition, RV 'with food' means taking their doses within 30 minutes before or 2 hours after a meal. The meal should be considered 'regular' as opposed to 'fat restricted' meal. It was not known that RV bioavailability is affected by coadministration of food. Therefore, Roche conducted a study to assess the relative bioavailability of a single 600 mg RV dose (three 200 mg tablets) in the presence and absence of food (58). This randomized, open-label, two-way crossover study in patients with hepatitis C infection showed an increase in $AUC_{0\text{-}192h}$ and C_{max} of 42% and 66%, respectively, when the RV tablets were taken with a high fat breakfast compared to being taken in the fasted state. In Roche's phase III combination studies, patients were instructed to take their twice-daily doses of RV with food. Similarly, patients in this study will be instructed to take their RV doses with food. As this is a 48 week treatment study, the meal content for individual patients will vary from day to day.

b. Dose Regimen and Dose Adjustment

After the first 30 patients enrolled have been treated for 4 weeks (or, if enrollment begins slowly, 3 months after the first patient is enrolled), there will be a safety checkpoint during which the DSMB will review all clinical and laboratory data for safety.

c. Investigator Dose Modification Guidelines for Intolerance

The intention of the protocol is that patients demonstrating a response to therapy remain on study drug until the completion of the study. However, it is possible that some patients will encounter adverse events during their participation in the trial necessitating study drug dosage adjustment. Decrement adjustments should be uniform across centers and patients. When appropriate, downward dose adjustments in one level increment should be considered. (see Manual of Procedures for PEG-2a and RV dosing guidelines).

Suggested dose adjustments to PEG-2a and RV are for guidelines to maintain consistency between centers. These guidelines are given below for neutropenia, thrombocytopenia, anemia, indirect hyperbilirubinemia, and elevated alanine aminotransferase (ALT) activity. When possible, abnormal lab results should be confirmed as soon as possible following notification. If laboratory abnormalities improve or resolve, the dose of PEG-2a or RV may be increased back to the original dose at the discretion of the investigator with continued close monitoring.

For other adverse effects considered to be possibly related to PEG-2a and/or RV investigators should utilize the "Toxicity Table for Grading Severity of Pediatric AEs" (see Manual of Procedures). Dose reduction for non-laboratory related adverse events will be based upon severity ratings in accordance with modified WHO criteria as noted in the Manual of Procedures.

- For grade 1 toxicity, no dose reduction is needed.
- For grade 2 toxicity that persists over two consecutive safety visits and does not respond to symptomatic management, a level 1 dose decrease of Peg-2a and/or a decrease in RV may be required.
- For grade 3 toxicity not responsive to adjunctive management, a level 1 dose decrease of PEG-2a and/or a decrease in RV may be required. Stepwise reduction should continue until symptoms resolve. Patients with grade 3 toxicity should be followed closely, visits at more frequent intervals or through close telephone contact to monitor in between regularly scheduled safety visits. If symptoms improve or resolve, return to previous dosing should be done at the discretion of the investigator.
- For grade 4 toxicity, treatment will be discontinued.

If adverse events continue at the same intensity despite maximal dose reductions, PEG-2a and/or RV may require discontinuation at the discretion of investigator. It should be noted that certain toxicities carry different levels of significance for each patient and therefore, the investigator should use these as guidelines only, within the context of clinical judgment. Growth factors may not be used to maintain normal levels of hemoglobin, neutrophils or platelets.

PEG-2a

Original Dose	One Level Adjustment	Two Level Adjustment	Three Level Adjustment
180 mcg/1.73 m^2	135 mcg/1.73 m^2	90 mcg/1.73 m^2	45 mcg/1.73 m^2
(104 mcg/m^2)	(78 mcg/m^2)	(52 mcg/m^2)	(26 mcg/m^2)

Ribavirin

Original Dose	Dose Reduction
15 mg/kg/day divided bid	7.5 mg/kg/day divided qd or bid based on dose

PEG-2a Dose Adjustments for Low Absolute Neutrophil Counts

Parameter	Response
ANC (cells/mm^3)	
1000	None
750-999	Week 1-2: Immediate 1 Level Adjustment Week 3-48: None
500-749	Week 1-2: Delay or hold dose until ≥750 then resume dose with a 1 Level Adjustment Assess weekly x 3 to verify WBC's ≥750 Week 3-48: Immediate 1 Level Adjustment
250-499	Week 1-2: Delay or hold dose until ≥750 then resume dose with a 2 Level Adjustment Week 3-48: Delay or hold dose until ≥750 then resume dose with a 1 Level Adjustment
<250 or febrile neutropenia	STOP DRUG

PEG-2a Dose Adjustments for Low Platelet Counts

Parameter	Response
Platelets (cells/mm^3)	
50,000	None
35,000-49,000	Delay or hold dose until 50,000 then resume dose with a Level 1 adjustment
25,000-34,000	Delay or hold dose until 50,000 then resume dose with a Level 2 adjustment
<25,000	STOP DRUG

Ribavirin Dose Adjustments for Anemia and Indirect Hyperbilirubinemia

Parameter	Response
Hemoglobin (gm/dl)	
<10 gm/dl	Reduce RV dose by one half. Follow weekly and increase dose when > 10 gm/dl
<8.5 gm/dl	Permanent discontinuation of RV
Indirect Bilirubin (mg/dl)	
>5 mg/dl	Stop drug, follow indirect bilirubin weekly. If reduces to <2.5, restart RV at one half dose. If <2.5 mg/dl for 4 weeks, increase to full dose. If recurs at > 5 mg/dl, reduce dose or stop for 1-2 weeks and restart at a reduced dose if indirect bilirubin is <2.5 mg/dl. Indirect bilirubin must remain at <2.5 mg/dl to continue RV even at a reduced dose.
>5 mg/dl for > 4 weeks	STOP DRUG

PEG-2a Dose Adjustments for Elevated Serum ALT

Parameter	Response
On Treatment Serum ALT	
< 5 X ULN	None
5 X ULN but < 10 X ULN	Repeat in 1 week **If ALT decreased**, continue at present dose, follow every 1-2 weeks to assure stability. **If ALT stable or increased but 10 X ULN**, Level 1 dose adjustment and follow weekly until stable or decreasing
10 X ULN	Repeat in 1 week **If ALT decreased below 10 X ULN but 5 X ULN**, Level 1 dose adjustment and follow weekly until stable or decreasing **If ALT still 10 X ULN** stop drug permanently

Screening and Management for Depression

All children undergoing screening for enrollment will be screened for depression using the Childhood Depression Inventory (CDI), published by Multi-Health Systems, Inc (59) (see copy of the questionnaire in the Manual of Procedures). This is a 10 minute questionnaire that has been validated for children ages 7-17 years; guardians can complete the questionnaire for children <7 years. Children that are unable to read the questionnaires may have them read to them by the study coordinator. The instrument can be used for 17 year old adolescents (59). (Analysis of the CDI data will be limited to children 7-17 years of age). In addition, each investigator will screen children at entry for a Major Depressive Episode using criteria from the American Psychiatric Association (See Table 7 Criteria for Major Depressive Episode).

Any child meeting the criteria for a major depressive episode at screening will not be enrolled in the study and will be referred to a mental health professional if the depression fits the criteria in Table 8A or to a psychiatrist if the depression fits the criteria in Table 8B (See Table 8 Indications for Mental Health Professional Care and Specialty Physician Care in Pediatric Patients with Depression).

Table 7. Criteria for Major Depressive Episode*

A major depressive episode is indicated by the presence of five or more of the following symptoms nearly every day during the same two-week period, representing a change from the previous level of functioning:

Depressed mood most of the day

Markedly diminished interest or pleasure in all or almost all activities

Clinically significant weight loss in the absence of dieting or weight gain (e.g., a change of more than 5 percent of body weight in a month) or a decrease in appetite †

Insomnia or hypersomnia

Observable psychomotor agitation or retardation

Fatigue or loss of energy

Feelings of worthlessness or excessive or inappropriate guilt

Diminished ability to think or concentrate or indecisiveness

Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

* Criteria are from the American Psychiatric Association. (58) (60)

† In children, this criterion includes the failure to make the expected growth-related weight gains.

The CDI/CES-D will be used to screen children for depression at the screening and baseline visits and at 12, 24,end of study drug, end of untreated follow up and at each of the annual visits. If the participant becomes 18 years of age or older during the study, then the CES-D depression screening instrument will be used. If depression develops as defined by the CDI manual (score >19) (59), or by the CES-D manual (score >15) (61), then the investigator will perform a more thorough evaluation to determine the validity of the screening test result. If the participant meets the criteria listed above for a Major Depressive Episode, and fits the criteria listed under 8A in Table 8, then he will be referred to a mental health professional and the PEG-2a or PEG-2a plus RV will be continued. If the management of the depression is not successful after eight weeks or if the participant develops criteria on Table 8B for referral to a specialty physician during that eight week period, the PEG-2a or PEG-2a plus RV will be stopped and the participant will be referred to a specialty physician. The participant will move to the first untreated follow-up visit. If the participant meets the criteria on Table 8B for indications for specialty physician care, he will be referred to a specialty physician for management, the PEG-2a or PEG-2a plus RV will be stopped and he will move to the first untreated follow-up visit.

Table 8. Indications for Mental Health Professional Care and Specialty Physician Care in Pediatric Patients with Depression*

8A. Indications for mental health professional care:

Initial episode of depression

Recent onset of depression

Absence of coexisting conditions

Ability to make no-suicide contract

High level of family discord

Chronic, recurrent depression

8B. Indications for specialty physician care and immediate withdrawal of study drug(s):

Lack of response to initial course of treatment**

Coexisting substance abuse**

Recent suicide attempt, current suicidal ideation with plan, or both**

Psychosis**

Bipolar disorder**

Inability of family to monitor patient's safety**

** The presence of this factor indicates the need for more urgent or more intensive care.

* Modified from Brent et al, Adolescent Depression (60)

d. Preparation and Administration of Study drug

Roche will provide all study drugs for this trial.

PEG-2a administration will be via the subcutaneous route utilizing sterile technique. RV will be given via the oral route. The first dose of study drug should be administered in the clinic under the supervision of study personnel. Before providing the guardian with study drug, the investigator or a qualified staff member will instruct the guardian on the proper methods of storage of the study drug, injections, and management and disposal of needles and syringes. The study drug will be issued to the guardian and the guardian will return any unused vials/bottles, partially used vials/bottles, and empty vials/bottles at each visit. The guardian will be instructed to keep accurate records of dosing date, time and amount of drug in mL for Peg-2a and number of tablets for RV or placebo. Standardized diaries will be provided to guardians so doses can be recorded as given.

e. Packaging and Labeling

Study drug:	Pegasys™ Peginterferon alfa-2a (R0 25-8310), 180 mcg vial Ribavirin (Roche): 100 mg tablets or "look alike" placebo
Packaging:	Pegasys™: a 4 week supply with one extra 4 pack of vials (for emergency use only) will be given at initial dispensing to allow for breakage (total of 8 vials). If necessary, another emergency pack can be given if the drug expires and/or if needed in the subsequent course of the study) Ribavirin: a 4 week supply: __ bottles supplied per patient
Storage:	Pegasys™ Peginterferon alfa-2a (Ro 25-8310): Refrigerate at 2°-8° C (36°-46°F) Ribavirin: Store at 15-30° C (59-86° F)

Each vial and bottle will be labeled according to FDA regulations. The study coordinator or research pharmacist will be responsible for inscribing each vial/bottle with the protocol number, patient identification number, patient 3 letter code and date dispensed. For PEG-2, the investigational pharmacist or study coordinator will calculate the ratio of child BSA/1.73 m^2. The guardian/patient will then be taught how to draw up the exact dose. The BSA will be recalculated after 24 weeks of therapy and the dose of PEG-2a adjusted accordingly. Doses will be rounded to the nearest 0.1ml.

Pegasys™ will be labeled with:

Vial Label
NV17424
PEGASYS ® (25-8310/J13), 180 mcg/mL
For Subcutaneous (Under the Skin) Injection
Store at 36° to 46°F (2° to 8°C)
Caution: New Drug – Limited by United States law to investigational use
Hoffmann-La Roche Inc., Nutley, NJ 07110
[Lot#] [ID#]

Packer Label
Patient Study No. _____ Patient Letter Code _____ Date Dispensed: _____
4 Vials Roche Protocol No. NV17424
PEGASYS ® (25-8310/J13), 180mcg/mL
For Subcutaneous (Under the Skin) Injection
Inject once each week, on the same day, as instructed by your health care provider
Store at 36° to 46°F (2° to 8°C)
DO NOT Become Pregnant During Therapy
This Container Must Be Returned at Your Next Visit
Keep Out of Reach of Children
Caution: New Drug – Limited by United States law to investigational use
Hoffmann-La Roche Inc., Nutley, NJ 07110
[Lot#] [ID#]

Ribavirin will be labeled with:

Bottle Label
84 Tablets Roche Protocol No. NV17424 MEDNO XXX
Patient Study No. _____ Patient Letter Code _____ Date Dispensed: _____
For Oral Administration
Take as instructed by your health care provider. 100mg ribavirin or placebo
Store at 59° to 86°F (15° to 30°C)
If you or your partner become pregnant, your use of ribavirin tablets could cause serious birth defects and harm (including death) to your unborn child. Refer to your informed consent for more information.
This Container Must Be Returned at Your Next Visit
Keep Out of Reach of Children
Caution: New Drug – Limited by United States law to investigational use
Hoffmann-La Roche Inc., Nutley, NJ 07110
[ID#]

In addition, Roche will include a neon yellow label with the warning: "Do not break, crush, or dissolve tablets."

f. Dispensing and Accountability of Study Drug

Accountability and subject compliance will be assessed by maintaining adequate drug dispensing logs and study drug return records.

Accountability of Drug Supplies

A drug dispensing log must be kept current and should contain the following information:

The identification of the patient to whom the drug was dispensed
The date(s) and quantity of the drug dispensed to the guardian
The date(s) and quantity of the drug returned by the guardian

The drug inventory (empty study drug vials/bottles, as well as partly used and unused study drug vials/bottles) must be available for inspection at every monitoring visit. All unused study drug must be returned by the guardian to the investigator at each visit. Drug supplies, including unused, partially used or empty vials/bottles and the drug dispensing logs, must be returned to Roche at the end of the study.

g. Assessment of Compliance

It is mandatory that each PEG-2a injection and RV dose be recorded. The guardian will be instructed to record the date and exact time of PEG-2a and RV in a patient diary. The person giving the PEG-2a injection must initial the patient diary. The date and exact time of PEG-2a and RV administration as well as the amount in ml of PEG-2a that was injected will be recorded in the source document and Case Report Form.

The date(s) and quantity of the drug returned by the guardian will be recorded by the research pharmacist to assess compliance.

The patient diary will also be used to collect self-reported adherence to therapy. Adherence information was adapted from the Adherence Modules developed by the Pediatric Acquired Imunodeficiency Syndrome Clinical Trial Group Adherence to Human Immunodeficiency Virus Therapy Subcommittee. The investigator or designee will review adherence information collected in the patient diary at each study visit.

It is the investigator's responsibility to ensure that each patient receives the appropriate dose of PEG-2a and RV.

5. Safety Issues

a. Safety Assessments

Safety assessments will be performed at each visit throughout the treatment period and the treatment-free follow-up period as outlined in the Schedule of Assessments. Measures of safety will consist of:

- For reasons of safety, breast-feeding and pregnant females are excluded as are sexually active females of child-bearing potential who do not use two methods of contraception during and up to 6 months after cessation of therapy.
- Hematology: Complete blood count (hemoglobin, hematocrit, WBC including differential, platelets)
- PT and PTT
- Clinical Chemistry: ALT, AST, total bilirubin, alkaline phosphatase, total protein, albumin, BUN, creatinine, creatinine phosphokinase, uric acid, calcium, phosphorus, cholesterol, triglycerides, glucose, sodium, chloride, and potassium
- Urinalysis: Dipstick with subsequent microscopic evaluation if positive for hemoglobin
- Thyroid Function Tests: TSH, Free T4, T4 total, T3 uptake
- Serum or urine pregnancy tests are to be performed in fertile or potentially fertile females only (age 10 years and older) within 24 hours prior to first tablet (RV/placebo) or at any time of a secondary amenorrhea of more than 1 week. Pregnancy test to be performed every 4 weeks during treatment and for the 6 months following completion of treatment
- Ophthalmologic exams
- Documentation of concomitant medication
- Depression screen
- Documentation of dose adjustment and premature withdrawals for safety reasons or intolerance
- Clinical adverse events

b. Adverse Events and Laboratory Abnormalities

1. Clinical Adverse Events

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product. Pre-existing conditions, which worsen during a study, are to be reported as adverse events.

All clinical adverse events (AEs) encountered during the clinical study will be reported on the AE page of the CRF. Intensity of adverse events will be graded on a scale (mild, moderate, severe, life threatening) and reported in detail as indicated on the CRF.

Mild: discomfort noticed but no disruption of normal daily activity
Moderate: discomfort sufficient to reduce or affect daily activity
Severe: inability to work or perform normal daily activity
Life threatening: represents an immediate threat to life

Relationship of the adverse event to the treatment will be assessed.

2. Laboratory Test Abnormalities

In the event of unexplained abnormal laboratory test values, the clinically significant laboratory values need to be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found.

If a laboratory abnormality has only triggered a dose reduction or a held dose, this should be handled and recorded as a laboratory abnormality and should NOT be recorded as an adverse event.
If a laboratory abnormality has received a specific treatment (e.g. elevated BUN and treatment of dehydration) this should be handled and recorded as an adverse event.
If a laboratory abnormality is serious, constituting a serious adverse event, this should be handled and recorded as an SAE.

If a laboratory abnormality has resulted in drug discontinuation, this should be handled and recorded as an adverse event.

Please see Manual of Procedures "Toxicity Table for Grading Severity of Pediatric (>3 months to 18 years of age) Adverse Experiences".

3. Serious Adverse Events (Immediately Reportable to Roche, Dr. Schwarz, MMRI, FDA, DSMB and NIDDK Project Scientist)

Any clinical adverse event or abnormal laboratory test value that is *serious* occurring during the course of the study, irrespective of the treatment received by the patient, must be reported by the individual investigator via telephone to Dr. Schwarz or the on call Pediatric GI physician within one working day of knowledge of the occurrence by the investigator. The individual investigator will also fax the completed SAE form to MMRI within one working day of occurrence. MMRI will automatically notify the Roche Drug Safety Group, the FDA and NIDDK electronically via a completed SAE form within one working day of knowledge of the occurrence by the investigator. MMRI will be responsible for submitting the follow-up SAE reports to FDA, Roche, the DSMB, NIDDK and the PI.. The Roche Drug Safety group will submit IND safety reporting (MedWatch) to the individual sites.

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution. With respect to human clinical experience, this includes any experience which:

Is fatal or life-threatening (NOTE: death is an outcome, not an event);
Requires inpatient hospitalization or prolongation of an existing hospitalization;
Results in persistent or significant disability/incapacity;
Is a congenital anomaly/birth defect;
Is medically significant or requires intervention to prevent one or other of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

For serious and all other adverse events, the following must be assessed and recorded on the adverse event page of the Case Report Form: intensity, relationship to test substance, action taken regarding test substance, and outcome to date.

In accordance with international and local laws and regulations, the investigator must promptly notify the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) of a serious adverse event.

All adverse event reports and all DSMB reports will be sent to the GCRC (if applicable).

c. Medical Safety Committee

This committee will be composed of the Director of MMRI as Chairman, a pediatric gastroenterologist who is not an investigator, the study coordinator and 3 other site coordinators on a rotating basis and the MMRI study coordinator as recording secretary. The committee will hold monthly teleconferences that will be arranged by MMRI. Systematic reviews of AE/SAE's will be performed and systematic reviews of the INS safety reports related to RV and PEG-2a will be reviewed for possible relevance to the study.

d. Criteria for Premature Withdrawal

Patients have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study if it is in the best interest of the patient. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

The investigator should contact the legal guardian either by telephone or through a personal visit, or a responsible relative must be contacted to determine as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patient is withdrawing from the study.

Follow-Up Schedule for Patients Prematurely Withdrawn

Investigators should make every attempt to follow patients who discontinue study drug permanently for whatever reason (e.g. AEs) for at least 24 weeks by the post treatment follow up schedule.

e. Replacement Policy

For Patients - No subject prematurely discontinued from the study for any reason will be replaced.

For Centers - A center may be replaced for the following administrative reasons:

Excessively slow recruitment
Poor protocol adherence

Potential problems: Although insufficient enrollment is a potential problem, the 11 centers now have 268 eligible patients so adequate numbers of patients should be available. Each center must enroll 5 patients and the centers may continue to enroll up to a total of 15 patients/center. (However, this policy will be reconsidered after six months of enrollment. If some sites are having difficulty in meeting the enrollment target and others are able to enroll more than 15 patients, the limitation of 15 patients per site will be removed.) Enrollment will stop at 112 patients. If for some reason, patient numbers are not adequate, then the study can be advertised in such places as the NASPGHAN web site and web site for PKIDS (Parents of Kids with Infectious Disease).

f. Warnings and Precautions

To date, no teratology or reproduction studies have been conducted in humans with PEG-2a. Primate teratology studies indicate an increased incidence of spontaneously aborted fetuses in pregnant rhesus monkeys (*Macaca mulatta*) receiving high doses of intramuscular Roferon-A. Studies with Roferon-A in non-pregnant rhesus monkeys have shown menstrual cycle irregularities, including prolonged menstrual periods. Male fertility and teratological evaluations have yielded no significant adverse effects to date.

Interferons are human proteins which show a substantial degree of species specificity making extrapolation of animal study data to humans of questionable value. Investigations have been conducted in normally cycling healthy women using non-recombinant human leukocyte interferon. Results demonstrate a significant reduction of serum estradiol and progesterone concentrations during the treatment interval.

There are no adequate, controlled studies of any IFN in pregnant women. Moreover, RV is a known teratogen (see below for information). Therefore, extreme care must be taken to avoid pregnancy during study in female patients, and female partners of male patients.

g. Carcinogenesis and Mutagenesis:

Adequate studies to assess the carcinogenic potential of RV in animals have not been conducted. However RV is a nucleoside analog that has produced positive findings in multiple in vitro and animal in vivo genotoxicity assays, and should be considered a potential carcinogen.

h. Pregnancy

RV has a significant teratogenic and/or embryocidal potential in all test animals. Malformations of the skull, palate, eye, jaw, limbs, skeleton, and GI tract were observed; the incidence and severity of teratogenic effects increasing with escalation of the drug dose. Survival of fetuses and offspring was reduced. In conventional embryotoxicity and teratogenicity studies in rates and rabbits, no effect dose levels were well below those for proposed clinical use. **Therefore, in order to avoid pregnancy, sexually active females of childbearing age and sexually active males should use two reliable forms of effective contraception through out the entire period of the study (treatment and follow-up):** These may include, but are not limited to, using birth control pills, IUDs, condoms, diaphragms, implants or being surgically sterilized. Note: Due to the potential for ribavirn to be transmitted in the semen it is recommended that one method should be a barrier-type e.g. condom.

A female subject must be instructed to stop taking the test drug/s and immediately inform the investigator if she becomes pregnant during the study. Pregnancies occurring up to 6 months after the completion of the test drug must also be reported to the investigator. The investigator should report all pregnancies within 24 hours to MMRI and the PI. In addition, any pregnancy that occurs in a female patient or the female partner of a male patient will be reported to the Ribavirin Pregnancy Registry (1-800-593-2214). The investigator should counsel the subject, discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

The patient must immediately report to the investigator (who will inform the MMRI safety monitor and the PI) any pregnancy occurring in a partner during the duration of the trial. Such patients may not continue to receive RV.

Serum pregnancy test is to be performed in fertile or potentially fertile females only (age 10 years and older), within 24 hours prior to first tablet (RV/placebo) or at any time of a secondary amenorrhea of more than 1 week. Pregnancy tests are to be performed every 4 weeks during treatment and for the 6 months following completion of treatment.

i. Ophthalmologic disorders

Decrease or loss of vision, retinopathy including macular edema, retinal artery or vein thrombosis, retinal hemorrhages and cotton wool spots, optic neuritis, and papilledema are induced or aggravated by treatment with Peg-2a or other alpha interferons. All patients should receive an eye examination at screening, week 24 and week 48. Patients in the Mono/Combo group will also receive an exam at week 76. Patients with preexisting ophthalmologic disorders (eg, diabetic or hypertensive retinopathy) should receive periodic ophthalmologic exams during interferon alpha treatment. Any patient who develops ocular symptoms should receive a prompt and complete eye examination. Peg-2a treatment should be discontinued in patients who develop new or worsening ophthalmologic disorders. Patients with preexisting severe retinopathy are excluded from the trial.

j. Adverse Reaction

The primary toxicity of RV is anemia. Reduction in hemoglobin levels generally occurs within the first 1-2 weeks of initiating therapy. Cardiac and pulmonary events associated with anemia may occur.

6. Statistical Considerations and Analytical Plan

The key elements of the study design are 1:1 randomization to PEG-2a plus placebo or PEG-2a plus RV and the decision point at week 24. Up to this point, patients and investigators will be blinded. At this time point, viral disappearance will be determined in all patients and the study will be unblinded to the HCV-RNA results. Participants randomized to PEG-2a monotherapy who do not exhibit viral disappearance (by the AMPLICOR™ HCV assay) at week 24 will be moved to a compassionate "mono/combo" arm to begin week 28 and be continued for 48 weeks if they exhibit viral disappearance at 24 weeks of combination therapy to end at week 76, followed by 24 weeks off therapy, ending at week 100. They will discontinue therapy after 24 weeks of combination therapy if they do not exhibit viral disappearance. For participants randomized to PEG-2a plus RV who do not exhibit viral disappearance at week 24 therapy will be discontinued. The basic study design is provided at the beginning of this Study Design Section.

a. Statistical Considerations in Design and Study Size

Based on the limited results available (see Background section above), we estimate that the PEG-2a alone group will have a primary outcome, a sustained viral response (i.e., non-detectable plasma HCV-RNA; SVR) at 72 weeks post baseline, of approximately 35%. We will use a standard Pearson chi-square for the main analysis of the primary outcome. If we vary the possible response rate in the combined PEG-2a + RV group from 60% to 70% as well as varying the response in the PEG-2a alone group and assume an α=0.05, power = 80%, we can calculate the sample sizes shown below, inflated for an estimated 15% drop-out rate (n*=n/(1-0.15):

Estimated Sample Sizes in Each Group for Comparison of Proportion of Participants with Non-Detectable Plasma HCV-RNA (ND HCV) at 24 weeks post treatment Assuming α =0.05, power = 80%, Drop-Out = 15%

Proportion of ND HCV in PEG-2a Alone Group	Proportion of ND HCV in Peg-2a+RV Group		
	0.60	0.65	0.70
0.30	49	37	28
0.35	73	51	37
0.40	114	73	49

The clinical centers indicate that, collectively, they are able to recruit approximately 112 children in the requisite time frame. Thus, we can see that, with 56 children in each group, we have approximately 80% power to detect a true difference between proportions of participants with non-detectable HCV-RNA in the PEG-2a alone group compared to participants with non-detectable HCV-RNA in the PEG-2a+RV group of 0.25-0.30.

b. Main Analysis of the Primary Outcome

The primary outcome for this study will be the proportion of participants with sustained viral response (i.e., non-detectable plasma HCV-RNA) 24 weeks post treatment (72 weeks after baseline visit)For clarification, children randomized to PEG-2a plus placebo who fail to exhibit viral disappearance at week 24 will be considered as non-responsive for SVR at week 72 in the intent to treat analysis. The SVR at week 72 will be compared between the two randomized arms: monotherapy and combination therapy. Because each patient will have a binary outcome (no detectable plasma HCV-RNA or detectable plasma HCV-RNA), the primary analysis will be a simple contingency table of treatment group and primary outcome (yes/no). We will use a Pearson chi-square test to analyze these tables, although we will verify that all cells in the contingency table have expected frequencies greater than 5, which would invalidate the use of the Pearson chi-square test and force us to use the Fisher's Exact Test.

Prior to performing the primary outcome analysis, the Breslow-Day test for homogeneity of the odds ratios will be used to assess if there is significant (at ∀ □=0.05) evidence of differences in the effect of treatment according to Clinical Site (62). The proposed strategy for dealing with the treatment x Clinical Site interactions should maintain the overall ∀ level of the primary outcome at 0.05 since the test for average treatment effect and for interaction effect will be statistically independent.

This clinic-treatment interaction is of concern for two reasons: (1) variation in treatment effect among clinics may reduce the power of the study; and (2) conceivably, a large treatment effect in a few clinics may results in an overall treatment effect in the study because of the particular patient population in those clinics.

It is difficult to specify appropriate hypotheses for this interaction test and to develop the appropriate formulations for power calculations since there are a virtually limitless number of possible outcomes by clinical site. A 10 df chi-square test has a critical value of 18.37, meaning that the sum of the individual cell chi-squares must be greater than 18.37. But this sum of the individual chi-square can occur in almost any way, including possibly an extreme treatment effect in one clinical site. The interplay between the overall reduction (i.e., the expected values in each cell) and the variation among the clinics make it impossible to present power for anything beyond a very simple case.

If an interaction between treatment and Clinical Site exists, the assumptions of the Mantel-Haenszel chi-square test of overall treatment effect will not be met and an overall estimate of the odds ratio does not make sense. In that case, we will review the Clinical Sites' performance and results to determine the nature of the differences in treatment effects. If there is no interaction, the Mantel-Haenszel chi-square procedure will be used to estimate the overall odds ratio across all Clinical Sites. Secondary analyses will be conducted using logistic models to adjust for other clinical factors, such as age and sex.

The main analysis will be an intention-to-treat analysis where every participant who was enrolled in the study and randomized to a treatment group will be included in the analysis in the treatment group to which they were assigned. Imputation of possible outcomes will be performed for those participants who did not return for the 24-week visit.

c. Interim Monitoring / Stopping Rules for the Primary Outcome

The DSMB will provide ongoing review of safety and ethics in accordance with the DSMP.

The DSMB will review the study protocol and consent forms prior to initiation of participant recruitment. Assuming a lag of approximately 2 weeks from the time of the last specimen collection for the primary outcome until all results are received and verified for a DSMB report and another 6 weeks to generate the report and distribute to the committee with enough lead time for the DSMB members to read the report, we anticipate the following DSMB meetings with the appropriate p-value for monitoring the primary outcome:

An adaptation of the Lan-DeMets(63) procedure will be used for assessing the primary study outcome when the interim "looks" of the data are taken. This adaptation is based on the work of Pampallona, Tsiatis and Kim(64,65) and allows for flexible interim monitoring while simultaneously preserving both the type-I error and type-II error of the study. Both alpha and beta spending functions will be used; alpha for efficacy and beta for futility(66). The rate at which the alpha and beta are spent is a function of the total information available at the time of the interim analysis (i.e., cumulative patient accrual). A stopping boundary that preserves the spirit of the O'Brien-Fleming stopping boundary(67) will be utilized. The software package East(68) will be used for the monitoring of the primary outcome.

Because the Pampallona, Tsiatis and Kim approach is flexible, the number of "looks" does not have to be specified in advance and the time interval between "looks" does not have to be the same throughout the course of the trial. The stopping boundaries and maximum information (total number of patients required for the analysis) determined at the design stage are based on the assumption that the number and spacing of the interim "looks" are known in advance. In reality this assumption is not likely to be satisfied. The Pampallona, Tsiatis and Kim

approach will allow for deviations from this assumption used at design. In other words, the Pampallona, Tsiatis and Kim approach provides flexibility to monitor the data at arbitrary time points with the ability to perform additional unplanned "looks" or possibly drop one or more planned "looks" while still preserving the type I error and the type II error of the study design.

Once actual data accumulates, the values of the cumulative accrual and test statistic of the current "look" of the data are used to compute the appropriate stopping boundaries for rejecting H0 and H1 in the East software. These newly computed stopping boundaries could be different than those determined at the design stage. If the current test statistic crosses a stopping boundary such that H0 is rejected (efficacy analysis) or H1 is rejected (futility analysis), an administrative determination would be made whether the study should stop or continue. In addition to the probability of rejection of H1, the futility analysis will take into account rate of recruitment to determine if that rate is sufficient to provide the information about study endpoints in the time frame set for the project.

For children who have been lost to follow-up or decline or are unable to return for their evaluation at 24 weeks post treatment, we will impute their outcome at the final assessment based on a logistic modeling approach to multiple imputation.(69) Adverse events will be monitored at every DSMB meeting, as well as recruitment, follow-up rates, data quality, and patient status in treatment. A complete description of the Data and Safety Monitoring Reports, including table shells for the reports, is contained in this Data and Safety Monitoring Plan.

We anticipate the following schedule of DSMB meetings (assuming that patients will be randomly allocated to treatment starting on 1/1/2005) with the appropriate proportion of participants with plasma HCV-RNA evaluations at the end of 24 week post treatment period. The p-values required for rejection of H0 at each visit are tentative and would be slightly modified depending on how much data are available at that visit. The p-values that will be used in practice will be generated by the East software as the study progresses.

Tentative Schedule of DSMB Meetings for Interim Monitoring

DSMB Meeting	Data Received As of Date	Expected Number of Participants Recruited/Followed	Primary Outcome Analysis Report	p-Value Required for Rejection of H_0
6/1/2005	4/1/2005	56 / 0	--	--
12/1/2005	10/1/2005	112 / 0	--	--
6/1/2006	4/1/2005	112 / 28	1	0.00161
12/1/2006	10/1/2006	112 / 56	2	0.00197
6/1/2007	4/1/2007	112 / 84	3	0.00221
12/1/2007	10/1/2007	112 / 112	Final	0.04829

d. Safety Analysis

The DSMB will receive routine reports on safety every 2-3 months. The first report will be generated after the first 30 patients have completed 4 weeks of therapy or, if recruitment is slower than expected, three months after the first patients is randomly allocated to treatment. The first report will be reviewed by the DSMB and, if there are serious concerns about safety, enrollment will be suspended pending further analysis and review. When the DSMB is satisfied with the safety of the treatment(s), enrollment will be resumed.

These reports will include detailed information on every adverse event (AE) and every serious adverse event (SAE), their relation to study medications, the duration of the event, and, for SAEs, a written narrative of the event. Summary tables will be prepared listing the frequency of both AEs and SAEs by body system and by type of event. This information will be presented by blinded treatment group for review by the DSMB.

If an SAE is found by clinic staff, it must be reported to the CRO within 72 hours. If the SAE is thought to be unexpected and related to study medication, it must be reported by the clinical center PI to Dr. Kathleen Schwarz within 24 hours as well as to the CRO. Dr. Schwarz will review the case with the clinical center PI to establish the relation to study medication. If it is related to study medication, it must be reported to the FDA, NIDDK, and to Roche within 72 hours.

Each AE and SAE will be reviewed by the Medical Safety Committee to determine classification of the event into a standard nomenclature (such as MedDRA [Medical Dictionary for Regulatory Activities]) for summarization and reporting purposes. The frequency of AEs and SAEs will be reported both as the total frequency, regardless of the number of times reported by an individual patient, and as a proportion of patients ever reporting the event. In each case, the proportion of events, both individual events and overall, will be tested for differences between the two treatment groups using a standard chi-square test with alpha=0.05. If, as indicated for the primary outcome, the expected frequencies within the contingency table of treatment x AE/SAE are insufficient to support the use of the standard Pearson chi-square test, we will use Fisher's Exact Test.

In addition to adverse events, other safety outcomes include vital signs and laboratory tests. These will be analyzed as indicated below for Secondary Outcomes.

e. Secondary Analyses of the Primary Outcome

The secondary analysis of the primary outcome, as described above, will be to adjust the treatment group comparison of the primary outcome for other potentially confounding factors, such as gender, age, parental education, baseline, week 12 and week 24 viral disappearance, and clinical site in a multiple logistic model. Furthermore, clinical sites will also be regrouped to 3-4 by regions of the country to effectively assess treatment site interaction by subset analysis. Co-variates will be determined prospectively or using combined data. Multiple logistic regression models can help to determine whether the apparent effect of one variable (e.g., treatment) on the outcome of interest (e.g., non-detectable plasma HCV-RNA) is accounted for by differences in other patient characteristics (e.g., age, gender, etc.). A typical logistic model might be:

$$\text{logit}(p) = \log(p/1-p) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots$$

with x_1 is treatment (coded 0=PEG-2a+placebo, 1=PEG-2a+RV), x_2 is age as a continuous variable (although we will investigate non-linearity of the relation between age and logit of the probability of SVR at the end of 72 weeks through the use of dummy variables for age), and other terms might include gender as a dummy variable (0=female, 1=male).

Additional analyses will be conducted to fully analyze the eventual four treatment groups: PEG-2a alone, PEG-2a + RV, PEG-22Aa followed by compassionate PEG-2a+RV, and PEG-2a+RV followed by compassionate PEG-2a + RV.

In general terms, continuous predictor variables will be included either as a single term or as a set of indicator (dummy) variables to model the effect of each category on the outcome. Binary and categorical predictor variables will also be included as predictor variables. For a set of indicator variables, we will use a global (Type III) test to assess the overall significance of the indictors and, if that test is significant, we will test the individual coefficients for significance. The goodness of fit for all models will be assessed, either through an F-test (for general linear models and GEE models discussed below) or through the Akaike Information Criterion and the –2 log likelihood statistic, for logistic models. Other goodness-of-fit tests for the logistic models, such as the Hosmer and Lemeshow goodness-of-fit test exist and may be used depending on the actual data.

These secondary analyses will help us to look for subgroups of patients for whom the treatment may be particularly beneficial or harmful. Examination of treatment by variable interactions is performed to identify such subgroups. For example, in the model above, since age may have an effect on response to treatment, a term for treatment by age interaction could be added to determine if treatment has the same effect on outcome in younger children compared to older children or adolescents.

Because of the multiple comparisons anticipated in secondary analyses, more stringent Type I error levels than the $p < 0.05$ used for the primary outcome analysis should be specified, for example, $p < 0.01$ to represent strong evidence of differences.

f. Analysis of Secondary and Safety Outcomes

The secondary outcomes will be sustained biochemical response defined as two consecutive normal serum ALT assessments taken at weeks 60 and 72. Safety outcomes will include vital signs, laboratory tests and clinical adverse events recorded throughout the study.

The analysis of the secondary outcome will be similar to that used for the primary outcome, since both will be binary variables recorded once for each participant. The safety outcomes, such as vital signs or laboratory values, will be modeled through longitudinal models, such as generalized estimating equations (GEE) (70) or random effects (71) models, since they are recorded at multiple times for each participant. These techniques are used to adjust for the inherent correlation among measurements on the same individual, since the existence of this correlation violates one of the basic assumptions of linear or logistic regression that each observation be independent of all of the others. Both continuous and binary (or ordinal) outcomes can be modeled with either GEE or random effects models.

g. Missing Data

Despite the best efforts of the clinical site staff, it may not be possible to collect all follow-up data for all patients. In addition, there may be missing data from the visits due to participant unwillingness to complete certain portions of the tests. To the extent possible, analysis approaches will be used to take into account these varying numbers of observations and missing data. However, most analytic techniques are 'complete data' techniques which require complete data for a participant to be included in the analysis. Further, since the primary analysis will be an intention to treat analysis, imputation for missing variables (and missing participants) should be carried out. It is clear that we need to account for all patients that are living at the time of the follow-up visit. We expect approximately 15% of the patients to drop-out of the study by the time of the 72-week follow-up visit. Although allowance for this level of drop-out has been made in the sample size calculations, imputation of this much data is troublesome because whatever assumptions are made in the imputation process may affect the outcome of the trial. Discussions of the approach to be taken will be discussed with the DSMB. A worst case scenario analysis will also be performed, assigning missing data as treatment failures in the active RV arm and as treatment successes in the placebo RV arm.

Although the primary outcome of the study is a binary variable, it is possible to impute a missing outcome using a multiple imputation approach (72). Using this approach, multiple data sets are created with a missing data point imputed from the distribution of the variable of interest chosen at random. The missing value can be predicted in a variety of ways, including a modeling approach. Since we will have previous measures of plasma HCV-RNA prior to 72 weeks, from the children with complete data, we should be able to model the possible outcome for children missing the 72 week data, based on their previous data and on baseline characteristics. After n complete data sets are generated, the selected statistical analysis is performed on each data set, yielding n sets of statistics. These statistics can then be summarized to yield results that reflect the underlying variability of the variable of interest. Software such as Solas (v. 3.0) and SAS (Version 9.0) contain procedures to perform multiple imputations. Again, the application of this technique to the primary outcomes, and the nature of those analyses, will be developed in consultation with the DSMB.

The analysis techniques that are proposed for this study can utilize whatever data are available from each

child without the requirement that all data be present, but give biased results if data from one treatment group are missing for reasons related to outcome. Additional analyses to investigate whether there is a bias in the missingness will model the missingness of the primary outcomes as predicted by a number of baseline (and potentially interim) factors, such as treatment group, gender, medical history, and age, as well as relevant interactions. For example, we may find that children who had the most illnesses prior to enrollment in the study may not be returning. Although we expect that the extent of missingness will be small, we will reassess our analysis strategies in light of the missingness analysis results.

h. Analysis of growth and body composition data

For analysis specific to growth and body composition, the primary outcome variables are change in weight for age z scores, linear growth velocity and lean body mass, all evaluated at 48 weeks (Tables 3, 3a and 3b Schedule of Assessments).

Data will be examined for skewness and log-transformed for analysis if necessary. Elementary methods (simple correlation, t-test, analysis of variance) will be used to determine the various correlates of changes in body composition during treatment with PEG-2a. Participants in the ancillary study will be compared with subjects in the parent trial who decline to participate, to confirm the absence of selection bias with respect to age, weight, disease activity, and other pertinent variables. Secondary outcome variables include percentage body fat and lean body mass (as measured by DXA) evaluated at 48 months (Tables 3, 3a and 3b Schedule of Assessments).

Hypothesis 1a. Children treated with PEG-2a will show negative changes in weight for age z scores, linear growth velocity and lean body mass after 48 weeks of therapy compared to baseline.

Hypothesis 1b. Children treated with PEG-2a plus RV will show greater pre to post treatment changes in weight for age z scores, linear growth velocity and lean body mass compared to children treated with PEG-2a alone.

Hypothesis 2a. Children treated with either therapy will exhibit improved weight for age z scores, linear growth velocity and lean body mass during years 2-5 after discontinuation of therapy compared to end of treatment.

Hypothesis 2b. In years 2-5 after discontinuation of therapy children with SVR will exhibit improved weight for age z scores, linear growth velocity and lean body mass compared to children who do not have an SVR.

To test hypotheses 1a and 1b, changes in weight for age z scores, linear growth velocity as well as lean body mass over the 48 week study period will be analyzed by paired t-test and tested for zero mean. The analysis will be refined by multiple regression analysis, using 48 week change as the dependent variable and controlling for covariates identified as above. To test hypotheses 2a and 2b we will also add those variables (alone and in combination) to the multiple regression model to determine whether they are significantly associated with the change in weight for age z scores and linear growth velocity, and if so which of these changes can “explain” the others.

To corroborate the results, we will repeat the multiple regression (a) with follow-up weight for age, z score and linear growth velocity and body fat lean body mass evaluated at 48 weeks (Table 3, 3a and 3b, Schedule of Assessments) as the dependent variable and the baseline measurement as a covariate, and (b) as repeated-measures analysis with time-varying covariates. The latter method will allow us to use the most data, because subjects with isolated missing values can be included (rather than eliminated because change cannot be calculated when one point is missing). The use of partial data adds precision and causes no bias so long as the missing values are not associated with the outcome or uncontrolled confounders (72).

The relationship among measurements of body composition made by DXA, BIA, and skinfold thickness will be analyzed by regression methods, aided by the Bland-Altman graphical technique of plotting difference vs average (73).

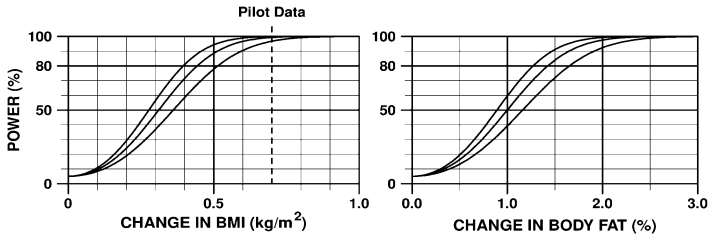


FIGURE 1. Power to detect 6-mo changes in BMI (*l.*)and percentage body fat (*r.*) in children receiving peginterferon for chronic hepatitis. In each panel, upper curve represents sample of 100; middle and lower curves, 80 and 60 respectively.

The power of the proposed trial to detect a given mean change Δ in any of the primary endpoints is determined by the formula $(\Delta/\sigma)\times n^{1/2} = t_{\alpha/2}+t_{\beta}$, where n is sample size; σ is the standard deviation of change among subjects; $t_{a/2}$ and t_{β} are Student t deviates corresponding to the Type I error rate $\propto$ and Type II error rate β; and power is $1-\beta$.

The parent study includes n=112 patients, of whom we expect at least 100 (89%) to consent to the growth and body composition part of the protocol. We also provide alternative calculations assuming 80 patients (71%) and a highly conservative 60 patients (54%). We use a=5% for each endpoint, following Glantz's guidelines for definition of familywise error rate (749). In Figure 1 the above formula is used to estimate the detectable changes in our two primary endpoints, weight for age z scores and linear growth velocity.

Pilot data on 31 children and adolescents treated with interferon (see Section C) showed BMI changing over 6 mo by an average of 0.7 kg/m2, with standard deviation σ =1.4 kg/m2. With those parameters, Figure 1 shows that a mean change of 0.5 kg/m2 (well under the change observed in pilot data), even under the most conservative estimate of participation, will be detectable with 80% power. With the anticipated sample of at least 80, or with a mean change closer to the observed pilot mean of 0.7 kg/m2, the power rises well above 90%.

Published data from normal children and adolescents give the standard deviation of percentage body fat as approximately 10% between subjects, as measured by DXA at one point in time (75). Because DXA estimates are strongly correlated with other measures of body fat, and the common measures of body fat show strong serial correlation — in our pilot data, ρ=0.93 between the subjects' two BMI measurements 6 mo apart — we estimate ρ=0.9 at least for two DXA measurements 48 weeks apart. Thus the standard deviation of change in body fat will be approximately $10\%\times (2(1-\rho))^{1/2} = 4.5\%$. With sample size and inferential parameters as above, Figure 1 shows changes as 1.5% body fat detectable with excellent power.

TABLE 9. Detectable correlations between changes in BMI or body fat and associated variables, such as change in energy intake or linear growth velocity.

Patients	Detectable correlation*
100	0.11
80	0.13
60	0.15

*Type I error rate 5%, power 80%.

Our secondary aims and hypotheses concern the association of changes in BMI and body composition with changes in energy intake and physical activity over the 48 weeks of observation and with linear growth velocity over that period. The smallest detectable correlation between two variables is given by $(1+(n-2)/(t_{\alpha/2}+t_{\beta})^2)^{-1/2}$, where all terms are as defined above. Table 9 shows that we have sufficient power to detect relatively subtle correlations, even if participation is not complete.

i. <u>QOL/Health Outcomes Sample Size Determination</u>

Soliday et al. (76) examined behavioral effects of corticosteroids using the CBCL. As the CBCL is our primary measure of psychological functioning, we will extrapolate from the changes in the CBCL T scores in the study by Soliday et al. to illustrate the power afforded by the proposed sample size for this part of the study. Iorio's study of IFN effects suggests that this is a conservative estimate of the effect we will observe (43). Assuming a standard deviation for pre and post CBCL scores of 8.9, a correlation for repeat testing of 0.80, and a standard deviation of the changes in scores of 5.7, we estimate needing only 18 patients to detect a 0.5 SD change (e.g., from 63 to 67.45) in our primary measure (CBCL) with two sided paired t test, 0.05 level of significance, with power set at .90. There will be a total of 112 subjects available to participate from the larger trial study. If we assume a 50%

participation rate and a 20% attrition rate, it will still be feasible to have an adequate number of participants. Since we are also interested in assessing covariates of the main effect, a sample size of 40 would be sufficient to test our secondary hypotheses as well.

j. QOL/Health Outcomes Analysis Plan

Hypothesis 1a. Children treated with PEG-2a will show negative changes in QOL, cognitive and developmental functioning, and psychosocial and/or behavioral functioning during and at the end of treatment when compared to their baseline evaluation.

Hypothesis 1b. Children treated with PEG-2a plus RV will show greater pre- to post- treatment change (i.e., lower QOL, lower cognitive and developmental functioning, and more psychosocial and/or behavioral difficulties) than children treated with PEG-2a alone.

To this end, the mean and standard deviation of change from baseline to post-IFN treatment (all assessment points reported in Tables 3, 3a and 3b) for all measures will be reported and compared using a repeated measures analyses of variance. A difference in the degree of change between the children in the PEG-2a alone and PEG-2a + RV groups can be tested by including an interaction term in the repeated measures ANOVA. The term would be treatment group X time; if it is statistically significant, it would tell us that the amount of change over time is not the same for the two treatment groups. For indices that are not normally distributed, appropriate nonparametric analyses, such as the Wilcoxon signed-rank test will be used.

Hypothesis 2a. Children treated with either therapy will exhibit improved QOL, cognitive and developmental functioning and psychosocial and/or behavioral functioning during years 2-5 after discontinuation of therapy compared to the end of treatment.

Hypothesis 2b. In Years 2-5 after discontinuation of therapy, children with SVR will exhibit improved QOL, cognitive and developmental functioning and psychosocial and/or behavioral functioning compared to children who do not have an SVR.

Post-hoc exploratory analyses will be conducted to examine bivariate associations between hypothesized variables (e.g., disease severity, parental health status, life events) and changes in primary outcome measures using linear regression analyses. This technique will also allow us to look for non-linear relationships, or cut points beyond which there is an effect.

7. Data Coordinating Center Role in Training of Study Personnel

Prior to the start of recruitment, MMRI staff will organize a multi-day training session for the staffs of the clinical sites to review the details of the study Protocol, data collection procedures, randomization and drug distribution procedures, CRA monitoring procedures, and laboratory specimen preparation procedures. MMRI staff will monitor each clinical site to assure that study coordinators at each site have completed the required training, Institutional Review Board clearances are obtained, and that all other steps necessary to begin patient follow-up are completed.

8. Receipt and Processing of Study Forms at the Data Coordinating Center (DCC)

a. Introduction

The main functions of the data management system are:

1. Electronic data entry of forms submitted by fax;
2. Editing of the data;
3. Preparation of protocol adherence aids; and,
4. Data extraction for analyses related to research objectives.

The data management system involves both clerical and computer components which are described below.

b. Data Entry

Clinical Centers will receive a notebook of data collection forms for each patient. 1) The first notebook will contain forms for screening to week 24 visits when all patients will complete the same visit sequence of forms. 2) The second notebook will be received after unblinding at the week 24 decision point and will include forms for visits at weeks 28 to 52. 3) The last notebook of forms will be received after the second decision point at week 52 when patients= viral response to study drug will dictate further treatment group re-assignments. All notebooks and forms will be labeled with the patient=s ID and letter code.

PEDS-C forms will be printed and distributed to the field center staff. Completed forms will be faxed to MMRI as outlined in the Manual of Procedures.

Data will processed using MMRI=s Comprehensive Data Entry System (CDE). PEDS-C forms will be formatted for use in this system which allows data entry through: 1) manual keying at MMRI, 2) faxing directly to the database server at MMRI for OCR/ICR scanning of data fields, or 3) scanning of the CRFs at MMRIdirectly into the database server for OCR/ICR scanning of data fields. PEDS-C data entry will use the CDE system based on Teleform data entry software. The data will be read from the form using ORC/IRC software, verified by a data manager, and a preliminary edit will be performed prior to moving the data into a transaction database. A comprehensive edit of the data in the transaction database will be performed and any questionable data items will be queried via e-mail to the Clinical Center field site prior to acceptance of the form data into the main study database. The form image will be stored for easy retrieval should a question arise about what was received by MMRI. Images associated with any corrections to the form will also be stored.

MMRI is currently using an MS SQL Server as the main database for large studies with edits and other procedures written in Visual Basic. The main study database will be housed on the MMRI database server running Windows 2000. Data entry will have quality control checks built into the system. A 10% sample of CRFs (regardless of input method) will be selected each month and verified manually against the main study database. A test set of forms with multiple errors will be run through the data edit system routinely to verify that errors are being queried and that correct items are not. The time between completion of a visit for a patient and when the forms are submitted will be tracked.

c. Data Editing

Current experience with Teleform data entry software at MMRI indicates that 100% of properly filled in bubbles will be read correctly. Before review by a data manager, over 90% of character data will be read accurately with virtually 100% accuracy after review, limited only by the handwriting on the form. Any data fields that cannot be read with certainty will be queried and returned to the Clinical Center staff for resolution.

The preliminary editing process will compare the patient ID number and letter code to the main study database containing the original patient registration information. If the identifying information is verified as correct, the remaining data are checked to verify that they are within pre-set ranges and are of the correct type. The main editing process will take place in three stages. First, each study CRF will be edited for completeness, internal consistency, numerical values within specified limits, and adherence to the treatment protocol. Second, all CRFs for each visit will be compared to identify discrepancies between data recorded on separate forms for the same patient. Third, all data for a patient will be compared longitudinally to identify any discrepancies between visits. Any discrepancies or questionable values will be returned to the Clinical Center for resolution. If a CRF fails to successfully pass the edit process, the data will be flagged as Afailed edit@ and may be excluded from the main database until the problem is resolved.

d. Electronic Transfer of Data from Central Laboratories

Virtually all of the laboratory data will be transferred electronically from the Central Laboratory (located at Covance Central Laboratory Services, Inc.). MMRI staff will work with laboratory staff to develop and implement a bar coding system to eliminate errors in the recording of identifying information. A mutually agreeable method and format of transmission will be developed, probably based on Covance's LabLink system of posting results on a secure Web-site for downloading at an appropriate time by MMRI staff. A tracking system will also be implemented to track the movement of specimens from the clinical sites to the central laboratory and the timely receipt of results from the central laboratory. This system will alert MMRI staff of any missing specimens or results on a daily basis for immediate follow-up. All laboratory data will also be subjected to an edit process that is similar in scope and comprehensiveness to the process for CRFs. Any problems with laboratory data will be immediately returned to the laboratory for verification and, if necessary, a reanalysis of the specimen.

e. Protocol Adherence Aids from Data Management System

MMRI staff will prepare and distribute protocol adherence aids from the data management system, including:

1. data collection schedules for children based on date of randomization; 2. list of visits that should be scheduled for the next month; and 3. list of forms delinquent based on the data collection schedule. The data collection schedule will be sent to the clinical site immediately after the child is randomized and enrolled in the study. This schedule, generated to reflect the schedule of visits in the protocol, will be customized to show the time window for each visit for that child and the data to be collected. The list of visits that should be scheduled for the next month will be a reminder for the clinical sites to schedule the visits and/or to verify that the children will be returning for their visit. The list of delinquent forms will also be sent as a reminder to the clinical sites to submit required forms for visits that should have been completed. This list will also be given to the site's Clinical Research Associate (CRA).

f. Data Extraction

Data will be extracted from the main study database using SAS/Access using form completion data as the selection criterion. This SAS module can read data directly from a database (including MS SQL Server) and store it as a SAS data set. SAS will be used to create the summary files to be used for analysis. Files that are created for a DSMB Report or for a publication (and related programs) will be permanently archived until the end of the study. All extracted files will be cross-checked with the original data table in the study database to verify that all records have been extracted and that data have been correctly transmitted. All summary files will be verified against the original data using both electronic comparisons and manual Quality Control (QC) comparisons.

At the appropriate time of database closure for a report, data which meet the selection criteria for the report will be extracted from the forms tables in the main study database and stored in the analysis section of the database for extraction by SAS/Access. If there are multiple versions of forms, they will be consolidated into a revision-independent format for analysis.

g. Data Backup

MMRI staff will utilize a variety of safeguards to protect the study from loss of data. MMRI staff will perform routine backup of all study files, including images of forms, the main study database, files created for analyses, and programs used to process and analyze data. The main study database will be completely archived on a daily basis. Other files and programs will be completely archived once every week with an incremental back-up of new or changed files every night. Every two weeks, the backup will be stored off site by Iron Mountain Storage Technologies. Analysis files for the routine reports will be backed up and kept for at least two cycles of the report. Backup copies of analysis files and programs used in the preparation of presentations and publications will be maintained until the end of the project.

h. Data Security and Patient Privacy

Due to the importance of protecting study data at MMRI, access to all computer files and programs will be restricted to authorized personnel through the use of passwords and automatic access control at the file level. For the protection of family privacy, the name, address, and other identifying information will not appear on the data forms sent to MMRI. All study records will be identified by the unique study ID number and any hard copies will be kept in locked file cabinets. Identification numbers are recorded on every page of the data forms in case one or more pages become separated (either electronically or physically). The study letter code will be used as a second level of identification and for matching with the child's ID number to verify correct identification. The letter code can be any combination of letters, but must be used consistently for that child throughout the study.

MMRI staff put the highest priority on guarding against an external threat to the study database. The MMRI intranet has the latest WatchGuard firewall protection and the latest MacAfee virus protection, both of which are updated daily. Within the intranet, no read/write external access is allowed to the study database. Read-only access is allowed for specific intranet users. There are no personal identifiers on any study database at MMRI. There is no dial-in access to the MMRI intranet.

i. Growth and Body Composition Data Management

Data management will be done by MMRI, acting as the Clinical Research Organization (CRO) and Dr. Shepherd at UCSF. Data will be collected on hard copy forms at each site under the supervision of the study project directors. All data forms from the sites will befaxed to MMRI as previously indicated. Project Directors will be responsible for assuring that all necessary data are collected in a timely and efficient fashion. MMRI will develop data entry procedures witha comprehensive edit checking process as previously indicated. These checks will include range checking, value table lookup, logical consistency checks, and entry verification for specified fields. Automated reports will provide a complete overview of the participant's status. Logic rules are programmed into the system to verify eligibility status and establish time windows for expected measurements and study visits after the participant is enrolled in the study. Each participant will have a master record of required measurements. A sequence of expected forms and dates of receipt of forms are generated by the initiation process. MMRI will review and track the receipt of all data forms, generate routine data submission status reports and develop and generate frequency distributions of study variables to provide timely and relevant feedback to project investigators regarding the accuracy and precision of data. Valid ranges and value checking protocols will be developed for each study variable during Phase I, and implemented on a routine basis. MMRI will work with field staff to resolve edit reports generated at the time of data entry and ensure the integrity of the database. A variety of standard summary reports will be generated from the central database to assist the field staff in the overall study data management.

j. QOL /Health Outcomes Data Management

Data will be collected at each participating center and sent to MMRIwhere it will be coded and stored. The primary hypotheses to be tested in this part of the study involve change over time, specifically, (1) whether children receiving IFN alone show short- and long-term changes in the variables of interest when compared to children receiving both IFN and RV, and (2) whether changes in the variables of interest occur over time. Since the data are longitudinal in nature and generated from a clinical population, it is subject to well-known difficulties like missing data, unplanned irregularly spaced measurement occasions, serial correlations, and questions about individual differences in time trends. Standard analyses, such as multivariate repeated measures analyses, will be used, in addition to generalized linear modeling when appropriate. Multilevel and multivariate repeated measures models will be used to explore time trends, treatment effects, and interactions between treatment and time on the outcomes of interest. Within each analysis, the baseline level of a variable will be used as a covariate in order to control for initial level of functioning on the variable.

9. HIPAA Compliance

To assure compliance with HIPAA regulations, each clinical site should include in their consent/assent forms specific language to permit the transmittal of patient information to MMRI, both in the form of CRFs and in the form of laboratory and genetic data. MMRI will review consent forms from each clinical site to verify the inclusion of the appropriate language and to notify the MMRI IRB of the compliance of the individual sites.

10. Monitoring Study Progress and Data Quality

a. Introduction

A primary concern of MMRI staff will be to assure the timely collection of complete and accurate data. There are two major steps associated with ensuring that accurate and complete data will be collected: 1) all persons associated with data collection should be properly trained and familiarized with the tasks that they are to perform (see Section 3 above); and 2) performance of the required procedures should be monitored and large deviations from study norms investigated. Monitoring reports on clinical site performance of recruitment and performance of follow-up procedures will be prepared at regular intervals.

b. Monitoring Patient Recruitment and Follow-up

MMRI staff will prepare weekly patient recruitment reports showing the number of patients recruited by each clinical site for that week and cumulatively. In addition, a comparison to recruitment goals will be included. Monthly follow-up reports will also be generated, showing the number of patients that have been seen as part of the follow-up by month, by visit, and cumulatively. These reports allow the study leadership to monitor the recruitment and follow-up efforts at each clinical site and to discuss with a clinical site director any problems with follow-up. In addition, each clinical site director will be aware of his or her standing with respect to the other clinical centers in the study. The CRA will also discuss recruitment and follow-up with the clinical site staff during the routine site visits.

It is important for the statistical power of the study to recruit all of the patients suggested by the sample size calculations and to obtain as much follow-up data as possible. It is just as important to achieve near complete follow-up to maintain the representativeness of the results from the two treatment groups. Every effort must be made to see, in one way or another, all of the children enrolled in each group and to collect basic information on them. This may involve home visits, increased incentives, or visits to local doctors.

c. Performance Reports

In addition to the recruitment and follow-up reports, a quarterly performance report will include information on the number and percent of forms delinquent for each clinical center and the edit status of individual forms. The percentage of follow-up procedures specified by the Protocol actually performed will also be reported. Selected baseline characteristics (e.g., gender, age) of patients will be summarized in the quarterly reports. The CRA will discuss these reports with the clinical site staff quarterly.

d. Quality Monitoring/Site Visits

Site monitoring will be performed using ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) and GCP (Good Clinical Practice) guidelines as outlined in the PEDS-C Monitoring Plan.

The CRA will prepare a full report after each site visit for regulatory compliance.

e. Quality Assurance Procedures

Quality assurance procedures will be implemented at every level of this study including clinical sites and theMMRI.

The quality control at the clinical site will be reviewed by the CRA, who will perform a review of study records/medical records in comparison to the data submitted to MMRI and will review selected patient's study charts for completeness, consistency with submitted data, quantity of missed visits, delinquent forms, and missing values on CRFs. The CRA will also review the reported condition of specimens received at the Central Laboratory.

A quality assurance procedure will also be implemented in the area of data entry, where certain key fields on each form will be reviewed by the data manager after processing by the CDE system. In addition, a sample of form images (including e-mail and Web data cast as a form image) will be printed out and compared with the data extracted from the fax image or against the original CRF by the CRA.

A report on quality assurance measures and activities will be prepared every six months for review by the Steering Committee and by the Data and Safety Monitoring Board. This report will include information (by clinical site and overall) on follow-up, protocol violations, missing and delinquent CRF submission and volumes of edit queries sent back to the clinical centers from MMRI.

f. Quality Control for Growth and Body Composition Data

Quality Control in Data Collection: Standard procedures to ensure the accurate and reliable collection of data include: well designed data forms with clear manuals of instruction; central training with hands-on practice and certification of staff; and on-going monitoring of all data collection activities. Data forms will be developed to ensure clarity and minimize missing information. Each form will also have question-by-question instructions. All data collection staff will be trained to conduct measurements. The training tool will be The NHANES III Anthropometric Procedures Video (Publisher: Health and Human Services Dept., Public Health Service, CDC, National Center for Health Statistics.) Standardized anthropometric procedures are demonstrated in this video for researchers involved in studies compiling data for multi-centered research studies. A copy of this video will be made available at each site, and the co-investigator at each site will be responsible for assuring that the data collection staff has reviewed this material. Staff will be instructed to use the Gulick II tape measure tension device, the standard for accurate body measurements, and Lange skinfold calipers.

Quality Control in Data Management: The data entry system provides for on-line editing of data, which includes range checking, table look-up for value accuracy, and intra and inter-form logical consistency checks. If an error occurs, the system automatically notifies the user, and if the error cannot be corrected immediately, errors will be printed out in hard copy for resolution by the study data manager.

Quality Control of Measurements: There will be a comprehensive quality assurance program for the DXA measurements in this study. This consists of four distinct activities: cross calibration, longitudinal calibration monitoring, centralized analysis, and technical consultation in DXA bone densitometry and body composition. The goal of this program is to have the data from all sites agree within 0.5% on each of the monitored variables over the course of the study.

1. *Cross Calibration:* UCSF maintains a stock of phantoms that have been shown to reflect the in vivo calibration of DXA devices. For this study, we will pass around a set of phantoms that include the Hologic Anthropomorphic Phantom, European Spine Phantom, and the Hologic whole body phantom. The phantoms travel as a set to each site and each scanned multiple times. In addition to the phantom scans, we will assure baseline device stability by acquiring radiographic uniformity scans. All scanners will be calibrated to one scanner using simple linear regression techniques and standardization relationships from the literature.

This study has several unique challenges. First, it contains different manufacturers (Hologic and Lunar) and different machine types (pencil beam, rectilinear fan beam, wide fan beam). There is limited literature on the in vivo relationships between all of these machine types for body composition. We will use the phantom results, available in vivo relationships, and possibly in vivo comparison at UCSF to insure each site is accurate to each other with regards to baseline measures and longitudinal changes.

2. *Longitudinal Monitoring:* UCSF recommends using the Hologic Whole Body Phantom (HWBP) to monitor body composition calibration. In this scenario, a unique HWBP would be resident at each site and measured at least three times a week. Calibration shifts will be monitored using the Cumulative sums technique described below. Because of the expense of these phantoms, approximately $5000 a piece, and financial constraints, less frequent phantom samples on fewer phantoms is sometimes necessary. Although reducing the sample points reduces sensitivity to calibration shifts, confidence in the data can still be shown if no shifts occur between samples. If a shift does occur, it may not be possible to correct for the shift and data may be lost. The HWBP has several advantages to other phantoms. It has bone samples for all regions of interests and thus is analyzed using the in vivo regions of interests, it has a mass that is as high as is practical for handling (70 lbs.), and provide the capability to monitor and correct for all whole body measures: Total Bone AREA, Bone Mineral Content (BMC), Bone Mineral Density (BMD), Fat Free Mass (FFM), Fat Mass (FM), percent Fat Mass (%FM), and Total Body Mass (TM). Only four of the seven variables are actually monitored since the other are derived from combinations of the others. UCSF will use the CUSUM method for determining longitudinal break points in the calibration. This approach has been previously detailed (77).

3. *Central Analysis*: Central analysis of all scans insures that there is uniformity in the placement of the region of interest lines as well as application of scan rejection criteria. UCSF research assistants are highly trained for the analysis of whole body scans. Training is supervised by experienced personnel and is a documented process. UCSF maintains a large collection of whole body scans for this purpose. Scans will be scored for the following error sources and:

(a) nonremovable objects
(b) removable objects not removed
(c) Amputations
(d) Excessive X-ray noise due to morbid obesity
(e) Hip/arm mass overlap
(f) Body parts off scanner
(g) Malpositioned subject
(h) Subject motion artifacts
(i) Scoliosis

This study has unique challenges regarding analysis. First, each differing device has unique analysis software and some of the devices have software that will not be upgraded except for bug fixes (Hologic QDR-4500A, Lunar DPX-IQ). In addition, the body composition software for children is evolving rapidly. For example, at the 2002 American Society for Bone and Mineral Research (ASBMR), Hologic announced new analysis algorithms for children that dramatically change its ability to detect bone. Thus, because of this evolution, it is likely that the scan results will need to be analyzed initially and then reanalyzed during the course of the study. UCSF maintains separate workstations for Hologic and Lunar devices and can insure that all scans are analyzed on the same version of software. UCSF also closely monitors changes in the software and will take advantage of software improvements as they become available.

4. *Technical Expertise*: UCSF will provide site manuals and procedures for acquiring the phantom and subject data. In addition, UCSF will provide procedures for data transfer from each site as well as consultation on how data accuracy is affected by software upgrades, machine malfunctions, and data pooling. UCSF's effort is lead by Professor Shepherd. His expertise is described in the Investigator's section.

The multi-center nature of our study also calls into question how comparable anthropometric measures are across the sites. Our use of the same personnel, training exercises, and anthropometric measuring devices at each site will improve the ability of our study to validly measure change in anthropometric measurements in individual subjects over time (our primary outcome) as well as to combine the data across sites.

Dietary questionnaires will be centrally analyzed.

Data from DXA measurements will be given to guardians at the end of the study.

11. Randomization and Study Drug Labeling

a. Randomization

At the screening visit the patient will be assigned a random three letter identification code by the study coordinator. The patient will be assigned an id number when a call is placed from the study site to the Automated Telephone Response System (ATRS) at MMRI. This system uses a touch-tone phone to enter information about a new participant, including age, gender, ethnicity, race, and a check on eligibility and informed consent. This system is available 24 hours per day, 7 days per week, with less than 50 hours of down time in over 10 years of functioning. In case of system failure, manual randomizations are available through a MMRI staff member who is available by pager at all times.

Access to the system is over an 800 number using assigned PIN numbers and passcodes. Each clinic coordinator (and any other designated staff at the clinical site who might enroll a participant in study) will be assigned a PIN and passcode. Each person assigned a PIN must perform at least one practice randomization to become certified in the system and to be allowed to perform a real randomization.

If the patient passes screening and is to be enrolled into the study, a second call to the ATRS system at the baseline visit will randomize them into the study. Randomization schedules will be generated within clinical sites with stratification by HCV genotypes (1 vs all others). The ratio of PEG-2a + RV to PEG-2a + placebo treatment assignments will be 1:1. The randomizations will be blocked using random blocking factors of 2 or 4, due to the relatively small sample size within a clinical site (approximately 10 participants for each of 11 sites). The resulting randomization schedules will be stored in the ATRS database for reference by the system. The randomization information telling the site which medication packages to dispense will be announced by the system to the caller and confirmatory faxes will be sent to the coordinator and to the clinical site pharmacy.

Study Medication Labeling

The study medications will be packaged with unique medication numbers on each package. Both treatment groups will receive PEG-2a, but the packaging will be labeled with a unique medication number as well. The local pharmacy at each clinical site will prepare the medications for distribution with the patient number and 3-letter identifier prior to distribution to the patient/caregiver.

12. Study Documentation, CRFs and Record Keeping

a. Preparation of Study Documents

MMRI staff will work with Dr. Schwarz and other investigators in coordinating the development of the study Manual of Procedures (MOP), reviewing drafts of the study MOP and case report forms (CRFs), and preparing sections of the Manual of Procedures related to follow-up procedures, data management, and statistical components of the trial.

Most of the CRFs developed for this study will be formatted to allow for ease of data entry using MMRI's Comprehensive Data Entry (CDE) system, a system that allows data entry through multiple entry points (as described below). However, the owners of some instruments, such as The Child Behavior Checklist (CBCL), may not permit reformatting. This instrument will be manually keyed at MMRI. If there is necessity for revisions of CRFs, they will be developed in a way to maximize the longitudinality of the data and minimize the introduction of new items or new coding schemes that are incompatible with previous versions.

For each patient enrolled, a Case Report Form must be completed and signed by the principal investigator or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the

study. If a patient withdraws from the study, the reason must be noted on the Case Report Form. If a patient is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using indelible ink, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his/her authorized delegate. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

The planned relationship and division of responsibilities between the PI (K Schwarz) and the MMRI is as detailed below:

b. The Transfer of Obligations Of The PI

PI hereby transfers responsibilities for the following obligations and MMRI hereby assumes such obligations.

(a) MMRI shall be responsible for obtaining the required regulatory documentation from qualified investigators, providing the investigators with the information they need to conduct an investigation properly ensuring proper monitoring of the investigation, and ensuring that the investigations are conducted in accordance with the general investigational plan and protocols contained in the Investigational New Drug Application ("IND"), MMRI shall also be responsible for maintaining adequate records, including:

Records and reports required by the FDA must be retained for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for the investigational use is discontinued and FDA has been so notified.

(b) MMRI shall be responsible for ensuring that the PI, the and Roche Drug Safety Monitoring Group, the FDA, the DSMB, and the NIDDK Project Scientist are notified within one working day of discovery of significant new or serious adverse effects or risks, or any unusual frequency of reactions with respect to the drug. MMRI shall be responsible for sending follow-up information to the PI and other groups to whom the original SAE report was sent as soon as the relevant information is available. .

13. Collaboration in Study Publications

MMRI staff will collaborate in the preparation of study publications and presentations to the scientific community, work with investigators to identify and carry out appropriate data analyses to answer study questions, and assist in writing manuscripts, with special responsibility for the accurate and effective presentation of study data, including both tabular presentations and graphical displays. MMRI staff will maintain a database of manuscript activity so that routine reports can be generated for the Publications Committee showing the status of each manuscript, how much additional work is required, what approvals are still pending, and the status of journal submission.

14. Publications Committee

The Publications Committee will consist of the PI, four investigators from the clinical sites, the NIDDK Program Officer, a representative of MMRI, and a representative from Roche.

E. PROTECTION OF HUMAN SUBJECTS - PERTINENT TO ALL SITES

1. Risks to the subjects

Human Subjects Involvement and Characteristics:
Number of subjects: 112
Number of centers: 11 centers qualified by training and experience in the management of pediatric patients with HCV
Age range: 5-18 years of age
Target Population:

Inclusion criteria

* Male or female patients who are 5-18 years of age
* HCV viremia (by any test) present on 2 tests separated by at least 6 months.
* Chronic liver disease, as indicated by inflammation and/or fibrosis, consistent with chronic hepatitis C virus infection on a liver biopsy obtained within 24 months of screening, as assessed by a qualified local pathologist, not consistent with other known liver disease and not normal
* Compensated liver disease (Child-Pugh Grade A clinical classification)
* Signed informed consent from legal guardian and willingness of legal guardian to abide by the requirements of the study.
* Hemoglobin values ≥11 g/dL for females;≥ 12 g/dL for males
* Normal TSH
* Able to swallow a 100 mg tablet
* Demonstration of ability to swallow a placebo tablet

Exclusion criteria

* Any prior treatment with Interferon or RV
* Receipt of any investigational drug <6 weeks prior to the first dose of study drug
* Any systemic antiviral therapy <6 weeks prior to the first dose of study drug. Exception: patients who have taken or are expected to require acyclovir for herpetic lesions
* Positive test at screening for anti-HAV IgM Ab, HBsAg, anti-HBc IgM Ab, or anti-HIV Ab
    * History or other evidence of a medical condition associated with chronic liver disease other than HCV (abnormal ceruloplasmin, alpha-1-antitrypsin, ANA>1:160, SMA>1:80, anti LKM antibody > 60 units)
* History or other evidence of bleeding from esophageal varices
* Decompensated liver disease (e.g. conjugated bilirubin >1.5mg/dl, ascites, varices, Child-Pugh Grade B or C clinical classification)
* History of autoimmune or immunologically mediated disease (e.g. inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hemolytic anemia, scleroderma, severe psoriasis, clinical evidence of rheumatoid arthritis)
* Absolute neutrophil count <1500 cells/mm^3 , Hgb <11 g/dL for females and <12 g/dL for males, WBC>17.5 x 10^9/L, or platelet count <90,000/mm^3
* Serum creatinine level >1.5 times the upper limit of normal for age
* Major depression according to the American Psychiatric Association (see Table 7, Criteria for Major Depressive Episode) , or a history of severe psychiatric disorder, such as major psychoses, suicidal ideation and/or suicidal attempt
* History or other evidence of chronic pulmonary or cardiac disease associated with functional limitation
* History of thyroid disease poorly controlled on prescribed medications, elevated thyroid stimulating hormone (TSH) concentrations with elevation of antibodies to thyroid peroxidase and any clinical manifestations of thyroid disease.
* Poorly controlled diabetes as defined by hemoglobin A1C of >8%
* History of solid organ or bone marrow transplantation
* Evidence of severe retinopathy
* Coagulopathy (INR>1.5)

* Evidence of an active or suspected cancer or a history of malignancy where the risk of recurrence is ≥20% within 2 years
* Hemoglobinopathy
* Hemophilia
* History of other evidence of severe illness or any other conditions which would make the patient, in the opinion of the investigator, unsuitable for the study
* Sexually active females of child-bearing potential (age 10 years and older) and sexually active males who are not practicing two forms of effective contraception during treatment and during the 6 months after treatment has been concluded
* Females who have a positive serum pregnancy test within 9 months of initiation of treatment or who are breast-feeding
* Males whose female partners are pregnant
* Active substance abuse
* A sibling and/or any other child living in the same household or sharing the same primary care giver enrolled in the study

Sources of Materials:

Medical records, blood and urine specimens, data. Use will be made of existing records to identify subjects for screening. From the screening visit onward, medical records, blood and urine will be obtained for purposes of research. For QOL and health related outcomes measurements all research material will be gathered for research purposes only and will include questionnaires completed by children and guardians. The project manager at the University of Florida Center for Behavioral Health Research in Organ Transplantation and Donation will be responsible for scoring and recording data for purposes of data management.

Potential Risks

Medical Risks of PEG-2a

Discomfort associated with injection and blood tests - likely, minimal risk
Common side effects of interferon include flu-like symptoms, loss of appetite, abdominal pain and diarrhea - likely, minimal risk
Temporary changes in laboratory tests including neutropenia, thrombocytopenia, ALT elevation - common - potentially serious without dosage reduction
Infrequently severe kidney problems requiring dialysis have been reported. - rare, serious
Depression, sleep disturbances, agitation, seizures and personality changes may also occur - uncommon, potentially serious
Possible risk to the fetus - rare, possibly serious
Lack of effect - common, minimal short-term risk
Decrease or loss of vision, retinopathy including macular edema, retinal artery or vein thrombosis, retinal hemorrhages and cotton wool spots, optic neuritis, and papilledema are induced or aggravated by treatment with Peg-2a or other alpha interferons – rare, potentially serious

Medical Risks of RV

Significant teratogenic and/or embryocidal effects - demonstrated in all animal species in which adequate studies have been conducted. These effects occurred at doses as low as one twentieth of the recommended human dose of RV - likely, serious

Hemolytic anemia - ~10% of patients treated in clinical trials. (Anemia has occurred within 1-2 weeks of initiation of RV therapy. Because of this initial acute drop in hemoglobin it is advised that complete blood counts should be obtained pretreatment and at week 1 and week 3 of therapy or more frequently if clinically indicated) – likely, minimal risk

Deterioration of cardiac function in subjects with hemolytic anemia – unlikely, potentially serious

Risks of Blood Drawing

Pain – likely, minimal risk
Bruise at the site – likely, minimal risk

In order to monitor the efficacy and potential toxicities of therapy, frequent blood sampling will be required. At each venipuncture, 10-30 ml of blood will be collected; however, no more than three milliliters per kilogram of body weight or a total of 50 mL will be drawn from any one patient during an eight-week period.

Risks Associated with DXA Scans

Each total body DXA scan (participants will have either 3 or 4 scans) delivers 1 mrem of radiation, less than the exposure during a transcontinental airline flight – likely, minimal risk

Risks Associated with dietary questionnaires and anthropometric measurements – unlikely, minimal risk

Legal Risks

NA

Financial Risks

NA

Alternative Treatments

Not to Treat

2. Adequacy of Protection Against Risks

Recruitment and Informed Consent:

Subjects will be recruited at each of the 11 pediatric liver centers by the caring physician and/or study coordinator who will identify potentially eligible subjects. Those guardians/caregivers will be contacted by phone by the study coordinator who will inform them about the study. If guardians/caregivers are interested a screening visit will be scheduled. At that visit, the study will be discussed in detail by the physician and study coordinator and the consent form (and age-appropriate assent forms) will be reviewed. The consent will include study purpose, description of procedures, risks and benefits, alternative treatments, costs, compensation, and issues of confidentiality. Signed informed consent/assent will be obtained from the guardian/caregiver and child and will be signed by the investigator and a witness. One copy of each consent/assent will be given to the family and one will be retained in the study records.

Protection Against Risk:

All children will be monitored closely at the pre-determined intervals discussed in the schedule of assessments. Frequent laboratory monitoring will alert the investigator to early signs of any adverse effects. Abnormal laboratory findings that are judged clinically significant by the investigator, will be recorded as an adverse event or serious adverse event. PEG-2a and RV will be stopped immediately if clinical signs, symptoms or laboratory abnormalities suggestive of a serious adverse event occur and/or an adverse event based on the clinical judgment of the investigator occurs. Dose modifications may be made by the investigator for adverse events. The toxicity table (see Manual of Procedures) will grade the event and the dosage will be adjusted based upon section D4c of the protocol. The informed consent will include procedures for withdrawal from and termination of the study.

Safety data will be shared with a DSMB who will judge the safety and tolerability of PEG-2a plus RV or placebo.. An investigator in this study may not be a member of the DSMB. Members of the DSMB will receive

safety data approximately every 3 months for review (schedule to be agreed upon between members of the DSMB and Roche). Safety assessments will consist of vital signs, AEs, and laboratory evaluations. A cumulative listing of patient withdrawals, dose adjustments, and serious adverse events (SAEs) will also be reviewed. DSMB members will be notified of all SAEs reported expeditiously to regulatory authorities. A copy of the DSMB charter is provided in the Manual of Procedures.

Regarding psychological risk, investigators will endeavor to address any concerns about negative impact on emotional well being expressed by the child or guardian. It is acknowledged that the questions included in the depression screening tools are the types of questions that are most likely to cause psychological risk. However, since screening for depression is a component of the data being collected, and the questions included in the assessment tools are the types of questions most likely to cause psychological risk, it is not appropriate to allow patients to 'skip' questions. The discomfort might be an indicator of depression and should prompt further investigation. Researchers will be instructed to closely monitor signs of discomfort, to provide information and support as needed.

Regarding social risk, the research team will carefully protect confidentiality of participants' assessment information. All researchers will be carefully trained about the importance of confidentiality and, consistent with all of our research projects, will be required to sign confidentiality agreements and to complete on-line instruction on human research participant protection. In addition, all paper-generated data will be stored in locked files in the research laboratory and all computer-generated data will be maintained in password-limited hard drive files (and floppy disk backup copies will be kept in locked files in the research laboratory). Participants will be told that they can discontinue participation in the sub-project at any time or refuse to answer any study questions.

3. Potential Benefits of the Proposed Research to the Subjects and Others

The subjects with CHC may experience permanent eradication of HCV, which is a leading cause of cirrhosis and hepatocellular carcinoma in adults. Information from this multicenter trial may lead to a safe and effective therapy for children with CHC. If PEG-2a plus placebo is as effective as the combination with RV, then the danger of teratogenicity and embrotoxicity can be avoided. Dr. Schwarz has submitted IND's 1511 and 1572 and Roche is authorizing the FDA to cross reference their IND's for PEGASYS™ and Ribavirin.

Subjects will have careful assessments of weight, other anthropometric measurements and body composition. At periodic times a dietician will be able to provide nutritional counseling and answer questions for the patient and family.

Learning the effects of PEG-2a on weight, body composition and linear growth will allow physicians to make more rational decisions about risk versus benefit in using this medication for chronic viral hepatitis in growing children. Recognition of changes in dietary intake during PEG-2a therapy will allow for better nutritional counseling and management of children in clinical practice who receive this treatment.

Participants in the proposed study may benefit by learning more about the QOL, cognitive, and psychological factors that may be associated with the child's medical treatment. Also, such information may be useful to guardians in identifying problems that may warrant psychological intervention. If as a result of the interviews and questionnaires, there is an indication of a clinically significant developmental, emotional, or behavioral problem, this information will be shared with the guardians, and families will be advised about further steps they could take to obtain other services. Participation in this study may advance knowledge about risks and benefits of treatment of HCV and will provide valuable information for current and future medical care. Overall, the risks to participating in this study are minimal in comparison to the potential benefits.

4. Importance of the Knowledge to be Gained

There are ~150,000 children in the United States with CHC and they are potentially at risk for end-stage liver disease, liver transplantation, and/or hepatocellular carcinoma, not to mention social stigmatization. If a safe, effective treatment can be identified then these potentially serious problems can be averted.

While the incidence of HCV in children is increasing, very little is known about effective treatments and their associated morbidity. The proposed study seeks to carefully evaluate the health-related QOL, cognitive performance, and psychological functioning of children with HCV being treated with either PEG-2a alone or in combination with RV. The findings of this study have important implications for enhancing our understanding of the morbidity associated with such treatment.

Collaborating Sites

OHRP assurance numbers and an assurance from each site that the four previous points have been addressed adequately at a level of attention that is at least as high as that documented at the applicant organization are provided in the letters of agreement from each co-investigator.

Inclusion of Women

Female children and adolescents up to the age of 18 years are part of the target population. For reasons of safety, breast-feeding and pregnant females are excluded as are sexually active females of child-bearing potential who do not use two methods of contraception during and up to 6 months after cessation of therapy.

Inclusion of Minorities

Minorities will be included consistent with the ethnic diversity of the patients with CHC seen in each of the 11 centers (which represent a broad geographic distribution.) Ethnicity and race will be established by self-reporting.

Inclusion of Children

Children 5-18 years of age are the target population. Children <5 years of age are excluded because of the possibility of spontaneous viral clearance and because of the increased risk of spastic diplegia in administration of high dose interferon to infants <1 year of age. Children and adolescents up to 18 years of age are included because there is already an FDA submission of PEG-2a alone and in combination with RV for subjects 18 years and older.

Description for Subject Selection Criteria (please see inclusion and exclusion criteria detailed above)

Description of the Rationale for Selection

Rebetol® (ribavirin, USP) Oral Solution to be used in combination with Intron A® has recently been FDA approved for the treatment of chronic hepatitis C among previously untreated pediatric patients as least three years of age and older. Although PEG-2a in combination with RV appears to be the most efficacious treatment in adults, this has not been assessed in children. Since RV may carry an increased teratogenic risk in children it is important to assess if RV is needed, or to assess strategies to minimize potential RV exposure. In adults, PEG-2a regimens are more effective when combined with RV than when given alone.

Available data suggests that children treated with unmodified interferon alone may respond better than similarly treated adults. It is then reasonable to speculate that children may do even better with PEG-2a but there are no current published data with the use of this agent in children. Results of recently published trials suggest that combination of unmodified interferon-RV therapy may enhance the virologic response in children with HCV infection but this is difficult to ascertain given the small number of children included in these studies. Furthermore, there are no controlled trials comparing these antiviral regimens in children. In summary, there is limited data regarding the treatment of childhood HCV infection. In particular, whether interferon alone is better than in combination with RV cannot be ascertained, which forms the basis for including a PEG-2a monotherapy arm. However, children in the monotherapy group who do not respond by 24 weeks of treatment (and are thus, unlikely to achieve a sustained virologic response) will have RV added since combination therapy is currently the preferred regimen in adults. We believe that this approach minimizes risks and maximizes benefits.

Children who have previously failed treatment with interferon alone or in combination with RV are very unlikely to benefit from the proposed therapy and are excluded for consideration.

Children who have relapsed after an initial response to interferon monotherapy may derive benefit from combination PEG-2a/RV therapy (based on adult data). These patients likely represent a unique group, which may bias our results and are thus, excluded for the purpose of this trial. However, children who have relapsed after a course of interferon should be considered for future study, depending on the outcome results of the current proposal.

Compelling rationale for proposed exclusion of any sex/gender or racial/ethnic group: none

Proposed dates of treatment and follow-up: January 2005 – November 2009
Proposed dates of enrollment: January 2005 – December 2005

Description of proposed outreach programs for recruiting women and minorities in clinical research as subjects: Clinical centers from across the country are participating to ensure the widest possible geographic, ethnic and racial diversity of subjects.

Targeted/Planned Enrollment Format Page

Data will be collected on ethnicity and race. Ethnicity and race will be established by self-reporting. Two separate questions will be the method used in collecting this data. Ethnicity data will be collected first. Respondents will be offered the option of selecting one or more racial designations. The number of respondents in each racial category who are Hispanic or Latino will be reported.

The questions will be asked to respondents as follows:

1. Do you consider yourself to be Hispanic or Latino?
Ethnic categories will include "Hispanic or Latino" and "Not Hispanic or Latino"

2. What race do you consider yourself to be? Select one or more of the following. Racial Categories will include "American Indian or Alaska Native", "Asian", "Black or African American" , "Native Hawaiian or Other Pacific", and "White"

Estimated total number of eligible patients (268) out of which at least 5 will be enrolled from each center for a total enrollment of 112 patients.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants

Study Title: Pegylated Interferon+/- Ribavirin for Children with HCV

Total Planned Enrollment: 112

TARGETED/ PLANNED ENROLLMENT: Number of Subjects eligible			
Ethnic Category	Sex/ Gender		
	Females	Males	Total
Hispanic or Latino	4	9	13
Not Hispanic or Latino	126	129	255
Ethnic Category Total of All Subjects	130	138	268

Racial Categories			
American Indian/Alaska Native	1	2	3
Asian	6	9	15
Native Hawaiian or Other Pacific Islander	2	2	4
Black or African American	13	20	33
White	108	106	214
Racial Categories: Total of All Subjects**	130	138	268

**The Ethic Category Total of All Subjects must be equal to the Racial Categories Total for all Subjects.

F. VERTEBRATE ANIMALS NA

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