SyNCH HCV Phase II:

A Multicenter, Randomized, Double-masked, Placebo-controlled Phase II Study to Assess the Safety and Efficacy of a Standardized Orally Administered Silymarin Preparation (Legalon®) for the Treatment of Patients with Chronic Hepatitis C Who Failed Conventional Antiviral Therapy

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Summary

Major advances have been made over the last decade in the field of antiviral therapy for chronic hepatitis C. Approximately 50% of patients treated with the combination of peginterferon and ribavirin achieve a sustained virological response. Unfortunately, the remainder either fails to respond or must discontinue treatment prematurely due to adverse events. In addition, a significant number of patients with chronic hepatitis C are never offered therapy because they have contraindications to the rigors of treatment with currently available medications. Additional therapeutic options are needed.

Herbal products have been used empirically for centuries as alternative medicines to treat a variety of human disorders. *Silybum marianum*, or silymarin, is primarily used for its purported beneficial effects in disorders of the liver, which include anti-inflammatory, anti-oxidant, and anti-fibrogenic activities. However, there is little evidence from clinical trials to support the use of silymarin as a treatment for diseases of the liver. Several major limitations of prior clinical investigations on the hepatoprotective effects of silymarin include: 1) the use of non-standardized silymarin extracts which confounds comparisons between trials since the concentrations of the four potentially active isomers of silymarin are known to vary; 2) the incomplete understanding of the relationship between silymarin dose and steady-state exposures to the potentially active isomers of silymarin, confounding the evaluation of safety and efficacy; and 3) the use of heterogeneous patient populations and variable endpoints to assess therapeutic response.

This proposal is a phase II study that will evaluate the safety and efficacy of silymarin for the treatment of subjects with chronic hepatitis C who did not respond to conventional antiviral therapy. The primary objectives of this study are to assess the safety and adverse event profile of silymarin over a range of doses compared to placebo and to assess efficacy of silymarin in normalizing serum aminotransferase activity in patients with chronic hepatitis C. Secondary objectives are to characterize the effect of silymarin on serum levels of HCV RNA and to explore relationships between silymarin therapy and serum biomarkers of HCV hepatic disease activity (oxidative stress, apoptosis, and fibrogenesis).

Eligible subjects will be randomized to treatment with placebo or one of two dosages selected from the phase I study: Legalon® 420 mg or 700 mg administered orally thrice daily.

Investigators and subjects will be masked to treatment assignment. The study design includes a screening period during which patients will undergo full medical evaluation to verify protocol eligibility and a treatment period of 24 weeks during which time clinic visits and laboratory studies will be performed every 2-4 weeks to monitor for safety and efficacy of therapy. Subjects will continue to be followed for an additional 12 weeks after the completion of study medication to monitor for adverse events and investigate post-treatment outcomes. Blood will be collected for pharmacokinetic analysis for confirming estimates of PK parameters obtained during phase I, for measuring biomarkers, and for storage for future studies.

1.0 Background

Current therapy for chronic hepatitis C is successful for many patients

Curing a chronic viral illness has been an elusive goal for clinicians, until recently. Tremendous advances in understanding the pathogenesis of hepatitis C infection and in strategies for treating this disease have led to unprecedented success in eradicating HCV infection for prolonged periods of time, if not permanently. Intention-to-treat analyses of two large phase III trials of peginterferon (alfa-2a or alfa-2b) in combination with ribavirin have shown sustained virological response rates, defined as undetectable HCV RNA in serum for six months after discontinuing interferon-based therapies, of 56% and 54%, respectively (1, 2). Indeed, it is well established that individuals who achieve sustained virological response rarely have clinical or virological relapse during lengthy follow-up (3-5). Based upon data from retrospective studies, patients who achieve SVR with interferon-based preparations have improved hepatic histology (1-3, 6), a decreased likelihood of developing hepatocellular carcinoma (5, 7, 8), a low likelihood of hepatic decompensation (5), and lower liver-related mortality (9). Therefore, sustained virological response remains the gold standard for evaluating the effects of antiviral agents for the treatment of chronic hepatitis C.

Some patients do not respond well to therapy: viral and host factors

Ironically, the therapeutic accomplishments described above, while celebrated, have also focused attention on the less fortunate individuals who fail to respond completely to the current generation of medications available to treat HCV infection. To date, a number of host and virological factors have been linked to diminished response to interferon/ribavirin combination therapy, although the mechanism by which these characteristics exert their influence remain poorly characterized (10). Among virological factors, hepatitis C genotype is the most important in determining response to treatment (1, 2, 11). In three registration trials, SVR for genotype 1 ranged between 41-52% when patients were treated for 48-weeks with peginterferon and ribavirin preparations, compared to rates approaching 80% for patients with genotypes 2 or 3 (1, 2, 12). Several host factors have also been implicated as having a significant effect on response to interferon-based therapies including cirrhosis, hepatic steatosis, and race (1, 2, 13-16). The relationship between hepatic steatosis and hepatitis C infection is complex. Individuals with the metabolic syndrome (obesity, hyperlipidemia, diabetes) have independent risk factors for development of hepatic steatosis, although an association between steatosis with hepatitis C genotype 3 also suggests a direct effect of HCV (13). A direct inhibitory effect of increased insulin levels on interferon response has recently been demonstrated in the replicon model, suggesting another mechanism by which obesity, insulin resistance, and hepatic steatosis could interact to hamper antiviral response(17). Race also has an impact on antiviral response (18); three prospective studies have recently demonstrated that African Americans have rates of sustained virological response to peginterferon and ribavirin that less than half those of Caucasians patients (19, 20).

Some patients are not candidates for antiviral therapy: need for alternative therapies

Several studies estimated that up to 86% of patients were deferred from IFN treatment for medical or psychiatric co-morbidities (21-23). Adverse events are common in patients with chronic hepatitis C treated with combination of peginterferon and ribavirin (24). Neuropsychiatric side effects, particularly mood disorders and depression, are the most common side effects that necessitate dose adjustments or premature discontinuation of therapy (1, 2, 24). Indeed, unstable psychiatric disorders or past suicide attempts, as well as medical

co-morbid conditions, such as coronary artery disease and autoimmune disease, are strong contraindications to therapy with existing agents due to the increased risks. It should be apparent from these data that the impressive therapeutic response rates from phase III clinical trials of peginterferon and ribavirin reflect a highly select patient population, those who are deemed to be ideal treatment candidates, with no co-morbidities that would hamper their ability to tolerate a rigorous therapy. The remainder of patients with chronic hepatitis C, those with absolute or relative contraindications and those who did not achieve sustained virological response with previous therapy, would potentially benefit from alternative therapies.

Important endpoints when sustained virological response is not possible

Until the next generation antivirals are available, alternative approaches must focus on other clinically important endpoints that could provide long-term benefits for patients with chronic hepatitis C when achieving sustained virological response is not possible. The most direct evidence that interferon can improve hepatic disease activity is derived from analyses of pre- and post-treatment liver biopsies. Studies of combination interferon and ribavirin have consistently demonstrated improvement in serum ALT activity, necroinflammatory activity, and even in some measures of hepatic fibrosis, with successful viral eradication (1, 2, 25-27). However, biochemical and histological improvement is also evident in many patients, even when sustained virological response has not been accomplished (1, 2, 28). One could infer that alternative therapeutic agents that could improve surrogate markers of disease activity and, perhaps, ameliorate complications of hepatic disease even without affecting HCV RNA, would represent an attractive adjunct to currently available medications. This provides a major rationale for rigorously studying an herbal product such as silymarin, as alternative therapy

Milk thistle (*Silybum marianum) extract*, commonly termed silymarin, is widely used as a natural treatment for liver disorders due to its hepatoprotectant and antioxidant properties.

Despite a large body of literature describing the use of silymarin over several decades as an alternative therapy (29), the published evidence of clinical efficacy in hepatic disease is equivocal. Silymarin, extracted from the dried fruits of *S. marianum*, consists of a variety of flavonolignans. Soil composition, sun exposure, water availability, and temperature, extraction, and processing influence the composition and potency of silymarin (30, 31) A standardized extract of *S. marianum* can be considered to contain approximately 60-70% silymarin with the rest mostly polymeric and oxidized polyphenolic compounds (32). The principal component, which constitutes approximately 50% the content of silymarin (SM), is silybin (SB). Other prominent isomers include silichristin (SC), silidianin (SD), and isosilybin (ISO), constituting approximately 20%, 10%, and 5% of silymarin, respectively. Others flavonolignans have been identified including taxifolin, dehydrosilybin, desoxysilycristin, desoxysilydianin, silandrin, silybinome, silyhermin, and neosilyhermin (33). For the purpose of this application, “silymarin” will be used to refer collectively to the four major silymarin isomers (i.e. silybin, isosilybin, silichristin, and silidianin) that are contained in extracts of milk thistle.

The relative contribution of individual isomers to overall activity is unclear

Kvasnicka and colleagues (33) examined the antioxidant effects of SB, ISO, SC and SD as well as a standardized mixture and various commercial products. A positive correlation was observed between increasing total flavonolignan content (0.1-1 mM) and antioxidant activity, and all SM isomers demonstrated antioxidant activity.

As the most abundant isomer, SB has been used almost exclusively as a marker of SM exposure in human subjects, while the contribution of the individual isomers to the potency and activity of SM is still unclear. Little information exists regarding the behavior of individual isomers *in vivo*. Schandalik et al assayed plasma and bile following a single low dose (equivalent to 120 mg SB) of silymarin extract (34). Although SD, SC, and ISO were not detected in plasma, SD and SC were detected in bile (peak concentrations ~1.5-3.5 ug/ml by 6 hours). While only a minor constituent of the extract, ISO concentrations were higher than SB in bile (peak concentrations ~25 ug/ml) (34). The two isomers of silybin display different pharmacokinetics (32). Since various SM isomers may have distinct pharmacokinetic properties, they should be evaluated separately to assess their contributions to the overall safety and efficacy of SM.

Silymarin's mechanism of action and efficacy in hepatic disease may depend upon achieving a critical concentration for antioxidant effects in the liver

The mechanisms of action that have been proposed for silymarin's hepatoprotective effects are diverse and have been reviewed elsewhere (AHRQ Evidence Report 21(35)). Of these, the antioxidant properties of polyphenolic flavonoids like silymarin are those most likely to attenuate the scope of biological effects initiated by oxidative stress and directly influence pathways of inflammation, necrosis, and fibrosis in chronic liver disease. For example, oxidative stress due to either mitochondrial dysfunction or reactive oxygen species (ROS) and lipid peroxidation, is thought to represent one of the several mechanisms responsible for initiation of apoptosis and necrosis in chronic liver diseases. Oxidative stress may also be an important initiating factor in fibrogenesis since ROS has recently been shown to stimulate HSC proliferation, collagen synthesis, and invasiveness (36).

The well-documented ability of polyphenolic flavonoids to scavenge reactive oxygen species (ROS) may only partially account for the antioxidant effects of silymarin. Arachidonic acid (AA) is a central lipid mediator of chronic inflammatory processes and is released from cellular stores by cytokine activation of PLA2 for conversion to prostanoids, thromboxanes, and leukotrienes in addition to its metabolism by cytochrome P450s that can result in formation of ROS and lipid peroxidation in the liver. Numerous lines of evidence suggest that altered or increased AA mobilization and turnover may be associated with progression of liver disease in HCV due to changes in the expression or activity of enzymes which metabolize AA (37). AA depletion is observed in mononuclear cells of HCV patients with fibrosis, a phenomenon that correlates to changes in lymphocyte and platelet counts, both biomarkers of disease progression (38). In addition, ratios of urinary AA metabolites are altered in HCV patients with cirrhosis (39). Silymarin has been reported to have immunomodulatory effects that may in part involve effects on AA metabolism. Silybin has been shown to inhibit the conversion of AA to leukotrienes by human granulocytes (IC_{50} = 15 uM), inhibit formation of PGE2 by human monocytes ((IC_{50} = 45 uM) and inhibit formation of thromboxane B2 ((IC_{50} = 15 uM) by human thrombocytes (40). Hepatic Kupffer cells reside in the liver and contribute to the severity and progression of the necroinflammatory activity and fibrogenesis in most chronic liver diseases due to their production of pro-inflammatory cytokines (IL-6), inflammatory prostaglandins (PGE2), death receptor ligands (TNF-α and FAS-L), and fibrogenic growth factors (TGF-β). SB concentrations between 15 to 80 uM have been shown to inhibit production of superoxide, nitric oxide, and leukotriene B(4) by activated hepatic Kupffer cells (41).

While SB concentrations in the range of 50-100 uM are not achievable in plasma even at higher than customary doses of silymarin, these concentrations might be achievable in the liver (see pharmacokinetics rationale below), which is the target organ for the Phase II study.

Silymarin has anti-inflammatory and immunomodulatory properties: another potential mechanism of action

In addition to being a potent antioxidant, silymarin has anti-inflammatory and immunomodulatory properties. In multiple cell types, silymarin strongly inhibits NF-κB- a transcription factor which regulates various inflammatory and immune response genes such as TNF-α, IFNγ, IL-2 and IL-6 (42). Consistent with its antioxidant effects, silymarin inhibits the generation of TNF induced ROS and lipid peroxidation which are implicated in TNF-induced NF-κB activation. In a mouse model of T cell dependent liver injury, SB was completely protective. Since SB did not affect concanavalin A-induced accumulation of CD4+ T cells within the liver, the proposed mechanism was inhibition of NF-κB activation with decreased intrahepatic synthesis of TNF-α, IFN-γ, IL-2 and inducible NO synthase and increased amounts of the anti-inflammatory cytokine IL-10. (43) Likewise, silymarin has proven to be protective in a mycotoxin mouse model of liver disease whose pathogenesis is modulated by TNF-α and IFN-γ.(44) Interestingly, serum concentrations of IL-2, IL-4, IL-10, IFN-γ and TNF-α are increased in chronic HCV infection(45). TNF-α is secreted by macrophages and lymphocytes and has proinflammatory, cytotoxic and direct anti-viral activity. In chronic HCV infection, serum TNFα and soluble TNF receptors (sTNFR) 55 and 75 are significantly elevated (46) .

Although the immunological mechanism responsible for progressive liver injury in chronic HCV infection is not completely known, the ongoing anti-viral immune response modulated by anti-viral cytokines such as IFN-γ and TNF-α as well as HCV-specific and nonspecific T cell results in damage to virally-infected as well as bystander hepatocytes. Conflicting results exist regarding silymarin's direct effect on T cells. *Ex vivo* studies performed on lymphocytes from alcoholic patients have revealed increased proliferation in response to mitogen after silymarin treatment but decreased cytotoxicity and reduced numbers of CD8+ T cells (47). Other studies have shown decreased T cell proliferation and cytotoxicity after silymarin exposure (48, 49). Kaempferol, another flavonoid compound, has been found to suppress Th1 cytokines IFN-γ and IL-2 and inhibit CD8+ T cell expansion and cytotoxicity in a mouse model of GVHD (50).

Dose Selection

The Phase I protocol indicated that decisions regarding dose selection for the Phase II trial would be primarily based upon consideration of safety and the PK relationships observed for the sum of parent (i.e. "Free") silymarin flavonolignan concentrations (i.e. ∑ Free silymarin). However, an unexpected finding from the Phase I study was that ∑ Free silymarin accounted for only 1.1% to 2.6% of the Total concentrations of silymarin flavonolignans found in the blood for all HCV dose groups (Appendix 1, Table 3). The remaining flavonolignan species that contribute to Total concentrations consist of glucuronide and sulfate conjugates (i.e. metabolites) of the parent flavonolignans.

Therefore, use of ∑ Free silymarin concentrations to assess drug exposures will be imprecise since they do not represent the total amount of drug entering the body. In addition, since conjugate concentrations in blood are approximately 11.5-times greater than "Free" concentrations, a small reduction in the extent of silymarin's metabolism to conjugates would be expected to greatly influence "Free" blood concentrations without changing "Total" blood concentrations or ∑ Total silymarin exposures. In addition, small changes in the extent of conjugation are not easily detected in "Total" concentrations because of the high %CV in these measures. Nonetheless, "Free" parent concentrations are expected to have influences on the efficacy and safety of silymarin that must be considered independent from those associated with

"Total" silymarin flavonolignan concentrations (i.e. unconjugated or "Free" flavonolignan plus all flavonolignan conjugates).

Parent ("Free") silymarin flavonolignans undergo extensive first-pass phase 2 metabolism to glucuronide and sulfate conjugates in the GI tract and in the liver. Therefore, "Total" flavonolignan concentration, which represents all species of a flavonolignan in blood, is the best measure of the amount of a flavonolignan entering the body following an oral dose. Because any or all of the silymarin flavonolignans may contribute partially or fully to the pharmacological activity of silymarin, ∑ silymarin concentration, which represents the sum of the concentrations of all six major silymarin flavonolignans, is the best measure of silymarin exposure for assessing safety and efficacy endpoints. Given these considerations, mean ∑ Total silymarin AUC was chosen for assessing dose-exposure proportionality in the Phase 1 trial and provides the basis for selecting 420 mg and 700 mg doses administered three times daily in this Phase 2 study.

Rationale for Dose Selection

High Dose: 700 mg silymarin three times daily
Dose-proportional increases in ∑ Total silymarin exposures were observed across all HCV dose groups (Appendix 1, Table 5). Therefore, ∑ Total silymarin exposures are predictable and reliable up to 700 mg in HCV patients. Also, a dose of 2100 mg daily (700 mg administered thrice daily) appeared to be safe and well tolerated in patients with HCV (Appendix 2). The 5 adverse events in 4 of 30 patients randomized to Legalon® were mild to moderate in severity and 4 of them were thought to be unrelated to treatment. Dizziness, the one adverse event considered to be possibly related to Legalon® was self-limiting and resolved in less than 1 day. There were no adverse events reported with doses above 840 mg/day. Since AEs were not observed at the highest exposures obtained with a dose of 700 mg, the silymarin dose of 700 mg administered three times daily that will be used as the high dose for this SyNCH Phase II trials is expected to provide reliable exposures and to be safe and well tolerated in the HCV population. In addition, this dose burden (5 pills three times daily) is the largest at which good patient compliance can be expected.

Low Dose: 420 mg silymarin three times daily
Since dose-exposure proportionality was observed over the dose range of 140 to 700 mg silymarin, a dose of 420 mg silymarin will be used as the lower dose in the Phase II trial as it represents a dose expected to result in 40% lower exposures which might be associated with clinically significant differences in pharmacodynamic endpoints when compared to those observed with a dose of 700 mg.

2.0 Study Objectives and Endpoints

2.1 Primary Objectives:

1. To assess the safety and adverse event profile of silymarin over a range of doses compared to placebo
2. To assess the efficacy of silymarin in improving serum alanine amino-transferase (ALT) to ≤ 45 IU/L or at least 50% decline to less than 65 IU/L

2.2 Secondary Objectives:

1. To characterize the changes in serum levels of HCV RNA during silymarin therapy

2. To explore relationships between silymarin therapy and serum biomarkers of HCV hepatic disease activity (oxidative stress, apoptosis, and fibrogenesis)
3. To characterize the population pharmacokinetics and pharmacodynamics of silymarin isomers including, silibinin A and B, isosilibinin A and B, silicristin, and silidianin following administration of silymarin to subjects with HCV.

2.3 Endpoints

Efficacy: The primary outcome variable (endpoint) for efficacy is whether or not serum ALT (mg/dl) is ≤ 45 IU/L (approximate normal range) or achieves at least 50% decline to less than 65 IU/L (approximately 1.5 times the upper limit of normal) at the end of the 24-week treatment period. The relationship between dose and change in $\log_{10}$(ALT) will also be investigated as a part of secondary analysis plans.

Safety: The primary outcome variable for safety is the occurrence of a dose-limiting toxicity during the 24-week treatment period. All adverse events (AE) will be categorized by severity.

Adherence: Will be measured using a summary of missed dose information obtained from patient diaries and dose counts and will be analyzed as both an outcome and as an explanatory variable in exploratory analyses of pharmacokinetic exposure and response.

Biomarkers: The following relationships will be explored: dose and change in biomarkers, changes in biomarkers and rate of treatment success, changes in biomarkers and rate of toxicity.

Rationale for using serum ALT and not liver biopsy as an endpoint:

Perhaps the most direct measure of improvement in liver disease is obtained by assessing the grade of necroinflammatory activity and stage of fibrosis in paired liver biopsies obtained before and after therapy. However, liver biopsies do incur some risks and discomfort to the subject and are costly to perform. Furthermore, it is possible that significant changes in hepatic histology could not be discerned during this relatively short treatment period, particularly in light of the small sample size and the inherent variability of biopsy sampling. The earliest studies of antiviral therapies for chronic hepatitis C, prior to the advent of qualitative assays for HCV RNA, utilized biochemical response as the primary endpoint of treatment (28, 51-53). Histologic improvement frequently accompanied biochemical improvement in these studies during interferon therapy (28). Thus, serum ALT activity can serve as an indirect marker for improvement in disease activity, although it is recognized that it is an imperfect association. Therefore, liver biopsy should be reserved for a phase III trial if this preliminary study provides some evidence of efficacy for silymarin. If a patient had a liver biopsy performed as part of standard of care practices, this information may be used to exclude patients with significant steatosis (see exclusions below). In addition, serum will be collected for a battery of serological tests that reflect fibrosis activity in the liver.

3.0 Study Design

3.1 Study Summary

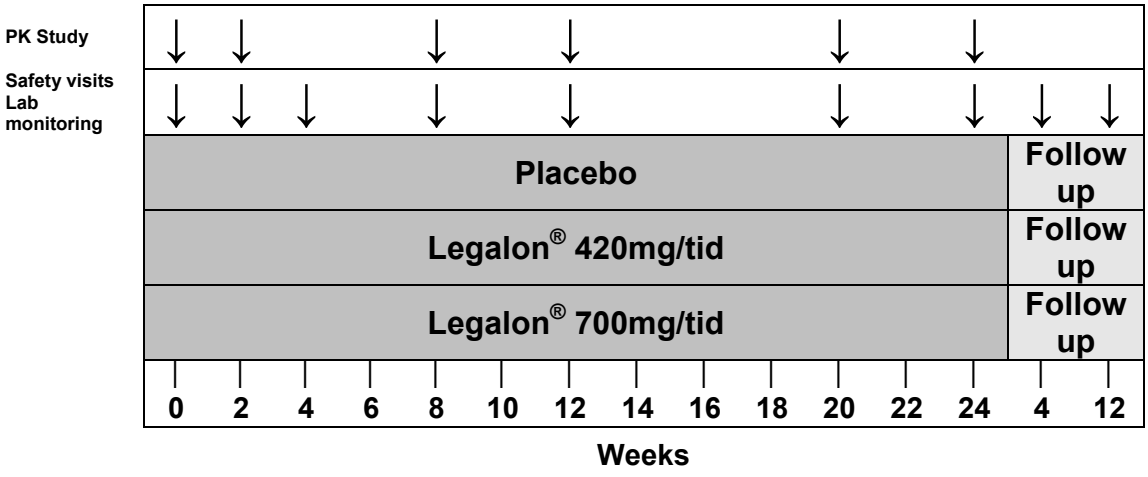
This will be a phase II, multicenter, randomized, double-masked, placebo-controlled study to assess the safety and efficacy of a standardized silymarin preparation (Legalon®) for treating chronic hepatitis C in subjects who failed to respond to conventional antiviral therapy.

Participating centers and Principal Investigators in the study are shown in Table 1. A total of 153 subjects who failed to achieve sustained virological response to a previous course of standard interferon or peginterferon with or without ribavirin for any duration will be enrolled at the 4 clinical centers. All subjects will be at least 18 years old, have serum ALT ≥ 65 IU/L, quantifiable (>600 IU/ml) HCV RNA,. After careful evaluation during a screening period, all eligible subjects will be randomized to treatment with one of two dosages of Legalon® or a placebo. Legalon® will be administered orally thrice daily for 24 weeks. Subjects will be evaluated regularly for adverse events and for monitoring efficacy through blood sampling. After completing therapy, subjects will be monitored for an additional 12 weeks to measure any sustained effects of study medication and to continue to monitor for resolution of any adverse events.

Table 1
Location of Participating Centers

Institution	Location	Principal Investigator	Type of Center
Beth-Israel Deaconess Medical Center	Boston, MA	Nezam Afdhal, MD	Clinical Center
Thomas Jefferson University	Philadelphia, PA	Victor Navarro, MD	Clinical Center
University of North Carolina	Chapel Hill, NC	Michael W. Fried, MD	Clinical Center
University of Pennsylvania	Philadelphia, PA	K. Rajender Reddy, MD	Clinical Center
University of Pittsburgh	Pittsburgh, PA	Steven H. Belle, PhD	Data Coordinating Center

A schematic of the study design is shown below. The required study visits and evaluations that will be performed at various time points are shown in subsequent tables.



3.2 Study Population

Definition of non-sustained response to previous therapy

Subjects will be considered as having non-sustained response to previous therapy if they received any interferon based therapy with or without ribavirin for any duration. Subjects whose HCV RNA remained detectable throughout therapy (non-responders) and those whose HCV RNA dropped below detectability during treatment but became detectable while still on therapy (break-through) or once therapy was discontinued (relapsers) will be eligible for participation. All genotypes will be eligible provided they meet the above virological definitions.

3.3 Entry Criteria

Inclusion Criteria

To be eligible for this trial, patients must meet the following inclusion criteria:

- Age at least 18 years at screening
- Serum HCV RNA above quantifiable level of detection by any assay after the end of previous therapy
- ALT $\geq$ 65 IU/L (i.e., approximately 1.5 X upper limit of normal) obtained during the screening period
- Previous treatment with any interferon-based therapy without sustained virological response
- Negative urine pregnancy test (for women of childbearing potential) documented within the 24-hour period prior to the first dose of silymarin. Females of childbearing potential must be using two reliable forms of effective contraception during the study (while on drug and during follow-up)

Exclusion Criteria

Patients with any of the following will not be eligible for participation:

- Use of silymarin or other milk thistle preparations within 30 days prior to screening
- Use of other antioxidants such as vitamin E, vitamin C, glutathione, alpha-tocopherol, or non-prescribed complementary alternative medications (including dietary supplements, megadose vitamins, herbal preparations, and special teas) within 30 days prior to screening. A multivitamin at standard doses will be allowed.
- Use of silymarin or other antioxidants or non-prescribed complementary alternative medications (as above) during the screening period or patient unwilling to refrain from taking these medications through completion of the study.
- Any antiviral therapy within 6 months prior to screening visit
- Known allergy/sensitivity to milk thistle or its preparations
- Evidence of poorly-controlled diabetes (defined as HbA1c > 8% in patients with diabetes)
- Use of warfarin, metronidazole or acetaminophen (greater than two grams per day) within 30 days of screening
- Lactose intolerance defined as patient reported inability to tolerate milk products
- Previous liver biopsy that demonstrated presence of moderate to severe steatosis or evidence of steatohepatitis
- Positive test for anti-HIV or HBsAg within 5 years of screening
- Average alcohol consumption of more than one drink or equivalent (>12 grams) per day or more than two (2) drinks on any one day over the 30 days prior to screening. Patients who met either criterion more than 30 days ago must have consumed a monthly average of 12 grams or less per day of alcohol for at least six months prior to screening.

- History of other chronic liver disease, including metabolic diseases, documented by appropriate test(s)
- Women with ongoing pregnancy or breast-feeding, or contemplating pregnancy
- Serum creatinine level 2.0 mg/dL or greater at screening or CrCl ≤ 60cc/min, or currently on dialysis. The creatinine clearance (CrCl) will be calculated according to Cockcroft-Gault.
- Evidence of drug abuse within 6 months prior to screening or during the screening period.
- Evidence of decompensated liver disease defined as any of the following: serum albumin <3.2 g/dl, total bilirubin > 1.5 mg/dl, or PT/INR > 1.3 times normal at screening, or history or presence of ascites or encephalopathy, or bleeding from esophageal varices
- History or other evidence of severe illness or any other conditions that would make the patient, in the opinion of the investigator, unsuitable for the study (such as poorly controlled psychiatric disease, coronary artery disease, or active gastrointestinal conditions that might interfere with drug absorption)
- History of immunologically mediated disease (e.g., inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune hepatitis, autoimmune hemolytic anemia, severe psoriasis, rheumatoid arthritis) that could affect inflammatory biomarkers
- History of solid organ or bone marrow transplantation
- History of thyroid disease poorly controlled on prescribed medications
- Use of oral steroids for more than 14 days within 30 days prior to screening
- Participation in a research drug trial, exclusive of the SyNCH Phase I trial, within 6 months of enrollment
- Inability or unwillingness to provide informed consent or abide by the study protocol

3.4 Study Treatment

All eligible participants will be randomized to treatment with either Legalon® 420 mg, or 700 mg or placebo administered thrice daily. The treatment period will be for 24 weeks and subjects will be followed off treatment for an additional 12 weeks. An IND (#74887) has been filed for the use of Legalon® in this study and a dossier reflecting additional preclinical and clinical information about this agent accompanies this protocol (Appendix 3).

Each patient will receive a total of 5 capsules as outlined in Table 2 for each orally administered dose that will consist of Legalon® and/or placebo in the appropriate capsule quantities in order to standardize pill burden, allow for an estimate of compliance, and to minimize the potential for variable absorption due to differential solubility in the gastrointestinal tract.

Legalon® (Rottapharm/Madaus) is a milk thistle fruit extract standardized to 140 mg silymarin per capsule (53% as total silybins). A Legalon® 140 capsule contains 180 mg dried extract of milk thistle fruits, or 140 mg silymarin which is the presumed active ingredients, or 108 mg silymarin quantitated as silybin by HPLC. The total weight of the capsule is 429 mg and includes: other milk thistle components that are not chemically well defined; components of the extraction process; excipients; and the capsule shell. The 108 mg of silymarin in Legalon® 140 capsules consists of the following potentially active ingredients: silybins A + B (~50 mg), silychristin (~20 mg), silydianin (~15mg), and isosilybins A + B (~15 mg).

Table 2
Dosing Strategy for Standardizing Pill Burden

Legalon® Dose	# Legalon® Caps	# Placebo Caps	Total # of Capsules
Placebo	0	5	5
420 mg	3	2	5
700 mg	5	0	5

3.4.1 Dose Adjustment Guidelines

Specific dose adjustment guidelines are provided to investigators for adverse effects considered to be possibly related to silymarin, including laboratory abnormalities, adverse events, and vital signs changes. Investigators should utilize the "General Dose Reduction Guidelines" (Section 3.4.2) below. When practical, abnormal laboratory results should be confirmed as soon as possible following notification of the investigator. If appropriate, downward adjustments in one level increment (see below) should be considered.

3.4.2 General Dose Reduction Guidelines

Dose reduction will be based upon severity ratings in accordance with the Common Toxicity Criteria for Adverse Events (CTCAE) (Appendix 4). Reporting of an abnormal ALT/AST results remains at the discretion of the clinical center Principal Investigator.

For grade 1, no dose reduction is needed. For grade 2 that persists over two consecutive safety visits and is not responsive to adjunctive symptomatic management, a level 1 dose decrease of study medication may occur at the discretion of the investigator. For grade 3 not responsive to adjunctive measures, a level 1 dose decrease of study medication is required. Subjects with grade 3 should be followed closely, possibly at more frequent intervals or via telephone contact to monitor in between regularly scheduled safety visits. If symptoms improve, return to previous dosing is allowed at the discretion of the investigator. For grade 4 , treatment will be discontinued. 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.

3.4.3 Dose-Specific Reduction Guidelines

Dose Reduction Guideline – Silymarin 700 mg dose level

AE Grade	Reduction?	Discontinue?	Silymarin	Placebo	Pill Burden
Mild	No	No	5	0	5
Moderate*	Yes	No	4	1	5
Severe**	Yes	Temp	3	2	5
Life Threatening	No	Yes	0	0	0

Dose Reduction Guideline – Silymarin 420 mg dose level

AE Grade	Reduction?	Discontinue?	Silymarin	Placebo	Pill Burden
Mild	No	No	3	2	5
Moderate*	Yes	No	2	3	5
Severe**	Yes	Temp	1	4	5

Life Threatening	No	Yes	0	0	0

*Moderate AE: Patient can resume original dose level if AE improves or resolves. NOTE: If the subject experiences a drug related AE and two dose reductions are not tolerated, the subject will stop the drug

**Severe AE: Discontinuation for 1 week. If SAE improves or resolves, restart at indicated dose level at the investigator's discretion. The dose level may be increased to original dose level at the discretion of the investigator with continued close monitoring.

3.4.4 Safety measurements

Laboratory evaluations: Participants will undergo laboratory tests according to the time and event schedule to monitor for medication toxicities.

Adverse event monitoring: Participants will be queried by study personnel at each study visit about the incidence of adverse events. These will be documented in the study record and classified according to duration, severity, relationship to study medication, and action with study medication. Serious adverse events will be reported to appropriate regulatory authorities, in addition to the DSMB as below, in a timely fashion.

In the event that an investigator considers an adverse event to be related to study medication, the investigator may submit an unmask request to the clinical center investigational pharmacist to unmask the study drug dose, per the study specific unmasking procedure.

Pregnancy: Women of child-bearing potential will have a urine pregnancy test performed within 24 hours of the first administration of study drugs, monthly for the duration of therapy, and at 1 month following completion of therapy.

3.5 Premature discontinuation

Treatment may be discontinued prematurely for the following reasons:
1. Participant requests termination of the study medication;
2. Participant is intolerant of the study medication despite predetermined dose reductions and side effect management strategies;
3. Safety concerns at the discretion of the investigator. These concerns will be documented in the source documents.

Participants who discontinue treatment prematurely for the above reasons will be encouraged to remain in the post-treatment follow-up period for an additional 12 weeks, unless they withdraw consent. For those discontinued prematurely, data and blood samples will be obtained as listed for the week 24 end-of-treatment visit.

4.0 Study visits (Appendix 5)

4.1 Screening Visit: The first screening visit will take place no more than 30 days prior to anticipated dose initiation. Written informed consent will be obtained prior to any screening evaluations. This initial screening visit will be used to determine patient eligibility as per the inclusion and exclusion criteria.

Table 3
Screening Visit 1

Informed Consent	Main study
Medical History and Complete Physical	
CBC/Differential	
Chemistry	ALT, AST, AlkPO4, total bilirubin, albumin, BUN, creatinine, sodium,
Coagulation Tests	PT or INR
Thyroid Test	TSH
Pregnancy Test	Urine
Viral Serology	Drawn at screening (historical acceptable if after end of previous therapy): HCV RNA quantitative Documented within 5 years: HBsAg, anti-HIV HCV genotype (historical acceptable)
Other Serology and Chemistry	Fe, TIBC, ferritin, iron saturation Anti-nuclear antibody (historical acceptable)
EKG	

4.1.1 Re-screen Criteria and Procedures: If the period between the initiation of screening and the Baseline visit is more than 30 days, but less than or equal to 6 weeks (42 days), a complete re-screen is not required. However, if the period exceeds 6 weeks (42 days), the screening evaluation must be repeated in its entirety.

Partial Re-screen (31-42 days elapsed between the initiation of screening and Baseline)

- The following laboratory tests must be repeated:
    - Liver function tests
    - CBC
    - Chem-7
    - Pregnancy test (where applicable)

Complete Re-screen (> 42 days elapsed between the initiation of screening and Baseline)

- Repeat the entire screening evaluation
- Repeat all labs required for screening evaluation
- Obtain blood samples for HCV-RNA test

4.2 Baseline visit (Day 0): Once the patient has been confirmed to be eligible and has completed the first screening visit, he/she will return for additional laboratory studies and comprehensive instruction in study procedures and medication administration by study personnel. These two visits, screening and baseline (day 0) will occur within 30 days. If the patient provides informed consent for the genetic component of the study, a 20ml sample of whole blood will be collected at the Baseline visit or at any of the subsequent protocol visits throughout the course of the study.

Table 4
Baseline (Day 0) visit

Interim Medical History	
CBC/Differential	
Chemistry	ALT, AST, AlkPO4, total bilirubin, fasting triglycerides and cholesterol, glucose, and insulin

Silymarin level	
HCV RNA	Serum 3ml (stored)
Sample storage	Fasting 10ml serum and 5ml plasma
Pregnancy Test	Urine, pre-dose
Adherence counseling	Use of MATI guidelines
Medication Education	Use of Medi-dose cups and patient diaries that record doses taken and alcohol use
Study Medication Administration	Supervised by research coordinator
Questionnaires	SF-36, CLDQ, CES-D, ASE

4.3 Treatment Period: Silymarin and/or placebo will be administered as a total of 5 capsules administered three times daily. Treatment will be continued for 24 weeks. During the treatment period, participants will return for safety and efficacy evaluations at treatment weeks 2, 4, 8, 12, 20, 24 (end of treatment). Phone calls will be made to the participants at weeks 6 and 16 to monitor safety, compliance with study medication and concurrent medications. There is an acceptable window of +/- 3 days for all visits. The following procedures will be performed at the study visits:

Table 5
Safety and Efficacy Visits

Interim Medical History	
Symptom-directed Physical Exam	Vital signs and weight at each visit; physical exam every 3 months or as needed
Adverse Event Assessment	Each visit
Concurrent Medication Assessment	Each visit
CBC/Differential	Each visit
Chemistry	Each visit; ALT, AST, AlkPO4, total bilirubin, albumin, BUN, creatinine, glucose. Week 12 and 24 only: fasting triglycerides, glucose, insulin
Pregnancy Test	Urine, each visit
TSH	Week 24
PT/INR	Week 24
Silymarin level	Weeks 2, 4, 12, 24
HCV RNA	Weeks 12, 24 (3 ml serum, stored)
EKG	Week 12
Sample Storage	Fasting 5ml serum and 3ml plasma at weeks 12 and 24 only
Pharmacokinetic sample	Each visit according to assigned schedule or random sample
Adverse Event Assessment for Mental Health	each visit
Questionnaires	Weeks 4, 12, 24: SF-36, CLDQ, CES-D, ASE
Adherence	Each visit

Monitoring and Counseling	Monitoring by dose counts and review of diary entries

4.3.1 Pharmacokinetic study

Specific goals of the study pharmacokinetic analysis will include 1) confirming estimates of PK parameters obtained during phase I, in which only a small number of chronic hepatitis C patients were enrolled, 2) a comparison with the NASH population, 3) identifying the sources and magnitude of intersubject pharmacokinetic variability on the basis of covariates, 4) observing changes in metabolite kinetics over time, and 5) potentially linking safety and outcome measures to pharmacokinetic profiles. The specific analyses are outlined below in section 6.4.

Subjects

The PK sub-study will provide samples according to the limited sampling strategy described below and will be used to develop a more robust population PK model and will also provide for an evaluation of the extent of intra-subject variability influencing covariates under investigation. All patients enrolled in the main study will be eligible to participate in the PK sub-study until the PK sub-study groups are filled. Of the 51 subjects in each dose group (3 dose groups = 153 total subjects), 10 subjects will initially be consented to participate in the PK sub-study and will have additional blood and urine samples collected according to the following design.

Replacement dropouts

The PK sub-study is using this limited subset of subjects to "predict" PK of the study population as a whole. For this reason participants who drop out the PK study will be replaced to successfully fill each dose group.

Sampling Design

A limited sampling strategy will be employed in which a total of 27-30 ml (9-10 samples x 3 ml/sample) of blood will be drawn per patient for quantifying silymarin isomers. Sampling time windows have been selected to optimize PK information from a sparse sampling schedule based on our preliminary model. The 10 subjects in each dose group will be randomly assigned by the Data Coordinating Center to either Schedule 1 or 2 which will require additional PK sampling at 3 or 4 of the clinic study visits; 5 of these subjects will be sampled on Weeks 0, 12, and 24, while the other 5 subjects will be sampled on Weeks 2, 8, 20. Actual sample collection times will be recorded on the case report forms and be utilized in the subsequent analyses.

Sequential blood samples will be obtained from each subject on the same day according to the sampling schedule into which the subject is assigned.

Schedule 1:	First Dose:	0-1 hours, 1-3 hours, 3-6 hours
	Week 12:	0 hour (trough), 1-2 hours, 2-4 hours
	Week 24, Last dose:	0 hour (trough), 4-6 hours
	Week 24, 18-24 hrs following last dose:	1 to 2 samples between 18-24 hours following last draw above*

* Time of visit relative to administration of last dose. If close to 18 hrs at time of visit then also collect a 22-24 hrs time point depending on length of stay. If 22-24 hrs at time of visit then only

Schedule 2:	Week 2:	0 hour (trough), 1-2 hours, 2-4 hours
	Week 8:	0 hour (trough), 1-2 hours, 2-4 hours
	Week 20:	0 hour (trough), 1-2 hours, 2-4 hours

Additional Random PK Samples from Subjects Not Participating in the PK Sub-Study

At the time of a scheduled study visit at baseline and treatment weeks 2, 4, 8, 12, 20 and 24, which can be any time during the day, 3 ml blood samples will be obtained from all other study subjects not participating in the PK sub-study. Study coordinators will record the time of the most recent dose from subject report and record the time of sample collection. The inclusion of this sampling scheme will be used to further develop a robust population PK model and will provide for an evaluation of the extent of inter-subject variability influencing the covariates under investigation. Plasma will be obtained for determination of silymarin (Legalon®) and silymarin metabolite concentrations (silibinin A and B, isosilibinin A and B, silicristin, and silidianin). Special attention will be paid to recording in source documents accurate sample collection times and timing of previous silymarin dose. Serum concentrations of free and total silymarin isomers will be quantified using LC/MS. Determination of the total fraction will be accomplished after hydrolysis with glucuronidase/arylsulfatase. The extent of phase 2 metabolism will be estimated from the quantitation of the amount of conjugated silymarin isomers that will be calculated as the difference been the total and free fractions for each isomer.

Blood

Blood will be collected from each subject for plasma silymarin concentration determination at the times indicated in the schedule of events tables. Plasma will be obtained from collected blood samples and processed and stored according to study procedures.

Urine

One 10 ml aliquot of urine from each visit will be taken and frozen for determination of silymarin concentrations.

Analysis of silymarin concentrations in plasma and urine will be performed using validated analytical methods. All biological samples will be processed and stored according to study procedures.

The label for each sample will identify the subject number and collection date and time.

4.4 Follow-up period: Participants will be followed for an additional 12 weeks after end of treatment. They will return for post-treatment visits at follow-up weeks 4 and 12. During these visits, participants will be evaluated for resolution of adverse events and laboratory studies will be drawn to measure efficacy.

Table 6
Follow-up Visit Procedures

Interim Medical History	
Symptom-directed Physical Exam	Vital signs, weight, and physical exam as needed

1 draw is needed. Prefer two draws in this window in order to determine the terminal half-life of the drug. The draws need to be at least 2 hrs apart.

Adverse Event Assessment	Each visit
Concurrent Medication Assessment	Each visit
CBC/Differential	Each visit
Chemistry	Each visit: ALT, AST, AlkPO4, total bilirubin, albumin, uric acid, BUN, creatinine, glucose
Pregnancy Test	Urine, Post-treatment week 4 only
Sample Storage	5ml serum and 3ml plasma at week 4 only
HCV RNA	Week 4
Questionnaires	Week 4: SF-36, CLDQ, CES-D ASE

4.5 Unscheduled visits: Participants who have changes in severity or initiation of new symptoms, or who need to have laboratory tests repeated, will be asked to return to the study site for unscheduled evaluations.

5.0 Adherence

It is recognized that there is a significant pill burden (15 capsules daily) for this study. Therefore, an effort will be made to measure adherence to the medication regimen in order to assist with the efficacy analysis and provide information that will be helpful if a phase III study is planned.

Adherence Measures

Procedures

Patient Education and Adherence Program
The adherence program for the study will consist of three central components: (a) informational-exchange, (b) skills development, and (c) social support enlistment. One of the tools utilized in this study will be the Medication Adherence Training Interview (i.e., MATI), a structured interview for medication education and adherence training. The MATI has been empirically tested in behavioral medical research in an HIV population and now has been modified for use in patients with chronic hepatitis C. The MATI will be reviewed by study coordinators and used as guidelines for on-going education and addressing medication adherence.

Baseline (day 0).
During this visit the study coordinator will also engage the participant in goal setting. Participants will be asked by the coordinator to set short-term goals (e.g., achieving a near-perfect adherence rate for the next 30 days) that may be important to the efficacy and safety of silymarin therapy. Participants will also be asked to create self-incentives for attaining the stated goals. The rationale is that individuals achieve greater self-directed change if they reward their successful efforts than if they provide no incentives for themselves.

Participants will be given a diary and asked to record the time of day they ingest each dose of study medication. The coordinator will assist the participant with planning a dosing schedule and integrating the medication times into lifestyle patterns. Participants will be encouraged to keep the portable diaries with them at all times or wherever they store their medications in their home or office. Participants will also be asked to track the number of alcoholic beverages consumed

on a daily basis. Participants will be encouraged to create a habit of completing the diary on a daily basis, preferably at the time of ingestion.

Study Visits. At subsequent study visits, participants will be asked about tolerance to medications and adherence will be stressed. The participant diary will be reviewed thoroughly and used as a tool to facilitate a discussion about adherence. Coordinators will apply strategies outlined in the original MATI to reinforce adherent behavior, and help participants problem-solve areas that need improvement. The tone of the discussion will be supportive and non-judgmental, while assisting the participant in identifying strategies to improve medication adherence. Coordinators will also review daily alcohol consumption and make recommendations when indicated.

Medication Adherence Outcomes

Primary: Cup counts. Patients will be asked to return all full cups to the coordinators. Missed doses (# of full cups returned) will be counted at each study visit.

Secondary: Patient self-report of missed doses in diaries. Patients will record the time of day for each dose ingestion in diaries. Study coordinators will record the number of self-reported missed doses on a monthly basis. In addition, silymarin levels measured at the specified visits will be used to identify non-adherent patients.

6.0 Statistical methods

Randomization: For the proposed multi-center parallel-arms experimental design with a total of N = 153 patients (approximately 38 per Clinical Center), adaptive allocation will be used to minimize the imbalance among doses (overall and within strata). Adaptive allocation will occur by a web-based system, with a manual backup system, devised by the DCC. Participants will be allocated to treatment arm within strata defined by site and history vs. lack of history of any milk thistle preparation use for greater than 30 days duration at any time in the past (except within the last 30 days prior to screening which is an exclusion criterion). Adaptive allocation will be performed via a web-based system located in a private and secure area of the server being used for the study. The web-based system will query the user for the information required to identify the stratum and other information to enable checks that the correct patient was assigned (e.g., patient ID, sex) and return the next treatment assignment in that stratum to the user.

Descriptive Analyses

Tabular and graphical descriptive statistical methods will be used to characterize the study sample at baseline in terms of demographic profile severity of disease and clinical measures. These results will be presented for each arm of the study. The numbers of subjects who drop out of the study or have incomplete data will be described. Descriptive statistical methods will also be used to summarize available measures of compliance recorded during the study by dose level.

6.1 Safety Analysis

To address the primary objective 1 (Section 2.1), adverse events will be tabulated and summarized by descriptive methods for each arm of the study. Estimates of the toxicity rate for each dose level of silymarin (θ_{420}, θ_{700}) and for placebo (θ_o) will be tabulated along with their 95% confidence intervals.

In safety analyses, the toxicity rates of the three arms of the study will be compared. A test of the null hypothesis H_{o2}: "$\theta_o = \theta_{420} = \theta_{700}$" is not anticipated to be adequately powered due to an

anticipated low toxicity rate and findings from the Phase I study, so it is not a primary hypothesis of this phase II study. An idea of the statistical power afforded by the study to test H_{o2} can be obtained by considering the simplified scenario in which stopping rules for toxicity are not used, and a chi-square test is used to test H_{o2}. In the absence of silymarin, the background rate (θ_o) during the treatment period may be as high as 0.05 (θ_o= 0.05). The maximum tolerated rate is θ_{max} = 0.10. If both of the toxicity rates for the silymarin arms are 0.10, then there is only a 12% chance that data will be collected which yields rejection of H_{o2} at α=0.05.

6.2 Efficacy analysis

To address the primary objective 2, for each arm of the study, an estimate of the response rate π will be tabulated along with a corresponding 95% confidence interval. Point and interval estimates of mean change in $\log_{10}$ (ALT) (a normalizing transformation) will also be tabulated.

The primary null hypothesis, H_{o1}: $\pi_o = \pi_{420} = \pi_{700}$, will be tested against the general alternative hypothesis, H_{a1} that response differs in at least one treatment arm via a chi-square test for association with α = 0.05. If H_{o1} is rejected, then pair wise comparisons of the arms will be examined using a sequentially rejective test procedure (Holm, 1979).

Computation of these results for the intent to treat (ITT) analysis will comprise the main results of this study. Similar results will also be examined for the compliant-per-protocol subgroup of participants, defined as patients taking at least 80% of the medication.

Secondary analyses of efficacy will include model-based analyses of the relationship between dose and change in $\log_{10}$(ALT). Appropriate statistical models will be used in which change in $\log_{10}$(ALT) is the dependent variable. Study personnel will also perform analyses designed to aid interpretation of the main results or designed to be exploratory for purposes of hypothesis generation. For example, study personnel will use logistic regression models to investigate the relationship of pharmacokinetic exposure to treatment response.

6.3 Power considerations

The treatment success rate for each dose level of silymarin (π_{420}, π_{700}) is to be compared to that of placebo (π_o), where the success is defined as the normalization of ALT (≤ 45 IU/L) or a 50% decline to less than 65 IU/L. The primary null hypothesis of interest is
H_{o1}: $\pi_o = \pi_{420} = \pi_{700}$.

In the absence of silymarin, it is anticipated that the background rate (π_o) of spontaneous ALT normalization during a 24-week period may be as high as 0.15 (π_o= 0.15). A clinically significant success rate would be in the neighborhood 0.40. An approximate idea of the statistical power afforded by the study to test H_{o1} can be obtained by considering the idealized scenario in which toxicity is not a concern, stopping rules for toxicity are not used, and a chi-square test is used to test H_{o1}. Assuming that there is no dose response relationship (i.e. $\pi_{420} = \pi_{700}$) and 40% success in each treatment arm with 153 patients (51 per arm) will provide 80% power to reject the null hypothesis at α=0.05 using a chi-square test. On the other hand, if there is a dose response relationship and it is assumed that the probabilities of success are 0.15, 0.30, and 0.50 respectively for placebo, 420mg and 700mg, then with 51 patients in each group, at α=0.05 will provide 94% power to reject the null hypothesis. For participants who drop out, the last observation carried forward method will be used to impute the final outcome so that the last

available ALT would be used to determine the treatment success. Therefore, no over-recruitment to account for loss to follow-up is necessary.

6.4 Pharmacokinetic data analysis

A limited sampling strategy has been devised based on empirical/noncompartmental and model-based approaches. This approach evaluates the family of single point and paired concentrations (observed and interpolated) in order to define the minimal sampling scheme to yield adequate prediction of drug exposure (plasma AUC). A regression-based approach will be utilized using various discrete concentration permutations as predictors and AUC as the response. Based on the availability of a suitable structural model, these results will be validated with a model-based approach based on D-optimal design theory once preliminary data from the Phase I study is available. All modeling and simulations will be performed using the NONMEM software (Version V, Level 1.1, Globomax LLC; Hanover, MD).

Population PK model parameters (fixed and random) will be estimated via nonlinear mixed-effects modeling using NONMEM. An appropriate compartmental model/structure model will be developed for silymarin isomers based on the PK data collected in this study. Data from the Phase I study may be added to improve model stability. The effects of clinical and demographic factors, including but not limited to weight, height, body surface area, body mass index, ALT, age, and gender on PK model parameters will be investigated using a backward elimination procedure in NONMEM.

Individual predicted PK parameter estimates based on the final model will be used to explore the relationship between various PK metrics and clinical outcomes. Using a logistic regression, the probability of positive (or negative) outcomes will be predicted based on various PK metric expressions (i.e. Cmax, AUC).

6.5 Data Management

Data will be submitted to the DCC via a distributed data entry system

DCC personnel will closely monitor clinical center adherence to study protocol and data collection practices to ensure complete and accurate research data. Monitoring will be performed via established data management procedures with on-site monitoring visits conducted at designated intervals or as needed to facilitate the smooth conduct of the study. At the time of the on-site visit, Data Coordinating Center personnel will have access to all study and patient documents as well as availability of clinical center personnel. All patient and study documents will be kept highly confidential.

Data Coordinating Center personnel meet weekly to discuss study status, recruitment, compliance, review data issues or interim analyses, clinical center participation, and other issues that arise during the course of the study.

7.0 Study Organization

7.1 Sites (See Table 1)

This study will be conducted at four clinical centers within the United States. The clinical centers are all tertiary care institutions and academic medical centers with extensive experience in the conduct of clinical trials of antiviral agents for the treatment of chronic hepatitis C. A Data

Coordinating Center will coordinate and oversee operations for the study, maintain the database and perform data analyses. This study will use the NIDDK repository. A central virology lab has yet to be selected.

7.2 NIH participants:

NIH participants will include project scientists and officials from NCCAM and NIDDK, as well as one or more consultants from NIDDK.

7.3 Committees:

A steering committee and an executive committee were formed to set policy and govern the conduct of the study.

Steering Committee: Serves as the primary governing body of the study; responsible for policy decisions; provides oversight in planning the overall study design, facilitates the conduct and monitoring of the study, and reporting study results; votes on and approves all major decisions, final protocol and subsequent amendments. Members consist of principal investigators of the clinical centers, the coordinating center, and the NCCAM and NIDDK project scientists. A chairperson is appointed by the NIH from among the clinical center PIs.

Executive Committee: Manages day-to-day issues of the study; makes decisions required between the Steering Committee meetings, as needed for efficient progress of the study, and reports its actions to the Steering Committee on a regular basis; organizes and sets agendas for Steering Committee meetings. Members consist of the Steering Committee chair, the Coordinating Center PI, and the NCCAM and NIDDK Project Scientists.

7.4 Subcommittees: Due to the small number of investigators and clinical sites involved in this study, all members will participate on ad hoc committees that will address issues that may arise during the course of the study including adherence, measurements, publications & presentations or ancillary studies. In addition, other subcommittees are as follows:

Coordinators Subcommittee: Attend to the day-to-day operations of the study including recruitment, protocol adherence, consistent and complete data collection at each clinical center. Make recommendations to the Steering Committee regarding any study issues that may require modification or resolution.

Exemption Subcommittee: Two hepatologists who are not recruiting participants into the study will review, on a case by case basis, protocol exemption petitions for subjects who do not meet one or more of the eligibility criteria (inclusion and exclusion) but are otherwise considered suitable candidates for enrollment. These exemptions will be considered only when they will not affect primary or secondary endpoints in the study. Examples might include liver biopsies or serological testing outside of predefined windows.

7.5 Data and Safety Monitoring Plan

Introduction

This phase II, randomized controlled trial aims to test the safety and efficacy of silymarin (Legalon®) in participants with chronic hepatitis C infection. The intervention poses greater than minimal risk to participants. Therefore, the data and safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigators (PI) and prompt reporting of

excessive adverse events and all serious adverse events to the National Center for Complementary and Alternative Medicine (NCCAM), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the Data and Safety Monitoring Board (DSMB) and to the participating centers' IRBs.

The Data Coordinating Center (DCC) will monitor clinical center performance (e.g., recruitment, retention, data completeness, timeliness of data collection and submission) and protocol compliance. These reports, with summaries of adverse event data, will be provided to the DSMB for their reports and calls or meetings, and to the Steering Committee at its annual meeting. DSMB reports will include both open and closed session reports, with only the latter including information by treatment arm.

Safety reports will be sent to the Principal Investigators and the NCCAM and NIDDK Project Officers. The Project Coordinator will be responsible for distributing these reports and assuring that all parties obtain copies of these reports.

The frequency of data review for this study differs according to the type of data and can be summarized in the following table:

Data type	Frequency of review
Recruitment Retention Protocol adherence(e.g., meeting inclusion/exclusion criteria, treatment compliance)	Quarterly reports for DSMB, NCCAM, and NIDDK
Adverse events	Quarterly reports for DSMB, NCCAM, and NIDDK
Serious Adverse Events (SAE)	Quarterly reports for DSMB, NCCAM, and NIDDK
Serious Adverse Events that meet expedited reporting criteria	As they occur for DSMB, NCCAM, and NIDDK
Laboratory data	Yearly

Data and Safety Monitoring Board

The DSMB members were appointed by the NCCAM and NIDDK. It consists of 5 members who have no financial, scientific, or other conflict of interest with the study. The DSMB acts as advisors to the NCCAM and NIDDK to monitor participant safety and study progress. The initial responsibility of the DSMB is to review the protocols and approve initiation of the study. Other responsibilities include reviewing informed consent documents; reviewing, commenting and approving the data and safety monitoring plan; assessing data quality, completeness, and timeliness; and evaluating recruitment and retention; monitoring risk versus benefit; consider factors external to the study when relevant information becomes available.

The DSMB will receive all MedWatch forms (FDA Form 3500A) submitted by the clinical centers for serious adverse events.

In the case of a serious adverse event requiring expedited reporting, the Data Coordinating Center will immediately notify NCCAM, the holder of the IND, the Project Scientists, and the DSMB. For the purposes of this study, reports of all deaths will be expedited, regardless of whether they are considered to be unexpected or related to study drug.

In accordance with IND safety reporting regulations, NCCAM, assisted by the Data Coordinating Center, will provide reports of serious adverse events requiring expedited reporting to the appropriate drug review division of the FDA and all participating investigators in writing within 15 calendar days of notification. Reports of serious, unexpected adverse events to the FDA will initially be conducted by telephone or fax within the required time frame (7 calendar days of notification by the clinical centers for telephone and fax safety reports). Following telephone/fax notification, written reports will be sent to the FDA.

The clinical centers are to report serious adverse events to the Data Coordinating Center within 24 hours of knowledge of the event so that notification can be provided as soon as possible following discovery of the event for reasons of patient safety. The clinical center PIs will be responsible for obtaining all information required to complete the adverse event report.

The DSMB will also review appropriate laboratory data annually.

Safety Monitoring

Adverse Events
The clinical centers will be instructed to collect information on all adverse events, defined as:

> "Any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product." (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *Guideline for Industry: Clinical SafetyData Management: Definitions and Standards for Expedited Reporting*, March 1995)

Adverse event information will be based on signs or symptoms obtained from the patient or family, during a physical examination, from results of laboratory testing, and clinical evaluation of the patient by the clinical center Principal Investigator (PI) and staff, and will include: the date and time of adverse event occurrence, severity, relation to medication administration, action taken, and outcome.

All serious adverse events, i.e., adverse events that result in, or are, any of the following:

- Death
- Life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Significant or permanent disability
- Congenital anomaly/birth defect
- Medical intervention to prevent permanent damage not requiring inpatient hospitalization
- Development of drug dependency or drug abuse

will be reported to the FDA and other entities (NCCAM, NIDDK, DSMB, IRBs) in cumulative fashion at designated time points (i.e., annual reports will be produced for the FDA and IRB; quarterly reports will be produced for the DSMB, NCCAM and NIDDK).

If the patient experiences a Serious Adverse Event:

1. Clinical center personnel will complete the FDA MedWatch form.

2. The completed MedWatch form must be faxed to the Coordinating Center at the University of Pittsburgh within 24 hours of knowledge of the event.
3. The Coordinating Center will immediately submit the MedWatch form to the chair of the DSMB if the SAE meets criteria for expedited reporting, and to the Project Scientists at NCCAM and NIDDK.
4. NCCAM, the holder of the IND, will report SAEs that meet criteria for expedited reporting to the FDA.

The Clinical Center Principal Investigators will be responsible for notifying their local Institutional Review Board.

Adverse Events Requiring Expedited Reporting

An adverse event will be subject to expedited reporting if it meets all of the following criteria, which are defined and discussed in detail below:

1) Serious in nature
2) Related to medicinal product
3) Unexpected

All deaths will be subject to expedited reporting, regardless of whether they are considered unexpected or related to medicinal product.

Related to Medicinal Product

The phrase "**related to the medicinal product**" implies causality or attributability to Legalon®. An adverse event should be considered to be related to Legalon® in situations in which a causal relationship cannot be ruled out. As noted above, the clinical center PIs will assess the degree of relatedness of the study medications and adverse events (i.e., unrelated, possibly related, probably related, definitely related).

Unexpected

The term **unexpected** refers to an adverse event that has not been previously observed or documented. A guideline is needed to define an adverse event as either expected or unexpected based on previous observation. The following documents or circumstances will be used to determine the expectedness of an adverse event:

- The Drug Label which contains the clinical and non-clinical data on therapy with Legalon®.
- The natural history of chronic hepatitis C.
- Reports, which add significant information on specificity or severity of an otherwise known and documented adverse event, associated with the use of Legalon®. Thus more severe or more specific adverse events than had previously been observed are considered unexpected.

Expedited reporting is also required when there is an increased rate of occurrence of expected, serious adverse events related to the medicinal product. Hence, the DCC will prepare reports of rates of serious adverse events for review at DSMB calls and meetings.

Sufficient data on adverse events requiring expedited reporting must be obtained by clinical center personnel to enable clinical center personnel to complete the MedWatch form (FDA Form 3500A).

The DCC will send copies of the initial notification and written reports on adverse events to the NCCAM and NIDDK project scientists, the Chairperson of the DSMB, the other clinical centers,

and Rottapharm/Madaus. The Data Coordinating Center Principal Investigator, project coordinator, and data managers will be notified of serious adverse events.

All adverse event information will be included in the annual IND report to the FDA. The initial annual report will be within 60 days of the anniversary date that the IND went into effect. In addition, the Data Coordinating Center will provide the clinical centers with updated information on adverse events that occur throughout the study. This information may be used to change the protocol or consent form if necessary. The clinical centers will also need to provide this information to their respective IRB offices annually with their renewals, or in the timeframes required by their IRBs.

The data sections of the FDA IND annual report will be prepared by the DCC and sent to NCCAM. NCCAM will compile the final report, and then submit it to the FDA.

Stopping rules

There will be no planned interim analysis. Safety information will be examined by the DSMB quarterly.

Participant Confidentiality

The central database of the study is on a server at the Epidemiology Data Center (EDC) in the Graduate School of Public Health at the University of Pittsburgh secured behind locked doors and an alarm with password access provided only to authorized personnel. Backups are performed daily to guard against data loss due to an equipment or power failure. Scheduled backups and archives at the EDC protect central and local information from hard disk failures. Tape backup volumes and CD-ROM copies of critical project files are located in a secured off-site storage area to prevent data loss due to catastrophic events. Routine virus detection is also enforced for all EDC computers involved in the study. All critical information regarding database transactions is logged and stored in journal files. In the event of accidental corruption of the project database, a previous database state may be restored from backup volumes or journal files. All servers used for this project are connected to uninterrupted power supplies to protect equipment against electrical surges and outages. A secured, raised-floor computer room in an area with a burglar alarm houses all project server equipment.

Subject confidentiality is preserved by assigning alphanumeric subject IDs at the clinical centers. Data sent to the DCC are identified by alphanumeric ID only. No reports of this study will use names or other identifying information such as social security numbers or addresses. Data, with alphanumeric ID only, will be stored at the DCC indefinitely. In addition, data and biospecimens, with alphanumeric ID only, will be stored indefinitely in the NIDDK data archives and biospecimen repository and may be used for future research.

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Appendix 1 = SyNCH Phase I- Pharmacokinetic Summary & Tables
Appendix 2 = Phase I Safety Summary Report
Appendix 3 = Legalon® Investigator's Brochure
Appendix 4 = Common Toxicity Criteria for Adverse Events (CTCAE) v3.0
Appendix 5 = Time Event Table