IRB Conditions Satisfied at the Protocol Level
Dec 03, 2018

CLINICAL STUDY PROTOCOL

A Phase II Multi-Center Open-Label Clinical Trial to Assess the Prevention of Liver Transplantation and/or Death Among Subjects Treated with Intravenous Silibinin (Legalon® SIL) for Amatoxin Induced Hepatic Failure

Protocol Number	**SB16A1.07**		
Product	Legalon® SIL (Silibinin-C-2′, 3-dihydrogensuccinate, disodium salt)		
Study Type	**Phase II**		
Version	**4.0**		
Protocol: Current Version / Date	**Version 4.0 dated 07 Sep 2018**	**Revision**	Amendment 4
Previous version / Date	Version 3.0 Dated 12 May 2009		
IND no.	105960		
Legal/Filing Sponsor	**Mylan Specialty LP** P.O. Box 4310 781 Chestnut Ridge Road Morgantown, WV 26504-4310, **USA**		

Confidential

SIGNATURE PAGE

Protocol Description	**SB16A1.07**
Product	Legalon® SIL (Silibinin-C-2', 3-dihydrogensuccinate, disodium salt)
Protocol Version	4.0
Protocol Version Date	07 Sep 2018

I have read this protocol and affirm that the information contained herein is complete and accurate.

Date: 17 September 2018

Global Lead, Safety Surveillance & Risk Management
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Date: 17 September 2018

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Date: 09/17/2018

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PRINCIPAL INVESTIGATOR ACCEPTANCE FORM

SIGNATURE OF INVESTIGATOR

I, the undersigned, as Investigator for this study, have read this protocol numbered SB16A1.07 and agree to conduct the study as outlined herein and in accordance with all applicable requirements of the country where the study is being conducted, the country where the study will be submitted, and the Sponsor's requirements. Applicable guidelines and regulations include, but are not limited to:

- Permission to allow the Sponsor and/or its agent or regulatory agencies to inspect study facilities and pertinent records at reasonable times and in a reasonable manner that ensures subject confidentiality.
- Immediate notification of the Sponsor and/or its agent of any regulatory inspection related to this study.
- Submission of the proposed clinical investigation, including the protocol and consent form, to a duly constituted IRB/Ethics Committee for approval, and acquisition of written approval for each, prior to study initiation.
- Use of IRB/Ethics Committee-approved written informed consent that is obtained prior to study initiation for each subject.
- Submission of any proposed change in or deviation from the protocol to the IRB/Ethics Committee using a signed formal amendment document prepared by the Sponsor and/or its agent. Any proposed change(s) or deviation(s) from the protocol require that the informed consent also reflect such change(s) or deviation(s), and that the revised informed consent be approved by the IRB/Ethics Committee.
- Documentation and explanation of the individual protocol deviations on the appropriate CRF page or other Sponsor-approved document.
- Submission of written reports of serious AEs, as defined in the protocol, to the Sponsor within 24 hours.
- Adherence to ICH GCP guidelines.

Signature (Investigator) and Date

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SYNOPSIS

Protocol Title	A Phase II Multi-Center Open-Label Clinical Trial to Assess the Prevention of Liver Transplantation and/or Death Among Subjects Treated with Intravenous Silibinin (Legalon® SIL) for Amatoxin Induced Hepatic Failure		
Product	Legalon® SIL (Silibinin-C-2′, 3-dihydrogensuccinate, disodium salt)		
Study Type	Interventional Clinical Trial		
Protocol Version / Date	Version 4.0 dated 07 Sep 2018		
Previous version / Date	Version 3.0 dated May 12, 2009	Revision	Amendment 4
EudraCT no.	NA		
IND no.	105960		
Legal/Filing Sponsor	**Mylan Specialty LP** P.O. Box 4310 781 Chestnut Ridge Road Morgantown, WV 26504-4310 **USA**		
Background and Rationale	Human mushroom poisonings are classified according to clinical symptoms. In the case of intoxication with Amanita species - the most relevant being Amanita phalloides - the summary of typical clinical symptoms is called phalloides syndrome. While severe mushroom poisonings are rare, phalloides syndrome is reported to account for about 90% of fatal mushroom poisonings [5]. There are no controlled clinical studies available due to ethical reasons, but uncontrolled trials and case reports describe successful treatment with intravenous silibinin (Legalon® SIL). In nearly 1,500 documented cases, the overall mortality in subjects treated with Legalon® SIL is less than 10% in comparison to more than 20% when using penicillin or a combination of silibinin and penicillin. Silibinin, a proven antioxidative and anti-inflammatory acting flavonolignan isolated from milk thistle extracts, has been shown to interact with specific hepatic transport proteins blocking cellular amatoxin re-uptake and thus interrupting enterohepatic circulation of the toxin. The addition of intravenous silibinin to aggressive intravenous fluid management serves to arrest and allow reversal of the manifestation of fulminant hepatic failure, even in severely poisoned subjects . These findings together with the available clinical experience justify the use of silibinin as Legalon® SIL in Amanita poisoning cases. (1a).		

	More than 90% of all fatal mushroom poisonings worldwide are due to amatoxin containing species that grow abundantly in Europe, South Asia, and the Indian subcontinent. Many cases have also been reported in North America (1,2,3). Initial symptoms of abdominal cramps, vomiting, and a severe cholera-like diarrhea generally do not manifest until at least six to eight hours following ingestion and can be followed by renal and hepatic failure. Outcomes range from complete recovery to fulminant organ failure and death which can sometimes be averted by liver transplant. Epidemiological data on mushroom poisoning, particularly poisoning with amatoxin-containing mushrooms, are rare. The true incidence of this intoxication is unknown since national data banks maintained at poison control centers have only incomplete data of this non-reportable illness [4]. Uncontrolled European studies and emergency use in the USA, suggest that treatment of amatoxin poisoning with Legalon® SIL drug may prevent severe morbidity (hepatic failure) and mortality. This is a Phase II Multi-Center Open-Label Clinical Trial to Assess the Prevention of Liver Transplantation and/or Death Among Subjects Treated with Intravenous Silibinin (Legalon® SIL) for Amatoxin Induced Hepatic Failure. A total of 139 subjects will be enrolled into this study. Legalon® SIL is formulated as a sterile lyophilisate in rubber-stoppered vials to be dissolved for intravenous infusion, containing 528.5 mg silibinin-C-2',3-dihydrogen succinate, disodium salt which is equivalent to 350 mg of silibinin according to DNPH testing or 315 mg of silibinin according to HPLC determination. The recommended daily dose is 20 mg silibinin/kg via continuous infusion over 24 hours, following a single loading dose of 5 mg silibinin/kg on the first day of treatment. Subjects will be screened for eligibility and, if confirmed to meet the study criteria, will be enrolled to receive treatment of Legalon® SIL. The study is comprised of a screening period of up to 24 hours from the time a potential study participant is identified. Treatment may be administered for up to 96 hours, or longer, following consultation with PPI. The final study assessment (Follow-up Visit) is to occur at 10 (+/-2) days from the last administered treatment. During screening, treatment and follow-up, assessments for medical history (only at screening), vital signs, labs and physical examination will be completed. Concomitant medications and adverse events will be recorded on every subject from the time of consent through the completion of the Follow-up Visit. Sponsor should be notified of any SAEs reported to the site and up to 30 days after discontinuation of study medication.
Primary Objectives	The primary objective is to evaluate the efficacy of Legalon® SIL in preventing hepatic failure and/or death resulting from amatoxin poisoning in a phase II clinical trial.

Primary endpoints	The primary endpoint is the percentage of subjects treated under this clinical trial without morbidity (liver transplantation) and or mortality (death).
Methodology and treatments	Legalon® SIL will be administered to subjects with amatoxin poisoning diagnosed by medical history, gastrointestinal symptoms, elevated liver enzymes (values higher than the upper limit of institution's normal for the age/sex at screening). Treatment consists of a 5 mg/kg loading dose followed by 20 mg/kg/day via continuous infusion (13). The treating physician is expected to administer supportive therapy of his/her choosing but consistent with best practices. Other measures to eliminate residual toxin, maintain perfusion and vital signs, and prevent coagulopathy will be as chosen by the treating physician. Legalon® SIL will be discontinued when prothrombin time and/or INR have returned to normal range, *and* when transaminases (AST and ALT) both are no more than 2 times the upper limit normal for the institution. If treatment is approaching 96 hours and the above criteria for discontinuation have not been met, the Primary Principal Investigator (PPI) and Onsite Investigator (OSI) must discuss the viability of continued study treatment. Upon confirmation of eligibility, subjects will receive a loading dose of 5 mg/kg of Legalon SIL intravenously (IV) over 1 hour via an infusion pump followed by 20 mg/kg continuous IV over 24 hours. Upon discharge from hospital, the subject should be advised to be seen for a physical examination and blood work (transaminases and PT/INR) approximately 10 days post discharge. The OSI or his designee is responsible to follow up with the subject for these results. The Protocol requires that the OSI and the PPI engage in daily telephone consultations once the subject is enrolled and for the duration of treatment with the study drug. All consultations will be documented and maintained on file by the PPI and OSI.

Inclusion/ exclusion criteria	Due to the potential life-threatening nature of Amatoxin poisoning and given the safety profile of Legalon® SIL, inclusion and exclusion criteria are minimal for the study. **Inclusion Criteria:** 1. Signed Informed Consent(s) for clinical trial participation (due to the potential critical status of the subject upon presentation, consent may need to be obtained from Legally Authorized Representative (LAR) per sites consenting policy and ICH/GCP guidance) Signed Informed Consent for Clinical Trial participation 2. History of eating foraged mushrooms 3. Gastrointestinal symptoms suggestive of amatoxin poisoning (cramping abdominal pain, nausea, vomiting, and / or watery diarrhea) usually 24-48 hours after of mushroom ingestion 4. Liver function tests suggestive of amatoxin poisoning: AST or ALT above the institutions upper limit of normal after mushroom ingestion **Exclusion criteria:** 1. Evidence of significant medical illness or any other abnormal laboratory finding that, in the Investigator's judgment, will substantially increase the risk associated with the subject's participation in, and completion of the study or could preclude the evaluation of the subject's response. Note: There are no adequate data from the use of Legalon® SIL in pregnant women and for this reason Legalon® SIL administration during pregnancy and lactation is not recommended. Based on the isolated report of fetal toxicity, it is recommended that the risks and benefits of Legalon® SIL therapy should be discussed with pregnant patients prior to use. In pregnant patients with a questionable exposure to amatoxin poisoning, it may therefore be reasonable to monitor carefully serial liver enzyme tests (e.g. every 4 hours) and defer initiation of Legalon® SIL until abnormalities are detected. Diagnosis of liver failure upon presentation based upon laboratory values and clinical picture. DIC at time of presentation. Evidence of significant medical illness or any other abnormal laboratory finding that, in the Investigator's judgment, will substantially increase the risk associated with the subject's participation in, and completion of, the study, or could preclude the evaluation of the subject 's response.
Sample size	**Sample Size:** A total of 139 enrolled subjects is considered sufficient to show the effect of the Legalon® SIL in preventing liver transplantation, and/or death resulting from amatoxin poisoning. Using the large sample normal

	approximation, a sample size of 139, provides a two-sided 95% confidence interval for proportion of subjects not meeting the outcome of interest (liver transplantation and/or death) within a half width of 5% from the observed proportion at an expected proportion of 90%.
Statistical Methods	**Demographic and Anamnestical Data:** All demographic and anamnestical data will be analyzed descriptively. For quantitative variables, the descriptive summary (means, medians, standard deviation, minimum and maximum) will be calculated. For qualitative data, the corresponding absolute and relative frequencies will be determined. **Efficacy Analysis:** The primary endpoint is the proportion of subjects treated with Legalon® SIL that do not exhibit morbidity (liver transplantation) and/or mortality (death). Proportion of subjects not meeting outcome of interest (liver transplantation and/or death) will be determined. Furthermore, the corresponding 95% confidence intervals will be determined. **Sensitivity Analyses:** Subject survival rate without hepatic transplantation and/or death will be calculated for all subjects and for each stage of disease. Moreover, the corresponding 95% confidence intervals for the rate of survival will be determined. The survival time will be described by means of Kaplan-Meier estimates. **Secondary endpoint (efficacy):** • All quantitative secondary endpoints will be analyzed descriptively. Means, medians, standard deviations, minimum and maximum will be calculated as measures of location and dispersion. • The time to normality for AST, ALT, bilirubin, PT/INR, and creatinine, if abnormal at presentation, will be determined for all subjects and for each stage of disease by means of Kaplan-Meier estimates. Additional summaries -- Assessment of Emergency Liver Transplantation Criteria in ALF due to Amanita Poisoning – using Ganzert's and Escudie's criteria. (13a. • Number (%) of subjects without hepatic transplantation by Ganzert's intoxication (pre-treatment) • Elapsed days between amanita poisoning and start of treatment

	• Number (%) of subjects without hepatic transplantation by Ganzert's intoxication (during treatment) • Number (%) of subjects without hepatic transplantation by INR severity criteria (INR < 2 and ≥2) • Number (%) of subjects without hepatic transplantation by Escudiè's intoxication grade transplantation criteria (INR < 6 and ≥6) • Safety analysis Clinical and laboratory adverse events will be analyzed by descriptive statistics. Moreover, the absolute and relative frequencies for laboratory variables within, above, or below the reference ranges will be determined. The frequencies and the corresponding 95% confidence intervals will be determined for MedDRA preferred terms and System Organ Classes.

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LIST OF COMMONLY USED ABBREVIATIONS

AE	Adverse event
ALF	Acute Liver Failure
ALT	Alanine transaminase
AST	Aspartate transaminase
CRF	Case Report Form
DIC	Disseminated Intravascular Coagulation
D5NS	5% Dextrose in Normal Saline
DNPH	2,4-dinitrophenylhydrazine
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HPLC	High-performance liquid chromatography (formerly referred to as high-pressure liquid chromatography
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent ethics committee
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
LAR	Legally Authorized Representative

MedDRA	Medical dictionary for regulatory activities
mg	milligram
mL	milliliter
ms	millisecond
NA	Not Applicable
OATP1B3	Organic Anion Transporting Polypeptide 1B3
OSI	Onsite Investigator
PPI	Primary Principal Investigator
PSRM	Product safety and risk management
PT	Prothrombin Time
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
TEAE	Treatment emergent adverse event
TNF	Tumor Necrosis Factor
US	United States

1 STUDY SCHEDULE OF ACTIVITIES

The Schedule of Activities table provides an overview of the protocol visits and procedures. Refer to the Study Conduct Section (6) for detailed information on each procedure and assessment required for compliance with the protocol.

Table 1 Study Schedule

Schedule of Events

PHASE	SCREENING PHASE		TREATMENT PHASE			FOLLOW-UP PHASE
Event	Screening/ Presentation	Pre -First dose*	Dosing	Every 6 hours post first dose until discharge or as indicated (+/-1 hour)	Every 24 hours post first dose (+/-2 hours)	At Follow-up, 10 (+/-2) Days after study drug discontinued
Informed consent	X					
History	X					
Physical/ Review of Systems	X	X			X	X
Vital Signs	X	X		X		X
CBC, Differential, Platelets	X	X		X		
Transaminases (AST, ALT)	X	X		X		X
ALP	X	X		X	X	X
Ammonia	X	X		X		
Lactate	X	X		X		
Amylase	X	X		X		X
Lipase	X	X		X		X
Bilirubin (total)	X	X		X	X	X
PT	X	X		X		X

PHASE	SCREENING PHASE		TREATMENT PHASE			FOLLOW-UP PHASE
Event	**Screening/ Presentation**	**Pre -First dose***	**Dosing**	**Every 6 hours post first dose until discharge or as indicated (+/-1 hour)**	**Every 24 hours post first dose (+/-2 hours)**	**At Follow-up, 10 (+/-2) Days after study drug discontinued**
INR	X	X		X		X
BUN, Creatinine, GFR	X	X		X	X	
Glucose	X	X		X**	X	
Electrolytes (NA, K, Cl, CO2)	X	X		X		
Serum Pregnancy test for woman of child bearing potential	X					
Loading dose Legalon-SIL 5mg/kg IV over 1 hour followed by continuous IV infusion of 20 mg silibinin/kg over 24 hours			X			
Adverse Events	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X

1 * All labs to be repeated if first dose occurs greater than 4 hours from last set of labs.

2 ** If subject is diabetic, then follow institution's standard for glycemic monitoring.

2 INTRODUCTION

2.1 Indication

Ingestion of amatoxin containing mushrooms may account for approximately 95% of deaths attributable to mushroom ingestion worldwide. In the United States, European invader Amanita phalloides and California native Amanita ocreata account for almost all cases reported on the West Coast (during the rainy season from September to April). Amanita bisporigera and Amanita virosa are responsible for poisonings in the Midwest and East Coast (April to September).

Amanita mushrooms are often mistaken for nonpoisonous species, even by experienced mushroom foragers, particularly immigrants familiar with similarly appearing mushrooms in their home environment. They are often described as delicious ("best tasting mushrooms I have ever eaten") and the onset of initial GI symptoms can be delayed by six or more hours.

The principal toxins (amanitins) are not destroyed by cooking, freezing, or drying, however, the mushrooms themselves are completely safe to handle (1). Amanitins are processed via enterohepatic circulation, are secreted in the bile, taken up by hepatocytes and tightly bound to RNA polymerase II, suppressing protein synthesis and resulting in severe acute hepatitis and fulminant hepatic failure. It is believed that prevention of the secretion of the toxin from the bile, by either gall bladder drainage or inhibition of gall bladder contractility, may help to mitigate the magnitude of the amanitins' toxicity.

2.2 Background and Rationale

The use of Legalon® SIL (silibinin) for amatoxin poisoning has a well-recognized biochemical mechanism of action: inhibition of OATP1B3 mediated amanitin uptake by hepatocytes (3, 4). This data also suggests that Penicillin G works by the same mechanism, although with a much less significant effect, and that combining Penicillin G with silibinin may lead to a reduced therapeutic effect. The undesirability of combining Penicillin G with silibinin was the subject of a paper published in Germany which suggested less favorable outcomes when Penicillin G combination therapy was used in comparison to silibinin monotherapy (5).

A second mechanism of action Legalon® SIL has also emerged. Amatoxin appears to induce fulminant hepatic failure via TNF mediated hepatocyte apoptosis. Building on earlier work it now appears that silibinin may inhibit TNF release in the injured liver (6, 7, 8, 9).

Legalon® SIL reduced death in amatoxin-poisoned dogs from 80% to 0% (10). This is the only substance that has been shown to be of benefit for post exposure prophylaxis against fulminant hepatic failure and death in this species.

Although there are no controlled studies of Legalon® SIL for amatoxin poisoning, open-label treatment in Europe compared to historical controls, suggests that mortality is markedly decreased from 22% to 10% among all subjects treated with Legalon® SIL (11, 12).

Diagnosis

Amatoxin poisoning is diagnosed by the clinical history and laboratory findings, specifically:

a. History of eating foraged mushrooms

b. Gastrointestinal symptoms consistent with amatoxin poisoning (cramping abdominal pain, nausea, vomiting, and / or watery diarrhea) usually 24-48 hours after mushroom ingestion
c. Liver function tests consistent with amatoxin poisoning: AST or ALT above the upper limit of normal after mushroom ingestion

Amatoxin Poisoning Clinical Picture and Laboratory Findings

Amatoxin Poisoning Clinical Picture and Laboratory Findings

Patients who consume amatoxin containing mushrooms exhibit signs and symptoms that typically occur in a progression through three stages.

Stage I:

There is an initial quiescent period (6-24 hours post ingestion) after which cramping abdominal pain, nausea, vomiting, and watery "cholera like" diarrhea develop. Routine laboratory values may reflect dehydration and electrolyte loss, but they may not accurately assess the magnitude of intoxication or predict the eventual outcome.

Stage II

The second stage of amatoxin poisoning is characterized by generalized clinical improvement (resolution of gastrointestinal symptoms) that begins 24 to 48 hrs after ingestion, albeit masking the hepatic deterioration that is occurring at the same time. The liver function tests show a progressive elevation of transaminases and an evolving coagulopathy. Early renal damage may be reflected by elevations in the serum creatinine and blood urea nitrogen levels.

Grade 1: Patients develop gastroenteritis-like symptoms several hours (6-36) after ingestion but do not develop severe biochemical indications of hepatitis (peak transaminases <1000 without coagulopathy) or renal dysfunction.

Grade 2: In addition to GI symptoms patients manifest a moderate (1000-5000 IU/L) rise in transaminases and a moderate (peak INR <2.0) coagulopathy.

Stage III

Transition into the third stage can occur quite suddenly. Hepatic failure may be fulminant and is manifested by a severe increase in serum transaminases and profound coagulopathy. Patients may experience a swift progression from Stage I to Stage III or IV. The presence of hypoglycemia is a poor prognostic sign reflecting massive hepatic necrosis. Normalization of transaminases in the setting of a rising INR, creatinine, ammonia level plus worsening symptoms, should be considered to be a sign of fulminant hepatic necrosis and, hence an indicator of poor prognosis. Renal failure, due to hepatorenal syndrome and/or direct nephrotoxicity of amanitins, may result in severe oliguria or anuria. Fifty percent of patients with amatoxin poisoning may also have laboratory evidence of pancreatitis (2).

Grade 3: Patients develop a marked elevation of transaminases (>5000 IU/L) and significant coagulopathy (INR>2.0). Grade 3 is divided into two subgroups according to bilirubin values. In Grade 3a: hyperbilirubinemia is mild or absent, while Grade 3b: shows a steep and continuous rise in bilirubin (greater than 5 mg/dL).

Grade 4: A steep rise in transaminases is accompanied by a corresponding steep decline in clotting function (INR>3.0) and renal dysfunction.

Grade 1 and Grade 2 patients have the best chance of surviving amatoxin poisoning and need symptomatic treatment only. Grade 3, particularly Grade 3b patients, are at risk and should be

transferred to a tertiary care center where liver transplant is available. Grade 4 patients have an extremely poor prognosis. There is a 90% probability of death in spite of intensive therapy. Survival usually requires a liver transplant.

Laboratory Abnormalities and Corresponding Prognostic Indication

Transaminases

Grade 1 patients show mild elevations of liver enzymes. Grade 2 patients develop a moderate rise in transaminases, usually below 5,000 IU/L but sometimes much higher. Grade 3 patients show a steep and massive rise in transaminases. There is no significant difference in the magnitude of transaminase results in Grade 4 relative to Grade 3, although in fatal cases the mean values are generally higher. A decrease of transaminase values on Day 4 is either a sign of improvement or a hepatic "burn-out" phenomenon. A massive rise in transaminases (Grade 3 and 4) does not necessarily predict a fatal outcome, whereas a mild rise in transaminases (Grade 2) generally indicates a good chance for survival.

Prothrombin Time

PT/INR is of high prognostic value. An early and steep prolongation of prothrombin time values suggests a poor prognosis. In Europe, those patients in whom prothrombin times could not be improved to values above 20-30% by substitution of clotting factors on Days 3-4 had >90% mortality. From 48 hrs after ingestion onwards, there is a significant difference between the prothrombin time of survivors in Grade 3 (>30 %) and non-survivors in Grade 4 (< 20%).

Bilirubin

On admission to the hospital, bilirubin values are normal in 90% of all cases. Elevation at this early stage (>3 mg/dL) suggests a poor prognosis. During the course of the intoxication, only about 20% of Grade 3 patients (Grade 3b) show a marked rise in bilirubin. It is possible to obtain information on the prognosis from the absolute values after Day 3. At that time, values < 5 mg/dL indicate a favorable prognosis, even if transaminases are high, while a value > 5 mg/dL indicates a severe or fatal course.

Impairment in kidney function

Early renal dysfunction signals a poor prognosis. Prerenal azotemia secondary to dehydration on initial presentation is a common finding that can usually be reversed by aggressive fluid replacement. Acute tubular necrosis may also occur in severely dehydrated patients and may represent a direct cytotoxic effect of amatoxin. A slight elevation of creatinine on Days 1 to 2 that does not respond to fluid replacement suggests that a direct toxic effect has occurred. About 70% of fatal cases show this early increase of creatinine. Hepatorenal syndrome occurs later and indicates an extremely poor prognosis.

In summary, Legalon® SIL will be administered to patients with amatoxin poisoning diagnosed by history, gastrointestinal symptoms, elevated liver enzymes. Treatment will consist of a 5 mg/kg loading dose followed by 20 mg/kg/day via continuous infusion (13). The treating physician is expected to administer supportive therapy of his/her choosing but consistent with best practices. Legalon® SIL will be discontinued when coagulopathy is no longer present, and when liver function tests have returned to no greater than 2 times the upper limit of normal or patient does not respond to treatment with Legalon® SIL. Patients will be followed 10 (+/-2) days after the end of Legalon® SIL therapy with follow-up laboratory studies and physical exam. If the Follow-up Visit is not feasible due to patient's

condition, reason for the missed visit must be clearly documented in the patient' medical record.

Complete information for Legalon® SIL may be found in the Legalon® SIL Investigator's Brochure.

2.2.1 Rationale for Dose Selection

A study was conducted aiming at the verification of Legalon® SIL reconstituted solution in infusion bags containing 5% glucose solution. The stability was tested up to 30 hours at 25°C/60% RH.

The design of the study was as follows:

- 4 times 35 ml were taken from a 500 ml Glucose (5%) infusion bag
- 4 vials of Legalon SIL were reconstituted with 35 ml each
- after reconstitution the 4 solutions were returned into the infusion bag
- the infusion bag (containing 4 vials of Legalon SIL) was stored under defined conditions for min. 24 hrs. (study report number 02VB0164_07/01).

This kind of preparation was intended to be used for a 24 hrs. infusion.

A stability of the solution of minimum 30 hrs. could be confirmed.

Based on the results, it was concluded that, the reconstituted solution of Legalon® SIL is stable at 25°C/60% RH, as shown by content of Silibinin mono- and dihydrogensuccinate (SHS), sodium salts.

Although there was a slight increase of the content of SHS, sodium salts, from 1.8% to 3.0% of batch number 120204 1nd from 1.3% to 2.4% of batch no. 163601 the values were still far below the specification limit of not more than 5.5%.

Therefore, the study verifies that the reconstituted solution of Legalon® SIL is from the analytical point of view stable at 25°C/60% RH for 24 hours.

No microbiological investigations were performed in this study, because such aspect depends more on the GCP application than on the product itself, which is sterile and is to be dissolved in sterile media.

3 OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary objective

The primary objective is to evaluate the efficacy of Legalon® SIL in preventing hepatic failure and/or death resulting from amatoxin poisoning in a phase II clinical trial

3.2 Endpoints

3.2.1 Primary Endpoints

The primary endpoint is the proportion of subjects treated with Legalon® SIL that do not exhibit morbidity (liver transplantation and/or mortality (death).

3.2.1.1 Safety

Safety based on the evaluation of changes in baseline laboratory and clinical presentation while on study treatment. The laboratory values to be followed are transaminases and renal function tests (AST, ALT, bilirubin, PT/INR, creatinine, lactic acid).

3.2.2 Secondary Efficacy

Efficacy as demonstrated by time to normality of the laboratory values from start of treatment with Legalon® SIL. The laboratory values to be followed are transaminases and renal function tests (AST, ALT, bilirubin, PT/INR, creatinine, lactic acid).

4 STUDY POPULATION

4.1 Study Population

Study will enroll 139 male and female subjects that meet all protocol defined eligibility criteria.

The period from Screening to completion of active treatment will be approximately 5 days. Patients will be required to have follow up physical examination and laboratory evaluations 7-14 days post-is continuation from study drug.

4.2 Sample Size Considerations

A total of 139 enrolled patents is considered sufficient to show the effect of the Legalon® SIL in preventing liver transplantation, and/or death resulting from amatoxin poisoning.

4.3 Inclusion and Exclusion Criteria

4.3.1 Inclusion Criteria

Subjects' eligibility should be reviewed and documented by the Investigator or a qualified designee before subjects are included in the study.

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Signed Informed Consent(s) for Clinical Trial participation
2. History of eating foraged mushrooms
3. Gastrointestinal symptoms consistent with amatoxin poisoning (cramping abdominal pain, nausea, vomiting, and / or watery diarrhea) usually 24-48 hours after mushroom ingestion
4. Liver function tests consistent with amatoxin poisoning: AST or ALT above the institutions upper limit of normal after mushroom ingestion

4.3.2 Exclusion Criteria

Subjects must not be enrolled in the study if they meet any of the following criteria:

1. Evidence of significant medical illness or any other abnormal laboratory finding that, in the Investigator's judgment, will substantially increase the risk associated with the patient's participation in, and completion of, the study, or could preclude the evaluation of the patient's response.

   Note: There are no adequate data from the use of Legalon® SIL in pregnant women and for this reason Legalon® SIL administration during pregnancy and lactation is not recommended. Based on the isolated report of fetal toxicity, it is recommended that the risks and benefits of Legalon® SIL therapy should be discussed with pregnant patients prior to use. In pregnant patients with a questionable exposure to amatoxin poisoning, it may therefore be reasonable to monitor carefully serial liver enzyme tests (e.g. every 4 hours) and defer initiation of Legalon® SIL until abnormalities are detected.

4.3.3 Randomization Criteria

This is an open label study and therefore randomization will not occur.

4.3.4 Criteria for study drug termination, withdrawal from the study and study termination

Subjects will be free to request termination of study drug or withdrawal from the study at any time for any reason.

Subjects must be withdrawn from the study prior to initiating treatment under the following circumstances:

- Laboratory values indicative of recovery prior to the start of Legalon® SIL

A subject must terminate study drug for safety reasons under the following circumstances:

- Development of a SAEs that is deemed attributable to Legalon® SIL by the OSI

A subject may be required to terminate study drug for other reasons including the following:

- Patients in whom, after 96 hours of starting treatment there is no significant improvement in the PT/INR and transaminases, termination of treatment may occur following consultation between the OSI and PPI.

- If it is in the subject's best interest based on decision of the OSI or his/her Sub-Investigator e.g. due to occurrence of an AE and/or other findings considered to present a safety concern to continued dosing with study drug.

- Despite education/reinforcement, the subject shows persistent inadequate compliance with required procedures, potentially compromising safety monitoring while on study drug.

Unless consent is withdrawn, subjects who prematurely terminate study drug and are discharged from the hospital, will have PT/INR, liver transaminases collected by local lab and have a physical examination performed by local physician 10 days post last dose of study drug. The OSI is responsible for obtaining the results of these procedures form the patient.

The Principal Investigator and/or Mylan reserves the right to terminate the study for any reason.

The study will be terminated early if there are significant safety concerns.

4.3.5 Females - Childbearing Potential

4.4 If the patient is pregnant appropriate counseling will be provided based on the risk/benefit condition. Pregnancy Testing

Serum or urinary pregnancy testing will be performed on all females of childbearing potential as described in the schedule of activities. In the event of a positive test, the patient will be informed that systematic data on the use of SIL in pregnancy and lactation are not available.

Note: There are no adequate data from the use of Legalon® SIL in pregnant women and for this reason Legalon® SIL administration during pregnancy and lactation is not recommended. Based on the isolated report of fetal toxicity, it is recommended that the risks and benefits of Legalon® SIL therapy should be discussed with pregnant patients prior to use. In pregnant patients with a questionable exposure to amatoxin poisoning, it may therefore be reasonable to monitor carefully serial liver enzyme tests (e.g. every 4 hours) and defer initiation of Legalon® SIL until abnormalities are detected. Any patient found to be pregnant at time of initiation of treatment, the pregnancy will be followed up and reported to the sponsor as per Section 9.2.6.

5 STUDY DRUG

5.1 Investigational Drug

Legalon® SIL is a fully developed and documented pharmaceutical formulation manufactured by Madaus (Cologne, Germany). Its active ingredient is silibinin-C-2',3-dihydrogen succinate, disodium salt Fig. (1). It has the molecular formula C33H28O16Na2 and a molecular mass of 726.56 g/mol. It is a microcrystalline powder that results from the esterification of silibinin with succinic anhydride to form its hydrosoluble disuccinic acid ester for parenteral application. Silibinin, a flavonolignan obtained by extraction of the seeds from milk thistle fruit (Ph. Eur.), is a mixture of two diastereomers, commonly named silibinin A and silibinin B. After the reaction of silibinin with succinic anhydride, silibinin-C-2',3-dihydrogen succinate, disodium salt is obtained as a mixture of cis- and trans diastereomers of silibinin A and B dihydrogen succinates. Legalon® SIL is currently registered and/or licensed in over a dozen European countries specifically for the treatment of Amanita phalloides intoxication.

Legalon® SIL

The drug is provided in carton containing four, rubber-stoppered vials. Each vial contains 528.5 mg of (equivalent to 350 mg of silibinin) powder for solution for infusion.

One vial contains:

Drug substance

Silibinin-C-2',3-dihydrogen succinate, disodium salt, 528.5 mg
[corresponding to 476 mg mono-, dihydrogensuccinate sodium salts (HPLC)]
equivalent to 350 mg (315 mg HPLC) of silibinin

Excipients

Inulin 70 mg USP
Nitrogen (as inert gas) Ph.Eur.
Total sum: 598.5 mg
Manufacturer: Madaus GmbH, Cologne (Germany)

5.1.1 Administration of Study Drugs

Preparation of infusion fluid

- Dissolve each of the 4 vials that are contained in a carton with 35 mL of 5% Dextrose or 0.9% sodium chloride solution. Upon reconstitution a total of 140 ml will be available across 4 vials.
- Remove 140 ml from a 500 mL IV container of Dextrose and replace with the 140 ml of reconstituted drug solution
- The initial loading dose is 5 mg/kg for one hour via infusion pump
- After the loading dose, 20 mg/kg/day is infused continuously via infusion pump

In the event of any significant dosing errors, the PPI and Mylan should be contacted immediately.

Since Legalon® SIL is potentially life-saving, reduction or elimination of dosing is to be avoided unless there is strong reason to think that an adverse event is due to Legalon® SIL, is clinically significant, and cannot be managed otherwise.

5.2 Drug Inventory

Legalon® SIL will be dispensed to enrolling sites from an institution/vendor designated by Mylan. Legalon SIL should not be stored above 25°C / 77°F.

The medical community will be alerted to the availability of Legalon® SIL via communication with poison control centers, mushroom organizations, and emergency room hospitals/physicians. There will be an emergency hotline number which the US physicians can call to initiate the process of obtaining Legalon® SIL.

Because amatoxin poisoning occurs episodically and randomly throughout the USA, most physicians wishing to enroll their patients on the study will not have prospective IRB approval of this protocol. The OSI will need to supply documentation of approval of this protocol from the IRB of his/her respective hospital.

5.3 Mylan will be responsible for furnishing study drug supply for distribution. Study Medication Complaints

In the event the patient has a complaint/concern during study participation regarding the supplied study medication, they should contact the site.

In the event of a complaint/concern during study participation, regarding the supplied study medication, the site should contact the sponsor. As a minimum the following information should be sent by the site as part of the complaint:

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- OSI name, site address, contact number
- Subject ID.
- Date of occurrence of incident/complaint.
- Description of incident/complaint (facts).
- Confirmation if the complaint caused or resulted in a SAE? If "Yes", confirmation that the SAE has been reported.

Additional information and potentially the return of study medication may be requested by Mylan in order to investigate the complaint.

5.4 Storage, Disposition of Unused Study Drug and Drug Accountability

The Investigator, or an approved representative, e.g. pharmacist, will ensure that all investigational products are stored in a secured area under recommended storage conditions and in accordance with applicable regulatory requirements while at the investigator site.

Study drug should be stored in accordance with the drug label. Storage conditions stated in the Investigator's Brochure may be superseded by the label storage.

Temperature of storage facilities should be monitored and recorded on a daily basis using validated devices that record maximum and minimum temperatures. Should the storage facility experience any excursion of temperature outside of the labelled storage condition this must be reported immediately to Mylan or designee

The investigator must maintain adequate records documenting the receipt, use, loss, or other disposition of the study drug. Mylan will supply drug accountability forms for this study. the form must be fully completed and identify the study drug, including batch or code numbers, and account for its disposition on a subject-by-subject basis, including specific dates and quantities. The forms must be signed by the individual who dispensed the drug, and copies must be provided to Mylan or designee.

Upon completion of each patient treatment at site, the site must return all used and unused vials as well as drug accountability log and administration records redacted of all personal identifiers with subject study identifier added.

5.5 Concomitant Medications

All concomitant medications taken during the study (from signing informed consent to discontinuation of study drug) must be recorded with indication, daily dose, and start and stop dates of administration in the CRF.

Medications taken within 28 days prior to screening and prior to dosing with study medication will be documented as a prior medication. Medications taken after dosing begins with study medication will be documented as concomitant medications.

There are no prohibited medications for this study.

6 STUDY CONDUCT

6.1 OSI and Site Requirement for Study Participation

Patients will be identified as potential study subject at the medical facility to which they present with symptoms. The treating physician at the local medical facility will either reach out to a Poison Control Hotline or will call the study designated hotline directly. Upon notification of the potential subject to the Mylan designated PPI, the following actions will take place:

- The designated hotline service for the study will contact the assigned coordinator for the study and be provided with contact information for immediate follow-up. This information includes
  - Treating physician's name
  - Contact details (telephone number and email) of treating physician
  - Medical facility name and address and phone number
- The assigned study coordinator will triage the call to assure there is suspicion of amatoxin poisoning and direct the case to the PPI
- The PPI will contact the treating physician to discuss the case(s) of amatoxin poisoning to determine eligibility for treatment under the protocol and complete the PPI Eligibility Confirmation Form(s). The information on the PPI eligibility confirmation form will include:
  - Date/Time of contact
  - Name and institution of referring physician
  - Referring physician contact phone number and email
  - Number of patients in cohort
  - If patient(s) included in study:
  - Written confirmation that the criteria for inclusion have all been met : mushroom eating history, Gastrointestinal symptoms, elevated liver function tests
  - If patient(s) excluded from study.
  - Reason for exclusion must be documented.
- Upon confirmation of eligibility for the subject(s), the treating physician must immediately contact the IRB for approval of treatment according to the protocol. At the least, an email or a memo with indication of IRB approval for the subject(s)' participation in the study must be provided to the PPI/SC prior to drug shipment.
- Upon receipt of the IRB approval email/memo, the PPI will direct dispatch of the study drug to be shipped to the medical facility
- The assigned SC, in the interim, will send the following documentation to be processed by the treating physician, now the prospective On-Site Investigator (OSI),
  - FDA Form 1572
  - OSI Commitment Form
  - OSI Financial Disclosure
  - Protocol
  - Informed Consent Template
  - Case Report Forms (CRFs) and Completion Guidelines
  - SAE Form
  - Drug Accountability Form

As soon as possible after enrollment of new patient into the study but no later than 24 hours, the Primary Study Coordinator will conduct a study training teleconference with the On-Site Investigator (OSI) and his designee(s). Training will include but not limited to the review of the protocol, safety reporting, and completion of CRFs.

6.2 Screening Phase (Screening/Presentation and Pre-first Dose)

a) Subjects eligible for study recruitment will have the nature, purpose, and risks of the study explained to them by the investigator. They will be provided with a written copy of the informed consent form (ICF) for the study and given sufficient time to consider the study's implications before deciding to participate. Subjects agreeing to participate in the study will sign the ICF and be given a duplicate copy before undergoing any screening or pre-screening (if required) procedures. Please note that due to the potential critical condition of subjects considered for this trial, informed consent may need to be obtained from the LAR or other responsible party per sites consenting policy and/or ICH GCP guidance. A unique subject identifier will be issued at the time of consent.

b) Due to the emergent treatment requirement of the study drug, a signed informed consent from the patient may be delayed, as the patient may be incapacitated. However, until this is possible, consent from the LAR must be obtained. Documentation of the consenting process, including interim consent obtained from the LAR, must be documented in the patient's medical record. Signed consent from the patient must be obtained at the earliest time possible. A signed copy must be given to the patient/LAR as well as sent electronically to the assigned SC. The original copy should be kept in the patients' medical record along with a copy of the protocol.

c) Once a subject enrolls in this trial the site will make every effort to retain the subject for the planned duration of the trial. In cases where the subject does not return for the post discharge Follow-up Visit or cannot be reached to reschedule the missed visit, the site should make every effort to regain contact with the subject so that they can appropriately be withdrawn from the study. All contact attempts should be documented in the subject's medical record. Should the subject continue to be unreachable, then and only then will he/she be considered to have withdrawn from the study with a primary reason of "Lost to Follow-up." For all other subjects withdrawing from the study, an alternative reason for discontinuation should be recorded in the CRF. Regardless of site plans to support and retain subjects within the trial, subjects may voluntarily withdraw from the trial for any reason and at any time.

For a subject that completes the study and all procedures it is anticipated that the duration of study would be approximately 20 days, inclusive of follow up visit.

6.2.1 Required Screening Study Procedures for Assessment and Confirmation of Eligibility for Treatment:

- Informed consent
- History

- Physical
- Vital signs
- Serum HCG in women of child bearing potential
- AST
- ALT
- Bilirubin
- PT
- INR
- BUN
- Creatinine
- Lactate
- Amylase
- Lipase
- Ammonia
- CBC with differential
- Platelet
- Glucose
- Na
- K
- Recovery of toxin (if possible)
- Baseline medical conditions
- Baseline medications

For details and timings of assessments, refer to Section 6.5.

The Protocol requires that the OSI contact the PPI for a daily telephone consultation once the patient is enrolled and for the duration of treatment with the study drug. All consultations will be documented and maintained on file by the PPI and OSI.

6.3 Treatment phase

Following receipt of study drug, if patient remains eligible for enrollment according to the protocol, treatment with Legalon® SIL should begin as soon as possible as a continuous infusion until the PT/INR have returned to the normal range set by the institution and transaminases have returned to no greater than 2 times the institutions upper limit of normal. If the laboratory tests and/or the patient clinical picture is not improving after 96 hours on treatment the PPI and OSI must specifically discuss the benefit of continued treatment. Full documentation and reason for decision to either continue or discontinue treatment must be documented in the patient's medical record as well as on the Daily Communication CRF.

Every effort should be made to ensure that the protocol required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside of the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator or designated representative will take all steps necessary to ensure the safety and well-being of the subject. When a protocol required test cannot be performed the investigator or designated representative will document the reason for this and any corrective and preventive actions which he/she has taken to ensure that normal processes are adhered to as soon as possible. The Mylan study team will be informed of these deviations in a timely fashion.

6.3.1 Required Daily Study Assessments/Procedures While on Study Treatment

- Physical
- Vital signs
- AST
- ALT
- ALP
- Bilirubin
- PT
- INR
- BUN
- Creatinine
- Lactate
- Amylase
- Ammonia
- CBC with differential

- Platelets
- Glucose
- Na
- K
- AEs
- Concomitant medications
- Infusion of Legalon® SIL
- Other measures to eliminate residual toxin, maintain perfusion and vital signs, and prevent coagulopathy will be at the discretion of the OSI.

6.3.2 Early Termination of study drug Termination

Subjects may request termination of study drug or withdrawal from the study at any time, or be required to withdraw or terminate study drug by the investigator or sponsor for reasons as per Section 4.3.4. If study drug is terminated or the subject withdraws or is withdrawn, the reason for termination/withdrawal should be established and recorded. If a subject does not return for the follow up visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document the outcome of subject contact attempt, if possible. The investigator will contact Mylan or designee, in the event that a subject fails to complete the study or violates the protocol.

If the subject withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

Unless consent is withdrawn, subjects who prematurely terminate study drug will be asked to return to the clinic for the follow up visit and will have this scheduled within 10 days of stopping the study drug.

6.4 Follow-up Phase

Patients will be followed 10 days +/-2 after stopping Legalon® SIL therapy unless they failed treatment and proceeded to transplant. Follow up will consist of a physical examination and blood work to assure recovery and to assess for any AEs.

Subjects will be reminded that SAEs should be reported to the study staff up to 30 days after the last dose of study medication.

If the patient recovers from the poisoning, upon discharge from the hospital, they should be given a prescription for follow up blood work to include PT/INR and liver function studies as well as instructed to follow with their local physician for a physical exam. The OSI or designee is responsible for following up with the patient for the results of the exam and blood work.

6.4.1 Safety Testing

6.4.1.1 Adverse Event Assessment

If a subject reports any symptoms that could preclude the drug administration before starting, they will be evaluated by medical staff and necessary measurements will be performed. The OSI will be notified before dosing to determine the course of action.

Findings from screening procedures, e.g., laboratory tests or physical examinations will be recorded as medical history. Clinically significant changes following completion of the screening procedures will be recorded as adverse events.

Subjects will be routinely queried in regards to the presence or absence of adverse events using open ended questions. The treating medical staff will provide documentation of any adverse events in the subject's CRF. The adverse event source documentation will minimally include the following information: date and time of assessment, description of the adverse event, date/time of onset, date/time of resolution if applicable, and identification of the staff member collecting the information.

There are no adverse events of special interest for this study.

6.4.1.2 Laboratory Safety

6.4.1.2.1 Blood Volume

Total blood volume collected per patient for this study will vary between treating institutions. The blood assessments required per the protocol approximate the sampling that would be conducted as standard of care for patients suffering from the effects of amatoxin poisoning and as such do not pose any additional risk to the patient. The following safety laboratory tests will be performed at times defined in the study schedules in Sections 1 and 6.

Table 2 Laboratory Safety Tests

Hematology	Chemistry	Other
Hemoglobin	Urea and Creatinine	Serum Pregnancy in women of childbearing potential (screening only)
Hematocrit	Glucose (non-fasting)	
RBC count	Sodium	
Platelet count	Potassium	
WBC count	AST, ALT	
Total neutrophils (Abs)	Total Bilirubin	
Eosinophils (Abs)	Alkaline phosphatase	
Monocytes (Abs)	Lactate	
Basophils (Abs)	Lipase	
Lymphocytes (Abs)	Amylase	
	Ammonia	

Hematology and chemistry will be analyzed by the local laboratory.

Any clinically significant findings in laboratory safety data following completion of screening should be recorded as an AE. Determination of clinical significance and seriousness will be based on the Investigator's medical judgment.

6.4.1.3 Blood Pressure and Pulse Rate

Blood pressure and pulse rate will be measured at times specified in Sections 1and 6. Additional collection times, or changes to collection times of blood pressure and pulse rate will be permitted, as necessary, to ensure appropriate collection of safety data.

Any clinically significant changes in blood pressure and pulse rate following completion of screening should be recorded as an AE. Determination of clinical significance and seriousness will be based on the OSI's medical judgment.

6.4.1.4 General Physical Examination

At screening, general physical examination will be performed which consists of an examination of the abdomen, cardiovascular system, lungs, lymph nodes, musculoskeletal and neurological systems, skin, extremities, head, ears, eyes, nose, and thyroid gland by trained medical personnel at the site.

At screening, any clinically significant abnormality should be recorded as a preexisting condition in the subject's medical history. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Height and weight will be assessed at screening. Physical examination results will not be recorded in the CRFs, but any clinically significant finding at Screening should be recorded under medical history and changes between Screening and subsequent examinations should be recorded as an AE. Determination of clinical significance and seriousness will be based on the OSI's medical judgment.

7 STATISTICAL ANALYSIS

7.1 Sample Size Calculations

The overall mortality rate for Legalon® SIL treated patients, regardless of the severity of liver damage, is less than 10%. This represents more than a 50% reduction in the reported mortality rate before Legalon® SIL was available.. For ethical and practical reasons there are no controlled clinical studies available for any amatoxin treatments but observational studies and case reports describe successful treatment with intravenous silibinin (Legalon® SIL).

A total of 139 enrolled patents is considered sufficient to show the effect of the Legalon® SIL in preventing liver transplantation, and/or death resulting from amatoxin poisoning. Using the large sample normal approximation, a sample size of 139, provides a two-sided 95% confidence interval for proportion of patients not meeting the outcome of interest (liver transplantation, and/or death) within a half width of 5% from the observed proportion at an expected proportion of 90%.

Published Clinical Experience with Legalon® SIL

Between 1982 and 2008, mostly single cases were published by various authors (Table7.1). Overall, out of these 1,136 reported patients treated with Legalon® SIL as monotherapy or in combination with mostly penicillin G, 1,064 patients (94%) survived. It was further reported that the severity of liver damage correlated with the time interval between mushroom intake and commencement of silibinin treatment. If silibinin was given within 48 hours after intoxication, a light or medium hepatic injury was expected. When silibinin treatment was delayed by more than 48 hours, then a severe clinical course with coagulation disorders and liver coma was more likely.

Table 3 Summary of Clinical Experience with Legalon® SIL in Amatoxin Poisoning

Reference	Treatment Regimen	No. of Patients	
		Treated	Survived
Lorenz *et al,* 1983 (unpublished)	PE + SIL	201	181
Strenge-Hesse *et al.*, 1996 (unpublished)	PE + SIL	154	139
Floersheim *et al.*, 1982 [29]	PE + SIL	16	16
Hruby 1983 [30]	PE + SIL	15	14
Marugg and Reutter 1985 [33]	PE + SIL	12	11
Smetana *et al.*, 1986 [34]	PE + SIL	2	2
Schenke *et al.*, 1987 [35]	SIL	2	2
Hruby 1987 [36]	SIL PE + SIL TH + SIL PE + TH + SIL	17 37 1 15	16 34 1 15
Kelbel and Weilemann 1989 [37]	PE + SIL	5	4
Nagy *et al.*, 1994 [38]	PE + SIL	4	3
Kleist-Retzow *et al.*, 1995 [39]	PE + SIL	2	2
Molling *et al.*, 1995 [40]	PE + SIL	2	2
Carducci *et al.*, 1996 [41]	PE + SIL	4	4
Alves *et al.*, 2001 [42]	PE + SIL	4	4
Boyer *et al.*, 2001 [43]	NAC + SIL	1	1
Enjalbert *et al.*, 2002 [28]	SIL or PE+SIL	624	589
Ganzert *et al.*, 2008 [31]	SIL or PE+SIL	367	339
Mitchell and Olson 2008 [32]	PE+NAC+SIL	6	5
Total		1,491	1,384

PE=penicillin, SIL=Legalon® SIL, NAC=N-acetylcysteine, TH=thioctic acid.

Based on the available clinical data (unpublished and published), from a total of 1,491 documented Amanita-poisoned patients treated with Legalon® SIL 1,384 survived, resulting in a survival rate of 93% (1a).

7.2 Analysis Set Definitions

The following analysis sets will be used for the statistical analyses:

Full Analysis Set (FAS): Full Analysis Set includes all treatment allocated subjects with least one post baseline evaluable efficacy assessment.

Safety Analysis Set (SAF): Safety Analysis Set includes all subjects who receive the study treatment.

Per-Protocol Set (PPS): Per-Protocol Set includes all subjects who receive study treatment, have evaluable efficacy assessment and have no major protocol deviations. A major protocol violation is defined as a protocol deviation that is considered to have an impact on the efficacy results. A list of possible major protocol deviations will be identified and documented in the SAP and the subjects having major protocol deviation will be finalized prior to the data base lock.

7.3 Primary Endpoint

The primary endpoint is the proportion of patients treated with Legalon® SIL that do not exhibit morbidity (liver transplantation,) and/or mortality (death) at the end of treatment period (96 hours).

7.4 Secondary Endpoint (s)

The secondary endpoints of the study are:

- Proportion of patients not needing liver transplantation
- The time to normality for AST, ALT, bilirubin, PT/INR, and creatinine during the treatment period
- Number (%) of patients without hepatic transplantation by Ganzert's intoxication (pre-treatment)
- Elapsed days between amanita poisoning and start of treatment
- Number (%) of patients without hepatic transplantation by Ganzert's intoxication (during treatment)
- Number (%) of patients without hepatic transplantation by INR severity criteria (INR < 2 and ≥ 2) (during treatment)
- Number (%) of patients without hepatic transplantation by Escudiè's intoxicion grade transplantation criteria (INR < 6 and ≥ 6) (during treatment)

7.5 Statistical Analysis Methodology

In general, data will be summarized using descriptive statistics by treatment group. Continuous variables will be summarized using the mean, standard deviation, median, minimum value and maximum value. Categorical variables will be summarized using frequency counts and percentages. All data will be presented in patient data listings. Any data excluded from the summaries and statistical analyses will be flagged accordingly. Baseline value is defined as the last valid value prior to the treatment, i.e.in other words, Baseline value is treatment specific value. If the data is missing at Baseline, then the Baseline will be imputed with the corresponding value at screening. Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP). This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment.

7.5.1 Patient Demographics, Patient Disposition and Protocol Deviations

Descriptive statistics will be presented for continuous demographic variables and number and percentage will be presented for categorical demographic variables. Subject's disease characteristics will be summarized as applicable.

Patient accountability will be tabulated for all patients by summarizing the number of patients who are assigned to treatment, complete the study, or prematurely discontinue, and the reason for early discontinuation by treatment. A listing will be presented to describe dates of completion or early withdrawal and the reason for early discontinuation, if applicable, for each patient. Listings of data collected during the study, e.g., inclusion/exclusion criteria responses, study treatment administration will be provided.

The protocol deviations in the study will be evaluated on a case to case basis and will be categorized as major or minor. A major protocol deviation is defined as any deviation that may affect the efficacy outcome or the treatment of the subject. The number and proportion of subjects meeting minor or major protocol deviations will be summarized by treatment group. The data will also be listed. The protocol deviation criteria and detailed list of major protocol deviations will be mentioned in the statistical analysis plan.

Treatment duration and compliance will be descriptively summarized.

7.5.2 Prior and Concomitant Medications

Concomitant medications will be coded by using WHO dictionary. Concomitant medications will be summarized by ATC class and WHO preferred term by treatment, and overall, and will be listed for all subjects in the Safety Population

7.5.3 Analysis of Primary Endpoint

The primary objective of the study is to compute the survival of the patients without liver transplantation, renal failure or death during the treatment period of 96 hours. The number and proportion of patients surviving without liver transplantation, renal failure and/or death at the end of treatment period will be computed, 95% CI for the proportion of patients surviving without liver transplantation, renal failure and/or death will be computed based on Newcombe-Wilson score method.

The analysis of primary endpoint will be performed based on Full Analysis Set.

7.5.4 Secondary/Sensitivity Analyses for Primary Endpoints

As a sensitivity analysis, the primary analysis will be repeated using the Per-Protocol Set.

In addition, the survival rates using Kaplan-Meier estimation method at 96 hours (end of treatment period) will be computed for the patients surviving without liver transplantation, renal failure or death. 95% CI will be computed for the survival rate by using Greenwoods formula. This analysis will be carried out using both Full Analysis and Per-Protocol Analysis sets.

7.5.5 Missing Data

In general, missing data will not be imputed. However, more analytical details about handling of missing data may be covered in the SAP.

7.5.6 Sub-Group Analyses

The primary and sensitivity analyses of primary endpoint will be repeated by the disease severity stages.

7.5.7 Analysis of Secondary Endpoint(s)

All secondary analysis will be carried out using the Full Analysis Set.

The endpoints of proportion of patients not needing liver transplantation, without hepatic transplantation by Ganzert's intoxication (pre-treatment), without hepatic transplantation by Ganzert's intoxication (during treatment), without hepatic transplantation by INR severity criteria (INR < 2 and ≥ 2) (during treatment), without hepatic transplantation by Escudiè's intoxicion grade transplantation criteria (INR < 6 and ≥ 6) (during treatment) will be analyzed in the same way as the analysis of primary endpoint. The number and proportion of patients meeting the criteria will be computed and presented along with 95% CI that is computed using Newcombe-Wilson score method.

Elapsed days between amanita poisoning and start of treatment will be summarized descriptively.

The time to normality for AST, ALT, bilirubin, PT/INR, and creatinine will be analyzed using Kaplan-Meier estimation method of estimation for the survival time. 95% CI will be computed for the survival rate by using Greenwoods formula. The analysis of time to normality for AST, ALT, bilirubin, PT/INR, and creatinine will be analyzed by the disease severity stages as well.

7.6 Interim Analyses

Interim Clinical Study Report summarizes the data related to the first 50 patients of the 54 recruited, as of December, 2012 into this study with protocol No. SB16A1.07, entitled "Prevention and Treatment of Amatoxin Induced Hepatic Failure with Intravenous Silibinin (Legalon® SIL): An Open Multicenter Clinical Trial", which was originally submitted to the FDA on 9 July 2009 under IND 105,960 and is being conducted in US investigational sites. Study Objectives and Endpoints: This study aims to assess the efficacy and safety of Legalon® SIL in the prevention of severe morbidity (defined as liver transplant) and death in amatoxin poisoned patients. The primary endpoint is the proportion of enrolled patients without liver transplant or death. Secondary assessments include AST, ALT, bilirubin, PT/INR, and creatinine as indicators of liver and renal function, adverse events and vital signs.

Design: Multicenter, open, uncontrolled study.

Study Population: Patients with a clinical diagnosis of amatoxin poisoning based on history, gastrointestinal symptoms, elevated liver enzymes.

Results: 42 of the 50 poisoned patients (84.0%; 95% CI 70.9% to 92.8%) were discharged home from the hospital with complete recovery. Conversely, 8 of the 50 patients (16%) had a severe morbidity, resulting in 5 deaths and 3 liver transplants. The negative outcome of these 8 patients was due to the progression of the liver and renal failure as a consequence of Amatoxin intoxication in the absence of an adequate aggressive hydration to preserve renal function and when treatment with Legalon® SIL was instituted started >96 hours after poisoning.

A total of 11 patients (22.0%) experienced 20 Adverse Events (AEs) during the course of the clinical trial, 11 of which were Serious Adverse Events (SAEs). All SAEs were regarded as severe AEs by the on-site investigator (OSI) and all of them were considered not related to Legalon® SIL treatment. Indeed, they were all signs of the severe amatoxin intoxication, responsible for severe liver failure leading these patients to die or to be liver transplanted.

Conclusions: Since silibinin strongly inhibits the hepatocyte uptake of amatoxin and the survival has been correlated with the time of initiation of therapy, Legalon® SIL treatment should be instituted as soon as a diagnosis has been made/suspected. As a matter of fact, all the patients who started treatment with the study drug >96 hours after poisoning had a negative outcome.

As long as renal function has been maintained with aggressive intravenous hydration, patients who receive intravenous Legalon® SIL appear to recover from amatoxin induced hepatotoxicity in a rapid, predictable, and reliable manner. Length of hospitalization for the successfully treated patients was relatively short, often well under 8 days. Legalon® SIL was not associated with any serious adverse events. It was easy to administer, safe, and was well tolerated in this patient population.

7.7 Safety Analyses

The safety analyses will be based on the Safety population.

The analysis set for safety summaries is defined as all subjects who received at least one dose of study medication.

7.7.1 Adverse events

Adverse events will be summarized and listed by Preferred Term (PT) and SOC using MedDRA vocabulary (most recent) by treatment. All AEs that occur after the first dose of study medication through 30 days after the last dose will be considered to be treatment emergent AEs. The number and percentage of subjects with at least one treatment emergent AE will be presented by treatment group and events further summarized by maximum severity and relationship to study medication. The number and percentage of patients with SAE, discontinuation of study treatment due to AE (DAE), and AEs of severe intensity will be summarized by treatment. The AEs will be summarized by outcome of the event as well. Furthermore, listings of SAEs and AEs that lead to withdrawal will be provided. Summary statistics (N, %) of AEs of special interest will be also be separately tabulated.

Descriptive statistics will be provided for the following safety data. No inferential analysis of this safety data is planned. Any ECG, BP, and pulse rate abnormalities of potential clinical concern will be described.

7.7.4 Laboratory Data

Clinical laboratory data consists of clinical chemistry, hematology, and other parameters. These data will be summarized by treatment. For clinical laboratory tests, listings of values for each patient will be presented with abnormal or out of range values flagged. The laboratory data will be presented in System International (SI) units. Numerical laboratory results will be summarized using standard summary statistics by timepoint of collection and treatment group. Baseline shift tables will be prepared for the laboratory findings ('Normal', 'Low' and 'High'). Subject listing will be prepared for the pregnancy test results for female patients.

8 ADMINISTRATIVE PROCEDURES

Access to Data/Source Documentation

The OSI or designated representative will permit full access to data and source documentation for the purpose of clinical monitoring, audits, IRB/IEC review and regulatory inspections.

8.1 Final Clinical Study Report and Case Report Forms (CRFs)

A written clinical study report will be provided in accordance with the International Conference on Harmonization (ICH) E-3 guidelines including Annex I (Synopsis) documenting the clinical execution of the study. This report will include a description of any protocol deviations. The final report will also include reasons for withdrawals and any necessary treatment(s). The report will also include tables presenting demographics (separate summary tables for enrolled and completed subjects), and adverse events recorded during the study. In addition, the clinical study report will include a Quality Assurance statement, documenting that the report has been reviewed for completeness, accuracy, and compliance with the protocol and applicable local and federal regulations. For final clinical reporting purposes only, adverse events deemed "definite", "probable" or "possible" will be included in the treatment-related summaries/listings.

Case Report Forms (CRFs) containing data transcribed from subject source documents (as appropriate) and copies of other source documents will be supplied by the clinical site. The OSI must sign each subject's CRF after completion of data entry, signifying that the data entered in the CRF is complete and accurate.

8.2 Adherence to Protocol

Except for an emergency situation, in which proper care for the protection, safety and well-being of the study subjects requires medical treatment, the study will be conducted as described in the approved protocol (and amendments, if applicable), GCP and applicable SOPs. In addition, the study will be conducted in accordance with the applicable regulatory requirements of the country where the study is being conducted as well as the country where the study will be submitted. Any deviation(s) from the protocol will be recorded and presented in the final report.

8.3 Data Handling and Record Retention

All clinical information shall be recorded, handled and stored in such a way that it can be accurately reported, interpreted and verified, while the confidentiality of records of the trial subjects remains protected.

A set of CRF is required to be completed for each subject receiving study medication. The CRF is property of the sponsor and the OSI must review all CRFs prior to submission to the sponsor.

All records pertaining to the receipt and return of study supplies (particularly study medication) and copies of final CRFs, worksheets, and other pertinent source documents must be retained in accordance with ICH-GCP and the applicable regulatory requirements of the country where the study is being conducted as well as the country where the study will be submitted.

The investigator must obtain in writing the sponsor's agreement to dispose of any records, even if the retention period has been reached.

8.4 Confidentiality

Information furnished to OSIs and IRBs/Ethics Committees will be maintained in confidence by the OSI and IRB/Ethics Committee. By signing this protocol, the OSI affirms to the Sponsor that he/she will maintain, in confidence, information furnished to the IRB/Ethics Committee relevant to this study under appropriate understanding of confidentiality with such IRB/Ethics Committee.

By signing the protocol, the OSI agrees that within local regulatory restrictions and institutional and ethical considerations, the Sponsor may consult and/or copy source documents (e.g., laboratory/X-ray reports, ECG tracings, workbooks, medical records) in order to verify CRF data.

8.5 Ethics and Regulatory Authorities

Guidelines will be followed with regards to the treatment of human subjects in the study, in accordance with the requirements of the Declaration of Helsinki and International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH-E6-R2 in addition to the regulatory requirements of the country where the study is being conducted as well as the country where the study will be submitted.

8.5.1 Institutional Review Board/Ethics Committee

The OSI is responsible for obtaining initial and continuing review (at intervals not more than once per year) of the study by an IRB/Ethics Committee, or in accordance with applicable government regulations of the country where the study is being conducted as well as the country where the study will be submitted. Ideally study will not enroll any subjects until the IRB/Ethics Committee provides written approval of the protocol and the informed consent to the investigator. Due to the critical condition of subjects with amatoxin poisoning verbal approval confirmed via email may suffice until formal written approval can be issued by the local IRB. In addition, a copy of the IRB/Ethics Committee approval documents (email and/or formal letter) must be provided to the sponsor prior to enrolling any subjects into the study.

8.5.2 Regulatory Authority

This clinical study protocol, title as well as other relevant documentation will be submitted to the local Regulatory Authorities for review and approval prior to trial start. Upon completion, the Regulatory Authorities will be notified the study has ended. The study will only be undertaken in compliance with the local regulatory requirements.

8.6 Informed Consent

A properly executed, written informed consent in compliance with current GCP guidelines and ICH guidelines shall be obtained from each patient or their legally authorized representative (LAR or next of kin) prior to entering the study. The investigator shall provide a copy of the signed and dated informed consent to the subject, and a signed and dated copy shall be maintained in the patient's medical record.

8.7 Disclosure and Publication of Clinical Study Data

The disclosure and publication of clinical study data will be detailed in the OSI Commitment Form.

8.8 End of Trial

The end of trial is considered to be the date of last subject last visit or the date of early termination of the study whichever is the later.

9 ADVERSE EVENT REPORTING

9.1 Assessment of Safety

9.1.1 Safety Parameters

Safety will be assessed by the monitoring of adverse clinical events, vital signs, clinical laboratory evaluations, and physical examinations. All adverse events occurring during the study must be recorded according to Section 9.2.2.

Findings from screening procedures, e.g., laboratory tests or physical examinations will be recorded as medical history. Clinically significant changes following the screening procedures will be recorded as adverse events.

The study period for the purpose of adverse event collection is defined as the period from the signing of a study specific informed consent to 30 days after the last dose of study medication. All adverse events that occur after the first dose of study medication through 30 days after the last dose will be considered to be treatment emergent adverse events. Any adverse event received by the clinical site for a subject who has previously exited the study needs to be forwarded to the sponsor for evaluation if within the aforementioned window.

9.2 Adverse Event Reporting

9.2.1 Definitions

Adapted from ICH Harmonised Tripartite Guideline: Clinical Safety Data Management Definition and Standards for Expedited Reporting: E2A

Adverse Event

Any untoward medical occurrence in a subject administered a medicinal 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 (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious Adverse Event

An event that is fatal, life-threatening, or leads to persistent or significant disability; one that requires or prolongs hospitalization; or one that results in a congenital anomaly, or one that is a significant medical event.

Medical events that are not fatal, life-threatening, or require hospitalization may be considered serious when, based on appropriate medical judgment, they jeopardize the subject and may require [urgent] medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.2.2 Documentation of Adverse Events

The investigator or designee must record all adverse events reported by subjects that have occurred during the adverse event collection period of the study. In addition, adverse events should be solicited from subjects though open-ended questions, and as appropriate, by examination. Subjects will be evaluated for adverse events from the time of consent through study follow up visit. The subject should also be queried for any previously unreported adverse event as part of study exit procedures. All adverse events must be followed until they have resolved or stabilized.

Where possible and appropriate, the investigator should record the most important medical event or specific diagnosis rather than individual signs and symptoms (all clearly related signs, symptoms, and abnormal diagnostic procedure results should be recorded in the source document, but should be grouped under one diagnosis on the AE page).

All adverse events (including laboratory abnormalities, if they are of clinical concern), *whether or not thought to be caused by the investigational drug*, will be recorded on the CRF. The information in the CRF will include:

- The date/time of onset of any new adverse event or the worsening of a previously observed adverse event or worsening of any previously existing disease
- The event in standard medical terminology
- The duration of the adverse event
- The relationship of the adverse event to the investigational drug(s)
- The severity of the adverse event
- Seriousness of the adverse event
- Description of action taken in treating the adverse event and/or change in investigational drug administration or dose

Clinically significant abnormal laboratory values that represent a change from baseline should be recorded diagnostically as AEs (e.g. for elevated serum glucose, the AE would be described diagnostically as "hyperglycemia"). Determination of clinical significance and seriousness is based on the investigator's medical judgment. The following are examples of laboratory abnormalities that would normally qualify as adverse events:

- The abnormality suggests a change in disease severity and/or organ toxicity

The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

9.2.3 Relationship of Adverse Experiences to the Investigational Drug

The Investigator is responsible for assessing relationship of adverse events to the investigational drug (e.g., causality assessment). Factors that need to be considered when making a causality assessment include: the temporal relationship (e.g., time to onset); the

clinical and pathological characteristics of the events; pharmacological plausibility; exclusion of confounding factors (medical and medication history); drug interactions; dechallenge/rechallenge, and dose relationship etc.

A suspected relationship (definite, probable, possible) between the events and the study medication means in general that there are facts (evidence) or arguments to suggest a causal relationship. Receipt of additional or clarifying information may warrant reassessment of causality.

The Investigator is responsible for assessing relationship of adverse events to the investigational drug in accordance with the following definitions:

Definite – causal relationship is certain (e.g., the temporal relationship between drug exposure and the AE onset/course is reasonable, there is a clinically compatible response to dechallenge, other causes have been eliminated and the event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary).

Probable – high degree of certainty for causal relationship (e.g., the temporal relationship between drug exposure and AE onset/course is reasonable, there is a clinically compatible response to dechallenge (rechallenge is not required), and other causes have been eliminated or are unlikely).

Possible – causal relationship is uncertain (e.g., the temporal relationship between drug exposure and the AE onset/course is reasonable or unknown, dechallenge information is either unknown or equivocal, and while other potential causes may or may not exist, a causal relationship to the investigational drug does not appear probable).

Unlikely – temporal relationship between drug administration and event onset is improbable, and/or another explanation is more likely such as disease, environment, other drugs etc. Does not represent a known reaction to the investigational drug.

Unrelated/Not related – no possible relationship (e.g., the temporal relationship between drug exposure and the AE onset/course is unreasonable or incompatible, or a causal relationship to the investigational drug is implausible).

9.2.4 Severity of the Event

Note that the term "severe" is a measure of intensity; thus, a severe AE is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious. Likewise, mild or moderate events could, under some circumstances, result in a serious outcome as defined above.

The intensity or severity of AEs will be graded as follows:

- **Mild** - awareness of sign or symptom, but easily tolerated. Not expected to have a clinically significant effect on the subject's overall health and well-being. Does not affect daily activities. Not likely to require medical attention.
- **Moderate** - discomfort enough to cause interference with usual daily activities or affects clinical status. May require medical intervention and/or prescription drug therapy.

- **Severe** - incapacitating or significantly affecting clinical status. Severely limits daily activities. Medical intervention, prescription drug therapy and/or hospitalization required.

9.2.5 Special Situations

Emergency Room Visits: Post discharge from the hospital and up to 30 days post discontinuation of study drug, events that result in emergency room visits that do not result in admission to the hospital are not routinely considered to be serious events; however, these events should be evaluated for one of the other serious outcomes (e.g., life-threatening, other serious [medically significant] events).

Neoplasms: For this protocol, any diagnosis of cancer or neoplasm is considered serious (significant medical event criterion).

Quality Complaints: Quality complaints related to study drug are not AEs, however, any AE or SAE experienced by the subject as a result of these should be documented in the CRF and SAEs sent to Mylan on the SAE reporting form. Please see Section 5.3 for further details.

Overdose: The protocol specified dose should not be exceeded. Overdose will be documented as a deviation in the source and communicated to the sponsor Clinical Scientist or designee. The subject should be monitored appropriately by the investigator for latent adverse effects. Any adverse event occurring as a result of overdose or medication error will be recorded as an AE in the CRF and if serious (per definition, Section 9.2.1) reported immediately to sponsor as per Section 9.2.7. Please see Section **Error! Reference source not found.** for further details.

9.2.6 Exposure to Investigational Drug During Pregnancy:

Pregnancy in a female subject is to be reported on the Pregnancy Report Form within 24 hours of awareness starting from first dose of study medication. The pregnancy should be followed to term or termination and updates submitted during the pregnancy at 3-monthly intervals via the Pregnancy Report Form.

While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of pregnancy for medical reasons will be recorded as an AE or a SAE depending upon the Investigator's medical judgment.

If a pregnancy occurs in a female partner of a male subject that has received at least one dose of investigational drug, the site should contact Mylan (or designate) to obtain a partner pregnancy informed consent form. While the occurrence of a partner pregnancy must be communicated to Mylan Product Safety & Risk Management, consent of the pregnant partner must be obtained before the site can collect any details of the pregnancy or share personal medical information of the partner with the sponsor. If the pregnant partner provides consent to have the pregnancy followed, the site should collect the information specified on the Pregnancy Report Form and forward the completed form to Mylan Product Safety & Risk Management in 3-monthly intervals until the pregnancy outcome has been obtained. No entry into the male subject's CRF is required for the partner pregnancy including adverse events experienced by the mother, fetus, or neonate.

A spontaneous abortion is always considered to be a SAE. Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered reasonably related in time to receipt of the investigational product by the investigator, will be reported to the sponsor. Serious pregnancy-related medical events and congenital anomalies are to be reported on the SAE Report Form.

Elective termination (i.e., without medical reasons) of an uncomplicated pregnancy is considered to be an elective procedure and not an adverse event; nevertheless, Mylan requests that the outcome (e.g. elective termination) be reported within 24 hours and sent as a follow-up on the Mylan Delivery and Infant Follow-up Form.

9.2.7 Notification – Immediately Reportable Events

In addition to standard documentation of adverse events in the CRF, serious adverse events and pregnancies require immediate notification to Mylan Product Safety and Risk Management Department (PSRM) on the Mylan Clinical Trials Serious Adverse Event/Pregnancy Reporting Forms. "Immediate" is defined as **within 24 hours** of the investigator's or site staff's knowledge of the event.

The SAE and Pregnancy Report Forms are to be completed for all serious AEs and pregnancies, signed by the Investigator, and emailed with supporting documentation (e.g. case report forms, hospital records, laboratory reports). Subject identity details (such as but not limited to name or clinic/hospital number) must not be visible on the forms or any supporting documentation provided by the Investigator. These should be "blacked out", signed and dated before submission to Mylan. The subject ID is to be provided on every document.

All SAEs/Pregnancies must be sent within 24 hours of awareness to:

Mylan Global Product Safety & Risk Management at:

pvclinical@mylan.com

Note: Email is the preferred method of communication

If an acknowledgment is not received from Mylan PSRM within 24 hours, report the SAE via:

Tel: +1.304.285.6409

The investigator will include a narrative description of the serious adverse event(s) that discusses:

- Investigational drug(s) administered, including dates
- Date and time of the onset of the adverse event
- Description of the reaction in standard medical terminology
- Date the event resolved or outcome of the event at last observation

- Investigator's assessment - the attribution of the adverse event to the investigational drug(s) or possible alternative etiologies for the event

- Description of action taken in treating the adverse event and/or change in investigational drug administration or dose

Follow-up information to a safety report shall be submitted to Mylan's Product Safety and Risk Management Department by the investigator as soon as the relevant information is available. Relevant information such as discharge summaries, autopsy reports, and medical consultations shall be reviewed in detail by the Investigator. The Investigator shall comment on any event, abnormal laboratory result, or any other finding, noting whether it shall be considered a serious or non-serious AE or considered part of the subject's history. In addition, the Investigator shall report on an SAE form all subsequent events or other findings determined to be relevant and shall state for each event or finding whether it is related to the investigational drug. All events determined to be non-serious shall be reported on the relevant CRF page and entered into the clinical database by the CRO or others as agreed in their contractual obligations.

Reconciliation of SAEs will be performed periodically (quarterly) or at the end of the trial, depending upon the duration of the study to ensure both the number of cases and information is consistent between both the Clinical Database and the Global Safety Database.

9.2.8 Reporting Timelines for Serious Adverse Events and Follow-up Information

The Principal Investigator (or designee) will report any AE classified as serious or pregnancy via email or telephone within 24 hours but no later than 1 calendar days after becoming aware. A complete description of the event (i.e. completed SAE or Pregnancy Report form) must be transmitted to the Sponsor, as follow-up data, in writing as soon as possible, but in any case, not later than 2 calendar days for all serious events after first knowledge of the SAE or pregnancy. The Investigator should also be aware of any special reporting requirements, such as institutional review boards or ethics committees. The Sponsor has strict regulatory reporting time lines for safety related information. Therefore, the above-mentioned reporting timeframes must be adhered to by the OSIs.

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11 PROTOCOL AMENDMENT DETAILS

Any significant changes in the study procedure or study design will only be affected upon mutual agreement between the sponsor and CRO and after obtaining a favorable opinion from the Ethics Committee and Regulatory Agency (if applicable). All such changes will be documented in the amended version of the protocol and a list of changes with reference to previous will be generated and submitted to the IEC/IRB along with amended version of the protocol.