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Continuous Infusion Terlipressin for Patients With Cirrhosis and Refractory Ascites

November 17, 2018

Protocol Edition #6

Safety and pharmacodynamic activity of low-dose terlipressin delivered by continuous intravenous infusion in patients with cirrhosis and refractory ascites requiring recurrent large volume paracentesis

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SIGNATURES OF AGREEMENT FOR PROTOCOL REVISION

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Clinical Investigator Date

Institution: _____

SIGNATURES OF AGREEMENT FOR PROTOCOL REVISION BY THE SPONSOR, BIOVIE INC.

_____

BioVie Inc. Consulting Medical Director November 17, 2018
Patrick D. Yeramian MD
Yeramian@comcast.net

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1. LIST OF ABBREVIATIONS

Abbreviation or specialist term	Explanation
8-LVP	8-lysine-vasopressin
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine amino transferase, also known as SGPT
ANCOVA	analysis of covariance
AST	aspartate amino transferase, also known as SGOT
AUC	area under the curve
BP	blood pressure
BUN	blood urine nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations
Cmax	maximum concentration
CMH	Cochran-Mantel-Haenszel
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CRF	case report form
d	day
DCF	data clarification form
dL	deciliter
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	electrocardiogram
Emax	maximum effect
EVH	esophageal variceal hemorrhage
FENa	fractional excretion of sodium
FDA	Food and Drug Administration
FU	follow-up
GCP	Good Clinical Practice

Abbreviation or specialist term	Explanation
GCRC	General Clinical Research Center
GFR	glomerular filtration rate
hr	hour
HR	heart rate
HRS	hepatorenal syndrome
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	intent-to-treat
IV	intravenous
IVRS	Interactive Voice Response System
MAP	mean arterial pressure
MedRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MDRD	modification of diet in renal disease
NSAIDs	nonsteroidal anti-inflammatory drugs
PCR	polymerase chain reaction
PK	pharmacokinetic
PD	pharmacodynamic
pp	per protocol
PPCD	post paracentesis circulatory dysfunction
q6h	once every six hours
RBC	red blood cell
RNA	ribonucleic acid
RRT	renal replacement therapy
SAE	serious adverse event
SAER	serious adverse event report
SBP	systolic blood pressure
SCr	serum creatinine
SGOT	serum glutamic-oxaloacetic transaminase, also known as

Abbreviation or specialist term	Explanation
SGPT	serum glutamic-pyruvic transaminase, also known as
SmPC	summary of product characteristics
SNPs	single nucleotide polymorphisms
TBD	to be determined
TIPS	transjugular intrahepatic portosystemic shunt
US	United States
UTI	urinary tract infection

2. SYNOPSIS

Title of Study:	Safety and pharmacodynamic activity of low-dose terlipressin delivered by continuous intravenous infusion in patients with cirrhosis and refractory ascites requiring recurrent large volume paracentesis

Objectives

Primary Objectives

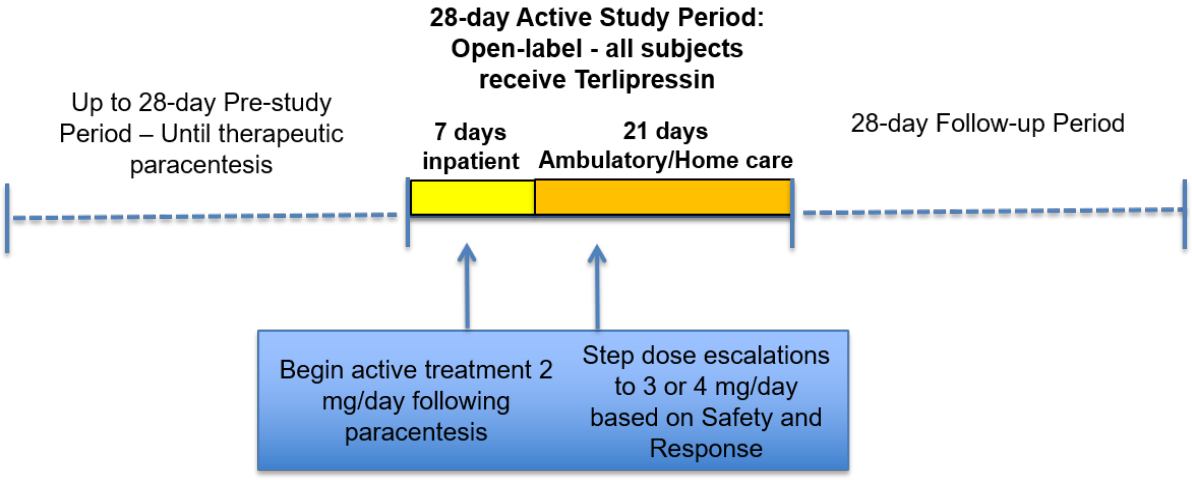
- To assess the safety and tolerability of continuous infusion of terlipressin for 28 days in cirrhotic patients with refractory ascites.
- To determine the steady state pharmacokinetics of terlipressin and 8-LVP in cirrhotic patients with refractory ascites.

Study Design:

The study is an open label trial of continuous infusion terlipressin.

The study will enroll 6 patients with cirrhosis and refractory ascites. Two sentinel patients with serum creatinine <1.5 mg/dL will be enrolled first in a safety lead-in Phase. Thereafter 4 additional patients will be enrolled, with no less than 2 patients with serum creatinine ≥ 1.5 and < 2.0 mg/dL

After being enrolled in the trial patients will be monitored for up to 28 days until they require a therapeutic paracentesis. Starting within 3 days of the paracentesis, patients will be treated with a continuous infusion of terlipressin over 7 days in-house and, if tolerated, continue treatment in the ambulatory setting for another 21 days. The initial Terlipressin infusion dose will be 2 mg/day, which may be escalated to 3 mg and to 4 mg based on effect of treatment on morning MAP and observed safety and therapeutic response. Upon completion of treatment, patients will be monitored for safety for 28 days in the ambulatory setting. The study design is outlined below:



Main Inclusion/Exclusion Criteria:

Criteria for Inclusion:

- Patients with cirrhosis
- Patient has diuretic-resistant or intractable ascites and required 3 or more large volume paracenteses in the previous 60 days
- Patient male and female age between 18-70 years
- Women of child bearing potential (e.g. not post-menopausal for at least one year or surgically sterile) must be neither pregnant or lactating and must agree to use adequate birth control or be abstinent for the duration of the study
- If patient is treated with an ACE inhibitors or beta blockers, has been on a stable dose for at least 2 months prior to enrollment and may be maintained on that dose for the trial duration.

Main Inclusion/Exclusion Criteria:

Criteria for Exclusion

- Ascites with causes other than cirrhosis such as cardiac, nephrogenic ascites or malignant ascites due to peritoneal carcinomatosis
- Total bilirubin > 5 mg/dL
- Blood clotting International normalized ratio (INR) > 2.5
- For two sentinel patients Serum creatinine < 1.5 mg/dL
- For other patients: Serum creatinine < 2.0 mg/dL
- Current or recent (within 3 months of consent) renal dialysis
- Hepatic encephalopathy grade 3 or 4
- Superimposed acute liver failure/injury due to factors including alcoholic hepatitis, acute viral hepatitis, drugs, medications (e.g., acetaminophen), or other toxins (e.g., mushroom [*Amanita*] poisoning)
- Current or recent treatment (within 7 days) with octreotide, midodrine, vasopressin, dopamine or other vasopressors
- Respiratory failure requiring PAP devices or intubation
- SIRS/sepsis episode in the previous 28 days from consent
- Episode of spontaneous bacterial peritonitis or gastrointestinal hemorrhage within 28 days of consent
- Ongoing documented or suspected infection
- Severe cardiovascular disease that are contraindication to terlipressin therapy such as advanced arteriosclerosis, arrhythmia, coronary insufficiency or uncontrolled hypertension
- Findings suggestive of severe organic renal disease (proteinuria, hematuria, or abnormal renal ultrasound suggestive of obstructive or other renal pathology)
- Severe comorbidity that in the opinion of the Investigator would affect short-term prognosis (such as for example, advanced progressive neoplasia such as hepatocellular carcinoma [confirmed with serum AFP testing], severe COPD or asthma)
- Alcoholics who have not been abstinent for the past 12 weeks
- Transjugular intrahepatic portosystemic shunt or other surgical shunt
- Known allergy or hypersensitivity to terlipressin
- For female patients: Confirmed pregnancy
- Participation in other clinical research studies involving treatment with other investigational drug or evaluation of implantable device within 30 days of

Study Evaluations: Refer to the Table in Section 6.1

3. INTRODUCTION

Cirrhotic patients that require paracentesis for mobilization of ascites, despite treatment with diuretics and sodium restriction, have a very poor prognosis and expedited referral for liver transplantation is recommended (Runyon, 2013). Since these patients are not hospitalized, outpatient therapies are desperately needed to manage ascites and decrease rate of complication and the need for hospitalization, in addition to improving quality of life. Such a therapy can act as a bridge to liver transplantation in those eligible for transplant and as palliative care in those ineligible for transplant.

A reduction of the splanchnic vasodilation associated with portal hypertension and sodium and water retention can lead to a decrease in ascites formation by means of an increase in effective blood volume and a decrease in activation of the renin-angiotensin system with resulting increase in renal sodium excretion (Kalambokis, 2005). Terlipressin (triglycyl lysine vasopressin; a long-acting synthetic analogue of vasopressin), binds to vasopressin 1 receptors and acts as a vasoconstrictor on the splanchnic circulation. A number of studies support the use of terlipressin to correct hemodynamic dysfunction and manage ascites in cirrhotic patients. Terlipressin has been shown to: 1) reduce portal hypertension in cirrhotic patients (Ding, 2013, Escorsell, 1997, Narahara, 2009, Baik, 2005); 2) improve renal function and induce natriuresis in cirrhotic patients with ascites without hepatorenal syndrome (HRS) (Krag, 2007); 3) increase GFR, urine volume and urine sodium while decreasing renin and aldosterone levels in HRS patients in combination with albumin (Ortega, 2002); 4) decrease the need for large volume paracentesis, increasing urinary sodium excretion when used in combination with albumin and diuretics in refractory ascites patients with normal renal function (Fimiani, 2011) and 5) reduce the volume of ascites and number of paracentesis procedures in refractory ascites patients treated for 28 days with terlipressin administered as a continuous infusion on an outpatient basis (Gow, 2016).

Currently, terlipressin is approved in Europe for the treatment of bleeding esophageal varices and HRS type 1. In two US clinical trials in patients with type 1 HRS terlipressin was administered as an IV bolus starting at 1 mg every 6 h and increased to 2 mg every 6 h (maximum 8 mg/day depending on response).

Recent studies support the increased safety and efficacy of low-dose terlipressin delivered by continuous infusion. While bolus dosing of terlipressin (1 or 2 mg) leads to a significant reduction of HVPG for 3-4 h, continuous infusion of terlipressin (4 mg/day) for 24 h results in a sustained reduction in portal venous pressure with no side effects (Ding, 2013). Continuous infusion allows for a significant reduction in the daily effective dose required for HRS reversal (2.23 mg/day versus 3.51 mg/day, $p < 0.0001$) with a concomitant reduction in AEs (35.29% continuous infusion vs 62.16% bolus regimen, $p < 0.025$) and SAEs (20.59% continuous infusion vs 44.4% bolus regimen, $p < 0.05$) (Cavallin, 2016). It thus appears that the improved safety of terlipressin delivered as a low-dose continuous infusion could enable its use in the outpatient setting in the prolonged treatment of patients with refractory ascites. In a pilot study, continuous outpatient infusion of terlipressin has been used successfully on an outpatient basis to reduce ascites and decrease the number of paracentesis procedures in 5/5 cirrhotic patients with refractory ascites (Gow, 2016).

There is limited PK data for terlipressin in humans despite being available since the early 1980s. Following IV bolus administration terlipressin plasma concentrations decline in a bi-exponential fashion. Terlipressin, which itself has vasopressive activity (Ryckwaert, 2009; Colson, 2016), reaches C_{max} of > 50 ng/ml after a single IV dose of 0.7 mg after approximately 5 minutes (Nilsson, 1990). Terlipressin is metabolized via enzymatic cleavage of its three glycine residues by endothelial peptidases into 8-lysine-vasopressin (8-LVP), its more biologically active component, with measurable levels of 8-LVP appearing approximately 30 minutes after bolus administration, peaking at between 1 and 2 h (Forsling, 1980, Nilsson, 1990). In 29 HRS patients, a dose of terlipressin of 1 mg every 6 h resulted in a mean C_{max} of terlipressin and 8-LVP of 62 ng/mL and 0.75 ng/mL, respectively (Lucassin AusPAR 130827-cer).

The pharmacokinetics and pharmacodynamics activity support the current bolus dosing regimen of every 4-6 h though significant reduction of portal hypertension lasts only half of a 6 hour dosing interval with a bolus dose of 1 mg (Escorsell, 1997) and effects on hemodynamic parameters, including portal hypertension, are intermittent with this regimen (Ding, 2013). To date PK data is not available for continuous infusion of low-dose terlipressin but this regimen would presumably result in sustained therapeutic levels of both peptides. PK projections for

plasma concentrations at steady state (C_{ss}) of both terlipressin and 8-LVP during continuous infusion of a dose of 2 mg/day/subject estimate steady state levels to be 2-3 ng/ml and 0.1 to 0.3 ng/ml, respectively. The projected C_{ss} of terlipressin is far less than the C_{max} obtained with bolus dosing (2-3 ng/ml vs 62 ng/ml) and might result in a reduction of the rate and severity of AEs related to the immediate severe hemodynamic effects of terlipressin as a bolus dose.

In our planned pilot study, low-dose continuous infusion of terlipressin will be administered via ambulatory pump to a cohort of cirrhotic patients with refractory ascites, initially for 7 days in a GCRC to establish safety and tolerability followed by transition to an outpatient setting for a further 21 days of treatment. Initially, 2 sentinel patients with SCr < 1.5 mg/dL will be enrolled followed by 4 additional patients with SCr < 2.0 mg/dL. An extensive PK analysis will be performed. The C_{ss} of both terlipressin and 8-LVP during continuous infusion of terlipressin will be determined. Successful management of ascites, as evidenced by a reduction in the need for paracentesis procedures and decreased ascites fluid volume, further supported by improvement in renal function, is anticipated to have a significant impact on the quality of life of these patients, including a decrease in the need for hospitalization.

4. OBJECTIVES

4.1. PRIMARY OBJECTIVES

The primary objectives of this study are:

- To assess the safety and tolerability of continuous infusion of terlipressin for 28 days in cirrhotic patients with refractory ascites.
- To determine the steady state pharmacokinetic of terlipressin and 8-LVP in cirrhotic patients with refractory ascites.

4.2. EXPLORATORY OBJECTIVES

- To assess the reduction in requirement of frequency and volume of paracentesis with continuous infusion of terlipressin.
- To assess the improvement in patient quality of life using the SF36 instrument with continuous infusion of terlipressin.

- To assess renal function based on the change in serum creatinine, glomerular filtration rate and sodium excretion with continuous infusion of terlipressin
- To assess the plasma renin activity (PRA) and plasma aldosterone concentration with continuous infusion of terlipressin

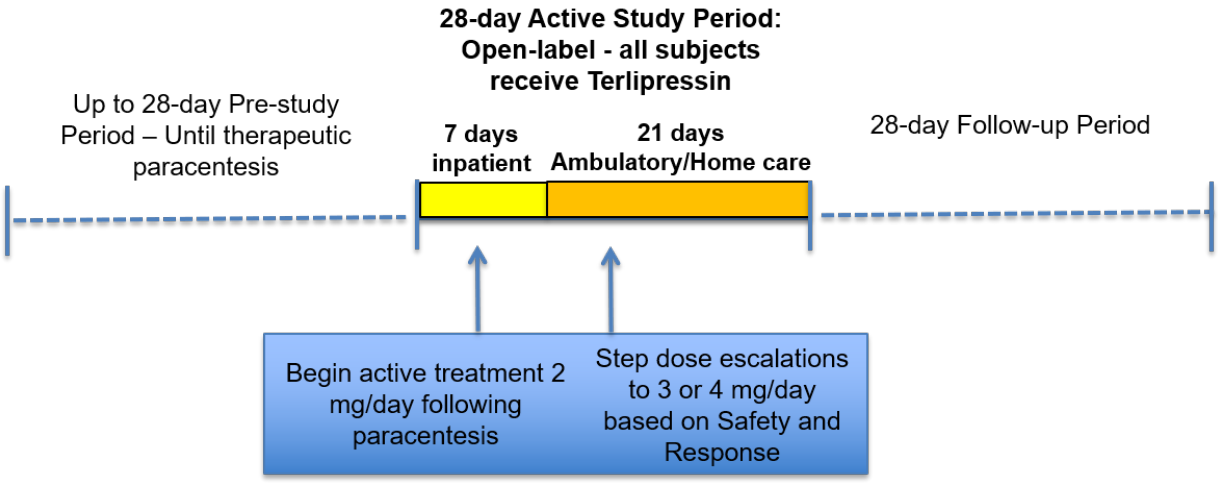
5. MATERIALS AND METHODS

5.1. STUDY DESIGN

The study is an open label single arm uncontrolled trial of continuous infusion terlipressin. The study will be completed in two sequential steps:

- Two sentinel patients with cirrhosis and refractory ascites with serum creatinine < 1.5 mg/dL will be enrolled and complete the 28 day infusion period.
- Then, 4 additional patients with cirrhosis and refractory ascites with serum creatinine < 2.0 mg/dL). No less than 2 patients should have a serum creatinine ≥ 1.5 and < 2.0 mg/dL.

After being consented and enrolled in the trial patients will be monitored without intervention for up to 28 days until they require a therapeutic paracentesis. Starting within 3 days after the paracentesis, patients will be admitted to the GCRC and be treated with a continuous infusion of terlipressin over 7 days in-house with step-wise dose escalation, and, if tolerated, continue treatment in the ambulatory setting for an additional 21 days. The initial terlipressin infusion dose will be 2 mg/day, which may be escalated to 3 mg and to 4 mg based on effect of treatment on morning MAP, overall safety and pharmacodynamic response as detailed in Section 6.4.2. Upon completion of treatment, patients will be monitored for safety for 28 days in the ambulatory setting. The study design is outlined below.



Patients will then enter a long-term observation only follow-up post-trial for an additional 3 months to monitor morbidity (transplant free survival, requirement for dialysis and need for therapeutic paracentesis).

5.2. STUDY SITES

Patients will be enrolled at up to 3 sites in the US.

5.3. STUDY POPULATION

5.3.1. Patient Screening and Enrollment

Informed consent will be obtained from each potential patient prior to screening for eligibility. A sufficient number of patients will be enrolled to ensure that a total of 6 patients complete the planned treatment periods.

5.3.2. Criteria for Inclusion

- Patients with cirrhosis.

  Note: The diagnosis of cirrhosis will based on liver biopsy (Ishak fibrosis stage 5–6) or, in the absence of a liver biopsy, on clinical diagnosis by the Investigator on the basis of the combination of unequivocal clinical data (splenomegaly, spider angiomata, palmar erythema, gynecomastia, and jaundice), and compatible laboratory (thrombocytopenia, leucopenia, hypoalbuminemia and hyper-bilirubinemia) or combination Indices with

better specificity such as a Bonacini cirrhosis discriminant score greater than 7 [Section 22] (Udell 2012), ultrasonography and endoscopic findings.

- Patient has diuretic-resistant or intractable ascites and required 3 or more large volume paracenteses in the previous 60 days [A written record of these paracentesis - date and volume - must be available in the patient medical file].

  Note: Diuretic-resistant ascites: Ascites that is unresponsive to sodium-restricted diet and high dose diuretic treatment (increasing doses of spironolactone up to 400 mg/day and addition of furosemide up to 160 mg/day) for a least 1 week.

  Note: Diuretic-intractable ascites: Ascites that cannot be treated with diuretics due to the development of clinically significant complications of diuretics, as determined by Investigator, e.g., encephalopathy, acute kidney injury, significant decreases in serum sodium or increases in serum potassium. (AASLD Practice Guideline 2012, Arroyo, 1996).

  Note: Large volume will be defined as a paracentesis ≥ 4 liters.

- Patient male and female age between 18-70 years
- Women of child bearing potential (e.g. not post-menopausal for at least one year or surgically sterile) must be neither pregnant or lactating and must agree to use adequate birth control or be abstinent for the duration of the study
- If patient is treated with an ACE inhibitors or beta blockers, has been on a stable dose for at least 2 months prior to enrollment and may be maintained on that dose for the trial duration.

5.3.3. Criteria for Exclusion

- Ascites with causes other than cirrhosis such as cardiac or nephrogenic ascites or malignant ascites due to peritoneal carcinomatosis

  Note: Cardiac ascites based on high serum-ascites albumin gradient (SAAG) greater than 1.1 g/dL and a high ascitic fluid total protein greater than 2.5 g/dL and compatible echocardiogram with an EF < 50%.

- Total bilirubin > 5 mg/dL
- Blood clotting International normalized ratio (INR) > 2.5
- For two sentinel patients: Serum creatinine < 1.5 mg/dL. For other patients: Serum creatinine < 2.0 mg/dL
- Current or recent (within 3 months of consent) renal dialysis
- Hepatic encephalopathy grade 3 or 4
- Superimposed acute liver failure/injury due to factors, including acute alcoholic hepatitis, acute viral hepatitis, drugs, medications (e.g., acetaminophen), or other toxins (e.g., mushroom [*Amanita*] poisoning)
- Current or recent treatment (within 7 days) with octreotide, midodrine, vasopressin, dopamine or other vasopressors
- Respiratory failure requiring PAP devices or intubation
- SIRS/sepsis episode in the previous 28 days from consent

  Note: SIRS is defined as the presence of 2 or more of the following findings: temperature > 38°C or < 36°C; heart rate > 90/min; respiratory rate of > 20/min or a $PaCO_2$ of < 32 mm Hg; white blood cell count of > 12,000 cells/μL or < 4,000/ μL

  Note: Sepsis: Documented infection and SIRS
- Episode of spontaneous bacterial peritonitis or gastrointestinal hemorrhage within 28 days of consent
- Ongoing documented or suspected infection
- Severe cardiovascular disease that are contraindication to terlipressin therapy such as advanced arteriosclerosis, arrhythmia, coronary insufficiency or uncontrolled hypertension
- Findings suggestive of severe organic renal disease (severe proteinuria/hematuria, or abnormal renal ultrasound suggestive of obstructive or other renal pathology)

- Severe comorbidity that in the opinion of the Investigator would affect short-term prognosis and/or disallow safe participation in the trial (such as for example, severe anemia or pancytopenia, advanced progressive neoplasia such as hepatocellular carcinoma [confirmed with serum AFP testing], severe COPD or asthma)
- Alcoholics who have not been abstinent for the past 12 weeks
- Transjugular intrahepatic portosystemic shunt or other surgical shunt
- Known allergy or hypersensitivity to terlipressin.
- For female patients: Ongoing pregnancy.
- Participation in other clinical research studies involving treatment with other investigational drug or evaluation of implantable device within 30 days of consent.

5.4. METHOD OF ASSIGNING TREATMENT NUMBERS TO PATIENTS

Subjects will receive a study number after they have signed an informed consent and their eligibility to enter the study has been confirmed. Patients will be assigned a three-digit study number in the order in which they are enrolled at a site, with the first digit to represent the site number. For example, the first patient enrolled at site #1 will carry the number 101.

5.5. REPLACEMENT OF PATIENTS

Patient who withdraw or drop-out of the study for a reason unrelated to drug safety prior to completion of planned continuous infusion with terlipressin will be replaced. The replacement subject will be assigned a new study number.

5.6. DESCRIPTION OF CLINICAL SUPPLIES

In this protocol terlipressin refers to terlipressin acetate, with approximately 85% terlipressin peptide content, i.e. a 2 mg terlipressin infusion, would refer to 1.7 mg terlipressin peptide equivalent.

Terlipressin acetate will be formulated as a sterile product in 0.9% sodium chloride and provided in a volume of 65 ml (65 mL volume in order to deliver 50 mL) in a 100 mL flexible IV bags at a concentration of 0.04 mg/ml, 0.06 mg/ml or 0.08 mg/ml) to be administered

intravenously by continuous infusion at a dose of 2, 3 or 4 mg/day respectively.

Terlipressin IV solution is received at the site pre-formulated and ready to administer. The solution is maintained at 2 – 8 C and should be warmed to RT prior to initiating administration. A Pharmacy Manual will be provided to the site research pharmacy to detail preparation and handling of the terlipressin IV solution.

IV infusion must be initiated within 72 hours of solution preparation.

The solution is to be administered over 24 hours (infusion rate 2.1 ml/hr) using a Curlin 6000 CMS Continuous Therapy ambulatory pump manufactured by Moog (see Moog Curlin 6000 series Clinicians Guide V2.01 and User Manual for use with the 6000 CMS™ and 6000 CMS™ IOD™ Ambulatory Infusion System in references, a copy of which will be provided to clinical sites). The solution must be administered using a Moog Curlin® Infusion Administration set with 1.2 micron filter and anti-siphon valve which is a non-DEHP microbore tubing with a non-vented bag spike. The ambulatory infusion pumps and administration sets will be provided to the site pharmacy by Coram Clinical Trials. A detailed study drug handling and administration manual will be provided to the site pharmacy and other site personnel. Fresh bags of solution are to be administered with new administration sets.

Inventory and destruction of supplies sent to the GCRC floor or to the hospital pharmacy will be handled locally per the hospital pharmacy procedures. Otherwise, infusion bags will be returned to Coram Pharmacy weekly as part of the Drug Inventory process and properly disposed of at Coram Pharmacy. Medical waste (needles, etc.) are placed in a Mailback Sharps container.

5.7. LABELING OF CLINICAL SUPPLIES

The label affixed to the Terlipressin bag will include the following information:

- Patient name
- Patient ID <number>
- Rx number
- Prescriber
- Terlipressin Acetate dose and volume <___mg/___mL>

- Discard after <date>
- Storage: Refrigerated
- Date prepared: <date>
- *Caution*: New *Drug*--Limited by Federal law to *investigational* use -____mg/mL terlipressin (as terlipressin acetate) in 0.9% sodium chloride solution - Bag contains 65 mL to deliver 50 mL over 24 hours - discard unused material via incineration

6. STUDY PLAN AND PROCEDURES

6.1. SCHEDULE OF OBSERVATIONS AND PROCEDURES

Study Assessment	Screening Period	Pre-Treatment Period Not over 28 days (Days prior terlipressin infusion) [1]		Treatment Period [2]				Follow-up Period (days after terlipressin infusion) [3]	
		StudyEntry	Day -28 to -1	At GCRC		Ambulatory	Site visits	Day +14 (± 2 days)	Day +28 (± 3 days)
				Baseline Day 0 [6]	Day 1 to 7	Day 8 to 28	Day 14±1 and 28±1		
Informed Consent	X								
Inclusion/Exclusion Criteria	X								
Assign Study Number		X							
Medical History		X							
Disease History – Child-Pugh score – Encephalopathy score		X							
Physical Exam		X		X			X		X
Pregnancy test			X						
Height		X							
Concomitant Medications		X							
Collect Treatment emergent AEs				X					
Record AEs of Interest				X	X (Day 7)		X	X	X
Weight / Abdominal circumference/ Temperature		X		X	X [14] [17]	X [8]	X	X	X
12-lead ECG		X		X	X [14]	X [16]		X	X
Vital Signs (MAP/BP, HR)		X	X [5]	X	X [15]	X [8]	X	X	X
Serum Creatinine & BUN		X	X [5]	X	X [14]		X	X	X
Serum Electrolytes, glucose, Calcium		X		X	X [13]		X	X	X

Study Assessment	Screening Period	Pre-Treatment Period Not over 28 days (Days prior terlipressin infusion) [1]		Treatment Period [2]				Follow-up Period (days after terlipressin infusion) [3]	
		StudyEntry	Day -28 to -1	At GCRC		Ambulatory	Site visits	Day +14 (± 2 days)	Day +28 (± 3 days)
				Baseline Day 0 [6]	Day 1 to 7	Day 8 to 28	Day 14±1 and 28±1		
ALT, AST, ALP, Protein, Albumin, Bilirubin, PT/INR		X		X	X [13]	X [16]			X
CBC and differential		X		X	X [18]		X		X
Plasma renin activity and aldosterone		X					X	X	X
Urinalysis		X		X			X	X	X
24 hr. sodium urine excretion			X [5]	X	X		X		
Spot urinary creatinine and sodium				X	X [13]		X	X	X
GFR (sponsor calculation)		X		X	X [13]		X	X	X
Fractional excretion of Sodium (sponsor calculation)				X	X [13]		X	X	X
MELDScore (sponsor calculation) [12]		X			X [13]		X		X
Terlipressin infusion					X [7] [15]	X [8]			
PK sampling					X [9] [10] [19]				
Therapeutic paracentesis			X [4]		X [4] [19]				
Short Form 36 and CLDQ QOL [11]		X		X			X		X
Sodium-intake daily tracker weekly monitoring		X		X				X	

1-Pre-treatment phase of up to 28 day duration, prior start of terlipressin infusion – Numbered Day -28 to -1

2-Treatment period, terlipressin infusion starts Day 1 – Numbered Day 1 to 28 – Day1-7 are administered at GCRC (patient discharge after Bag #7 started), while Day 8-28 are administered in ambulatory setting under home supervision by a nurse.

3-Post-treatment follow-up period, after completion of terlipressin infusion – Numbered +1 to +28

4-As required during pre-treatment, treatment and post-treatment period – date of procedures and volume removed will be recorded. Ascites fluid will be inoculated into blood culture bottles at the bedside and examined by microscopy for a neutrophil count to rule out spontaneous bacterial peritonitis (SBP). Date/volume removed of paracentesis following Day 28 discharge will be collected from the patient file to compute intervals between paracentesis. Requirement for dialysis during that period will also be recorded.

5-Pre-paracentesis baseline serum creatinine, 24hr sodium urine excretion and MAP will be established by collection two values at least a week apart, one should be at time of therapeutic paracentesis performed prior to terlipressin infusion

6-Day 0 is day of admission to GCRC

7-Terlipressin dosing will start on Day 1 at 2 mg/day administered as IV continuous infusion via an infusion pump. The Terlipressin infusion rate may be raised step-wise. The first dose escalation may no earlier than Hour 48 to 3 mg/day Terlipressin; the second dose escalation no earlier than Hour 96 to 4mg/day Terlipressin. (Dose escalation based on observed MAP response, safety and pharmacodynamic response - Refer to Section 6.4.2)

8-Ambulatory, managed by daily home visit by study nurse

9- PK sampling times (Refer to Section 6.7): [Blood samples on Day 1 will be collected in the arm contralateral to the terlipressin infusion, if possible, or below the terlipression infusion site in the same arm.]

- Prior to the start of the terlipressin infusion on day 1 and 0.5, 0.75, 1, 1.5, 2, 3, 6, and 24 h after the start of the continuous infusion.
- Prior to the change in infusion rate and 1, 2 and 8 hours after the change to 3 mg, if it occurs.
- Prior to the change in infusion rate and 1, 2 and 4 hours after the change to 4 mg, if it occurs
- Morning on days 7, 14 and 28.

10-If infusion is discontinued due to AE, an additional blood sample will be obtained prior to interruption of terlipressin infusion

11-Short Form 36 and CLDQ QOL instruments - Refer to Section 21

12-Refer to Section 17

13-Done on Day 3, 5 (i.e. prior to potential dose adjustments at hour 48 and 96) and at discharge

14-Done Daily

15-Terlipressin dose changes are based on MAP computed from first BP measurement in the morning (average from 2 morning measurement of MAP in sitting position – Refer to Section 15), safety and pharmacodynamic response (See Section 6.4.2. BP and HR compiled before Terlipressin start of infusion or dose adjustment(s) and at 0.5, 1, 2, 3, and 6 hours thereafter, then twice a day.

16-Once weekly during outpatient treatment

17-Done first thing in the morning prior to breakfast

18-Done upon discharge from GCRC (Day 7)

19-If a LVP occurs during terlipressin infusion, then blood samples, 4-ml, will be collected in the arm contralateral to the terlipressin infusion, if possible, or below the terlipressin infusion site in the same arm immediately before the paracentesis, 30 minutes after completion of the paracentesis and if feasible 6 hours after completion of the paracentesis

6.2. ETHICS AND REGULATORY CONSIDERATIONS

This study will be conducted according to Good Clinical Practice, the Declaration of Helsinki, and U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards. Written informed consent for the study must be obtained from all patients before protocol-specific procedures are carried out. Patients will be informed of their right to withdraw from the study at any time.

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations. Each patient will receive an oral and a written explanation of the purposes, procedures and potential hazards of this study and will be offer the opportunity to ask questions. Consent will be documented by the dated signature of the subject. The signature confirms that the consent is based on the information that has been understood. Each subject's signed informed consent will be kept on file by the investigator for possible inspection by sponsor representatives and regulatory authorities.

6.3. SCREENING AND PRE-TREATMENT PERIOD

6.3.1. Screening Procedures

To determine whether a patient meets the eligibility criteria the site investigator or representative will examine medical records of patients who consult at the clinic for resistant ascites or patients who require therapeutic paracentesis, including cirrhosis history, recent safety laboratory results and medication records and may contact patients who appear to meet eligibility criteria.

6.3.2. Informed Consent

Obtain the written informed consent from the patient prior to performing any trial mandated activities and tests.

Collect appropriate contact information to conduct follow-up assessment (i.e. name, mailing address, Email and phone number of patient, contact for caregiver and physicians). This confidential information should NOT be transmitted to BioVie Inc. personnel and should be stored accordingly.

6.3.3. Study Entry Visit

The following assessments will be performed:

- Significant medical history over the past 5 years and current medical conditions
- Cirrhosis disease history
- Collate Child-Pugh and encephalopathy score
- Physical exam, with vitals (MAP/BP, HR), body height/weight, temperature, abdominal circumference
- Electrocardiogram
- Collect blood samples for clinical laboratory assessments including: Hematology (CBC with differential), Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR), PRA and plasma aldosterone.
- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein.
- List concomitant medications
- Administer the Short Form 36 and CLDQ QOL

6.3.4. Enrollment of Patients

Patients who meet inclusion/exclusion criteria will be assigned a study number and are also to be identified by first, middle and last initials. If the patient has no middle initial, a dash is to be used. For patients screened but found non-eligible the reason will be recorded in a screening log.

6.3.5. Pre-Treatment Observation Period

Patients will be monitored for up to 28 days after enrollment into the study. They will continue their standard medical care, including dietary restriction and stable therapy with diuretics (i.e. spironolactone, furosemide).

A pre-paracentesis baseline serum creatinine, 24 hr Na+ urine excretion and MAP will be established by collection of two values at least a week apart, one should be close to time of therapeutic paracentesis performed prior to terlipressin infusion.

Admission to GCRC and start of Terlipressin infusion will be dependent on when a therapeutic paracentesis is warranted, as terlipressin treatment should start within 3 days of a paracentesis.

If the patient does not require a paracentesis within this 28 days observation period then the patient will be terminated from the trial and will be replaced. The volume of ascites drained at paracentesis will be recorded. The ascites fluid will be tested for evidence of spontaneous bacterial peritonitis (SBP) (PMN count, culture). A patient found to have SBP will be withdrawn from the trial and not receive terlipressin infusion. Such patients will be replaced.

At the time of the paracentesis, female patients will have a serum pregnancy test to confirm they are not pregnant. The patient will be admitted to the GCRC so that terlipressin infusion may be initiated within 1 and 3 days of completion of the paracentesis.

6.4. TREATMENT PERIOD

6.4.1. Baseline - Admission

Patient will be admitted to the GCRC the day prior to planned initiation of terlipressin infusion. A peripherally inserted central catheter (PICC) or a midline catheter per site usual practice will be positioned for administration of terlipressin.

The following tests will be completed prior to start of terlipressin infusion:

- Sign and symptoms directed physical exam, with vitals, body weight, temperature, abdominal circumference
- Electrocardiogram
- Collect blood samples for clinical laboratory assessments including: Hematology (CBC with differential), Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR).

- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein.
- 24 hour urinary sodium excretion
- List concomitant medications
- Administer the Short Form 36 and CLDQ QOL
- Evaluate AE of interest pre-treatment

The patient should be hemodynamically stable prior to starting the terlipressin infusion. BP/HR and MAP should be determined at least twice and terlipressin infusion may be delayed within the 72-hour window according to the inclusion criteria if the patient is found hemodynamically unstable.

6.4.2. In-House Treatment of Patients

Terlipressin will be administered by continuous intravenous infusion. Start of infusion should be within 1-3 day from completion of the first therapeutic paracentesis that occurs during the Pre-Treatment Period.

Initial terlipressin dosing will be 2 mg/day administered via an ambulatory infusion pump. Refer to Section 5.6 for detailed pharmacy and preparation instructions. The following assessments will be obtained prior to starting terlipressin infusion in the morning:

- Estimated MAP (computed as 2/3[diastolic] + 1/3[systolic] taken from the earliest morning BP measurement repeated once 10 minutes apart in a sitting position - Refer to Section 15.
- Spot urinary creatinine and sodium
- Vitals, including MAP/BP and HR will be monitored for safety during the infusion of terlipressin and after each dose adjustments (See below) at 0.5, 1, 2, 3 and 6 hours.

A first dose escalation may occur no earlier than Day 3 (Bag #3) to 3 mg/day terlipressin; A dose escalation will occur unless the following is observed:

- Estimated MAP (computed as 2/3[diastolic] + 1/3[systolic] taken from the earliest morning BP measurement is increased compared from pre-dose escalation or pre-treatment values by more than 5 mmHg. Note: Estimated MAP will be obtained from the average of 2 BP measurements taken 10 minutes apart in a sitting position - Refer to Section 15.
- Based on investigator judgment a dose increase is not advisable (including for example: Treatment emergent elevation of systolic BP over 120 mmHg, clinically significant signs or symptoms of vasoconstriction, headache, and arrhythmia or other treatment emergent ECG anomalies, treatment emergent gastrointestinal symptom, treatment emergent hyponatremia / hypokalemia or fluid imbalance, other clinically significant AE deemed probably related to treatment by the investigator). The reason(s) for not escalating the infusion rate will be documented on the CRFs.

A second dose escalation may occur to 4 mg no earlier than Day 5 (Bag #5) if based on Investigator judgement a dose increase is advisable:

- Treatment has been well tolerated and no concerning treatment emergent anomalies have occurred, such as: Sustained of morning MAP by more than 5mmHg from pre-treatment values, treatment emergent elevation of systolic BP over 120 mmHg, clinically significant signs or symptoms of vasoconstriction, headache, and arrhythmia or other treatment emergent ECG anomalies, treatment emergent gastrointestinal symptom, treatment emergent hyponatremia / hypokalemia or fluid imbalance, signs of dehydration, signs of reduced renal function, and other clinically significant AE deemed probably related to treatment in the opinion of the investigator).
- There has been no sign of a pharmacodynamic response to terlipressin therapy, such as no clinically significant increased sodium excretion, no reduction in weight or abdomen circumference, volume of paracentesis or ascites accumulation appears unchanged from baseline.

Should the dose escalation to 4 mg be decided by the Investigator after discharge form the GCRC (Refer to Section 6.4.4) then the patient will be monitored in-house for safety for no less

than 6 hours. Vitals, including MAP/BP and HR will be monitored for safety after the dose adjustments to 4 mg/day at 0.5, 1, 2, 3 and 6 hours.

Bag for Terlipressin continuous infusion will be changed daily.

6.4.3. Day 1-7 in-house monitoring

The following assessments will be performed daily:

- Body weight, abdominal circumference, temperature - first thing in the morning prior to breakfast
- Vitals, including MAP/BP and HR (twice daily – the morning measurements [in duplicate] serves to adjust terlipressin dose)
- Electrocardiogram
- Collect blood sample for serum creatinine and BUN (except when a full metabolic panel is done – see below)
- Collect treatment emergent AEs and concomitant medications

The following assessment will be repeated on Day 3 and 5 (i.e. prior to Terlipressin dose adjustments):

- Collect blood for Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR)
- Spot urinary creatinine and sodium

6.4.4. Discharge from GCRC

The patient will be discharge from the unit on Day 7 (after Bag #7 has been started). However, when the 3 mg dose of terlipressin infusion has been maintained for no less than 24 hours the patient may at the Investigator discretion, be discharged home for the night and return for day monitoring at the GCRC in the morning.

The following assessments will be performed on Day 7:

- 24 hour urinary sodium excretion

- Electrocardiogram
- Collect blood samples for clinical laboratory assessments including: Hematology (CBC with differential), Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR).
- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein, and spot urinary creatinine/sodium.
- Collect treatment emergent AE and concomitant medications
- Evaluate AE of interest

6.4.5. Ambulatory Treatment and Monitoring of Patients – Day 8-28

Patient will continue treatment with terlipressin in the ambulatory setting. A licensed healthcare worker (RN, NP or MD) will make home visits twice a day for the first 7 days after discharge and once a day thereafter. The nurse will review with the patient his/her compliance with diet recommendation and salt restriction and spot evidences of possible intestinal bleeding (black stools).

The following assessments will be performed:

- Change terlipressin bag (morning visit only)
- Body weight, abdominal circumference, temperature (morning visit only)
- Monitor access line and infusion rate per instructions
- Vitals, including BP and HR
- Collect 24 hour urine for 24 hour urinary sodium excretion test prior to Visits at Day 14 and 28
- Monitor patient for emergent clinical complaints and emergent symptoms or AEs

An electrocardiogram will be performed at weekly interval (refer also to visits at Day 14 and 28 below).

Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR) will be performed at weekly interval (refer also to visits at Day 14 and 28 below).

6.4.6. Interim Visits at Day 14 and 28

The following assessments will be performed:

- Sign and symptoms directed physical exam, with vitals, body weight, temperature, abdominal circumference
- Electrocardiogram
- Collect blood samples for clinical laboratory assessments including: Hematology (CBC with differential), Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin, PT/INR), PRA and plasma aldosterone.
- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein, and spot urinary creatinine/sodium.
- 24 hour urinary sodium excretion
- Collect treatment emergent AEs and concomitant medications
- Evaluate AEs of interest
- Administer the Short Form 36 and CLDQ QOL (Day 28 Visit only)

The terlipressin infusion will be terminated at the Visit on Day 28 and the IV line removed.

6.4.7. Follow-up Period

Patients will continue monitoring for emergence of AEs for 28 days after discontinuation of Terlipressin infusion. Two post-treatment visits are scheduled, 14 and 28 days (±2 days) after therapy.

The following assessments will be performed at the visit on Day 14:

- Vitals, body weight, temperature, abdominal circumference
- Electrocardiogram

- Collect blood samples for metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium) and PRA and aldosterone.
- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein, and spot urinary creatinine/sodium.
- Collect treatment emergent AEs and concomitant medications
- Evaluate AEs of interest

The following assessments will be performed at the final follow-up visit on Day 28:

- Sign and symptoms directed physical exam, with vitals, body weight, temperature, abdominal circumference
- Electrocardiogram
- Collect blood samples for clinical laboratory assessments including: Hematology (CBC with differential), Metabolic Panel (creatinine, BUN, electrolytes [sodium, potassium, CO2, chloride], glucose, calcium), Liver function test (ALT/AST, alkaline phosphatase, total6.5 protein, albumin, total and direct bilirubin, PT/INR) and PRA and aldosterone.
- Collect urine sample for urinalysis including: pH, glucose, bilirubin/urobilirubin, RBC/blood, WBC, protein, and spot urinary creatinine/sodium.
- Collect treatment emergent AEs and concomitant medications
- Evaluate AEs of interest
- Administer the Short Form 36 and CLDQ QOL

6.5. RECOMMENDED CONCOMITANT MEDICATION

Diuretics will be maintained at the dose established in the pre-treatment period unless patient develops an adverse event, regardless of relationship attribution, that requires diuretic discontinuation (hypotension, acute kidney injury, GI hemorrhage). Patients will be maintained on the same dose of other medications (e.g. lactulose, rifaximin).

If patients are on ACE inhibitors or beta blockers they will be required to be on a stable dose for at least 2 months prior to enrollment and maintained on that dose for the trial duration.

Albumin is recommended for all subjects per current standard of care after paracentesis to prevent post-paracentesis circulatory dysfunction (PPCD) and as clinically indicated. It is recommended (if clinically appropriate) that the albumin dose administered to prevent PPCD (usually 6-8 g/L of ascites fluid removed) is kept constant for each LVP that might be required during the study period. Albumin use will be recorded in the CRF as a concomitant medication.

6.6. PROHIBITED CONCOMITANT MEDICATIONS

The following medications are prohibited or strongly discouraged during terlipressin infusion.

Prohibited Concomitant Medications
Midodrine and other vasopressive drugs including vasopressin, dopamine, dobutamine,norepinephrine
Octreotide
Prostaglandin analogs (e.g., misoprostol)
NSAIDs (e.g., ibuprofen, naproxen, diclofenac)

6.7. PHARMACOKINETICS OF TERLIPRESSIN

In order to characterize the pharmacokinetics and examine the influence of potential covariates, serial blood samples will be obtained from subjects. The pharmacokinetics of terlipressin and its metabolite, 8-lysine vasopressin, will be assessed following the start of the of the 2 mg/kg infusion on Day 1, at changes in infusion dose during the 7-day in-house stay, at interim visits while the infusion continues and prior to the discontinuation of the infusion due to an adverse effect. Blood samples will be collected in the arm contralateral to the terlipressin infusion, if possible, or below the terlipressin infusion site in the same arm.

- Prior to the start of the terlipressin infusion on Day 1 and 0.5, 0.75, 1, 1.5, 2, 3, 6, and 24h after the start of the continuous infusion.
- Prior to the change in infusion rate and 1, 2 and 8 hours after the dose change to 3 mg (if such dose change occurs).
- Prior to the change in infusion rate and 1, 2 and 4 hour after the dose change to 4 mg (if such dose change occurs)

- Morning on days 7, 14 and 28.
- Prior to discontinuation of terlipressin for an adverse effect

Samples on Day 1 up to 6 hr will be drawn through an intravenous catheter. The catheter will be kept patent between blood draws with normal saline until 6 hr. A waste sample, approximately 1-2 mL, will be collected through the catheter and discarded prior to each sample. The 24 hr, weekly and adverse effect prompted blood draws will be performed by needle stick. Refer to Section 14 for instruction for sample processing.

6.8. INFLUENCE OF PARACENTESIS ON TERLIPRESSIN PHARMACOKINETICS.

The influence of removal of ascitic fluid on the pharmacokinetics of terlipressin and its metabolite, 8-lysine vasopressin, will be assessed. If a LVP occurs during terlipressin infusion, then blood samples, 4-ml, will be collected in the arm contralateral to the terlipressin infusion (if possible or below the terlipressin infusion site in the same arm) immediately before the paracentesis, 30 minutes after completion of the paracentesis and if feasible 6 hours after completion of the paracentesis. The total volume of ascites fluid removed during paracentesis will be measured and a 10-ml ascites fluid aliquot saved for assay.

6.9. DIET

Patient will remain on their regular diet with usual sodium restriction during the duration of the study per AASLD Practice Guideline 2012. Patients will be requested to complete a daily-sodium tracker form during the duration of the trial (American Heart Association Sodium Tracker) to be reviewed at weekly interval by a nutritionist to monitor their adherence to their diet.

6.10. PATIENT EDUCATION

Patient consented into the trial will receive dieticians' guidance and information leaflets to assist in re-educating patient and relatives regarding salt restriction to a target of 90 mmol/day and helping the adoption of a no-added salt diet and avoidance of pre-prepared foodstuffs (for example, pies).

Patients will also be educated to spot any evidence of intestinal bleeding (blood in stool or black stool).

6.11. THERAPEUTIC PARACENTESIS DURING AND AFTER THE STUDY PERIOD

Paracentesis should be done as indicated by the patient condition when the following conditions are met:

- Presence of moderate to severe ascites upon medical exam with patient discomfort (shortness of breath or umbilical hernia or abdominal pain and/or distension, and/or limitation of activity) and patient's request for a repeat LVP
- Weight gain to 90-100% of LVP delta weight loss (reference weight taken prior to initial LVP). For example, if the patient loss is 10kg immediately after LVP the patient must regain 9-10 kg (90%) of 10kg at the time of repeat paracentesis
- Increase in abdominal girth to 90-100% of LVP delta abdominal girth (prior to initial LVP).

Reason for deviations from the standardized triggers detailed above will be noted in the CRF.

For each paracentesis performed in the course of the trial, the date and volume collected will be recorded. Ascites fluid will be inoculated into blood culture bottles at the bedside and examined by microscopy for a neutrophil count to rule out spontaneous bacterial peritonitis (SBP).

A 10mL sample will be collected, labelled (Patient Number, Date of collection) and stored frozen for later determination of albumin and terlipressin ascites concentration if such determination is warranted.

Adverse events following paracentesis including for example infection, post-paracentesis circulatory dysfunction (PPCD) and infection will be recorded, together with preventative measures such as administration of albumin.

6.12. DATA COLLECTION AFTER THE STUDY PERIOD

To better estimate the durability of the terlipressin effect and explore the possibility of a rebound effect, the date/volume of paracenteses occurring within 3 months after discharge from the trial will be collected from the patient file. The requirement for dialysis, hospitalization and death will also be recorded.

6.13. CRITERIA FOR WITHDRAWAL FROM STUDY

Subjects have the right to discontinue treatment and/or withdraw from the study at any time without prejudice. The investigator may discontinue any subject at any time for any reason.

If study treatment or protocol-specified assessments are discontinued, the reason will be recorded on the CRFs and the sponsor should be notified promptly.

Reasons for terminating participation in the clinical study may include the following:

- Patient did not require a LVP during the up to 28 day Pre-Treatment Period.
- Patient with evidence of SBP based on baseline ascites fluid examination.
- Any clinical adverse event (AE), laboratory abnormality, requirement for a concomitant medication, concurrent illness, or other medical condition or situation occurs such that, in the opinion of the Investigator, continued participation in the study would not be in the best interest of the subject.
- Serious protocol violation
- Patient is non-compliant or lost to follow-up
- Patient withdrawal of consent
- Patient dies

A patient may choose to discontinue participation in the study at any time. However, the Site Investigator or designee will encourage subjects who have initiated terlipressin treatment to continue with safety follow-up for 28 days after discontinuation of terlipressin infusion.

If a patient permanently discontinues terlipressin infusion for safety reason the patient should continue safety follow-up as detailed by the protocol for 28 days and the adverse event causing withdrawal should be followed until the event resolves or stabilizes.

6.14. PATIENT REPLACEMENT

If a treated patient withdraws for a reason unrelated to drug toxicity, or is determined to be ineligible before dosing (based upon the requirements of this protocol), that patient will be replaced by the next eligible patient.

Loss to follow-up should be prevented whenever possible and every attempt should be made to re-contact the patient.

Patients who permanently discontinue terlipressin infusion and who request to not continue safety monitoring per the study schedule should, whenever possible, complete an early study drug termination visit per protocol (Day 28 Visit).

6.15. DISCONTINUATION OF TREATMENT FOR ADVERSE EVENTS

Treatment with Terlipressin will be discontinued for the following adverse events.

Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified [a]			
CTCAE System/Organ/ Class (SOC)	ADVERSE EVENT	AGENT	ACTION
Gastro-Intestinal	Persistent diarrhea: Persistence of several (>5) loose, watery stools for more than 3 days after the start of the treatment associated with the need of rehydration therapy. Clinical evidence of intestinal ischemia with or without hematochezia or positive fecal occult blood test.	Terlipressin infusion	Discontinue terlipressin infusion, go to follow-up phase
Underlying disease related events	Treatment emergent spontaneous bacterial peritonitis (SBP) Gastrointestinal hemorrhage - variceal bleeding Treatment emergent moderate hepatic encephalopathy		
Cardiovascular	Arterial hypertension: Arterial blood pressure >150/90 at least in three measurements during treatment (at any time in patients randomized to continuous i.v. infusion of terlipressin, within 2 hours after the bolus in those who were randomized to i.v. boluses of terlipressin) Circulatory overload: Shortness of breath associated with one among the following: a) tachypnea with rales, b) dilatation of the jugular veins, c) a central venous pressure, when available, higher than 18 cm H2O, or d) radiological signs of pulmonary edema Peripheral ischemia: Onset of pain associated with one among the following: a) pallor, b) coldness or c) pulselessness Angina pectoris: A thoracic pain suggestive of ischemic origin (retrosternal or across the anterior chest sometimes radiating to the arms, neck, or shoulders) or any thoracic pain associated with the new onset ECG signs of myocardial ischemia Arrhythmia: Onset of one among the following: a) bradyarrhythmia (an heart rate that was less than 45 beats per minute), b) tachyarrhythmia (an heart rate higher than 120 beats per minute) or c) atrial fibrillation.Intestinal ischemia		

[a] http:/tep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified [a]			
CTCAE System/Organ/ Class (SOC)	ADVERSE EVENT	AGENT	ACTION
	Myocardial infarction Prolonged QTc: At least Grade 2 with QTc prolongation as 471–500 ms or >60 ms change from baseline		
Acute liver injury	Sustained (>1 week) doubling of AST or ALT levels to no less than 4xULN Or sign of increasing functional liver impairment with >2x baseline increase in total bilirubin or an ≥INR Or increased AST or ALT levels above 8xULN		
Renal and urinary disorders	Confirmed increase serum creatinine within 48 hr. consistent with acute kidney injury – such as > 50% baseline increase in serum creatinine. New onset hepatorenal syndrome (HRS) Type 1		
All other adverse events	≥Grade 3		

Patient who discontinue treatment will be monitored as outlined in Section 6.4.7 in the Follow-up Period.

6.16. AEs OF INTEREST

The emergence of the following AEs of interest will be specifically monitored on a regular basis during treatment:

Intestinal ischemia: Persistent moderate to severe abdominal pain with or without hematochezia or positive fecal occult blood test.

Arterial hypertension: Arterial blood pressure >140/90 at least in three measurements during terlipressin treatment.

Circulatory overload: Shortness of breath associated with one among the following: a) tachypnea with rales, b) dilatation of the jugular veins, c) a central venous pressure, when available, higher than 18 cm H2O, or d) radiological signs of pulmonary edema

Peripheral ischemia: Onset of pain in fingers or toes associated with one among the following: a) pallor, b) coldness or c) mottling.

Angina pectoris: A thoracic pain suggestive of ischemic origin (retrosternal or across the anterior chest sometimes radiating to the arms, neck, or shoulders) or any thoracic pain associated with the new onset ECG signs of myocardial ischemia.

Arrhythmia: Onset of one among the following: a) bradyarrhythmia (heart rate that was less than 45 beats per minute), b) tachyarrhythmia (heart rate higher than 120 beats per minute) or c) atrial fibrillation.

6.17. MANAGEMENT OF AEs AND TREATMENT DOSE MODIFICATION

Initial management of treatment emergent AEs occurring during the in-house phase of the treatment and that do not meet the threshold for discontinuation of terlipressin infusion as detailed above, may be managed by a temporary interruption of terlipressin infusion based on clinical judgement and at the discretion of the Investigator. After dose interruption, if the AE has abated by 48 hr., terlipressin may be restarted at the starting dose of 2 mg terlipressin, at the discretion of the Investigator. After restarting terlipressin, the dose may NOT be increased to the previously reached dose level.

There may be only one dose interruption for intercurrent AE. If the dose interruption is longer than 48 hr, then treatment will be discontinued and patient will start monitoring as outlined in the Follow-up Period in Section 6.4.7.

Terlipressin infusion will also be briefly interrupted during and following paracentesis if one is required (refer to Section 6.11).

Suspected acute liver injury (drug or ischemic induced) with elevated aminotransferases to more than 3xULN should be followed with a repeat liver panel testing within 48 hr (ALT. AST, alkaline phosphatase, total bilirubin). If the repeat testing shows AST or ALT >3xULN for subjects with normal baseline measures or 2-fold increases above baseline values for subjects with elevated values before drug exposure then close observation will be initiated including repeat liver enzyme testing every 3 days for no less than a week, ruling out viral hepatitis or ischemic or alcoholic liver injury and review history of concomitant drug use or other agents.

Rising blood pressure during terlipressin period in the hospitalization period should be monitored closely. If the SBP increases above 130 mmHg (or a SBP increase of > 20 mmHg if

pre-terlipressin baseline was elevated) the BP should be closely monitored with taking BP/HR at regular interval, no less than hourly, until stable.

Sign of dehydration or rapid increase in sodium excretion possibly related to terlipressin response should be monitored closely.

6.18. SAFETY MONITORING RULES

The rates of AE of interest, SAEs, therapy interruptions for safety, and grade 3 or 4 treatment-related clinically significant toxicities (NCI Common Terminology Criteria for Adverse Events version 3.0) will be monitored. The first two patients in the trial (sentinel patients with baseline SCr < 1.5 mg/dL) will be monitored for emergence of these AEs until completion of terlipressin infusion. These patients may be replaced if the study treatment is interrupted for reason other than safety. Once at least 2 patients have been observed for at least 4 weeks and safely complete terlipressin infusion, enrollment of subsequent patients may then resume at the discretion of the Principal Investigator.

During the course of the trial, observance of 1 or more unexpected, clinically-significant AE as described above that is possibly, probably, or definitely related to protocol therapy should prompt consideration to amend or terminate the protocol.

7. STATISTICS

7.1. JUSTIFICATION OF SAMPLE SIZE

This study is a proof-of-concept study and the study is not powered for inferential statistics. The sample size chosen of this study is based on observations feasibility, data from a recently published study (Gow, 2016) and historical first-in-man studies. However, the assumptions in this study include the following:

- Patients are expected to have ascites requiring large volume paracentesis. It is assumed that the ascites volume pre-treatment is at least 5 L per paracentesis per patient (average volume of ascites 7 L) as per Gow 2016 study).
- Patients are expected to require a minimum of 1.5 paracentesis procedures per month with an average of 2 per month.

- The pre-treatment period will include the pre-treatment LVP before the terlipressin infusion is initiated. This LVP will occur when the patient would have normally needed it.
- The total volume of ascites fluid removed during the pre-treatment period may be calculated as the product of the average ascites volume per paracentesis (7 L) and the average number of paracentesis procedures during the course of the pre-treatment period, including the pre-treatment LVP.

7.2. SAMPLE SIZE

This is a Phase I safety and feasibility study and no formal power analysis has been performed.

7.3. DESCRIPTION OF STATISTICAL METHODS

All patients who received terlipressin will be included in the analysis of safety and tolerability. All patients who have a result for the baseline and a follow-up will be included for the analysis of pharmacodynamics end-points.

7.3.1. Demographics, Baseline and Other Characteristics

Subject characteristics (demographic information and pre-treatment measurements) will be summarized. All medical history and physical examination findings will be coded as appropriate and listed by subject.

7.3.2. Safety and Tolerability

7.3.2.1. Adverse Event Analysis

All Investigator-reported verbatim terms for AEs will be coded using the most current version of MedDRA coding and will be presented by primary system organ class (SOC) and preferred term (PRT). An overall summary of AEs will be presented to include the overall incidence and frequency of the following events: AE, related AE, severe AE, SAE, AE leading to discontinuation of study medication, and AE leading to interruption of study medication.

7.3.2.2. Safety Laboratory

Adverse events will be coded for purposes of summary by body system and preferred term. The incidence of treatment emergent adverse events will be summarized and tabulated by severity

and attribution. Serious adverse events and those leading to study withdrawal will also be tabulated.

The results of laboratory tests results at each study visit will be listed by patient. Values outside the normal range will be identified, with special attention given to those values the PI or Medical Monitor flags as clinically significant. Shift table or scatter plots of numeric laboratory parameters will be provided, depicting changes on values, as appropriate. Normal ranges will be incorporated.

7.3.3. Pharmacokinetics

Pharmacokinetic analysis of terlipressin will be performed by fitting the plasma concentration-time data for terlipressin and lysine vasopressin from patients to the following models (Gibaldi, 1975 and Rowland, 1989):

$$C_{terlipressin} = \frac{k_0}{CL}(1 - e^{-K \times t})$$

$$\frac{dC_{lysine\ vasopressin}}{dt} = CL_f \times C_{terlipressin} - CL_{lysine\ vasopressin} \times C_{lysine\ vasopressin}$$

Where:
$C_{terlipressin}$ = plasma concentration of terlipressin
K_0 = terlipressin infusion rate
CL = terlipressin clearance
K = terlipressin terminal elimination rate constant
t = time
$C_{lysine\ vasopressin}$ = plasma concentration of lysine vasopressin
CL_f = formation rate CL of lysine vasopressin from terlipressin
$CL_{lysine\ vasopressin}$ = CL for lysine vasopressin

The pharmacokinetic analysis will involve individual fitting of the data from the subjects. Individual pharmacokinetic analysis will be performed using the nonlinear mixed effects software program, NONMEM (vs 7.3, ICON Development Solutions, Hanover, Maryland). Pharmacokinetic parameters obtained from the model fitting include CL, K, and CL_f. The model building and validation will follow previously described procedures (Fischer, 2014).

Secondary (derived) parameters include: volume of distribution of terlipressin (V), area under the terlipressin plasma concentration-time curve from 0 to 24 hours ($AUC_{Terlipressin\ 0\text{-}24}$), area under the lysine vasopressin plasma concentration-time curve from 0 to 24 hours ($AUC_{lysine\ vasopressin\ 0\text{-}24}$), and average steady-state plasma concentrations ($C_{ss\text{-}ave}$) for terlipressin and lysine vasopressin.

Pharmacokinetic parameters will be summarized using standard descriptive statistics, including mean, median, SD, min, max, and CV%. Qualitative comparison of parameters between subjects with and without renal impairment will be performed.

The individual PK analysis will be reported as a separate study report to be produced independently of the clinical study report.

7.3.4. Evaluation of Pharmacokinetic Design

Simulations were used to evaluate the ability of the study to provide precise estimates of the pharmacokinetic parameters. The simulations were performed in NONMEM (vs 7.3, ICON Development Solutions, Hanover, Maryland) utilizing the one-compartment model with first order elimination and zero order infusion shown in equation 1. Estimates of the pharmacokinetic parameters, inter-individual variability and residual variability were obtained from the literature (Forsling ML, Aziz LA, Miller M, Davis R, Donovan B. Conversion of triglycylvasopressin to lysine-vasopressin in man. J Endocr. 1980; 85:237-244; Nilsson G, Lindbom P, Ohlin M, Berling R, Vernersson E. Pharmacokinetics of terlipressin after single IV. doses to healthy volunteers. Drugs Exptl Clin Res. 1990; 16:307-314; Population pharmacokinetics report study OT-401). Fixed and random effect parameter values were 537 ml/min for CL, 0.011 min^{-1} for k, 0.168 for the inter-individual variance for CL, 0.267 for the inter-individual variance (IIV) for k and 0.123 for residual error. Simulations provided 300 replicates of the pharmacokinetic dataset (number of subjects and sampling times) specified in Section 6.7 Each simulated dataset was fit to the model.

The mean, median and 95% confidence intervals from the model fitting of the replicated simulated datasets are shown in the table below. The means and medians of the replications from the simulations closely agree with actual values, with relative differences of -2.8% and -4.8% for CL and 6.8% and 5.2 % for k. Similarly, the modest coefficients of variation and narrow 95% confidence show the precision of parameter estimates.

Pharmacokinetic Parameters from Simulations of Dataset				
Parameter	Mean	Median	95% Confidence Interval	Coefficient of Variation (%)
CL (ml/min)	522	511	367, 738	17.5
Interindividual Variance for CL	0.125	0.125	0.0149, 0.353	30.7
k (min^{-1})	0.012	0.012	0.00830, 0.0181	18.3
Interindividual Variance for k	0.189	0.182	0.034, 0.57	34.0

Our analysis supports the ability of the study design to provide accurate and precise estimates of the pharmacokinetic parameters.

7.3.5. Efficacy

This is an open label no-controlled trial and all patients will receive terlipressin infusion.

Efficacy parameters include:

- Total volume of ascites fluid removed by paracentesis, the volume of ascites fluid removed at each paracentesis, and the total number of paracentesis procedures during the course of the study
- Time to recurrence of ascites (defined as requirement of large volume paracentesis during the study period compared to pre-study period)
- Changes from baseline for:
  - Weight and abdominal girth
  - Mean arterial blood pressure
  - Serum creatinine level
  - Serum/urinary sodium levels and 24 hour sodium clearance
  - Computed GFR
  - MELD score

- PRA and aldosterone level and ratio
- PRO (patient reported outcome- quality of life)

Efficacy parameters including total volume of ascites fluid removed by paracentesis, the volume of ascites fluid removed at each paracentesis, the total number of paracentesis procedures, and the number/duration of hospital visits, during the course of the study will be analyzed by descriptive statistics including number of patients (N), mean, standard error (SE), median, minimum and maximum, and 95% confidence intervals (95%CI). Comparative statistics between pre-study period and study period will be conducted by student's t-test, wherein patients would serve as their own control. No sensitivity analysis is planned for this study.

8. SERIOUS ADVERSE EVENT REPORTING

8.1. DESCRIPTION OF ADVERSE EVENTS

The term "adverse event" could include any of the following events that develop or increase in severity during the course of the study:

1. Any signs or symptoms, regardless of severity, and whether or not ascribed to the test article;
2. Any clinically significant laboratory abnormality, and;
3. Any abnormality detected during physical examination.

The Clinical Investigator will follow all subjects withdrawn from the study due to any adverse event, until the outcome is determined and where appropriate, additional written reports will be provided.

Adverse events are graded using the CTCAE. If the CTCAE grading does not exist for an adverse event, the severity of mild (1), moderate (2), severe (3), life-threatening (4), and death related to an adverse event (5) will be used. Adverse event monitoring should be continued until adverse event resolution/stabilization (whichever is later).

Medical conditions/diseases present before consenting the patient are only considered adverse events if they worsen after receiving any study drug. All laboratory values are to be reviewed by the Investigator and abnormal values will be graded according to the CTCAE as well.

A laboratory abnormality is considered an adverse event if it results in

1. Discontinuation from study drug,
2. Necessitates therapeutic medical intervention,
3. If the Investigator assesses the abnormality as an adverse event, or
4. Any laboratory test that is clinically significant or meets the definition of an SAE

8.2. Relationship to Study Drug or Study Intervention

The relationship of each adverse event to study drug or study intervention will be defined as "not related" or "related". The Investigator is responsible for determining the relationship for an adverse event that occurs during the study.

Not related: The temporal relationship of the clinical event to study intervention **makes a causal relationship unlikely**, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

Related: The temporal relationship of the clinical event to study intervention **makes a causal relationship possible**, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

8.3. Description of and Procedural Requirements for Serious Adverse Events

A serious adverse event is defined in general as an untoward (unfavorable) adverse event which:

1. Is fatal or life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
2. Requires or prolongs hospitalization;
3. Is significantly or permanently disabling or incapacitating;
4. Constitutes a congenital anomaly or a birth defect; or
5. May jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above (Examples of such events include, but are

not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization).

Suspected transmission of an infectious agent (eg, any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.

Although pregnancy, overdose and cancer are not always serious by regulatory definition, these events must be handled as SAEs for data transmission purposes to BioVie Inc.

The following hospitalizations are not considered SAEs in BioVie Inc. clinical studies:

1. A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered "important medical event" or event life threatening)
2. Elective surgery, planned prior to signing consent
3. Admissions as per protocol for a planned medical/surgical procedure
4. Routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
5. Medical/surgical admission for purpose other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases
6. Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

8.4. Reporting Responsibility For Serious AE

The study site team will monitor for and track serious adverse events whether related or not to the study procedures/interventions and/or to participation in the study. Such events will be reported to the Clinical Investigator and reported to IRB per their requirements and pre-established written procedures, as required by 45 CFR 46

Any serious adverse event occurring in a subject after he/she has provided informed consent and HIPAA authorization, and while in the study must be reported.

Each serious adverse event must be reported by the Clinical Investigator to the BioVie Inc. Medical Monitor or designee, within 24 hours of learning of its occurrence, even if it is not felt to be related to study drug.

The report must include the adverse event term, subject identifier, attribution, description, concomitant medication used to treat the adverse event, and any other relevant information per BioVie Inc. SAE form. Follow-up information about a previously reported serious adverse event must also be reported to BioVie Inc. within 24 hours of receiving the information. BioVie Inc., or its designee, may contact the Investigator to obtain further information about a reported serious adverse event. If warranted, an Investigator Alert may be issued to inform all Investigators involved in any study with the same study drug that a serious adverse event has been reported.

8.5. REPORTING PROCEDURES

The Investigator must complete the Serious Adverse Event Report Form in English, assess the causal relationship to study procedure/intervention, and send the completed form to BioVie Inc. by Facsimile or Email, to BioVie Inc. Medical Monitor or its designee. The study monitor will review the Serious Adverse Event Report Form and the supporting source documents during monitoring visits. Follow-up information should be sent as well, within 24 hours of the time the information is known. Either a new Serious Adverse Event Report Form is faxed (indicating that the information is a follow-up), or the original form may be re-faxed (with the new information highlighted and a new date provided). The follow-up report should describe whether the serious adverse event has resolved or is continuing, if and how it was treated, and whether the subject continued or permanently discontinued study participation. The form(s) and FAX confirmation sheet(s) must be retained in the investigational site study file. The Investigator is responsible for informing the Institutional Review Board/Independent Ethics Committee (IRB/IEC) of the serious adverse event and providing them with all relevant initial and follow-up information about the event. BioVie Inc. medical monitor or designee will communicate serious adverse events to the study sites as required by regulatory authorities. SAEs must be recorded on the BioVie Inc. SAE Report Form; pregnancies on a BioVie Inc. Pregnancy Surveillance Form. These original BioVie Inc. Forms are to remain on site. SAEs, whether related or not related to

study intervention/procedures, and pregnancies must be reported to BioVie Inc. within 24 hours via a scanned and reported via electronic mail to:

SAE Email Address: yeramian@comcast.net

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BioVie Inc. using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

9. ETHICAL ASPECTS

9.1. ETHICS AND GOOD CLINICAL PRACTICE

This trial will be conducted in compliance with the appropriate protocol, ICH GCP guidelines, the principles of the Declaration of Helsinki, and all other applicable regulatory requirements. Participating sites must obtain written approval of the study protocol, consent form, other supporting documents, and any advertising for participant recruitment from their local institutional review board (IRB) in order to participate in the study. Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Ethics Review Committee (ERC) or IRB. Any amendments to the protocol or consent materials must be approved before they are implemented.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical license, debarment). All potential serious breaches must be reported to BioVie Inc. immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

The regulatory files at the site should contain all required regulatory documents, study-specific documents, and important communications. Regulatory files will be checked at each participating site for regulatory compliance prior to study initiation, throughout the study, as well as at the study closure.

9.2. CONFIDENTIALITY REGARDING STUDY SUBJECTS

Investigators must assure that the privacy of subjects, including their personal identity and all personal medical information, will be protected at all times, as required by law. Study sites may be required by their institutions to obtain authorization from participants for use of protected health information. Sites will be responsible for communicating with their IRBs or Privacy Boards and obtaining the appropriate approvals or waivers to be in regulatory compliance with HIPAA. In CRFs and other study documents submitted to BioVie Inc. or its designee, subjects will be identified by their initials, subject number, date of birth, and gender. Personal medical information may be reviewed and/or copied for research, quality assurance, and/or data analysis. This review may be conducted by the study monitor, properly authorized persons on behalf of BioVie Inc., an independent auditor, IRBs/IECs or regulatory authorities. Personal medical information will always be treated as confidential.

9.3. FINANCIAL DISCLOSURE

All investigators will comply with the requirements of 42 CFR Part 54, Subpart F to ensure that the design, conduct, and reporting of the research will not be biased by any conflicting financial interest. Everyone with decision-making responsibilities regarding the protocol will have an up-to date signed financial disclosure form on file with the sponsor.

9.4. SAFETY MONITORING COMMITTEE

An independent Safety Monitoring Committee (SMC) composed of two physicians, with expertise with hepatology, cardiology and/or intensive care will be assembled for the duration of the trial. The SMC receives the summary reports of the frequency of all clinical adverse events and safety laboratory tests for planned periodic meetings throughout the study (after each 2 patients complete treatment). Meetings will be held via teleconference. In addition, an SMC member may call ad hoc meetings.

Summaries of safety monitoring, adverse events and enrollment will be provided to the SMC prior to each meeting by the BioVie Medical Director. In addition, clinically significant events (including AEs of interest and interruptions of infusion for safety reason) and any severe (Grade 3 or above) or serious adverse events are considered events of interest and will be reported in real-time (within 1 business day of sponsor becoming informed) to the SMC. All adverse events and abnormal laboratory values results will be listed and will be completely identified (using MedDRA adverse reaction codes) by subject.

The SMC will review safety data throughout the trial. Recommendations for modification or termination of the trial based on safety data will be made by the SMC to the Protocol Steering Committee (PSC) that will include the Principal Investigator and the BioVie Medical Director and Clinical Manager. The PSC will be responsible for communication with the sites and IRBs.

9.5. INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE

Before implementing this study, the protocol, the proposed ICF, and other information provided to subjects must be reviewed by an IRB/IEC. A signed and dated statement that the protocol, and ICF, subject recruitment materials/process (eg, advertisements), and any other written information to be provided to subjects have been approved by the IRB/IEC must be given to BioVie Inc. before study initiation. The name and occupation of the chairperson and the members of the IRB/IEC (preferred) or the IRB's Health and Human Safety Assurance number must be supplied to BioVie Inc. or its designee. This committee, as required by local law or procedure, will approve any amendments to the protocol that need formal approval. The IRB/IEC will also be notified of all other administrative amendments (i.e., administrative changes). The investigator or sponsor should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling, information to be provided to subjects and any updates. The investigator or sponsor should provide the IRB/IEC with reports, updates and other information (eg, expedited safety reports, amendments and administrative letters) according to regulatory requirements or institution procedures.

Study sites will be responsible for maintaining signed consent forms as source documents for quality assurance review and regulatory compliance.

9.6. INFORMED CONSENT

Investigators must ensure that subjects, or, in those situations where consent cannot be given by subjects, their legally acceptable representatives, are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. The Investigator, or designee, will explain to each subject (or legally authorized representative) the nature of the research study, its purpose, the procedures involved, the expected duration of subject participation, alternative treatment, potential risks and benefits involved, and any discomfort that may occur during the subject's participation in the study. Each subject will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician. This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it and should be given a copy of the signed document. No subject can enter the study and no study-related procedures can be done before his/her informed consent has been obtained. The Investigator must submit the ICF with the protocol for IRB/IEC approval.

BioVie Inc. supplies a proposed ICF template that complies with regulatory requirements, includes all elements required by ICH, GCP and applicable regulatory requirements, and is considered appropriate for the study. The sample informed consent form will adhere to the ethical principles that have their origin in the Declaration of Helsinki. Any changes to the proposed ICF suggested by the Investigator must be agreed to by BioVie Inc. or its designee before submission to the IRB/IEC, and a copy of the approved version must be provided to the BioVie Inc. study monitor after IRB/IEC approval.

Investigators must:

1. Provide a copy of the consent form and written information about the study in the language in which the subject is most proficient prior to clinical study participation. The language must be non-technical and easily understood.
2. Allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study.

3. Obtain an informed consent signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion.
4. Obtain the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects, prior to the beginning of the study, and after any revisions are completed for new information.
5. If informed consent is initially given by a subject's legally acceptable representative or legal guardian, and the subject subsequently becomes capable of making and communicating their informed consent during the study, then consent must additionally be obtained from the subject.
6. Revise the informed consent whenever important new information becomes available that is relevant to the subject's consent. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative or legal guardian, of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

The consent form must also include a statement that BioVie Inc. and regulatory authorities have direct access to subject records. Subjects unable to give their written consent (eg, stroke patients, or subjects with severe dementia) may only be enrolled in the study with the consent of a legally acceptable representative. The subject must also be informed about the nature of the study to the extent compatible with the subjects' understanding, and should they become capable, personally sign and date the consent form as soon as possible. The explicit wish of a subject unable to give his or her written consent, who is capable of forming an opinion and assessing this information to refuse participation in, or to be withdrawn from, the clinical study at any time should be considered by the investigator. The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

10. ADMINISTRATIVE REQUIREMENTS

10.1. PROTOCOL AMENDMENTS

Any change or modification to this protocol requires a written protocol amendment that must be approved by BioVie Inc. medical monitor before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation, or the scientific quality of the study require additional approval by the IRB/IEC of all centers and, in some countries, by the regulatory authority. A copy of the written approval of the IRB/IEC must be given to the BioVie Inc. study monitor, or their designee. Examples of amendments requiring such approval are:

1. Increase in drug dosage or duration of exposure of subjects, or any significant increase in the number of subjects under study;
2. Significant change in the study design (e.g., addition or deletion of a control group);
3. Increase in the number of procedures to which subjects are exposed; or
4. Addition or deletion of a test procedure intended to improve safety monitoring.

These requirements for approval should in no way prevent any immediate action from being taken by the Investigator or by BioVie Inc. medical monitor in the interests of preserving the safety of all subjects included in the study. If an immediate change to the protocol is felt by the Investigator to be necessary and is implemented by him/her for safety reasons, BioVie Inc. medical monitor should be notified and the IRB/IEC for the center should be informed within 1 working day. Any significant deviation must be documented in the CRF.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion
- BioVie Inc. medical monitor
- Regulatory Authority(ies), if required by local regulations

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to BioVie Inc. If an amendment substantially alters the study design or increases the

potential risk to the subject: (1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

If the revision is an administrative letter, investigators must inform their IRB(s)/IEC(s).

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC approval; however, the IRB/IEC for each center must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB/IEC approval that can be treated as administrative amendments include, but are not limited to:

1. Changes in the staff used to monitor studies (e.g., BioVie Inc. staff versus a contract research organization); and
2. Minor changes (within regulatory guidelines) in the packaging or labeling of study drug.

10.2. MONITORING PROCEDURES

Before study initiation, at an initiation visit conducted on-site or by video conference or at an Investigator's meeting, a BioVie Inc. representative will review the protocol, CRFs, and other study documents with the Investigators and their staff. During the study, the BioVie Inc. study monitor, or designee, will visit the site regularly to check the completeness of subject records, accuracy of entries on the CRFs, adherence to the protocol and to GCP, progress of enrollment, and also to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The Investigator must give the study monitor access to relevant hospital or clinical records to confirm their consistency with the CRF entries. No information in these records about the identity of the subjects will leave the study center. BioVie Inc. monitoring standards require full verification for the presence of informed consent, HIPAA authorization, adherence to the inclusion/exclusion criteria, documentation of serious adverse events, and recording of efficacy and safety variables. Additional checks of the consistency of source data with the CRFs are

performed according to the study-specific monitoring plan. Representatives of BioVie Inc. must be allowed to visit all study site locations periodically to assess the data quality and study integrity. On site they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain acceptable.

In addition, the study may be evaluated by BioVie Inc. internal auditors and government inspectors who must be allowed access to CRFs, source documents, other study files, and study facilities. BioVie Inc. audit reports will be kept confidential. The investigator must notify BioVie Inc. promptly of any inspections scheduled by regulatory authorities, and promptly forward copies of inspection reports to BioVie Inc.

10.3. PROTOCOL DEVIATION AND VIOLATION

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP). The noncompliance may be either on the part of the subject, the Site Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All deviations from the protocol must be addressed in the subject's source documents. Protocol deviations must be sent to the local IRB per their guidelines and entered in the Protocol Deviations Log CRF.

10.4. INVESTIGATIONAL SITE TRAINING

BioVie Inc. may provide quality investigational staff training prior to study initiation. Training topics will include but are not limited to: GCP, AE reporting, study details and procedure, electronic CRFs, study documentation, informed consent, and enrollment of WOCBP as applicable.

10.5. RECORDING OF DATA AND RETENTION OF DOCUMENTS

All information required by the protocol should be provided; any omissions or corrections should be explained. All CRFs should be completed and available for collection within a timely manner, preferably no more than 10 days after the subject's visit (except for the last visit of the last subject, which should be completed in a timely manner, preferably within 5 working days),

so that the study monitor may check the entries for completeness, accuracy and legibility, ensure the CRF is signed by the Investigator and transmit the data to BioVie Inc. or its designee. All entries to the CRF must be made clearly in black ball-point pen to ensure the legibility of self-copying or photocopied pages. Corrections will be made by placing a single horizontal line through the incorrect entry, so that the original entry can still be seen, and placing the revised entry beside it. The revised entry must be initialed and dated by a member of the Investigator's research team authorized to make CRF entries. Correction fluid must not be used. If Electronic Data Capture (EDC) system is deployed, the CRF will be completed by the authorized study site personnel. Electronic queries will be used to communicate eligible discrepant data with the study sites.

The Investigator must maintain source documents for each subject in the study. All information on CRFs will be traceable to these source documents, which are generally maintained in the subject's file. The source documents will contain all demographic and medical information, including laboratory data, ECGs, etc., and also a copy of the signed informed consent/HIPAA authorization, which should indicate the study number and title of the study. Essential documents, as listed below, will be retained by the Investigator for the maximum period required to comply with national and international regulations, or institutional procedures, or for the period specified by the sponsor, whichever is longer. BioVie Inc. will notify the Investigator(s)/institution(s) when study-related records are no longer required to be retained. The Investigator agrees to adhere to the document retention procedures by signing the protocol. The investigator must contact BioVie Inc. prior to destroying any records associated with the study.

If the investigator withdraws from the study (eg, relocation, retirement), the records shall be transferred to a mutually agreed upon designee (eg, another investigator, IRB). Notice of such transfer will be given in writing to BioVie Inc. Essential documents include:

1. Signed protocol and all amendments;
2. IRB/IEC approvals for the study protocol and all amendments;
3. All source documents and laboratory records;

4. CRF copies;
5. Subjects' ICF/HIPAA authorization; and
6. Any other pertinent study documents.

10.6. CASE REPORT FORMS

An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated or entered as a control in the investigation. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

The investigator will maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on CRFs.

The completed CRF, including any paper SAE/pregnancy CRFs, must be promptly reviewed, signed, and dated by a qualified physician who is an investigator or sub-Investigator.

The investigator must retain a copy of the CRFs including records of the changes and corrections.

10.7. AUDITING PROCEDURES

In addition to the routine monitoring procedures, BioVie Inc. or its designees, may conduct audits of clinical research activities in accordance with internal SOPs to evaluate compliance with the principles of GCP. BioVie Inc., its designee, or a regulatory authority may wish to conduct an inspection (during the study or after its completion). If an inspection is requested by a regulatory authority, the Investigator will inform BioVie Inc. immediately that this request has been made.

10.8. DISCLOSURE AND CONFIDENTIALITY

By signing the protocol, the Investigator agrees to keep all information generated in connection with the study or provided by BioVie Inc. or its designee in strict confidence and to request

similar confidentiality from his/her staff and the IRB/IEC. Study documents provided by BioVie Inc. (protocols, Investigators' Brochures, CRFs, and other material) will be stored appropriately to ensure their confidentiality. Such confidential information may not be disclosed to others without direct written authorization from BioVie Inc., except to the extent necessary to obtain informed consent/HIPAA authorization from subjects who wish to participate in the study.

10.9. DISCONTINUATION OF STUDY

BioVie Inc. reserves the right to discontinue the study for any reason at any time.

This study may be also prematurely terminated if, in the opinion of the SMC or Principal Investigator, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to BioVie Inc.

Circumstances that may warrant termination include, include but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Enrollment is unsatisfactory.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Plans to modify, suspend or discontinue the development of the study drug.

If the study is prematurely terminated or suspended, the sponsor will promptly inform the Investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the Investigator/institution, as specified by the applicable regulatory requirement(s).

11. DATA MANAGEMENT

11.1. DATA COLLECTION

Investigators must enter the information required by the protocol onto the BioVie Inc. CRFs. BioVie Inc. study monitors or designees will review the CRFs for completeness and accuracy, and instruct site personnel to make any required corrections or additions. The CRFs will be forwarded to BioVie Inc., or its designee, with one copy retained at the study site. If Electronic

Data Capture (EDC) system is deployed, CRF will be completed by the authorized study site personnel. An electronic version of the final CRF book for each subject will be forwarded to the study sites for record keeping at the study site closure.

11.2. DATA CLARIFICATION

Data items from the CRFs may be entered into the study database using double data entry with verifications. Obvious CRF errors will be corrected by BioVie Inc. personnel, or its designee. Other errors, omissions, or requests for clarification will be queried; queries will be returned to the study site for resolution using a Data Clarification Form (DCF). The original completed and signed DCF will be returned to BioVie Inc. while a copy will be kept with the CRFs at the site.

12. REFERENCES

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13. APPENDIX 1: OVERDOSE INSTRUCTIONS

If overdose or serious adverse reaction related to overdose or terlipressin accumulation is suspected:

1. Admit patient to the hospital for observation
2. Assure IV access present with adequate hydration status
3. Monitor vital signs hourly or more frequently as clinically indicated
4. PI will manage patient, including administration of corticosteroid, anaphylaxis medications, and seizure medications as needed
5. Notify hospital of potential need for ICU admission.
6. Admit patient to hospital ICU if clinically indicated.

14. APPENDIX 2 - INSTRUCTIONS FOR THE COLLECTION, HANDLING AND SHIPPING OF SAMPLES

14.1. COLLECTION OF BLOOD SAMPLES

Sample Collection and Processing Procedures

Blood will be collected at the specified time points (refer to study protocol) using a 10 mL K2EDTA Vacutainer® blood collection tube. Collect samples within ± 2 minutes from the time stated on the protocol, or indicate reason for deviation from planned schedule. At successive time points, the same order of sampling shall be followed. Collect 4 mL of blood at the specified time. After drawing the blood, gently invert the tube approximately 8 times immediately after collection and immediately place in an ice bath for transport to a centrifuge.

14.2. PROCESSING AT SITE

Blood samples must be kept on ice and processed into plasma within 1 hour after collection. Record any draw deviations and reason(s) for deviation(s) on the Sample Processing Log if the clinical site has. Place the blood samples in a refrigerated centrifuge within 1 hour of collection and spin at high speed (approximately 2700 - 3000 RPM or approximately 1000 g) for approximately 10 minutes at 4˚C. As soon as the centrifuge stops, return the samples to an ice bath. Withdraw about 2.5 mL of plasma into a appropriately labeled 5-mL polypropylene cryovial as aliquot 1; transfer the rest of the plasma (transfer as much as possible) to a second appropriately labeled 5-mL polypropylene cryovial as aliquot 2. NOTE: Fill out sample label and affix on the tube before placing plasma into the tube. Affix a piece of transparent lab tape over the affixed label before transferring the plasma.

14.3. SHIPPING OF SAMPLES FOR CENTRAL ANALYSIS

All specimens will be labeled with Protocol Number, Subject number, and time points (as indicated in the study protocol), aliquot # and stored in the same location until shipped. The tubes are to be labeled with freezer-safe labels and/or marked by permanent marker. Sort the samples by subject and place them in a box or small zipper bag and place the samples in the freezer (-70ºC). Plasma samples should be immediately frozen in an upright position to keep the plasma in the bottom of the tube and record the time placed in the freezer in the sample accountability record.

Both aliquots from each set of specimens will be sent to Frontage Laboratories, Inc. for concentration measurement.

A copy of the Sample Processing Log should be included in the sample shipment. Specimens must be shipped in accordance with rules and laws governing the shipment of human diagnostic specimens. Shipments are to be sent only on Monday, Tuesday or Wednesday of any given week and must be shipped by overnight delivery. There should be adequate amount of dry ice included in the shipment to last for three days of shipping.

These samples will be shipped to:

Sample management
Frontage Laboratories, Inc.
700 Pennsylvania Drive
Exton, PA 19341 USA
Tel. 484-348-4799/484-348-4790
Fax. 610-232-0101
samplemanagement@frontagelab.com

Notify Frontage Laboratories at least 1 day prior to the arrival of the sample, providing shipping details and tracking numbers for the shipment. The site will e-mail Mira Hong (Executive Director, Project Management) at mhong@frontagelab.com and Joseph Falcone and Maulika Patel at samplemanagement@frontagelab.com. The e-mail must specify the study number, the number of pharmacokinetic samples, the time of shipment pick-up and include an electronic sample inventory.

Important Note: SHIPMENTS MUST BE SENT ON MONDAY – WEDNESDAY, AS THERE MAY NOT BE SOMEONE AVAILABLE TO RECEIVE SHIPMENTS ON THE WEEKEND.

Prior to shipment, prepare a sample shipment list (or Inventory list) containing the details of each sample / label identification included in the shipment. All of the sample details on this list must correspond with the details included on the individual sample labels, as each sample label will be checked against the list by Frontage sample coordination personnel. Any discrepancies

between information on the sample tubes and information on the e-rosters will be verified and noted in the e-rosters prior to shipping. All sample correspondence must contain the Study Number, Study Drug, and Site references (including emergency contact details and responsible shipment coordinator).

15. APPENDIX 3 – BLOOD PRESSURE MEASUREMENT

- Ensure the patient has been sitting for at least 10 minutes and is relaxed.
- Explain the procedure and obtain consent.
- Ask the patient to remove any tight clothing from around their arm.
- Ensure the arm is supported at the level of the heart - on a pillow, for example.
- Select an appropriately sized cuff: its bladder should encircle at least 80% of the arm but no more than 100%.
- Place the cuff snugly onto the patient's arm with the center of the bladder over the brachial artery. Most cuffs have a 'brachial artery indicator', an arrow that can be aligned with the artery.
- Ask the patient to refrain from talking or eating during the procedure as this can result in an inaccurate higher blood pressure measurement being recorded.
- Switch on the automated device and press start.
- Document the systolic and diastolic blood pressures on the patient's observation chart.
- Compute the MAP.
- Let the patient sit for 5 minutes.
- Proceed to determine the repeat MAP measure.
- Enter the average of the two MAPS in the CRF.

16. APPENDIX 4 – CHILD-PUGH SCORE

The score employs five clinical measures of liver disease. Each measure is scored 1-3, with 3 indicating most severe derangement.

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time, prolongation (s)	<4.0	4.0-6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Chronic liver disease is classified into Child-Pugh class A to C, employing the added score from above.

Points	Class
5-6	A
7-9	B
10-15	C

17. APPENDIX 5 – MELD SCORE

MELD(i) uses the patient's values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival. It is calculated according to the following formula:

$$\text{MELD(i)} = 3.78\times\ln[\text{serum bilirubin (mg/dL)}] + 11.2\times\ln[\text{INR}] + 9.57\times\ln[\text{serum creatinine (mg/dL)}] + 6.43$$

MELD scores are reported as whole numbers, so the result of the equation above is rounded.

UNOS has made the following modifications to the score:[5]

- If the patient has been dialyzed twice within the last 7 days, then the factor for serum creatinine used should be 4.0
- Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8 a value of 1.0 is used) to prevent the occurrence of scores below 0

In January 2016, OPTN Policy 9.1 (MELD Score) was updated to include serum sodium as a factor in the calculation of the MELD score.

$$\text{MELD Score (2016)} = \text{MELD(i)} + 1.32*(137-\text{Na}) - [0.033*\text{MELD(i)}*(137-\text{Na})]$$

Note: Sodium has a range of 125-137 mEq/L

18. APPENDIX 6 - FRACTIONAL SODIUM EXCRETION RATE

The **fractional excretion of sodium** (FE_{Na}) is the percentage of the sodium filtered by the kidney which is excreted in the urine.

$$FE_{Na} = 100 \times (Na_{urinary} \times Creatinine_{plasma}) / (Sodium_{plasma} \times Creatinine_{urinary})$$

19. APPENDIX 7 – HEPATIC ENCEPHALOPATHY SCORE

The severity of hepatic encephalopathy is graded with the West Haven Criteria; this is based on the level of impairment of autonomy, changes in consciousness, intellectual function, behavior, and the dependence on therapy.

- Grade 1 - Trivial lack of awareness; euphoria or anxiety; shortened attention span; impaired performance of addition or subtraction
- Grade 2 - Lethargy or apathy; minimal disorientation for time or place; subtle personality change; inappropriate behaviour
- Grade 3 - Somnolence to semistupor, but responsive to verbal stimuli; confusion; gross disorientation
- Grade 4 - Coma

20. APPENDIX 8 – COMPUTATION OF GLOMERULAR FILTRATION RATE

CKD-EPI Creatinine Equation (2009) [b]

Expressed as a single equation:

$$eGFR = 141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if Black]}$$

Abbreviations / Units

- eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2
- S_{Cr} (standardized serum creatinine) = mg/dL
- κ = 0.7 (females) or 0.9 (males)
- α = -0.329 (females) or -0.411 (males)
- min = indicates the minimum of S_{Cr}/κ or 1
- max = indicates the maximum of S_{Cr}/κ or 1
- age = years

[b] https://www.kidney.org/professionals/kdoqi/gfr_calculator

21. APPENDIX 9 – SHORT FORM 36 AND CLDQ

RAND HEALTH

RAND > RAND Health > Surveys > RAND Medical Outcomes Study > 36-Item Short Form Survey (SF-36) >

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
14. **Accomplished less** than you would like	○ 1	○ 2
15. Were limited in the **kind** of work or other activities	○ 1	○ 2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	○ 1	○ 2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
18. **Accomplished less** than you would like	○ 1	○ 2
19. Didn't do work or other activities as **carefully** as usual	○ 1	○ 2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- ○ 1 - Not at all
- ○ 2 - Slightly
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- 1 - None
- 2 - Very mild
- 3 - Mild
- 4 - Moderate
- 5 - Severe
- 6 - Very severe

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- 1 - Not at all
- 2 - A little bit
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
27. Did you have a lot of energy?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
28. Have you felt downhearted and blue?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
29. Did you feel worn out?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
30. Have you been a happy person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
31. Did you feel tired?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- ○ 1 - All of the time
- ○ 2 - Most of the time
- ○ 3 - Some of the time
- ○ 4 - A little of the time
- ○ 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	○ 1	○ 2	○ 3	○ 4	○ 5
34. I am as healthy as anybody I know	○ 1	○ 2	○ 3	○ 4	○ 5
35. I expect my health to get worse	○ 1	○ 2	○ 3	○ 4	○ 5
36. My health is excellent	○ 1	○ 2	○ 3	○ 4	○ 5

ABOUT

The RAND Corporation is a research organization that develops solutions to public policy challenges to help make communities throughout the world safer and more secure, healthier and more prosperous. RAND is nonprofit, nonpartisan, and committed to the public interest.

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THE CHRONIC LIVER DISEASE QUESTIONNAIRE (CLDQ)—QUALITY OF LIFE INDEX

FOR PATIENTS WITH CHRONIC LIVER DISEASE

TODAYS DATE _____ INITIALS_____ STUDY ID_____

This questionnaire is designed to find out how you have been feeling during the last two weeks. You will be asked about your symptoms related to your liver disease, how you have been affected in doing activities, and how your mood has been.

Please complete all of the questions and select only one response for each question.

1. How much of the time during the last two weeks have you been troubled by a feeling of abdominal bloating?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

2. How much of the time have you been tired or fatigued during the last two weeks?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

3. How much of the time during the last two weeks have you experienced bodily pain?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

4. How often during the last two weeks have you felt sleepy during the day?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

5. How much of the time during the last two weeks have you experienced abdominal pain?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

6. How much of the time during the last two weeks has shortness of breath been a problem for you in your daily activities?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

7. How much of the time during the last two weeks have you not been able to eat as much as you would like?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

8. How much of the time in the last two weeks have you been bothered by having decreased strength?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

9. How often during the last two weeks have you had trouble lifting or carrying heavy objects?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

10. How often during the last two weeks have you felt anxious?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

11. How often during the last two weeks have you felt a decreased level of energy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

12. How much of the time during the last two weeks have you felt unhappy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

13. How often during the last two weeks have you felt drowsy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

14. How much of the time during the last two weeks have you been bothered by a limitation of your diet?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

15. How often during the last two weeks have you been irritable?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

16. How much of the time during the last two weeks have you had difficulty sleeping at night?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

17. How much of the time during the last two weeks have you been troubled by a feeling of abdominal discomfort?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

18. How much of the time during the last two weeks have you been worried about the impact your liver disease has on your family?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

19. How much of the time during the last two weeks have you had mood swings?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

20. How much of the time during the last two weeks have you been unable to fall asleep at night?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

21. How often during the last two weeks have you had muscle cramps?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

22. How much of the time during the last two weeks have you been worried that your symptoms will develop into major problems?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

23. How much of the time during the last two weeks have you had a dry mouth?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

24. How much of the time during the last two weeks have you felt depressed?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

25. How much of the time during the last two weeks have you been worried about your condition getting worse?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

26. How much of the time during the last two weeks have you had problems concentrating?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

27. How much of the time have you been troubled by itching during the last two weeks?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

28. How much of the time during the last two weeks have you been worried about never feeling any better?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

29. How much of the time during the last two weeks have you been concerned about the availability of a liver if you need a liver transplant?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

Appendix 10 – Bonacini cirrhosis discriminant score

Score	Platelets (x 10^3 /µL)	ALT:AST ratio	INR
0	>340	>1.7	<1.1
1	280-340	1.2-1.7	1.1-1.4
2	220-279	0.6-1.19	>1.4
3	160-219	<0.6	
4	100-159		
5	40-99		
6	<40		

The modified Bonacini CDA has a range of possible values from 0 to 11; higher scores identify patients with higher likelihood of cirrhosis.