Protocol

A Monocentric, Open-Label, Proof of Concept Study to Evaluate the Safety and Efficacy of NTZ at 500mg Twice Daily on Collagen Turnover in Plasma in NASH Patients with Fibrosis Stage 2 or 3.

NCT Number: 03656068

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List of Abbreviations

ACR	Albumin to Creatinine Ratio
AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
AST	Aspartate aminotransferase
BMI	Body Mass Index
CDAA	Choline-deficient L-amino Acid Defined Diet
CFR	Code of Federal Regulations
CSR	Clinical Study Report
CPK	Creatine phosphokinase
CRF	Case Report/Record Form
CRN	Clinical Research Network
CTCAE	Common Terminology Criteria for Adverse Events
D_2O	Deuterated Water
DILI	Drug Induce Liver Injury
DSMB	Data Safety Monitoring Board
GGT	Gamma-glutamyltransferase
eCRF	Electronic case report form
eGFR	estimated Glomerular Filtration Rate
EC	Ethics Committee
ECG	Electrocardiogram
ET	Early Termination
EOS	End Of Study
EU	European Union
FDA	Food and Drug Administration
FSR	Fractional Synthesis Rate
GCP	Good Clinical Practice
Hb	Hemoglobin
HBV	Hepatitis B Virus
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDPE	High-density polyethylene
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HOMA	Homeostasis Model Assessment
HSC	Hepatic Stellate Cell
hs-CRP	High Sensitivity C-reactive protein
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational medical product

INR	International Normalized Ratio
IRB	Institutional Review Board
LDL	Low density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activity
MRE	Magnetic Resonance Elastography
MRI	Magnetic Resonance Imaging
NAFLD	Non Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non Alcoholic Steatohepatitis
NTZ	Nitazoxanide
PI	Principal Investigator
PK	Pharmacokinetic
PT/INR	Prothrombin Time/International Normalized Ratio
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TQT	Thorough-QT study

Study Synopsis

Protocol Number:	NTZ-218-1
Study Title:	A Monocentric, Open-Label, Proof of Concept Study to Evaluate the Safety and Efficacy of NTZ at 500mg Twice Daily on Collagen Turnover in Plasma in NASH Patients with Fibrosis Stage 2 or 3.
Phase:	Proof of Concept
Study Drug:	Nitazoxanide
Indication:	NASH induced fibrosis
Principal Investigator & Sponsor	[redacted] Pinnacle Clinical Research 5109 Medical Drive, Suite 200 San Antonio, TX 78229
Study Duration:	34 weeks
Objectives:	The primary objective of the study is: • To evaluate the safety and tolerability of NTZ 500mg bid after 24 weeks of treatment in patients with NASH-induced stage 2 or stage 3 fibrosis The secondary objectives of the study are: • To determine the effect of oral administration of NTZ 500mg bid on de novo collagen synthesis through Fractional Synthesis Rate (FSR) of circulating plasma proteins in patients with NASH-induced stage 2 or stage 3 fibrosis. • To assess changes in liver stiffness as measured by FibroScan® and by Magnetic Resonance Elastography (MRE). • To assess the change from baseline in non-invasive markers of fibrosis: ELF™ test score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin,, ,miRNA, ProC3, ProC6, FGF19, FGF21. • To assess changes in additional non-invasive tests: fibrosis scores: NAFLD fibrosis score, FIB-4
Study Design:	This is a Phase 2 proof of concept study to evaluate the safety and efficacy of open-label NTZ 500mg bid in a NASH population with NASH induced Stage 2 or 3 fibrosis. The study will include a screening visit within 6 weeks of randomization (Day 1) followed by a 24-week treatment period consisting of daily, BID, oral administration of NTZ and an end of treatment follow up period of 4 weeks. Patients will come to the research center and complete the following visits: • Screening Visit within 6 weeks of Randomization • Day -14 Deuterated Water Run-In ○ Day-14, Day-11, Day-7 and Day 1: sampling for labeling of Deuterated water during run-in, • Repeat baseline labs for ALT, AST, total bilirubin, ALP, CPK collected at least 4 weeks from initial screening.

	• Day 1 randomization: start of treatment
	• Every 4 weeks after randomization until 20 weeks post randomization
	• 22 weeks post randomization and Deuterated Water Administration Period
	  ○ 22 weeks plus 3 days
	  ○ sampling for labeling of Deuterated water on Day 155, Day 158, Day 162 and Day 169
	• 24 weeks post randomization: End of Treatment
	• 4 Week Post Treatment Follow Up

Number of Sites:	1 site
Number of Subjects:	20 evaluable subjects
Drug Dosage / Frequency:	500 mg NTZ BID
Route of Administration:	Oral Administration
Inclusion Criteria:	Patients must meet all of the following inclusion criteria to be eligible for enrollment in the trial: 1. Males or females aged from 18 to 75 years inclusive at the Screening Visit. 2. Must provide signed written informed consent and agree to comply with the study protocol. 3. Females participating in this study must be of non-childbearing potential or using highly efficient contraception for the full duration of the study. Childbearing potential refers to those female subjects who have not had a hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Female subjects are considered postmenopausal and not of child-bearing potential if they are >55 years of age, or >50 years of age with >12 months of amenorrhea or have serum follicle stimulating hormone (FSH) values >40 mIU/ml with >12 months of amenorrhea. Highly efficient contraception is defined as the use of one of the following methods of birth control in addition to a male partner using a condom (unless surgically sterile) from Screening to 90 days after the last dose of study medication: (1) hormone-containing contraceptive (2) intrauterine device (3) cervical cap or diaphragm with spermicidal agent 4. Histological confirmation of steatohepatitis on a diagnostic liver biopsy (biopsy obtained within 6 months prior to Screening or during the Screening Period) with at least 1 in each component of the NAS (steatosis scored 0-3, ballooning degeneration scored 0-2, and lobular inflammation scored 0-3). 5. Fibrosis stage of 2 or 3, according to the NASH CRN fibrosis staging system on a diagnostic liver biopsy (biopsy obtained within 6 months prior to Screening or during the Screening Period). 6. Two assessments of ALT, AST, Total bilirubin, ALP, CPK will be collected during screening at least 4 weeks apart. To be eligible the second value cannot be ≥2x the first value.
Exclusion Criteria:	Patients who meet any of the following criteria will be excluded from entering the study:

	1. History of efficient bariatric surgery within 5 years prior to Screening, or planned bariatric surgery in the course of the study. 2. Patients with HbA1c >10.0%. If abnormal at the first Screening Visit, the HbA1c measurement can be repeated. A repeated abnormal HbA1c (HbA1c >10.0%) leads to exclusion. 3. Patients with a history of clinically significant acute cardiac event, or cerebrovascular event within 6 months prior to Screening such as: stroke, transient ischemic attack, or coronary heart disease (angina pectoris, myocardial infarction, revascularization procedures). 4. Weight loss of more than 10% within 6 months prior to Randomization. 5. Patient with any history or presence of cirrhosis. 6. Current or recent history (within a <1 year prior to screening) of significant alcohol consumption. For men, significant consumption is typically defined as higher than 30 g pure alcohol per day. For women, it is typically defined as higher than 20 g pure alcohol per day. 7. Current or history of other substance abuse within 1 year prior to screening. 8. Pregnant or lactating females or females planning to become pregnant during the study period. 9. Other well documented causes of chronic liver disease according to standard diagnostic procedures including, but not restricted to: a. Positive hepatitis B surface antigen (HBsAg) b. Positive HCV RNA, (tested for in case of known cured HCV infection, or positive HCV Ab at Screening) c. Suspicion of drug-induced liver disease d. Alcoholic liver disease e. Autoimmune hepatitis f. Wilson's disease g. Primary biliary cirrhosis h. Primary sclerosing cholangitis i. Genetic homozygous hemochromatosis j. Known or suspected HCC k. History or planned liver transplant, or current MELD score >15. 10. Patients who cannot be contacted in case of emergency. 11. Known hypersensitivity to the investigational product or any of its formulation excipients. 12. Patients who are taking warfarin or other highly plasma protein-bound drugs with narrow therapeutic indices. 13. Patients who are currently participating in, plan to participate in, or have participated in an investigational drug trial or medical device trial containing active substance within 30 days or five half-lives, whichever is longer, prior to Screening. Patients who have screened for, and failed to qualify, for another clinical trial are eligible for screening for the NTZ-218-1, even if the Screening Visit for the NTZ-218-1 study is less than 30 days from the failed study screening visit, provided that the subject received no investigational product within 30 days of 5 half-lives of the investigational product prior to screening for NTZ-218-1.

	14. Evidence of any other unstable or, untreated clinically significant immunological, endocrine, hematological, gastrointestinal, neurological, neoplastic, or psychiatric disease. 15. Mental instability or incompetence, such that the validity of informed consent or ability to be compliant with the study is uncertain. 16. History of noncompliance with medical regimens, or patients who are considered to be unreliable 17. Positive anti-human immunodeficiency virus (HIV) antibody. 18. AST and/or ALT >5 x upper limit of normal (ULN). 19. Total bilirubin >1.3 mg/dL due to altered hepatic function. 20. Direct bilirubin > ULN Note: Gilbert Disease patients are allowed into the study. 21. INR >1.2 in the absence of anticoagulant therapy. 22. Platelet count <150,000/mm^3 in the context of portal hypertension. 23. Significant renal disease, including nephritic syndrome, chronic kidney disease (defined as patients with markers of kidney damage or eGFR of less than 60 ml/min/1.73 m2).
Evaluation Criteria	Primary Endpoint: To assess the safety and tolerability of NTZ after 24 weeks of treatment **Secondary Endpoints:** To assess the % change in FSR from baseline to end of treatment (24 weeks of treatment) To assess the change in liver stiffness by MRE from baseline to 12 weeks and 24 weeks of treatment To assess the change in liver stiffness by fibroscan from baseline to 24 weeks of treatment To assess the change from baseline to 12 weeks and 24 weeks of treatment of fibrosis serum biomarkers and scores.

TABLE 1: Schedule of events

	Screening period				**Treatment period**											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Window		*+3*	*+1*	*+1*	*+3*	*+3*	*+3*	*+3*	*+3*	*+3*	*+3*	*+1*	*+1*	*+1*	*+3*	
Week	*-6*	*-2*			*0*	*4*	*8*	*12*	*16*	*20*	*22*			*24*	*28*	
Informed Consent	X															
Demographics	X															
Medical History	X															
Inclusion Exclusion Criteria	X				X											
Height	X															
Weight, BMI	X				X			X						X	X	X
Physical Exam	X				X			X						X	X	X
12-Lead ECG	X													X		X
Vital Signs	X				X			X						X	X	X
Con Meds	X	X			X	X	X	X	X	X	X	X	X	X	X	X
AEs	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Liver Biopsy[1]	X															
MRE					X			X						X		X
Fibroscan					X									X		X
Dispense deuterated water		X									X					
FSR		X	X	X	X						X	X	X	X		
Study Drug Dispense					X	X	X	X	X	X						
Study Drug Compliance						X	X	X	X	X				X		X

[1] Required only if there is no history of a liver biopsy within the past 6 months.

TABLE 2: Biological assessments

	Screening period				**Treatment period**											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Week	*-6*	*-2*			*0*	*4*	*8*	*12*	*16*	*20*	*22*			*24*	*28*	
Labs - Haematology haemoglobin, haematocrit, RBC, WBC, differential count, platelet count, reticulocytes count, MCV	X				X	X	X	X	X	X				X	X	X
Coagulation (PT (INR))	X				X	X	X	X	X	X				X	X	X
Labs- Urinary Pregnancy tests[2]	X				X	X	X	X	X	X				X	X	X
Labs – Serology HIV, HBs and HCV serology	X															

[2] If Urine pregnancy test is positive, a serum β-hCG is required.

	Screening period				Treatment period											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Labs – Biochemistry HbA1c, fasting plasma glucose, insulin, HOMA-IR, fructosamine, alkaline phosphatase, ALT, AST, GGT, CPK, total and conjugated bilirubin, ferritin, creatinine, eGFR, uric acid, BUN, total cholesterol, HDL-C, LDL-C, triglycerides, albumin, lipase, amylase, electrolytes (sodium, potassium, chloride, calcium), MELD	X			X[3]	X	X	X	X	X	X				X	X	X
Inflammatory markers hsCRP, fibrinogen, haptoglobin, resistin, TNF-α, TGF-β, IL-6, PAI-1	X				X			X						X	X	X
Biomarkers of fibrosis ELF Score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin,, ,miRNA, ProC3, ProC6, FGF19, FGF21	X				X			X						X	X	X
NAFLD Fibrosis Score (NFS) & FIB-4					X			X						X		X

	Screening period				Treatment period											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Labs – Urinalysis dipstick Specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrite, leukocytes	X				X	X	X	X	X	X				X	X	X
Safety markers Serum Cystatin C, troponin T, NT proBNP Urinary albumin, urinary creatinine, urinary ACR	X				X	X	X	X	X	X				X	X	X
Labs -- FSR		X	X	X	X						X	X	X	X		
Sampling for additional parameters	X				X			X		X				X	X	X

[3] ALT, AST, Total bilirubin, Alkaline phosphatase and CPK must be retested at least 4 weeks after initial screening labs. . If these values at SV4 are ≥2x Screening values SV, then the patient cannot be randomized. A repeat test may be performed if a screening value is considered an error.

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1. Introduction

1.1. Background

Non-alcoholic fatty liver disease (NAFLD) has become one of the most prominent forms of chronic liver disease worldwide, reflecting the epidemic of global obesity. Those with the progressive variant of NAFLD, non-alcoholic steatohepatitis (NASH), are at significantly increased risk of multisystem morbidity and mortality. However, there are currently no approved pharmacologic therapies for NASH (Konerman et al, 2018). The World Health Organization estimates the prevalence of obesity has more than doubled since 1980 with >600 million people (13%) having a body mass index (BMI)) ≥30 (Konerman et al, 2018). In this setting, the global prevalence of diabetes among adults has also increased from 4.7% in 1980 to 8.5% as of 2014 (WHO Fact Sheet 312) . Prevalence rates of NAFLD among those with metabolic disease are notably higher. Approximately a third of patients with hypertension, half of patients with dyslipidaemia, up to two-thirds of patients with type II diabetes, and >90% of patients undergoing bariatric surgery had evidence of NAFLD (Leite et al, 2009; Fruci et al, 2013; Assy et al, 2000; Wu et al, 2016; Sasaki et al, 2014 Subichin et al; 2015 ; Donati et al ,2004).

The underlying pathophysiologic mechanisms that contribute to the development and progression of NAFLD and NASH are complex and include modulation of metabolic pathways, inflammatory cascades, and/or mechanisms impacting fibrosis. During disease progression fibrosis may worsen, while features of steatohepatitis resolve or ‘‘burnout”. As a result, NASH and fibrosis need to be evaluated independently to ensure a beneficial impact on one parameter does not simultaneously result in a negative impact on another endpoint of interest, particularly given that individual treatments tend to focus on one primary mechanism of action (i.e. metabolic or NASH disease modifier vs. antifibrotic) (Konerman et al, 2018).

Persistent and progressive fibrosis is a characteristic of many chronic liver diseases in which excessive accumulation of collagen-rich extracellular matrix is a key feature.

Following liver injury, quiescent Hepatic Stellate Cells (HSC) undergo a process of activation that is characterized by a differentiation into (α-SMA)-positive contractile myofibroblasts which secrete high amounts of collagen and play a central role in the fibrogenic process (Friedman et al, 2008, Carpino et al, 2005). Nitazoxanide may be a potent antifibrotic agent that can potentially be used for the treatment of different fibrotic pathologies, including liver fibrosis. (Belanger et al;2017)

1.2. General Information for Nitazoxanide

1.2.1. Nitazoxanide (NTZ)

Nitazoxanide (NTZ) is a broad-spectrum antiparasitic and broad-spectrum antiviral drug used for the treatment of various helminthic, protozoal, and viral infections. It was approved in the United States by the Food and Drug Administration (Alinia®, Romark Laboratories) as an Orphan Drug for treatment of diarrhea caused by *C. parvum* and *Giardia intestinalis* in adults and children at least 12 months of age [Rossignol, 2014]. The approved Alinia dose for adults is 500 mg twice daily (BID) for three days.

1.2.2. Clinical Safety & Toxicology

Nitazoxanide is rapidly metabolized in the liver to an active metabolite tizoxanide (TZ) in vivo. TZ undergoes conjugation to form tizoxanide glucuronide. Nitazoxanide is not detectable in the serum following oral administration. Peak concentrations in blood plasma of TZ and TZ glucuronide are seen between 1-4 hours. NTZ is excreted in both Urine (~33%) and feces (~67%).

The safety of ALINIA was evaluated in 2177 HIV-uninfected subjects 12 months of age and older who received ALINIA Tablets or ALINIA for Oral Suspension at the recommended dose for at least three days. In pooled controlled clinical trials involving 536 HIV-uninfected subjects treated with ALINIA Tablets or ALINIA for Oral Suspension, the most common adverse reactions were abdominal pain, headache, chromaturia and nausea (≥2%).[Alinia USPI]

Safety data were analyzed separately for 280 HIV-uninfected subjects ≥12 years of age receiving Alinia at the recommended dose for at least 3 days in 5 placebo-controlled clinical trials and for 256 HIV-uninfected subjects 1 through 11 years of age in 7 controlled clinical trials. There were no differences between the adverse reactions reported for Alinia-treated subjects based upon age [Alinia USPI].

Twenty-one clinical studies of NTZ were conducted in patients with chronic hepatitis C (14 studies: Rossignol et al, 2008; Rossignol et al, 2009; Bacon et al, 2010, Rossignol et al, 2010; Shiffman et al, 2010 ; Laufer et al, 2011; Kabil et al, 2011 ; Amorosa et al, 2013; Shebab et al, 2014; Macias et al, 2015; Kohla et al, 2016; Basu et al, 2013; NCT01185028; NCT01770483), hepatic encephalopathy in chronic liver failure (1 study: Basu et al, 2008), acquired immune deficiency syndrome (AIDS)-related cryptosporidiosis (3 studies: Rossignol et al, 1998; Rossignol et al, 2006; Zulu et al, 2005), or Helicobacter pylori (2 studies: Abd-Elsalam et al, 2016; Shehata et al., 2017), and Clostridium difficile infections (1 study: Musher et al, 2009) in the literature. The dose of NTZ administered in 19 studies was 500 mg BID; in one study 1000 mg NTZ BID was administered, whereas in one compassionate-use study the NTZ dose administered ranged from 500 to 1500 mg BID. Most studies administered NTZ treatment for 48 to 60 weeks, whereas the duration of treatment in the compassionate-use study ranged from 1 day to 4 years.

The studies identified from the literature review demonstrate the safety of NTZ in the treatment of chronic hepatitis C, hepatic encephalopathy in chronic liver failure, AIDS-related cryptosporidiosis, persistent diarrhea in patients with AIDS, and Helicobacter pylori and Clostridium difficile infections.

The adverse event (AE) profile of NTZ was predominantly GI in nature and included nausea, vomiting, diarrhea, abdominal pain, dyspepsia, constipation, flatulence, and dysphagia. These GI AEs were generally mild in intensity and transient. Other AEs related to NTZ treatment were fatigue, rash, headache, myalgia, and arthralgia. When

reported, no significant changes were observed in the electrocardiograms (ECGs), vital signs, and clinical laboratory parameters after administration with NTZ [Stockis et al, 2002a; Stockis et al, 2002b]. A formal International Council on Harmonization (ICH) E14 compliant thorough QT (TQT) study [Taubel et al, 2014] completed the cardiac safety evaluation and showed that neither a therapeutic single dose of 675 mg nor a 2700 mg (supra-therapeutic) dose of NTZ prolonged the QT interval in healthy male and female volunteers.

The safety profile of NTZ reported in all these studies therefore provides supporting evidence for the potential use of NTZ for the treatment of liver fibrosis in patients with NASH.

The following adverse reactions have been identified during post approval use of ALINIA as described in the Package Insert:

- Gastrointestinal disorders: diarrhea, gastroesophageal reflux disease
- Nervous System disorders: dizziness
- Respiratory, thoracic and mediastinal disorders: dyspnea
- Skin and subcutaneous tissue disorders: rash, urticaria

1.2.3. Non clinical pharmacology

Nonclinical pharmacology studies were conducted to determine whether NTZ and its main metabolites (TZ and TZ glucuronide [TZ glu]) exhibit anti-fibrotic properties *in vitro*, and *in vivo*.

NTZ and its main metabolite, TZ, significantly ($p<0.001$ from 0.3 µM) inhibited, in a dose-dependent manner, the expression of αSMA protein in TGF-β-induced human HSCs, but TZ glu had no anti-fibrotic properties. NTZ and TZ demonstrated good potency as the IC50 was in the micromolar range.

The CDAA diet-induced rodent model develops a fibrosing NASH within a relatively short period [Takeuchi-Yorimoto et al, 2013], which makes it a suitable model for studying the treatment of both NASH pathology and hepatic fibrosis.

In studies to determine the effects of NTZ on liver fibrosis in C57BL/6J mice induced by a CDAA diet supplemented with 1% cholesterol, microscopic examination revealed that NTZ at doses of 11.4 mg/kg/day and 33.9 mg/kg/day tended to reduce, in a dose-dependent manner, CDAA + 1% cholesterol diet-induced NASH. Although NTZ did not have a beneficial effect on hepatic steatosis and lobular inflammation grade, a tendency to reduce hepatocyte ballooning was observed, which resulted in a slight reduction in NASH prevalence.

At 12 weeks, NTZ significantly prevented, in a dose-dependent manner, hepatic collagen storage induced by the CDAA + 1% cholesterol diet. However, upon histological examination, this beneficial effect was not reflected by a dose-dependent improvement of the fibrosis grade. A significant decrease of the fibrosis area was observed with NTZ at doses of 11.4 mg/kg/day ($p<0.05$) and 78.1 mg/kg/day ($p<0.001$) and correlated with the significant decrease of the hepatic collagen content.

The CDAA + 1% cholesterol diet notably induces a significant augmentation in circulating markers of liver injury such as AST, ALT, and PIIINP, which is directly associated with fibrosis [Tanwar et al, 2013]. Moreover, the hepatic content is dramatically modified as illustrated by the significant increase of collagen. Mice treated with NTZ at 78.1 mg/kg/day exhibited lower plasma levels of ALT (-17%, $p>0.05$), AST (-24%, $p<0.05$), and PIIINP (-25%, $p<0.01$). These data suggest a potential preventive action of NTZ on liver damage induced by the CDAA + 1% cholesterol diet in mice.

The hepatic expression of fibrotic markers (collagens, MMP2), but also inflammatory markers (TNFα) were enhanced in the animals fed the CDAA + 1% cholesterol diet as compared with animals fed a CSAA control diet. Chronic administration of NTZ at 78.1 mg/kg/day to mice fed a CDAA + 1% cholesterol diet led to the inhibition of the

Col1α1 (-17%, $p>0.05$), Col1α2 (-25%, $p<0.05$), MMP2 (-32%, $p<0.05$), and TNFα (-28%, $p<0.01$) gene expression.

In the studies with CCl4-treated mice, NTZ significantly prevented hepatic fibrosis. This was shown at the dose of 95.3 mg/kg/day on the hepatic collagen content decrease in the first study [SSR_NTZ_PHA1_004], whereas no significant improvement was observed upon histological examination. Conversely, in the second study [SSR_NTZ_PHA1_005], no significant improvement of CCl4-induced hepatic collagen content was observed, whereas the histological evaluation of hepatic fibrosis revealed a significant effect of NTZ at 32 and 104 mg/kg/day.

There was no significant beneficial effect of NTZ treatment on plasma markers of hepatotoxicity in CCl4-treated mice.

These studies demonstrate the anti-fibrotic effect of NTZ, which suggests that NTZ may have potential as an anti-fibrotic drug. These studies also suggest that NTZ may show some beneficial therapeutic properties for NASH, such as a tendency to reduce hepatocyte ballooning.

1.3. Rationale for This Study

Based on the anti-fibrotic properties demonstrated in the animal models of fibrosis, this proof of concept clinical study aims at evaluating NTZ in patients with non-alcoholic steatohepatitis (NASH) and fibrosis stage 2 and 3.Although NTZ has been evaluated in liver disease populations (viral hepatitis C) up to 60 weeks, this is the first study evaluating NTZ treatment in a population with NASH induced stage 2 and 3 fibrosis. The aim of this study is to evaluate the safety and tolerability of NTZ 500 mg BID after 24 weeks of treatment in this population. This proof of concept study will also evaluate the anti-fibrotic effect of NTZ as a secondary objective.

The methods of evaluation of fibrosis will include an innovative method of metabolic labeling. This approach is based on the concept that liver status can be determined by measuring the ratio of newly synthesized/pre-existing proteins. The turn-over rate of newly synthesized collagen and proteins represents the hepatic fibrogenic disease activity. Patients will be given "heavy water" to drink. Heavy water contains D_20, deuterium being a stable isotope of hydrogen. Mass spectrometry is used to identify individual proteins and to quantify the ratio of labeled protein to total protein. The results are expressed as fractional synthesis rate of these proteins (FSR). This method has been previously published (Decaris et al, 2017).

Other non-invasive methods will be used to evaluate the liver stiffness changes after NTZ treatment: Magnetic Resonance Elastography (MRE) and FibroScan®.

2. Objectives

2.1. Primary Objective

The primary objective of the study is:

- To evaluate the safety and tolerability of NTZ 500mg bid after 24 weeks of treatment in patients with NASH induced Stage 2 or Stage 3 fibrosis.

2.2. Secondary Objectives:

The secondary objectives of the study are:

- To determine the effect of daily oral administration of NTZ 500mg bid on de novo collagen synthesis through Fractional Synthesis Rate (FSR) of circulating plasma proteins in patients with NASH-induced Stage 2 or Stage 3 fibrosis.
- To evaluate changes in liver stiffness as measured by FibroScan® and by Magnetic Resonance Elastography (MRE).
- To assess the change from baseline in non-invasive markers of fibrosis: ELF™ test score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin,, ,miRNA, ProC3, ProC6, FGF19, FGF21.
- To assess changes in additional non-invasive tests: fibrosis scores: NAFLD fibrosis score, FIB-4

3. Study Design

3.1. Study Design

This is a phase 2 proof of concept study to evaluate the efficacy and safety of open-label NTZ 500mg bid in a NASH population with NASH induced Stage 2 or 3 fibrosis.

The study will include a screening visit within 6 weeks of randomization (Day 1) followed by a 24-week treatment period consisting of daily, BID, oral administration of NTZ and an end of treatment follow up period of 4 weeks.

Patients will come to the research center and complete the following visits:

- Screening Visit within 6 weeks of Randomization
- Day -14 Deuterated Water Run-In
    - Day-14, Day-11, Day-7 and Day 1: sampling for labelling of Deuterated water during run-in
- Repeat baseline labs for ALT, AST, total bilirubin, ALP, CPK collected at least 4 weeks from initial screening.
- Day 1 Randomization: start of treatment
- Every 4 weeks after randomization until 20 weeks post randomization

- 22 weeks post randomization and Deuterated Water Administration Period:
  - 22 weeks plus 3 days
  - sampling for labelling of Deuterated water on Day 155, 158, 162, and Day 169/24 weeks post randomization: End of Treatment
- 4 Week Post Treatment Follow Up

3.2. Treatment Plan and Regimen

NTZ will be administered in an open label fashion to participating subjects as 500mg bid, for 24 weeks.

4. Subject Population

4.1. Number of Subjects & Subject Selection

Twenty male and female subjects with histologically confirmed NASH and fibrosis stage 2 or 3 will be enrolled in the study at a single site in the United States.

4.2. Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment in the trial:

1. Males or females aged from 18 to 75 years inclusive the Screening Visit.
2. Must provide signed written informed consent and agree to comply with the study protocol.
3. Females participating in this study must be of non-childbearing potential or using highly efficient contraception for the full duration of the study. Childbearing potential refers to those female subjects who have not had a hysterectomy, bilateral oophorectomy, or medically-documented ovarian failure. Female subjects are considered postmenopausal and not of child-bearing potential if they are >55 years of age, or >50 years of age with >12 months of amenorrhea, or have serum follicle stimulating hormone (FSH) values >40 mIU/ml with >12 months of amenorrhea. Highly efficient contraception is defined as the use of one of the

following methods of birth control in addition to a male partner using a condom (unless surgically sterile) from Screening to 90 days after the last dose of study medication:

- hormone-containing contraceptive
- intrauterine device
- cervical cap or diaphragm with spermicidal agent

4. Histological confirmation of steatohepatitis on a diagnostic liver biopsy (biopsy obtained within 6 months prior to Screening or during the Screening Period) with at least 1 in each component of the NAS (steatosis scored 0-3, ballooning degeneration scored 0-2, and lobular inflammation scored 0-3).
5. Fibrosis stage of 2 or 3, according to the NASH CRN fibrosis staging system on a diagnostic liver biopsy (biopsy obtained within 6 months prior to Screening or during the Screening Period).
6. Two assessments of ALT, AST, Total bilirubin, ALP, CPK will be collected during screening at least 4 weeks apart. To be eligible the second value cannot be ≥2x the first value.

4.3. Exclusion Criteria

Patients who meet any of the following criteria will be excluded from entering the study:

1. History of efficient bariatric surgery within 5 years prior to Screening, or planned bariatric surgery in the course of the study.
2. Patients with HbA1c >10.0%. If abnormal at the first Screening Visit, the HbA1c measurement can be repeated. A repeated abnormal HbA1c (HbA1c >10.0%) leads to exclusion.
3. Patients with a history of clinically significant acute cardiac event, or cerebrovascular event, within 6 months prior to Screening such as: stroke, transient ischemic attack, or coronary heart disease (angina pectoris, myocardial infarction, revascularization procedures).
4. Weight loss of more than 10% within 6 months prior to Randomization.
5. Patient with any history or presence of cirrhosis.

6. Current or recent history (within a year of screening) of significant alcohol consumption. For men, significant consumption is typically defined as higher than 30 g pure alcohol per day. For women, it is typically defined as higher than 20 g pure alcohol per day.
7. Current or history of other substance abuse within 1 year prior to screening.
8. Pregnant or lactating females or females planning to become pregnant during the study period.
9. Other well documented causes of chronic liver disease according to standard diagnostic procedures including, but not restricted to:
    a) Positive hepatitis B surface antigen (HBsAg)
    b) Positive HCV RNA, (tested for in case of known cured HCV infection, or positive HCV Ab at Screening)
    c) Suspicion of drug-induced liver disease
    d) Alcoholic liver disease
    e) Autoimmune hepatitis
    f) Wilson's disease
    g) Primary biliary cirrhosis
    h) Primary sclerosing cholangitis
    i) Genetic homozygous hemochromatosis
    j) Known or suspected HCC
    k) History or planned liver transplant, or current MELD score >15.
10. Patients who cannot be contacted in case of emergency.
11. Known hypersensitivity to the investigation product or any of its formulation excipients.
12. Patients who are taking warfarin or other highly plasma protein-bound drugs with narrow therapeutic indices.
13. Patients who are currently participating in, plan to participate in, or have participated in an investigational drug trial or medical device trial containing active substance within 30 days or five half-lives, whichever is longer, prior to

Screening. Patients who have screened for, and failed to qualify, for another clinical trial are eligible for screening for the NTZ-218-1, even if the Screening Visit for the NTZ-218-1 study is less than 30 days from the failed study screening visit, provided that the subject received no investigational product within 30 days of 5 half-lives of the investigational product prior to screening for NTZ-218-1.

14. Evidence of any other unstable or, untreated clinically significant immunological, endocrine, hematological, gastrointestinal, neurological, neoplastic, or psychiatric disease.
15. Mental instability or incompetence, such that the validity of informed consent or ability to be compliant with the study is uncertain.
16. History of noncompliance with medical regimens, or patients who are considered to be unreliable.
17. Positive anti-human immunodeficiency virus (HIV) antibody.
18. AST and/or ALT >5 x upper limit of normal (ULN).
19. Total bilirubin >1.3 mg/dL due to altered hepatic function.
20. Direct bilirubin > ULN

Note: Gilbert Disease patients are allowed into the study.

21. INR >1.2 in the absence of anticoagulant therapy.
22. Platelet count <150,000/mm3 in the context of portal hypertension.
23. Significant renal disease, including nephritic syndrome, chronic kidney disease (defined as patients with markers of kidney damage or eGFR of less than 60 ml/min/1.73 m2).

5. Investigational Medicinal Products

Patients will receive 500 mg NTZ (ALINIA®) twice daily for 24 weeks.

5.1. Description

NTZ will be supplied as 500 mg of ALINIA® (nitazoxanide) tablets, for oral use. The tables are round, yellow, film-coated and debossed with ALINIA on one side and 500 on the other side. Each tablet contains 500 mg of nitazoxanide.

For additional information see the Package Insert for ALINIA®.

5.2. Packaging

ALINIA® tablets are packaged in HDPE bottles of 12 tablets. Period boxes will be delivered every 4 weeks, each box containing 6 bottles.

5.3. Labeling

All labels for study drugs meet all applicable requirements of the US Food and Drug Administration (FDA) and the EU annex 13 of Good Manufacturing Practices: Manufacture of Investigational Medicinal Products (February 2010) and /or other local regulations, as applicable.

Distribution of study drug will be performed according to the Good Distribution Practices.

Product boxes for each period will be labeled with the protocol number, Sponsor's name and address, description of contents, storage conditions, expiry date, dosage instructions, and any other applicable items required by national and regional guidelines/regulations. The label will contain the statements "Caution – new drug, limited by Federal Law to investigational use" or other similar/appropriate statements as well as the following instructions "Please return empty packaging and unused products to your doctor at your next visit."

5.4. Storage and Handling

The tablets are to be stored at 20-25^{o}C (68-77^{o}F). Storage conditions are specified on the label.

5.5. Dosage and Administration

Patients will be instructed to take one tablet of 500mg NTZ mg orally, twice a day with water during a meal, approximately 12 hours apart.

The study staff will administer the first dose of the medication at the research center. Patients will self-administer the evening dose on Day 1 and twice a day thereafter, when not attending

a visit at the research center. On scheduled follow up visit days while the patient is being treated with study drug, the patient will be directed to bring back all used and unused study medication containers. Compliance will be checked by the Investigator or designee during those visits and registered in the electronic Case Report Form (eCRF).

If treatment is interrupted, whatever the cause, duration and reason of the interruption will be documented.

5.6. Dose Adjustment

Enrolled subjects who experience treatment emergent adverse events may have their dose adjusted, at the discretion of the Principal Investigator, in accordance with section 6.6.3.

5.7. Prior and Concomitant Medications

In a general manner, patients should be discouraged from starting any new medication without consulting the Investigator unless the new medication is required for emergency purpose. In the same way, any qualitative or quantitative change in concomitant therapy should be avoided, when possible. In the event that it becomes necessary during the study, this should be recorded by the Investigator or designee in the eCRF (including concomitant medications taken within 6 months prior to Screening). This includes drugs used on a chronic as well as on an “as needed” basis.

5.7.1. Non-permitted medication

The following medications are not allowed within the timeframe given:

- Warfarin or other highly plasma protein-bound drugs with narrow therapeutic indices are not permitted from Day 1 until end of treatment

5.7.2. Permitted Medications

Any medications other than those listed above are permitted. However, the dosage of a current medication for a chronic disease should remain unchanged as far as possible in order to reduce the risk of unknown drug-drug interactions.

In the event that additional concomitant therapy becomes necessary during the study, this will be recorded by the Investigator in the eCRF. This includes drugs used on a chronic as well as on

an “as-needed” basis. Patients should be discouraged from starting any new medication without consulting the Investigator unless the new medication is required for emergency purpose.

5.8. Study Drug Accountability

The Investigator will acknowledge receipt for each study treatment on the day of receipt. A drug accountability record will be maintained by the person responsible for dispensing the trial medication to the patient.

All partially used or unused treatments will be inventoried during and at the conclusion of the study.

The Drug Distribution Center will organize the retrieval of all treatments (used or unused) and will proceed to their destruction only after written authorization and review of accountability and distribution records.

6. Study Procedures

The procedures performed at each visit are summarized in the Schedule of Events (Table 1).

A patient may be seen at any time for reasons of safety.

During each visit, safety evaluations will be done, and the patient will be queried in the form of an open question regarding new or continuing events.

6.1. Duration of Study Participation

The estimated duration of the study will be approximately 34 weeks inclusive of the screening period.

6.2. Screening Assessment

Screening Visit

The following screening procedures will be performed for all potential patients at the Screening Visit and prior to Day 1:

- Informed consent witnessed by the Investigator or designated person.

- Medical history & demographics.
- Assessment of inclusion/exclusion criteria
- Complete physical examination.
- Record vital signs
- Record height, weight, BMI
- Check concomitant/prior medication (within 6 months prior to Screening)
- 12-lead ECG
- Liver biopsy. A historical liver biopsy with confirmed NASH and fibrosis is acceptable if performed within 6 months prior to the Screening Visit.
- Adverse Events assessment
- Blood samples will be collected for
    - Biochemistry
    - Hematology
    - Serology (HIV, HBs, HCV). In the event of a positive HCV Ab, HCV RNA can be tested for confirmation. In case of known cured HCV infection, HCV RNA can be tested directly at Screening.
- Repeat baseline labs for ALT, AST, total bilirubin, ALP, CPK collected at least 4 weeks from initial screening.
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).

If needed, a retesting of any laboratory results may be performed during the screening window to determine the eligibility for the study.

6.3. Day -14 Deuterated Water Run In

Potential Subjects who complete all of the Screening Visit assessments and who continue to be eligible for the study, will return to the research center to complete the Deuterated Water Run In. The visit is to be done on Day -14 $\pm$ 3 days, provided that the subject completes 7 consecutive days of deuterated water administration. The following procedures will be performed at the Day -14 Visit:

- Dispense deuterated water (D_2O). Instruct patient to drink 50ml of deuterated water 3 times per day starting on Day-14 for 7 consecutive days (until day-8).
- Blood sample for baseline labeling by D_2O (kinetic biomarkers) (patients should be instructed to fast the evening prior to the visit to ensure a 10-hour fast)
- Adverse Events assessment
- Record concomitant medication taken

Patients will return at Day-11, Day-7 (± 1 day) and Day 1 for blood sampling (labeled D_2O)

6.4. Treatment Period Assessments

6.4.1. Day 1 Visit

The following procedures will be performed at the Day 1 Visit prior to dose administration:

- Review inclusion/exclusion criteria
- Complete physical examination
- Weight, BMI
- Record vital signs
- Record concomitant medication taken
- Adverse Events assessment
- MRE (may be done within ± 5 days of the visit)
- Fibroscan
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).
- Dispense the study drug as directed
- Review study drug administration instructions with subject
- Administer 500 mg of NTZ with water & food

6.4.2. Week 4 and Week 8 Visit ± 3 days

The following procedures will be performed at these Visits:

- Record concomitant medication taken
- Adverse Events assessment
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).
- Dispense the study drug as directed
- Review study drug compliance and drug administration instructions with subject
- Reconcile study drug administration using pill counts
- Administer 500 mg of NTZ with water & food

6.4.3. Week 12 Visit ± 3 days

The following procedures will be performed at the Visit:

- Complete physical examination
- Weight, BMI
- Record vital signs
- Record concomitant medication taken
- Adverse Events assessment
- MRE (may be done within ± 5 days of the visit)
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).
- Dispense the study drug as directed
- Review study drug compliance and drug administration instructions with subject
- Reconcile study drug administration using pill counts
- Administer 500 mg of NTZ with water and food

6.4.4. Week 16 and Week 20 visits ±3 days

The following procedures will be performed at these Visits:

- Record concomitant medication taken
- Adverse Events assessment
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).
- Dispense the study drug as directed
- Review study drug compliance and drug administration instructions with subject
- Reconcile study drug administration using pill counts
- Administer 500 mg of NTZ with water & food

6.4.5. Week 22 Visit ± 3 days (Day 155)

The following procedures will be performed at the Visit:

- Record concomitant medication taken
- Adverse Events assessment
- Blood samples will be collected for D2O labeling
- Urinary pregnancy test (for women of childbearing potential only [WOCBP])
    - Dispense Deuterated water. Instruct patient to drink 50ml of deuterated water 3 times per day starting on Day 155 for 7 consecutive days (until day 162).
    - Blood sample for kinetic biomarkers analysis (patients should be instructed to fast the evening prior to the visit to ensure a 10-hour fast)

Patients will return for blood sampling for kinetic biomarker analysis at 22weeks/± 3 days), Day 158, Day 162 (± 1 day), and at day 169 (Week 24).

6.4.6. Week 24 Visit ±1

The following procedures will be performed at the visit:

- Complete physical examination
- 12-lead ECG
- Record vital signs
- Record weight, BMI

- Record concomitant medication taken
- MRE (may be done within ± 5 days of the visit)
- Fibroscan
- Adverse Events assessment
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).
- Review study drug compliance and drug administration instructions with subject
- Reconcile study drug administration using pill counts

6.5. Follow Up Period

6.5.1. 4-Week Post Treatment Follow up (EOS – End of study visit) ±3 days

The following procedures will be performed at the visit:

- Complete physical examination
- Weight, BMI
- Record vital signs
- Record concomitant medication taken
- Adverse Events assessment
- Blood samples will be collected Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP]).

6.5.2. Early Termination (ET)

Patients who have discontinued from the study after the first dose of study medication and for any reason will have the following procedures completed:

- Complete physical examination
- Record vital signs
- Weight, BMI
- ECG
- Record concomitant medication taken
- MRE (may be done within ± 5 days of the visit)

- Fibroscan
- Adverse Events assessment
- Blood samples will be collected
- Urine samples will be collected for Urinalysis
- Urinary pregnancy test (for women of childbearing potential only [WOCBP])
- Review study drug compliance with subject
- Reconcile study drug administration using pill counts

6.5.3. Unscheduled Visits

An unscheduled visit is defined as any visit to the study unit outside of the protocol-evaluation time points where the patient is seen by study unit personnel, e.g., when follow-up assessments are required for safety reasons or when repeat measurements are required out of the screening period (either to confirm a measurement or in case of errors, measuring device failure, etc.).

Unscheduled visits will be needed for patients who may require further follow-up due to safety.

6.6. Assessment for Premature Discontinuation from Study

Study medication may be discontinued in the following instances:

- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree. Following resolution of intercurrent illness, the subject may resume study dosing at the discretion of the investigator.
- Subject noncompliance
- Investigator discretion
- Study medication must be discontinued in the following instances:
    - Unacceptable toxicity, or toxicity that, in the judgment of the investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
    - Subject request to discontinue for any reason

- Pregnancy
- Discontinuation of the study at the request of a regulatory agency or an institutional review board or independent ethics committee (IRB/IEC)

Pre-specified stopping criteria are defined at the patient level and at the study level. These are described in the following sections 6.6.1., 6.6.2., and 6.6.3. In all cases, when the stopping criteria are met, the Data Safety Monitoring Board will perform causality assessment, and decide whether the study drug/study trial may be continued (See section 6.6.4.).

6.6.1. Pre-specified Patient Discontinuation rules

If a patient experiences a Grade IV Common Terminology Criteria for Adverse Event (CTCAE) or meets liver injury criteria as listed in section 6.6.3., the study drug should be interrupted. After causality assessment by the DSMB, if the AE or lab abnormality is considered unrelated to study drug, and the event has resolved or returned to baseline, then the study drug may be re-started. If the event reoccurs after restart of study drug, then the study drug will be permanently discontinued.

6.6.2. Pre-specified Trial Discontinuation rules

If one patient experiences a Grade V CTCAE, or 2 patients experience the same Grade IV CTCAE, or 3 patients experience the same Grade III CTCAE, or 4 patients experience the same Grade II CTCAE, the trial will be paused. The DSMB will assess causality of these adverse events; if the events are deemed unrelated to study drug, the trial will continue.

6.6.3. Dose Adjustment

Enrolled subjects who experience treatment emergent adverse events should be monitored closely and may have the NTZ dose adjusted at the Principal Investigator's discretion. The following procedure will be followed for dose adjustments:

1. Patient will be asked to hold study drug for 3 days.
2. After the 3 day drug hold, patients will be restarted on a reduced dose of 500 mg NTZ, once a day for 7 days.
3. Patients will be evaluated for treatment emergent adverse events and consideration for resuming the dosage of 500 mg NTZ twice a day after 7 day of reduced dose. PI

may determine that the patient should remain at the 500 mg NTZ once a day following 7 days of reduced dose. In such scenarios, the patient will be monitored and consideration for resuming the dosage of 500 mg NTZ twice daily will be evaluated at each subsequent visit.

6.6.4. Specific Liver Function Monitoring after Start of Treatment

Liver function monitoring guidelines are detailed below. The cases will be reviewed by the DSMB, who will assess potential Drug Induced Liver Injury (DILI), and apply discontinuation rules as described in 6.6.1., 6.6.2., and in this section.

Study drug interruption

Study drug must be interrupted in the following cases:

- ALT >8x ULN (if ALT is normal at baseline)
- ALT > 5x Baseline (if ALT is elevated at baseline) OR ALT>500 U/L
- ALT >5x ULN (if ALT is normal at baseline) AND Total Bilirubin>2xULN
- ALT >3x Baseline (if ALT is elevated at baseline) OR ALT>300 U/L AND total bilirubin>2xULN
- ALT >5x ULN (if ALT is normal at baseline) AND liver-associated symptoms
- ALT >3x Baseline (if ALT is elevated at baseline) OR ALT>300 U/L AND liver-associated symptoms

In all these cases, close monitoring should be initiated, with repeat liver biochemistries within 24-72 hours. Continue monitoring twice/week until liver biochemistries resolve, stabilize, or return within baseline values. If abnormalities stabilize and patient is asymptomatic, then monitoring should be performed once a week. History of symptoms, disease, concomitant medication, or other conditions such as alcohol consumption or alcohol hepatitis should be obtained for competing etiologies. The cases will be reviewed by the DSMB, who will determine if the patient may restart study drug (if causality assessment is deemed to be not related to study drug).

Management of liver biochemistries elevations

In the following cases of liver enzyme increase, liver biochemistries including ALT, AST, Total bilirubin, ALP, should be repeated within 2-5 days. Follow up of symptoms should be carefully monitored. Frequency of monitoring should continue once/week until liver abnormalities resolve, stabilize, or return to values below the thresholds mentioned below:

- ALT >5x ULN (if ALT is normal at baseline)
- ALT >3x Baseline (if ALT is elevated at baseline) OR ALT >300 U/L

NOTE: Laboratory assessments should be done centrally. If the patient lives remotely, he/she can have laboratory tests performed locally, and the results communicated to the investigator promptly.

6.6.5. Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) composed of 3 liver experts will review the safety of patients enrolled in the trial. The DSMB will perform a safety data review every 3 months, the first review occurring 3 months after first patient randomized. A DSMB charter will define the role, responsibilities, rules, and tasks of the DSMB.

The DSMB will ensure that the patient and trial discontinuation rules are respected and will determine the causality assessment of the events described in sections 6.6.1, 6.6.2., and 6.6.3., in order to make the recommendation for trial /patient discontinuation. If the events are deemed unrelated to the study drug by the DSMB, then the patient or the trial will continue.

6.7. Description of Assessments

6.7.1. Histological Assessments

All possible attempts should be made to acquire a liver biopsy specimen of at least 2.0 cm in length, from a 16 gauge needle to ensure accurate staging of fibrosis and other histological lesions. A historical biopsy within 6 months of the Screening visit may be accepted as the Screening biopsy. The liver biopsy sample must be deemed adequate for evaluation by the pathologist for inclusion. Liver biopsies will be assessed by a local reader. This assessment will include an assessment of the adequacy of the specimen as well as the fibrosis stage and a determination that the biopsy is consistent with NASH. If either the historical or screening liver biopsy is deemed unacceptable, it may be repeated.

6.7.2. Assessment of newly synthesized proteins

Kinetic biomarkers will be analyzed to evaluate the pharmacodynamic effect of NTZ on fibrogenesis. Blood samples analyses will be performed after deuterated water intake before and after 24 weeks of treatment. The assessment will involve the analysis of the change (absolute and relative) from baseline between the post-dose and pre-dose deuterated water loading periods.

6.7.3. Biological Assessments (Table 2)

Chemistry:

HbA1c, fasting plasma glucose, insulin, HOMA-IR, fructosamine, alkaline phosphatase, ALT, AST, GGT, CPK, total and conjugated bilirubin, ferritin, creatinine, eGFR, uric acid, BUN, total cholesterol, HDL-C, LDL-C, triglycerides, albumin, lipase, amylase, electrolytes (sodium, potassium, chloride, calcium), MELD

Hematology:

Hematocrit (Hct), hemoglobin (Hb), platelet count, red blood cell count (RBC), white blood cell count (WBC) with differential (absolute and percentage) including lymphocytes, monocytes,

neutrophils, eosinophils, and basophils and, reticulocyte count and mean corpuscular volume (MCV).

Inflammatory markers:

hsCRP, fibrinogen, haptoglobin, resistin, TNF-α, TGF-β, IL-6, PAI-1

Coagulation Panel:

Prothrombin time (PT), and international normalized ratio (INR)

Pregnancy Tests:

Urine β-hCG (if positive, requires immediate confirmation with Serum β-hCG)

Urinary Tests:

Urinary dipstick analysis, urinary albumin, urinary creatinine, urinary ACR

Additional Tests:

Serology (HIV-1, HBsAg, HCVAb & HCV RNA (as needed)), serum cystatin C, troponin T, NT proBNP

Fibrosis Biomarker tests:

ELF™ test score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin, miRNA, FGF19, FGF21, ProC3 and ProC6.

A laboratory manual will be provided

The manual will outline the collection and processing requirements for the laboratory. Blood sampling will be performed by trained personnel. Blood samples will be processed and shipped as outlined in the laboratory manual. Refer to the laboratory manual for exact amounts of blood required for each test.

The option to retest during the study is left to the Investigator's judgment.

Additional serum and plasma samples will be maintained for future analysis

Table 2 Biological assessments

	Screening period				**Treatment period**											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Week	*-6*	*-2*			*0*	*4*	*8*	*12*	*16*	*20*	*22*			*24*	*28*	
Labs - Haematology haemoglobin, haematocrit, RBC, WBC, differential count, platelet count, reticulocytes count, MCV)	X				X	X	X	X	X	X				X	X	X
Coagulatio (PT, INR)	X				X	X	X	X	X	X				X	X	X
Labs- Urinary Pregnancy tests	X					X	X	X	X	X				X	X	X
Labs – Serology HIV, HBs and HCV serology	X															
Labs – Biochemistry HbA1c, fasting plasma glucose, insulin, HOMA-IR, fructosamine, alkaline phosphatase, ALT, AST, GGT, CPK, total and conjugated bilirubin, ferritin, creatinine, eGFR, uric acid, BUN, total cholesterol, HDL-C, LDL-C, triglycerides, albumin, lipase, amylase, electrolytes (sodium, potassium, chloride, calcium), MELD	X			X[1]	X	X	X	X	X	X				X	X	X
Inflammatory markers hsCRP, fibrinogen, haptoglobin, resistin, TNF-α, TGF-β, IL-6, PAI-1	X				X			X						X	X	X

	Screening period				Treatment period											
Visit	**SV**	**SV2**	**SV3**	**SV4**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**EOS**	**ET**
Day	*-42*	*-14*	*-11*	*-7*	*1*	*29*	*57*	*85*	*113*	*141*	*155*	*158*	*162*	*169*	*+28 days*	
Biomarkers of fibrosis ELF Score, PIIINP, Hyaluronic acid, CK18, TIMP-1, YKL-40, Alpha 2 macroglobulin,, ,miRNA, ProC3, ProC6, FGF19, FGF21	X				X			X						X	X	X
NAFLD Fibrosis Score (NAS) & FIB-4					X			X						X		X
Labs – Urinalysis dipstick Specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrite, leukocytes	X				X	X	X	X	X	X				X	X	X
Safety markers Serum Cystatin C, troponin T, NT proBNP Urinary albumin, urinary creatinine, urinary ACR	X				X	X	X	X	X	X				X	X	X
Labs -- FSR		X	X	X	X						X	X	X	X		
Sampling for additional parameters	X				X			X		X				X	X	X

6.7.4. Electrocardiogram

Standard 12-lead electrocardiogram (ECG) assessments will be performed. The Investigator will review the ECGs for any clinically significant abnormalities to ensure subject safety. Abnormal ECG findings that are considered clinically significant by the Investigator and meet the definition of an AE should be reported and recorded in the AE eCRF page.

6.7.5. MRE

Liver stiffness will be assessed by MRE. It is recommended that each subject's radiological assessment is performed using the same procedure for each study visit. Exploratory measures for MRI/MRE sequence development will be maintained for future analysis.

6.7.6. Fibroscan

Liver stiffness will be assessed by FibroScan®. It is required that each subject's FibroScan® assessments be done with the same type of probe at each study visit.

6.7.7. Medical History

Medical history including details regarding illnesses and allergies, date(s) of onset, and whether condition(s) is currently ongoing, and medication history, including nicotine and alcohol use, will be collected on all subjects during screening.

6.7.8. Physical Examination

A complete physical examination should include source documentation of general appearance, and the following body systems: head, neck, and thyroid; eyes, ears, nose, throat, mouth, and tongue; chest (excluding breasts); respiratory; cardiovascular; lymph nodes; abdomen; skin, hair, nails; musculoskeletal and neurological.

The patient's weight will be measured under the same conditions at each visit where the procedure is required. Where possible, the scale for weight must be the same for a given patient throughout the visits.

6.7.9. Vital Signs

Blood pressure (mmHg) and pulse rate (beats per minute) will be measured at each visit using automated blood pressure equipment.

Important points for clinical blood pressure measurement

- The patient should be seated comfortably with the back supported and the upper arm bare without constrictive clothing. The legs should not be crossed.
- The arm should be supported at heart level, and the bladder of the cuff should encircle at least 80% of the arm circumference.

- Neither the patient nor the observer should talk during the measurement.

Systolic BP and diastolic BP will be measured after 5 minutes rest in the seating. Where possible, the blood pressure equipment should be the same for a given patient throughout the visits.

6.7.10. Pregnancy Testing

Urinary pregnancy tests will be done on WOCBP.

7. Adverse Events and Toxicity Management

7.1. Definitions of Adverse Events, Serious Adverse Events and Unexpected Events

7.1.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal product, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. AEs may also include pre-or post-treatment complications that occur as a result of protocol specified procedures, overdose, drug abuse/misuse reports, or occupational exposure. Preexisting events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an adverse event and must be reported
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the Screening visit that do not worsen
- Situations where an untoward medical occurrence has not occurred (eg., hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae.

- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history eCRF.

7.1.2. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

Laboratory abnormalities without clinical significance are not recorded as AEs or SAEs. However, laboratory abnormalities (eg, clinical chemistry, hematology, and urinalysis) that require medical or surgical intervention or lead to study drug interruption, modification, or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (eg, electrocardiogram, x-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE as described above. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (eg, anemia), not the laboratory result (ie, decreased hemoglobin).

7.1.3. Serious Adverse Events

A serious adverse event (SAE) is defined as an event that, at any dose, results in the following:

- Death
- Life-threatening (Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- A medically important event or reaction: such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the

other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is a reportable under expedited reporting rules. Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and subject to expedited reporting requirements.

7.1.4. Unexpected adverse event

Expectedness is assessed by the Sponsor-Investigator. An unexpected AE is defined as an event that has a nature of severity or specificity that is not consistent with the applicable Package Insert or that is symptomatically and pathophysiologically related to a known toxicity but differs because of a greater severity or specificity.

“Unexpected” refers to an event that has not been previously observed and reported rather than an event that has not been anticipated based on the properties of the drug.

7.2. Assessment of Adverse Events & Serious Adverse Events

The Investigator will establish whether or not any AE have occurred at each visit from the date of consent. The patient will be questioned in a general manner to determine specific symptoms without offering the patient any suggestion. The investigator or qualified sub-investigator is responsible for assessing AEs and SAEs for causality and severity, and for final review and confirmation of accuracy of event information and assessments.

7.2.1. Intensity assessment

The intensity of the AE will be graded as follows:

- Mild: Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities.

Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.

- Moderate: Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- Severe: Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.

7.2.2. Relation to the study treatment

The Investigator will make a clinical and scientific judgment regarding whether or not the AE was related to study treatment. The Investigator will evaluate any changes in laboratory values, make a determination as to whether or not the change is clinically important, and whether or not the changes were related to study drug. However, even if the Investigator feels there is no relationship to the study drug, the AE or clinically significant laboratory abnormality must be recorded in the eCRF.

The Investigator will record the relation to the study treatment according to the following causality terms:

- Related: the AE follows a reasonable temporal sequence from the time of drug administration and it cannot be explained by the patient's clinical state or the study procedures/conditions. The AE abates upon discontinuation of the study drug and reappears when the study drug is introduced.
- Possibly related: the AE follows a reasonable temporal sequence from the time of drug administration, but could have been produced by the patient's clinical state or the study procedures/conditions.

- Unlikely related: the temporal association between the AE and the study drug is such that the study drug is not likely to have any reasonable association with the AE. The relationship is not likely because of other plausible explanations.
- Not related: the AE must definitely be caused by the patient's clinical state or the study procedure/conditions. A reasonable explanation must be given, e.g., no investigational product taken, preplanned elective medical intervention, or incompatible temporal relationship.
- Not assessable: the report suggesting an adverse reaction cannot be judged because information is insufficient or contradictory and data cannot be supplemented or verified.

7.2.3. Action taken and outcome

The Investigator will record the action taken with drug and outcome of the event for each AE according to the following:

Action taken with investigational drug

- Drug permanently withdrawn – in case a patient is permanently withdrawn from the study drug
- Drug temporarily withdrawn – in case the study drug is temporarily withdrawn
- Dose not changed – in case no action is taken regarding the study drug
- Unknown
- Not applicable – an AE started before initiation of treatment with study drug, the treatment had been completed prior to reaction/event, or the patient has died.

Outcome

- Recovered/resolved

- Recovering/resolving
- Not recovered/not resolved
- Recovered/resolved with sequelae
- Fatal
- Unknown.

Note: In case of irreversible congenital anomalies the choice not recovered/not resolved should be used. “Fatal” should be used when death is possibly related to the reaction/event.

7.3. Reporting

7.3.1. Reporting an adverse event

All AEs regardless of seriousness or relationship to study drug, including those occurring during the Screening Period, are to be recorded on the corresponding page(s) of the eCRF and in the patient’s medical record from the ICF signature until study end for each patient. Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The Investigator should specify the date of onset, maximal intensity, action taken with respect to study drug, corrective therapy given, outcome, and his/her opinion as to whether there is a reasonable possibility that the AE was caused by the study drug.

Adverse event reporting begins from signature of the patient ICF at the first Screening Visit and ends at study end for each patient.

7.3.2. Initial reports

All SAEs occurring from the time of informed consent until 30 days following the last administration of study drug must be reported to Medpace Clinical Safety within 24 hours of the knowledge of the occurrence (this refers to any adverse event that meets any of the aforementioned serious criteria). All SAEs that the Investigator considers related to study drug occurring after the 30-day follow-up period must be reported to the Sponsor.

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, Medpace Safety

personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Safety at medpace safetynotification@medpace.com or call the Medpace SAE hotline (phone number listed below), and fax the completed paper SAE form to Medpace (fax number listed below) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

7.3.3. Follow-up reports

The Investigator must continue to follow the patient until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the patient dies.

Within 24 hours of receipt of follow-up information, the Investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (eg, patient discharge summary or autopsy reports) to Medpace Clinical Safety via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

All SAEs independent of the circumstances or suspected cause must be reported on a SAE Form/captured in EDC. The SAE Form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

It is critical that the information provided on the initial or follow-up SAE Form matches the information recorded in the source documents and the eCRF for the same event.

Any unexpected safety issue that changes the risk benefit analysis and is likely to have an impact on the patients who have participated in the trial will be reported as soon as possible to the Competent Authority(ies) concerned together with proposed actions.

7.3.4. Follow-up

The Investigator should take all appropriate measures to ensure the safety of the patients, notably he/she should follow up the outcome of any AE until the return to normal or until stabilization of the patient's condition.

The patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This information should be documented in the patient's medical records.

7.4. Post Study Reporting Requirements

Any SAEs and deaths that occur within 30 days of the last dose of the study drug, regardless of causality, should be reported.

Any SAE that is brought to the attention of the Investigator at any time after the reporting period and which is considered by him/her to be caused by the study drug within a reasonable possibility, should be reported.

7.5. Special Situation Reports

Special situations reports include pregnancy reports, reports of medication error, abuse, misuse or overdose, and reports associated with product complaints.

7.5.1. Pregnancy

In case of pregnancy, the Investigator will notify regulatory authorities and IRB within 24 hours of his/her knowledge of the pregnancy.

Female patients must be instructed to discontinue the study drug immediately and inform the Investigator as soon as possible once they are aware of being pregnant or suspect that they are pregnant during the study or within 30 days of the last dose of the study drug.

Female patients will be requested, as part of the general ICF, to provide informed consent to allow reasonable attempts to be made to obtain information on any possible medicinal product exposure to an embryo or fetus and to follow up on the outcome of the pregnancy.

The Investigator will contact the patient at the expected time of delivery for follow-up.

7.5.2. Medication error

Medication error is defined as an unintentional error in the prescribing, dispensing, or administration of a medicinal product while in the control of the healthcare professional, patient, or consumer. All medication errors will be documented in the eCRF and, in case of any potential risk to patient safety, would be reported as appropriate.

7.5.3. Misuse

This refers to situations where the medicinal product is intentionally and inappropriately used not in accordance with the authorized product information and will be reported in the eCRF. All misuse will be documented in the eCRF and, in case of any potential risk to patient safety, would be reported as appropriate.

7.5.4. Overdose

This refers to the administration of a quantity of a medicinal product given per administration or cumulatively, which is above the maximum recommended dose according to the authorized product information. Clinical judgment should always be applied. Doses >4000 mg in one day are considered overdose.

7.5.5. Abuse

This corresponds to the persistent or sporadic, intentional excessive use of a medicinal product, which is accompanied by harmful physical or psychological effects.

8. Statistical Considerations

This section is an overview of the principal elements of the statistical analysis for this study. Further details will be contained in a separate statistical analysis plan (SAP).

8.1. Analysis Endpoints

8.1.1. Primary Endpoint

The primary endpoint is to assess the safety and tolerability of NTZ after 24 weeks of treatment

8.1.2. Secondary Endpoints

The secondary endpoints are:

- To assess the % change in FSR from baseline to end of treatment (24 weeks of treatment)
- To assess the change in liver stiffness by MRE from baseline to 12 weeks and 24 weeks of treatment
- To assess the change in liver stiffness by fibroscan from baseline to 24 weeks of treatment
- To assess the change from baseline to 12 weeks and 24 weeks of treatment of fibrosis serum biomarkers and scores

8.2. Analysis Sets

The Full Analysis Set will consist of all subjects that meet the eligibility criteria and enroll into the study (Visit 1 Day 1).

The Safety Set will consist of all enrolled subjects who receive at least one dose of study drug.

8.3. Data Handling Conventions

Continuous variables will be summarized with descriptive statistics (i.e., sample size, mean, standard deviation, minimum, maximum, median, and quintiles). Categorical variables will be summarized with descriptive statistics (i.e., sample size, frequency counts, and percentage). Statistical testing on change from baseline values will be performed using analysis of variance techniques with a term for baseline in the model. Statistical significance will be considered to have been reached at an alpha level of 0.05.

Baseline value is measured on Day 1 of the treatment period before the administration of NTZ.

8.4. Demographic Data and Baseline Characteristics

Demographic and baseline characteristics will be descriptively summarized. Quantitative and/or categorical summaries will be presented for demographics and other baseline characteristics. Demographic and baseline characteristics will be summarized using the Full Analysis Set.

8.5. Primary Safety Analysis

Safety and tolerability will be assessed by the following parameters:

AEs including TEAEs and SAEs, laboratory evaluations, vital signs, ECGs, and physical examinations.

8.5.1. Adverse Events

Treatment-emergent adverse events (TEAEs) are defined as AEs that worsen or commence on or after the time of start of first study drug administration. AEs, including SAEs, will be coded using MedDRA. The number and percentage of subjects experiencing TEAE, including TEAEs leading to study drug discontinuation and SAEs, will be summarized for each system organ class (SOC) and Preferred Term. In addition, AEs will be tabulated according to severity, causality, and relation to the study drug.

Individual data listings for AEs will be presented for each subject.

8.5.2. Laboratory Evaluations

Absolute and change from baseline values for laboratory parameters will be summarized by visit. In addition, shift from baseline tables will be presented for selected laboratory abnormalities.

8.5.3. Other Safety Evaluations

Observed values and change from baseline will be summarized descriptively for all vital sign parameters and ECG parameters by visit. Individual listings will be presented for each subject. Individual data listings for physical examination results will be presented for each subject.

Safety data will be summarized using descriptive statistics. Further details will be provided in the SAP.

All safety analyses will be conducted using the Safety Population.

8.6. Secondary Efficacy Analyses

8.6.1. FSR

Fractional Synthesis Rate of circulating plasma proteins will be measured at Baseline, and after 24 weeks of treatment.

The change in FSR (absolute and relative) from baseline to end of treatment between the first and second deuterated water loading periods will be assessed to determine the effects of NTZ 500mg BID on de novo collagen synthesis in the Full Analysis Set.

Wilcoxon signed-rank test will be used for statistical inference. Estimated mean, standard error and 95% confidence interval of change in FSR (absolute and relative) change from baseline in FSR will be reported and evaluated at a significance level of 0.05. Exploratory analyses may also be performed to evaluate the association of individual exploratory biomarkers or combination of biomarkers with clinical measurements and other risk factors.

Additional details will be provided in the SAP.

8.6.2. Liver stiffness

The change in liver stiffness from baseline at 24 weeks as measured by FibroScan, and at 12 weeks and 24 weeks by MRE, the change in non-invasive markers of fibrosis from baseline to 24 weeks, and the change in fibrosis scores from baseline to 12 weeks, to 24 weeks for the Full Analysis Set will be assessed. Summary statistics for the Full Analysis Set will be provided at all visits and the change from baseline will be provided.

8.6.3. Exploratory Analysis

Additional serum and plasma samples will be maintained for future analysis. Exploratory measures for MRI/MRE sequence development will be maintained for future analysis.

8.6.4. Interim Analysis

An interim analysis is not planned.

8.7. Sample Size

The sample size of this Phase 2 proof of concept study is based on clinical assessment. Twenty male and female subjects with histologically confirmed NASH and fibrosis stage 2 or 3) will provide sufficient information to assess the safety and tolerability of NTZ 500mg BID over 24 weeks.

9. Responsibilities

9.1. Investigator Responsibilities

By signing this protocol, the Investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of GCP; and all applicable local laws, rules and regulations relating to the conduct of the clinical study.

The Investigator also agrees to allow monitoring, audits, IRB review and regulatory agency inspection of trial-related documents and procedures and provide for direct access to all study related source data and documents.

The Investigator shall prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable local laws, rules and regulations; and, for each subject participating in the study, provide all data, and upon completion or termination of the clinical study submit any other reports to the Funder as required by this protocol or as otherwise required pursuant to any agreement with the Funder.

Study documentation will be promptly and fully disclosed to regulatory authorities by the Investigator upon request and also shall be made available at the Investigator's site upon request for inspection, copying, review and audit at reasonable times by representatives any regulatory agencies.

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on this study.

9.2. Good Clinical Practice

The investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), International Conference on Harmonisation (ICH) guidelines. These standards are consistent with the European Union Clinical Trials Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC. The investigator will ensure adherence to the basic principles of Good Clinical Practice, as outlined in 21CFR312, subpart D, "Responsibilities of Sponsors and Investigators," 21CFR, part50, 1998, and 21CFR, part56, 1998. The investigator and all applicable sub investigators will comply with 21CFR, Part54, 1998, providing documentation of

their financial interest or arrangements with any study funder, or proprietary interests in the investigational drug under study. This documentation must be provided prior to the investigator's (and any sub investigator's) participation in the study. The investigator and sub investigator agree to notify study funder of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes the protocol-defined activities

9.3. Institutional Review Board Review & Approval

The investigator will submit this protocol, informed consent form, and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) to an IRB/IEC. The investigator will not begin any study subject activities until approval from the IRB/IEC has been documented and provided as a letter to the investigator. Before implementation, the investigator will submit to and receive documented approval from the IRB/IEC any modifications made to the protocol or any accompanying material to be provided to the subject after initial approval, with the exception of those necessary to reduce immediate risk to study subjects.

9.4. Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The investigator must use the most current IRB/IEC approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject and the person conducting the consent discussion, and also by an impartial witness if required by local requirements.

9.5. Confidentiality

The investigator will assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, another unique identifier (as allowed by local law) and an identification code will be recorded on any form or biological sample submitted to the study funder, IRB/IEC or laboratory. Laboratory

specimens will be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. Refer to specific laboratory instructions for further details. NOTE: The investigator will keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations. The investigator agrees that all information received from the study funder, including but not limited to the Package Insert, the IMP, and any other study information, remain the sole and exclusive property of the study funder during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from the study funder. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

9.6. Study Files and Retention of Records

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study master file, and (2) subject clinical source documents. The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB/IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence. The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender);
- Documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);
- Documentation of the reason(s) a consented subject is not enrolled
- Participation in study (including study number);

- Study discussed and date of informed consent;
- Dates of all visits;
- Documentation that protocol specific procedures were performed;
- Results of efficacy parameters, as required by the protocol;
- Start and end date (including dose regimen) of IMP, including dates of dispensing and return;
- Record of all adverse events and other safety parameters (start and end date, and including causality and severity);
- Concomitant medication (including start and end date, dose if relevant; dose changes);
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the investigator until at least 2years or according to local laws, whichever is longer, after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan) and until there are no pending or planned marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with study funder. The investigator must notify study funder before destroying any clinical study records. Should the investigator wish to assign the study records to another party or move them to another location, study funder must be notified in advance. If the investigator cannot provide for this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and study funder to store these records securely away from the site so that they can be returned sealed to the investigator in case of an inspection. When source documents are required for the continued care of the subject, appropriate copies should be made for storage away from the site.

9.7. Electronic Case Report Forms

For each subject consented, an eCRF will be completed by an authorized study staff member whose training for this function is documented according to study procedures. The eCRF will be completed in a timely manner. Original entries as well as any changes to data fields will be stored in the audit trail of the system. At the conclusion of the trial, a read-only archive copy of the data entered will be generated. This archive will be stored in accordance with the records retention requirements.

9.8. Investigational Medicinal Product Accountability and Return

The investigator or designee is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgment of receipt of each shipment of study product (quantity and condition), subject dispensing records, and returned or destroyed study product. Dispensing records will document quantities received from study funder and quantities dispensed to subjects, including lot number, date dispensed, subject identifier number, subject initials, and the initials of the person dispensing the medication. Drug may be returned or destroyed on an ongoing basis during the study, if appropriate. At the end of the study, following final drug inventory reconciliation, the study site will arrange for the return of unused investigational medicinal product supplies. All drug supplies and associated documentation will be periodically reviewed and verified by the study funder over the course of the study.

9.9. Inspections

The investigator understands that source documents for this trial should be made available to appropriately qualified personnel from study funder and its representatives, to IRBs/IECs, or to regulatory authority or health authority inspectors.

9.10. Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

9.11. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made by the investigator in collaboration with the study funder. All protocol modifications must be submitted to the IRB/IEC in accordance with local requirements. Approval must be obtained before changes can be implemented.

9.12. Study Reports

A clinical study report (CSR) will be prepared and provided to the regulatory agency (ies). The Investigator will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICHE3). Note that an abbreviated report may be prepared in certain cases. After conclusion of the study, Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met:

- The results of the study in their entirety have been publicly disclosed by or with the consent of the study funder in an abstract, manuscript, or presentation form.

9.13. Study Termination

The Investigator reserves the right to terminate the study at any time. Should this be necessary, the investigator will arrange discontinuation procedures and notify the appropriate regulatory authorities, IRBs, IECs, and the Funder. In terminating the study, the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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10.2. WHO Fact Sheet 312

World Health Organization. Media Centre- Diabetes.
http://www.who.int/mediacentre/factsheets/fs312/en/

10.3. Studies on ClinicalTrials.gov

NCT01185028. A safety and tolerability study of nitazoxanide in HIV-HCV treatment failures. Last updated: March 21, 2018

https://clinicaltrials.gov/ct2/show/results/NCT01185028?sect=X430156#othr

NCT01770483. The role of nitazoxanide, interferon alfa and ribavirin in treatment of hepatitis C infected Type 2 diabetic patients (HEP-C-FM). Last updated: September 20, 2013

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INVESTIGATOR PROTOCOL REVIEW AND SIGNATURE FORM

Protocol Number: NTZ-218-1

Protocol Title: "A Monocentric, Open-Label, Proof of Concept Study to Evaluate the Safety and Efficacy of NTZ at 500mg Twice Daily on Collagen Turnover in Plasma in NASH Patients with Fibrosis Stage 2 or 3."

I have read the above-mentioned Protocol Amendment dated 2 March 2019. I agree to conduct the study as detailed herein and in compliance with ICH Guidelines for Good Clinical Practice and applicable regulatory requirements, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

[redacted]

Principal Investigator (Please PRINT)

[redacted] 9-6-2019

Principal Investigator (Signature) **Date**