CLINICAL TRIAL PROTOCOL

A Randomized, Double-Blind, Placebo Controlled, Parallel-Group, Phase II trial of JKB-121 for the Treatment of Nonalcoholic Steatohepatitis (NASH)

PROTOCOL NUMBER: JKB-121-001

DUKE IRB PROTOCOL NUMBER: Pro00062677

FDA IND NUMBER: IND 124,082

STUDY MEDICATION: JKB-121 (nalmefene hydrochloride)

SPONSOR: Investigator Initiated Study

PRINCIPLE INVESTIGATOR: Manal F. Abdelmalek, MD, MPH

SPONSOR FOR STUDY DRUG: TaiwanJ Pharmaceuticals Co., Ltd.
Zhupei, Hsinchu County 302
Taiwan.

PROTOCOL VERSION: Version 4.0

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1. PROTOCOL SYNOPSIS

NAME OF PRINCIPAL INVESTIGATOR: Manal F. Abdelmalek, MD, MPH	**PROTOCOL NUMBER**: JKB121-001 (Version 4.0) **IND 124,082**
NAME OF SPONSOR TaiwanJ Pharmaceuticals Co., Ltd	
NAME OF FINISHED PRODUCT: JKB-121	
NAME OF ACTIVE INGREDIENT: Nalmefene hydrochloride	

Study Title	A Randomized, Double-Blind, Placebo Controlled, Parallel-Group, Phase II Trial of JKB-121 for the Treatment of Nonalcoholic Steatohepatitis (NASH).
Lead Investigator:	Manal F. Abdelmalek, MD, MPH Duke University
Number of Centers:	Approximately 12 sites—United States only
Study Phase	II
Study Objectives:	
Primary Objectives:	To evaluate the safety and potential efficacy of two dose levels of JKB-121 (5 mg twice daily and 10 mg twice daily) in reducing liver fat compared to placebo.
Secondary Objective:	The secondary objectives are • Determine the pharmacokinetic profile of JKB-121 in NASH • Assess the impact of treatment with JKB-121 on metabolic markers • Assess the impact of treatment on liver enzymes (serum ALT) over 24 weeks. • Establish the recommended dose for future NASH treatment studies. • To evaluate changes of NASH related biomarkers such as adiponectin, leptin, ghrelin, TNF-alpha, TGF-beta, hyaluronic acid and MMP-2.
Exploratory Objective	Investigate the impact of treatment of potentially relevant inflammatory and metabolic biomarkers associated with NASH
Study Endpoints:	
Primary Endpoints:	Safety: Incidence and severity of adverse events and changes in vital signs and clinical laboratory tests Efficacy: Comparative changes in • Mean changes from baseline in the percentage fat content of the liver measured by magnetic resonance imaging (MRI) at week 24.
Secondary Endpoints:	Pharmacokinetics: • Serum concentration of JKB-121 over time including but not limited to maximum observed concentrations (Cmax), minimum observed concentation (Cmin) and area under the concentration time curve (AUC) and if feasible half-life (t½) Metabolic markers: • Body mass index (BMI)

	• HbA1c and the homeostatic model assessment of insulin resistance (HOMA-IR) • Serum lipid profile: total cholesterol, triglycerides, low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions. <u>Liver Function:</u> • Mean change from baseline in serum ALT at week 24. • Mean serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) at weeks 4, 8, 12, 16, 20, and 24. • Proportion of subjects whose ALT level at week 24 is within normal range defined as < 20 U/L for women and < 30 U/L for men.
Exploratory Outcome Measures:	• Mean serum concentrations of lipopolysaccharide (LPS), c-reactive protein (CRP), cytokeratin (CK)-18 fragments, proinsulin c-peptide, glucagon-like polypeptide (GLP-1) and adiponectin. • Relative levels of inflammatory cytokines, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) • Relative levels of regulatory T cells in the peripheral blood mononuclear cells (PBMC) samples including CD4, CD8, CD 25, Fox P3, NKT, and CD62 T cells.
Methodology:	
Study design	Randomized double blind, placebo control 3-arm parallel group, multi-dose single center study
Study duration	Up to 28 days for screening, 24 weeks of treatment and 28 days of follow-up. Subjects terminating study drug will be followed-up post-treatment for 28 days.
Number of Subjects:	The study will aim to recruit 60 subjects, randomized 1:1:1
Stratification:	Treatment allocation will be stratified by known diagnosis of diabetes status. Nondiabetic status will be defined as HbA1c ≤ 6.0 and absence of any medications to treatment diabetes. Diabetic status will be defined as HbA1c > 6.0 and/or requiring medication for treatment of diabetes mellitus.
Study Drug:	JKB-121 oral tablet
Control Group	Placebo to match
Dose Regimens	Arm A: 5 mg JKB-121 twice daily. Arm B: 10 mg JKB-121 twice daily Arm C: Matching placebo twice daily All treatment dose regimens are administered per orally
Administration: Schedule:	Twice daily for 24 weeks

Study Population:	Adults with biopsy-proven nonalcoholic steatohepatitis (NASH)
Inclusion Criteria:	1. Age ≥ 18 years 2. Provision of written informed consent 3. Biopsy-proven NASH within 12 months prior to randomization verified by central pathology reading with: • NASH activity score (NAS) of 4 or more • 10% or more macrovesicular steatosis • cytologic ballooning score of at least 1 point • hepatic fibrosis of at least 1 point • hematoxylin and eosin (H&E) stained slides and/or paraffin block available for independent assessment 4. ALT > 40 U/L for women and > 60 U/L for men at screening and at least once in the previous 12 months. 5. MRI with > 6% hepatic steatosis 6. HbA1c of ≤ 9.0 7. Lipid lowering agents (i.e. statins) and allowable antidiabetic treatment (i.e. metformin or sulfonylurea) must be stable at screening and during the study. 8. Agree to use of effective contraceptive measures if female of child bearing potential.
Exclusion Criteria:	1. Any chronic liver disease other than NASH (i.e., drug-induced, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, A1AT deficiency, Wilsons disease) 2. Cirrhosis, as assessed clinically or histologically (fibrosis stage 4) 3. Presence of vascular liver disease 4. BMI ≤ 25 kg/m^2 5. Excessive alcohol use (> 20 g/day) within the past 2 years 6. AST or ALT > 250 U/L 7. Type 1 diabetes mellitus 8. Bariatric surgery in the past 5 years. 9. Weight gain or weight loss of > 5% in past 6 months or > 10% change in past 12 months. 10. Contraindication to MRI 11. Inadequate venous access 12. HIV antibody positive, hepatitis B surface antigen positive (HBsAg), or HCV RNA positive. 13. Receiving an elemental diet or parenteral nutrition 14. Chronic pancreatitis or pancreatic insufficiency 15. Any history of complications of cirrhosis (i.e. ascites, hepatic encephalopathy, or portal hypertensive bleeding), even if absent or optimized with medical management at time of screening 16. Concurrent conditions: a) Inflammatory bowel disease b) Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of screening c) Ongoing infectious, immune mediated disease within previously 1 years d) Any malignant disease (other than basal cell carcinoma of the skin) within previous 2 years e) Prior solid organ transplant

	f) Any other concurrent condition which, in the opinion of the investigator, could impact adversely on the subject participation or the interpretation of the study data. 17. Concurrent medications including: a) Anti-NASH therapy(s) initiated ***after*** the liver biopsy diagnosing NASH. Anti-NASH therapies include S-adenosyl methionine (SAMe), milk thistle, and vitamin E. b) Antidiabetic medication which may impact NASH histology initiated ***after*** the liver biopsy diagnosing NASH including thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or glucagon-like peptide 1 analogs. c) Immune modulatory agents including systemic steroids, methotrexate, anti-TNF-α therapies (infliximab, adalimumab, etanercept) or anti-integrin therapy (namixilab). d) UGT2B7 enzyme inhibitors (e.g., diclofenac, fluconazole, medroxyprogesterone acetate, meclofenamic acid) e) UGT2B7 inducers (e.g., dexamethasone, phenobarbital, rifampicin, omeprazole) f) Chronic opioid use within 28 days prior to screening (Day -28) 18. Self-reported or known marijuana or illicit drug use within 28 days before the screening 19. The following laboratory abnormalities: a) HbA1c > 9.0% b) Neutrophil count < 1.0 x 10^9 / L c) Platelets < 100 10^9 / L d) Hemoglobin < 10 g/dl e) Albumin < 3.5 g f) International normalized ratio (INR) >1.3 g) Any elevation of total bilirubin above normal (unless Gilbert's syndrome or extrahepatic source as denoted by increased indirect bilirubin fraction). h) Serum creatinine > 1.5 mg/dl i) Creatinine clearance ≤ 50 ml/minute calculated by Crockroft-Gault. 20. Pregnancy or breastfeeding. 21. Women, of childbearing age, who are not willing to practice effective contraception (i.e., barrier, oral contraceptives, or past medical history of hysterectomy) for the 48-week duration of the trial and for 1 month after the last administration of the drug. Women with documented (not reported) oophorectomy or hysterectomy are considered sterile. Women with tubal ligation are at small risk and should have pregnancy test at screening. 22. Participation in an investigational drug study within past 30 days.

Study Procedures and Visit Schedule:	Refer to Table 1 for the schedule of study procedures. Subjects who provide voluntary written informed consent will be screened for eligibility. Subjects meeting all the inclusion and none of the exclusion criteria will be eligible to participate. The maximum time on study drug for each subject is 24 weeks with an additional 4 weeks of follow-up. Eligible subjects will be randomized at the baseline visit to receive one of three study treatments twice daily for a period of 24 weeks. Each subject will return for assessment and required study procedures on days 14, 28 and every 4 weeks thereafter until week 24. A final follow-up visit will be conducted at week 28 if the subject ceases treatment at week 24. Investigational product dispensing and accounting will be conducted on a 4 week basis. The actual visit schedule will be as follows: • Screening period, culminating in randomization (screening assessments must be completed no more than 4 weeks prior to enrollment. • Baseline visit and every 4 weeks afterward for total of 24 weeks • Week 8, 16 and 24 (± 4 days) visits will include measure of metabolic (glucose, lipids, glycosylated hemoglobin, HOMA-IR, C-reactive protein etc.) • Week 12 and 24 (± 4 days) will include measures of hepatic steatosis and exploratory measures (i.e. CK-18, adiponection, leptin, ghrelin, TNF-α, TGF-β, hyaluronic acid, cytokines, and MMP-2 etc) • Follow-up visit at week 28 (±4 days). • Unscheduled or early withdrawal visits may be necessary.
Safety Assessment	
Safety Parameters:	The following measures of safety and tolerability will be conducted at screening, baseline and every 4 weeks during the study: • Adverse event monitoring • Physical examination including vital signs • Safety laboratory assessments • Treatment compliance • Urine pregnancy test (women only) The following measures of safety and tolerability will be conducted at screening, baseline and every 12 weeks during the study: • Electrocardiogram (EKG) • Urinalysis
Other Parameters:	The following measures of immunological profile will be explored in this study: • Insulin resistance (HOMA-IR, insulin and glucose levels,HbA1c) • Lipid levels • Special laboratory functions panel include CK-18, adiponectin, leptin, ghrelin, TNF-α, TGF-β, hyaluronic acid, and MMP-2.

Statistical Considerations:	
Sample Size Determination:	An approximate total of 60 subjects will be enrolled. Subjects will be randomized into three treatment groups in a ratio of Group 1 (5 mg twice daily): Group 2 (10 mg twice daily): Group 3 (placebo twice daily) = 1:1:1. No empirical power calculation is performed. The sample size is based on the best medical judgment. The power to detect a difference between placebo and active treatment with respect to change from baseline in liver fat content and serum ALT has been determined for a sample size of 20 patients per arm to assess safety and potential efficacy. Fat quantification by MRI is very sensitive with as small as a 5% detectable change in fat quantification. Further, since the endpoint is the change from baseline, plausible estimates of the degree of correlation between pre-and post-baseline measurements will be incorporated into the variance estimates. Given the wide range of results observed in the literature, several "what-if" scenarios were examined. As such, the current sample size was considered sufficient for the exploratory objectives of the study for the given range of plausible treatment effects and variance estimates. Due to the exploratory objects of the protocol, no adjustment for the two co-primary endpoints was made. Additionally, there will be no adjustments for multiple comparisons between the 3 treatment groups. Assuming the observed difference between two treatment groups is 20%, then the 95% confidence interval for the true mean is given by (2.5%, 37.5%) with 20 subjects in each group. <u>Fat Liver Content Change from Baseline:</u> The current sample size of 20 subjects per group should yield approximately 85% power to detect a treatment effect of at least ~ 5% with respect to the difference in the mean change from baseline between one of the JKB-121 treatment groups and placebo <u>ALT change from Baseline:</u> Patients will be stratified into treatment arms based on threshold of baseline elevation of ALT (i.e. ALT < 2x ULN and ALT ≥ 2 x ULN, but < 5 x ULN). The current sample size of 20 subjects per group should yield approximately 73% power to detect a treatment effect of at least 30 U/L assuming a SE of 50 U/L (α= 0.05; two tailed; unpaired t-test). The SD of 50 U/L for the change from baseline endpoint was derived assuming SDs of 40 for the pre-and post-baseline measurements with a correlation of 0.25. Sample size and power estimates have not been adjusted for multiple comparisons or multiple endpoints, dropouts or stratifications. The analysis of each primary endpoint will be based on a linear model that incorporates the baseline measures as a covariate, the stratification factor and randomization treatment group. Pairwise comparisons of each active treatment group to control will be conducted vial linear contrasts.

Statistical Analysis	The populations for analysis will be as follows: Full Analysis Set (FAS) for Efficacy: includes all subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received may be excluded. In the event of allocation errors, subjects will be analyzed for efficacy according to the treatment to which they were randomized. Safety Analysis Set: includes all subjects who received at least one dose of study medication. Only subjects with clear documentation that no study medication was received may be excluded. In the event of treatment allocation errors, subjects will be analyzed for efficacy according to the treatment in which they were randomized. Per Protocol Analysis Set includes all subjects who complete 24 weeks of study treatment and have MRI and ALT measured at baseline and at week 24 and no major protocol violations, determined prior to break of the treatment blind. All hypotheses will be tested for statistical significance with two-tailed p-values. Results of all tests will be considered statistically significant if their p-value is less than or equal to 0.05, except results of the tests for interaction will be considered statistically significant if the p-value is less than or equal to 0.10
Efficacy:	The primary endpoint of this study is the safety and potential efficacy of two dose levels of JKB-121 (5 mg twice daily and 10 mg twice daily) in reducing liver fat compared to placebo. The secondary endpoint of the study is the time to remission (TTR) is defined as time in weeks from randomization to liver aminotransferase remission as defined as two consecutive ALT values within normal range (< 20 U/L for women and < 30 U/L for men) or a 20% reduction from the baseline during study period. A Cox proportional hazard model will be used to compare the TTR duration between treatment arms. Median duration of TTR and the hazard ratio with 95% confidence limits will be estimated using Kaplan-Meier survival methodology. TTR from randomization will be compared using estimates from the Kaplan-Meier survival curves. A detailed comparison of the treatment effects will be analyzed by fitting a Cox proportional hazard model with baseline variables as covariates to assess the benefit-risk ratio.
Safety:	The primary safety endpoints will be the rates of patients who experience (1) one or more AEs; (2) SAEs; and (3) AEs leading to discontinuation of study medication. Treatment effect will be explored by inspection of observed means or rates for the dose groups within each treatment regimen. Other safety parameters such as laboratory test, vital signs, and other safety related variables will be summarized regarding each scheduled visit. Shift tables will be presented for laboratory values based on the reference laboratory normal ranges.

1.1 TABLE OF EVENTS

	Screening Period	Enrollment	Treatment period						End of Treatment	Follow-up Visit
Visit	0	1	2	3	4	5	6	7	8	9
Week	up to 4 wks	0	2	4	8	12	16	20	24	28
Day (Visit Window)	-1 to -28	1	14 (±4)	28 (±4)	56 (±4)	84 (±4)	112 (±4)	140 (±4)	168 (±4)	196 (±4)
Determination of eligibility	X	X								
Review of medical history	X	X								
Obtain informed consent	X									
Randomization		X								
Study drug dispensing		X	X	X	X	X	X			
Managing drug accountability			X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X		X	X	X	X	X	X	X
Serum hCG (if applicable)	X									
Urine hCG (if applicable)		X		X	X	X	X	X	X	X
Hematology panel	X	X	X	X	X	X	X	X	X	X
Comprehensive metabolic panel	X	X	X	X	X	X	X	X	X	X
Lipid panel		X		X	X	X	X	X	X	X
Glycosylated hemoglobin	X	X		X	X	X	X	X	X	X
HOMA-IR	X	X		X	X	X	X	X	X	X
Urinalysis	X					X			X	X
Electrocardiogram	X					X			X	
Liver biopsy (if prior biopsy > 12 mo)	X									
PK study		X*			X		X		X	
Review concomitant meds	X	X	X	X	X	X	X	X	X	X
Review adverse events **		X**	X	X	X	X**	X	X	X**	X
MRI for hepatic steatosis	X					X			X	
CK-18		X				X			X	X
Stored serum for exploratory measures and noninvasive biomarkers of fibrosis		X				X			X	X
Exit Interview										X

* Frequent sampling PK study on Day 1 for Substudy subjects only. All patients to have a pre-dose trough level drug measures (6 ml blood) at treatment Weeks 8, 16, and 24 (Visits 4, 6, and 8).
** Hospital Anxiety and Depression Scale and Columbia Suicide Severity Scale

2. ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALD	Alcoholic liver disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CK-18	Cytokeratin 18
CRF	Case report form
CRO	Contract research organization
CTCAE	Common terminology criteria for adverse events
EC	Ethics committee
EKG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
ß-HCG	ß-human chorionic gonadotropin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSC	Hepatic stellate cells
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDRB	Independent Data Review Board
IL	Interleukin
IND	Investigational new drug
INR	International normalized ratio
IRB	Institutional Review Board
ITT	Intent-to-treat
KO	Knock-out
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MedDRA	Medical dictionary for regulatory activities
MCD	Methionine choline deficient
MCP-1	Monocyte chemotactic protein 1
MMP-1	Matrix metallopeptidase 2
MRI	Magnetic resonance imaging
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
NCI	National Cancer Institute

NFK-β	Nuclear factor-kappa beta
SAE	Serious adverse event
TNF-α	Tumor necrosis factor alpha
TLR-4	Toll like receptor 4
TIR	Toll/IL-1 receptor
TLR	Toll-Like Receptors
TTR	Time to remission

3.0 BACKGROUND

3.1 Nonalcoholic Steatohepatitis (NASH)

The emerging epidemic of obesity and metabolic syndrome has contributed to the increased prevalence of nonalcoholic steatohepatitis (NASH) as what is now considered to be the leading cause of chronic liver disease in the Western World [1]. An estimated 25% of the industrialized world has hepatic steatosis and fatty liver, an entity that has been termed nonalcoholic fatty liver disease (NAFLD). NAFLD encompasses a spectrum of liver pathology with different clinical prognoses. The simple accumulation of triglyceride within hepatocytes (hepatic steatosis) is on the most clinically-benign extreme of the spectrum. On the opposite, most clinically-ominous extreme, are cirrhosis and primary liver cancer. The risk of developing cirrhosis is extremely low in individuals with chronic hepatic steatosis, but increases as steatosis becomes complicated by histologically-conspicuous hepatocyte death and inflammation, i.e., nonalcoholic steatohepatitis (NASH). NASH affects approximately 20-25% of all NAFLD patient, which equates approximately 6.0% of the overall population [2]. NASH resembles alcoholic liver disease but occurs in people who drink little or no alcohol [3].

Most subjects with NAFLD are asymptomatic. The diagnosis is often made when abnormal liver aminotransferases or features of fatty liver are noted during an evaluation performed for other reasons. NAFLD may also be diagnosed during the work-up of vague right upper quadrant abdominal pain, hepatomegaly, or an abnormal appearing liver at time of abdominal surgery. The association of NAFLD with obesity, diabetes, hypertriglyceridemia, hypertension, and cardiovascular disease is well known. NAFLD is an independent risk factor for metabolic syndrome and type 2 diabetes mellitus [4]. In addition, approximately 50-90% of patients with obesity may have NAFLD while 50% of patients with diabetes may have biopsy proven NASH and are at increased risk for progressive hepatic fibrosis and cirrhosis [5, 6].

The mechanisms underlying NAFLD pathogenesis and progression are not entirely clear. The best-understood mechanisms pertain to hepatic steatosis. Hepatic steatosis results when hepatocyte mechanisms for triglyceride synthesis (e.g., lipid uptake and *de novo* lipogenesis) overwhelm mechanisms for triglyceride disposal (e.g., degradative metabolism and lipoprotein export), leading to accumulation of fat (i.e., triglyceride) within hepatocytes. Obesity stimulates hepatocyte triglyceride accumulation by altering the intestinal microbiota to enhance both energy harvest from dietary sources and intestinal permeability. Reduced intestinal barrier function increases hepatic exposure to gut-derived products which stimulate liver cells to generate inflammatory mediators that inhibit insulin action and cause oxidative or inflammatory stress. Increased delivery of triglyceride precursors (e.g., fatty acids and diacylglycerols) and metabolic by-products (e.g., reactive oxygen species) may damage hepatocytes, leading to hepatocyte lipotoxicity. Lipotoxicity also triggers the generation of other factors (e.g., inflammatory cytokines, hormonal mediators) that deregulate systems that normally maintain hepatocyte viability and result in progressive chronic liver injury.

Weight reduction with diet and exercise is the only therapeutic strategy that has been shown to be beneficial in NASH. Unfortunately, most patients are unable to achieve and maintain the required lifestyle change for any significant period of time. Bariatric surgery is effective for

weight loss, but has not been specifically assessed as a treatment for NASH and may be contraindicated in those patients with advanced liver disease attributable to NAFLD. Insulin sensitizing agents such as metformin and glitazones have been used experimentally with some limited evidence of short term benefit; however, the benefits are not sustained. Experimental approaches under clinical evaluation in patients with NASH include antioxidants, newer antidiabetic medications and antifibrotic agents. In two pilot trials, pentoxifylline [7, 8], which inhibits Tumor necrosis factor alpha (TNF-α) production, was reported to show beneficial effects in improving aminotransferase levels, hepatic steatosis, fibrosis and necroinflammation among patients with NASH. *Currently, there are no FDA-approved treatments for NAFLD and NASH.*

3.2 Toll-Like Receptor 4

Toll-like receptor 4 (TLR-4) is a cell surface, type 1 trans-membrane protein that recognizes bacterial lipopolysaccharides (LPS) and endotoxins, among other ligands, a key mediator of the release of proinflammatory cytokines and inflammatory responses, and a member of TLR family which are mainstays of the innate immune system and best known for their role in host defenses against infections. Lipopolysaccharide (LPS), an endotoxin, is a specific agonist for TLR-4. Activation of inflammatory signaling pathways is of central importance in the pathogenesis of alcoholic liver disease (ALD) and NASH. Recent studies demonstrated that Toll-like receptors, the sensors of microbial and endogenous danger signals, are expressed and activated in innate immune cells as well as in parenchymal liver cells involved in hepatic fibrosis (hepatic stellate cells and Kupffer cells) and thereby contribute to ALD and NASH [9]. The importance of gut-derived endotoxin and its recognition by TLR4 in the liver, the significance of TLR-induced intracellular signaling pathways and cytokine production as well as the contribution of TLR-4 signaling to the induction of liver fibrosis and to the progression of liver pathology suggest that a TLR-4 antagonist for therapeutic target for the treatment of chronic liver disease [10].

3.3 Obesity, Insulin resistance and TLR-4

Chronic low-grade inflammation is a hallmark of obesity and thought to contribute to the development of obesity-related insulin resistance. TLR-4 is a key mediator of pro-inflammatory responses. Mice lacking TLR-4s are protected from diet-induced insulin resistance and inflammation. Mice deficient in hepatocyte TLR-4 (TLT4KO) exhibit improved glucose tolerance, enhanced insulin sensitivity and ameliorated hepatic steatosis despite the development of obesity after a high-fat diet (HFD) challenge [11]. Furthermore, TLR4KO mice have reduced macrophage content in white adipose tissue, as well as decreased tissue and circulating inflammatory markers. TLR4 are activated by fatty acids and endotoxinemia (a marker of gut permeability), features of both obesity and metabolic syndrome, resulting in activation of nuclear factor-kappa beta (NFK-β) and increased release of inflammatory biomediators such as IL-6, IL-1, TNF-α, and monocyte chemotactic protein-1 (MCP-1), which play a role in the pathophysiology of obesity and metabolic syndrome [12]

Such data indicate that the activation of TLR-4 on hepatocytes contributes to obesity-associated inflammation and insulin resistance, and suggest that targeting hepatocyte TLR-4 might be a useful therapeutic strategy for the treatment of type 2 diabetes [13]. Given the very strong

association of metabolic syndrome and obesity in that pathogenesis of NAFLD and NASH, targeting TLRs can potentially forestall the end-organ complication of insulin resistance syndrome, including the development obesity-related chronic liver disease.

3.4 Endotoxemia, LPS and NASH

Emerging data have shown a close association between compositional changes in gut microbiota and the development of NAFLD [14, 15]. The change in gut microbiota may alter nutritional absorption and storage. In addition, gut microbiota are a source of TLR ligands, and their compositional change can also increase the amount of TLR ligands delivered to the liver. TLR ligands can stimulate liver cells to produce proinflammatory cytokines. Therefore, the gut-liver axis has attracted much interest, particularly regarding the pathogenesis of NAFLD [16]. The abundance of the major gut microbiota, including Firmicutes and Bacteroidetes, has been considered a potential underlying mechanism of obesity and NAFLD, but the role of these microbiota in NAFLD remains unknown [17]. Several reports have demonstrated that certain gut microbes are associated with the development of obesity and NAFLD. For instance, a decrease in Akkermansia muciniphila causes a thinner intestinal mucus layer and promotes gut permeability, which allows the leakage of bacterial components. Interventions to increase Akkermansia muciniphila improve the metabolic parameters in obesity and NAFLD. In children, the levels of Escherichia were significantly increased in NASH compared with those in obese control. Escherichia can produce ethanol, which promotes gut permeability [18]. Another possibility by which the altered intestinal microbiota may promote obesity and fatty liver is an increase in LPS-containing microbiota [19] and increased intestinal permeability to LPS [20]. Bacterial LPS stimulates Kupffer cells and participates in the pathogenesis of hepatic fibrogenesis [21]. Thus, normalization of gut microbiota using probiotics or prebiotics is a promising treatment option for NAFLD. In addition, TLR signaling in the liver is activated, and its downstream molecules, such as proinflammatory cytokines, are increased in NAFLD. To date, TLR2, TLR4, TLR5, and TLR9 have been shown to be associated with the pathogenesis of NAFLD. Therefore, gut microbiota and TLRs are targets for NAFLD treatment. Thus, plausible evidence exists to support the use of a TLR-4 antagonist in the treatment of NASH.

3.5 JKB-121

JKB-121, a methylenemorphinandiol compound, is a long-acting small molecule that is efficacious as a weak antagonist at the TLR-4 receptor, thus reducing the likelihood of affecting the innate immune system. It is an off-patent FDA-approved drug (as a non-selective opioid antagonist) with no known safety issues.

JKB-121 has been shown to prevent the LPS induced inflammatory liver injury in a methionine/choline deficient (MCD) diet fed rat model of NAFLD [22]. In vitro, JKB-121 neutralized or reduced the LPS-induced release of IL-6 and IL-12 in murine macrophages, deactivated hepatic stellate cells (HSCs), inhibited HSCs proliferation, collagen expression and α-SMA expression in cultured HSCs (spontaneously activated) [23]. In vivo, JKB-121 neutralized the stimulated release of TNF-a, IL-6, and IL-18 by LPS/D-Gal in mice. Moreover, JKB-121 also neutralized LPS-induced p38 phosphorylation and TNF-α in isolated rat Kupffer cells [22]. The data supports that JKB-121, a TLR4 antagonist, exhibits the potential for treating

NASH. Based on the evidence reviewed above, inhibition of the TLR4 signaling pathway may provide an effective therapy in the prevention of inflammatory hepatic injury in NAFLD, the progression to NASH and fibrosis, and ultimately cirrhosis.

4. STUDY CONDUCT STATEMENT

This study will be conducted in compliance with the protocol, according to current United States federal regulations (21, Code of Federal Regulations [CFR] Parts 50, 56 and 312D) and the principles of International Conference on Harmonization (ICH) (ICH E6 1997) Good Clinical Practice (GCP), Food and Drug Administration (FDA) guidelines and the Declaration of Helsinki.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

To evaluate the safety and potential efficacy of two dose levels of JKB-121 (5 mg twice daily and 10 mg twice daily) in reducing liver fat compared to placebo.

5.1.2 Secondary Objective(s)

The secondary objectives are:

- Determine the pharmacokinetic profile of JKB-121 in NASH
- Assess the impact of treatment with JKB-121 on metabolic markers
- Assess the impact of treatment on liver enzymes (serum ALT) over 24 weeks.
- Establish the recommended dose for future NASH treatment studies.
- To evaluate changes of NASH related biomarkers such as adiponectin, leptin, ghrelin, TNF-alpha, TGF-beta, hyaluronic acid and MMP-2.

5.1.3 *Exploratory Object(s)*

Investigate the impact of treatment of potentially relevant inflammatory and metabolic biomarkers associated with NASH.

5. 2 Endpoints

5.2.1 Primary Endpoint

The primary endpoint of this study is safety and potential efficacy of two dose levels of JKB-121 (5 mg twice daily and 10 mg twice daily) in reducing liver fat compared to placebo.

5.2.2 Secondary Endpoint(s)

The secondary endpoint of the study is time to remission (TTR). TTR is defined as time in weeks from randomization to liver function remission as defined as two consecutive ALT values within normal range (< 20 U/mL for woman and < 30 U/L for men) or 20% reduction from the baseline during study period.

- TTR rate in ALT at the end of the treatment period.
- Assess the pharmacokinetic profile of JKB-121 in NASH

- Change on metabolic markers associated with NASH
- Change in liver enzymes (serum ALT) over 24 weeks.
- Change of NASH related biomarkers such as adiponectin, leptin, ghrelin, TNF-alpha, TGF-beta, hyaluronic acid and MMP-2.

6. STUDY DESIGN

This is a randomized, double-blind, placebo controlled, parallel-group, phase II trial of JKB-121 in treating subjects with biopsy-proven NASH.

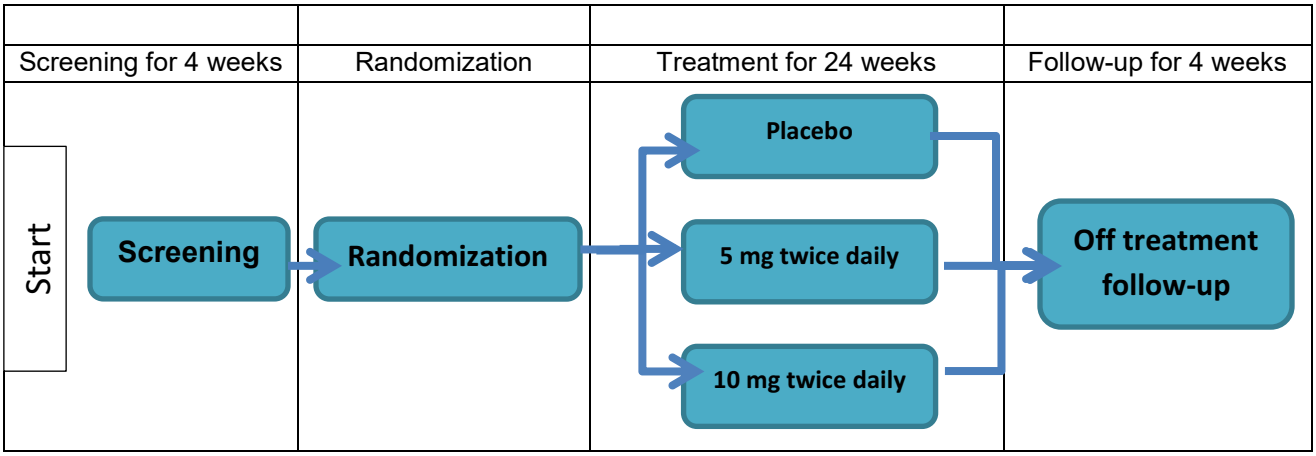
Subjects will be randomized at study entry to placebo twice daily, 5 mg twice daily, or 10 mg twice daily of JKB-121 orally to improve NASH with regard to biochemical and MRI/MRS features of NASH in a 1:1:1 ratio.

- Treatment Group 1: placebo twice a day orally
- Treatment Group 2: 5mg of JKB-121 twice a day orally
- Treatment Group 3: 10 mg of JKB-121 twice a day orally

The maximum time on study drug for each subject is 24 weeks with an additional 4 weeks of follow-up.

This allocation is shown diagrammatically in Figure 1

Figure 1: Study Schema



6.1 Duration of the Study

Subjects will be on treatment for up to 24 weeks. Subjects who experience adverse reaction will discontinue study drug but will continue to complete all protocol-specified follow-up visits, assessments and evaluations. They will then continue to be followed up for the remaining duration of the study. Unless otherwise recommended by the Independent Data Review Board (IDRB) or regulatory authorities, the study will continue until it accrues a minimum of 60 subjects complete the study.

Until formal conclusion of the study, subjects, investigators and all site study personnel will be remain blinded as to treatment allocation, except in the event of a medical emergency which necessitates unblinding.

6.2 Sponsor's Termination of Study

Although TaiwanJ Pharmaceuticals has the intention of completing the study, the company reserves the right to discontinue the study at any time for clinical or administrative reasons. If ≥ 30% of subjects received study treatments has a hepatic AE of > Grade 3 or a non-hepatic, drug-related AE of ≥ Grade 3, then the study will be terminated.

6.3 Reasons for Subject Withdrawal

Any subject may withdraw his or her consent at any time.

Subjects will be monitored carefully during the study. Any subject who experiences an increase in ALT value greater than 3X baseline during the treatment period will be monitored weekly. If ALT values increase to greater than 5X the baseline ALT will be rechecked within 48 hours and if value remains > 5X baseline, the subject will be withdrawn from the study. The subject will then be followed, and ALT, AST, prothrombin time, and total bilirubin will be monitored every 2 weeks thereafter until the value that led to the subject's discontinuation of therapy has returned to the baseline value (Baseline for prothrombin time and total bilirubin is the value obtained at the screening).

Other reasons for withdrawal from the study may include, but are not limited to the following:

- Subject is in violation of the protocol, defined as refusal or inability to adhere to prescribed dosing and/or follow-up regimen.
- Subject is lost to follow-up.
- Subject experiences a serious or intolerable AE that, in the medical opinion of the principal investigator, warrants discontinuation.
- Subject has laboratory safety assessments that reveal clinically significant worsening from baseline values.
- Subject develops, during the course of the study, symptoms or conditions listed in the exclusion criteria.
- Subject requires a medication that is prohibited by the protocol.
- Investigator or TaiwanJ Pharmaceuticals requests an early discontinuation of the trial for any reason.

The investigator may also withdraw a subject upon the request of TaiwanJ Pharmaceuticals or if TaiwanJ Pharmaceuticals terminates the study. Upon occurrence of a serious or intolerable AE, the principal investigator will confer with TaiwanJ Pharmaceuticals. If a subject is discontinued because of an AE, the event will be followed until it is resolved or until the principal investigator or sub-investigator deems the event to be chronic or the subject to be stable.

6.3.1 Handling of Withdrawals

Subjects should be encouraged to complete all study procedures; however, all subjects are free to withdraw from the study at any time. Subject participation in the trial may be stopped at any time at the discretion of the investigator or at the request of TaiwanJ Pharmaceuticals.

When a subject withdraws from the study, the reason(s) for withdrawal shall be recorded by the investigator in the source documents and on the relevant page of the CRF. Whenever possible, all subjects who withdraw from the study prematurely will undergo all end-of-study (Visit 8) and follow-up (Visit 9) assessments. Subjects who fail to return for final assessments will be contacted by the site in an attempt to have them comply with the protocol. Follow-up assessment for early withdrawals will be attempted through 2 documented telephone calls to the subject followed by 1 registered letter. All attempted contacts should be detailed thoroughly in the source documentation.

It is vital to obtain follow-up data on any subject withdrawn because of an AE or serious adverse event (SAE). In any case, every effort must be made to undertake protocol-specified safety follow-up procedures.

6.3.2 Replacements

Subjects who are withdrawn from the study will not be replaced.

7. *STUDY* POPULATION

7.1. Number of Subjects

A target of 60 subjects will be enrolled. It is envisioned that recruitment of subjects will occur at approximately 12 sites. Subjects who discontinue treatment per protocol or withdraw consent after initiating treatment will not be replaced. Any subject who fails a screening investigation may, at the discretion of the investigator, be re-tested, providing that they remain able to complete screening assessments within the protocol-specified screening period (up to 4 weeks).

7.2. Assignment of Subjects Numbers

Patients will be randomized in the order in which they are enrolled into this double-blind study by using a computer-generated randomization schedule. TaiwanJ Pharmaceuticals will prepare the schedule prior to the start of the study. This number will designate which allocated treatment a patient will receive. The randomization schedule will be a ratio of 1:1:1 to one of the following three treatment groups:

- JKB-121 5 mg tablet twice daily orally
- JKB-121 10 mg tablet twice daily orally
- Placebo as tablet twice daily orally

The patient number will be used to identify the patient throughout the duration of the study and will be used on the CRF.

8. SUBJECT SELECTION

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

Eligibility criteria may not be waived by the investigator and are subject to review in the case of a Good Clinical Practices (GCP) or a regulatory authority audit. Any questions regarding a subject's eligibility should be discussed with TaiwanJ site management or study management personnel (see protocol cover page) prior to enrollment.

8.1. Subject Inclusion Criteria

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

1. Age ≥ 18 years
2. Provision of written informed consent
3. Biopsy-proven NASH within 12 months prior to randomization as reviewed by central pathologist with:
    - NASH activity score (NAS) of 4 or more
    - 10% or more macrovesicular steatosis
    - cytologic ballooning score of at least 1 point
    - hepatic fibrosis of at least 1 point
    - hematoxylin and eosin (H&E) stained slides and/or paraffin block available for independent assessment
4. ALT > 40 U/L for women and > 60 U/L for men at screening and at least once in the previous 12 months.
5. MRI with > 6% hepatic steatosis
6. HbA1c of ≤ 9.0
7. Lipid lowering agents (i.e. statins) and allowable antidiabetic treatment (i.e. metformin or sulfonylurea) must be stable at screening and during the study.
8. Agree to use of effective contraceptive measures if female of child bearing potential.

8.2. Subject Exclusion Criteria

1. The presence of any of the following will exclude a subject from study enrollment: Any chronic liver disease other than NASH (i.e., drug-induced, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis, A1AT deficiency, Wilsons disease)
2. Cirrhosis, as assessed clinically or histologically
3. Presence of vascular liver disease
4. BMI ≤ 25 kg/m^2
5. Excessive alcohol use (> 20 g/day) within the past 2 years

6. AST or ALT > 250 U/L.
7. Type 1 diabetes mellitus
8. Bariatric surgery in the past 5 years.
9. Weight gain or weight loss of > 5% in past 6 months or > 10% change in past 12 months.
10. Contraindication to MRI
11. Inadequate venous access
12. HIV antibody positive, hepatitis B surface antigen positive (HBsAg), or HCV RNA positive.
13. Receiving an elemental diet or parenteral nutrition
14. Chronic pancreatitis or pancreatic insufficiency
15. Any history of complications of cirrhosis (i.e. ascites, hepatic encephalopathy, or portal hypertensive bleeding), even if absent or optimized with medical management at time of screening
16. Concurrent conditions:
    a) Inflammatory bowel disease
    b) Unstable angina, myocardial infarction, transient ischemic events, or stroke within 24 weeks of screening
    c) Ongoing infectious, immune mediated disease within previously 1 years
    d) Any malignant disease (other than basal cell carcinoma of the skin) within previous 2 years
    e) Prior solid organ transplant
    f) Any other concurrent condition which, in the opinion of the investigator, could impact adversely on the subject participating or the interpretation of the study data.
17. Concurrent medications including:
    a) Anti-NASH therapy(s) initiated ***after*** the liver biopsy diagnosing NASH. Anti-NASH therapies include S-adenosyl methionine (SAMe), milk thistle, and vitamin E.
    b) Antidiabetic mediation which may impact NASH histology initiated ***after*** the liver biopsy diagnosing NASH including thiazolidinediones (glitazones), dipeptidyl peptidase 4 inhibitors (gliptins) or glucagon-like peptide 1 analogs.
    c) Immune modulatory agents including systemic steroids, methotrexate, anti-TNF-α therapies (infliximab, adalimumab, etanercept) or anti-integrin therapy (namixilab).
    d) UGT2B7 enzyme inhibitors (e.g., diclofenac, fluconazole, medroxyprogesterone acetate, meclofenamic acid)
    e) UGT2B7 inducers (e.g., dexamethasone, phenobarbital, rifampicin, omeprazole)
    f) Chronic opioid use within 28 days prior to screening (Day -28)
18. Self-reported or known marijuana or illicit drug use 30 days before the screening
19. The following laboratory abnormalities:
    a) HbA1c > 9.0%
    b) Neutrophil count < 1.0×10^9 / L
    c) Platelets < 100 10^9 / L
    d) Hemoglobin < 10 g/dl
    e) Albumin < 3.5 g

f) International normalized ratio (INR) > 1.3
g) Any elevation of bilirubin above normal (unless Gilbert's syndrome or extrahepatic source as denoted by increased indirect bilirubin fraction).
h) Serum creatinine > 1.5 mg/dl
i) Creatinine clearance ≤ 50 ml/minute calculated by Crockroft-Gault

20. Pregnancy or breastfeeding.
21. Women, of childbearing age, who are not willing to practice effective contraception (i.e., barrier, oral contraceptives, or past medical history of hysterectomy) for the 48-week duration of the trial and for 1 month after the last administration of the drug. Women with documented (not reported) oophorectomy or hysterectomy are considered sterile. Women with tubal ligation are at small risk and should have pregnancy test at screening.
22. Participation in an investigational drug study within past 30 days.

9. STUDY MEDICATION

9.1 Study Medication Identification

Investigational drug supplies will be provided by TaiwanJ Pharmaceuticals and will consist of the following three treatment arms:

1. Placebo tablet twice daily
2. JKB-121 5mg tablet twice daily
3. JKB-121 10mg tablet twice daily

9.1.1 Investigational Drug

JKB-121 is a tablet which contains nalmefene (as hydrochloride) in different strength of 5mg, 10mg and with GRAS (generally recognized as safe) excipients. The tablet is white to off white, round and plain biconvex tablet.

9.1.2 Reference Drug:

Placebo will be supplied as a similar appearing tablet which also contains lactose.

9.2 Justification for the dose chosen

JKB-121 is effective at a dose of 1 mg/kg in reducing elevated serum liver enzyme ALT in D-Gal/LPS mouse model (Szabo *et al.* Alcohol Clin Exp Res 2005). Using the FDA guidelines (fda/cder/guidance/5541fnlcln1.doc 07/06/05, page 29), 1 mg/kg dose in mouse corresponds to 6.4 mg for an 80 Kg patient. In light of these results, the JKB-121 doses at 5, and 10 mg bid have been chosen, based on safety and potential efficacy, as the ideal doses for this study.

The overall previous human safety profile of chronically administered nalmefene is very positive. Trials on alcohol dependence were conducted with once or twice a day tablet, while those on pruritus were treated with tablet twice or three times a day. The duration of testing in at least one study in cholestatic pruritus patients lasted for over 26 months at 10 mg and higher twice daily, while maximal dose of 120 mg bid was given to 2 patients for 4 months. The daily administered doses in these trials were 2 to 12 times higher than the 5mg and 10 mg bid in proposed JKB-121 IND application (Refer to Investigational Brochure pages 15-17).

9.3 Rationale for a placebo group

In order to assess the efficacy of an agent in NASH, a placebo-arm is needed to determine its relative efficacy in improving NASH histology beyond that achieved with a placebo [24]. Currently, there are no FDA approved therapies for NASH. The recently completed PIVENS trial for NASH [25] did not find either vitamin E or pioglitazone to be uniformly effective. The study demonstrated that 43% of vitamin E treated patients and 34% of pioglitazone treated patients met a pre-determined histological endpoint compared to 19% of placebo treated patients (P = 0.001 and P = 0.04 for each drug respectively) [25]. Previous non-randomized and pilot studies have shown the efficacy of several agents such as ursodiol [26] and betaine [27] in the treatment of NASH, but follow-up randomized, placebo controlled studies failed to show improvement in liver histology in patients with NASH beyond that observed in placebo groups. To have the highest quality of evidence to test our hypothesis, the proposed JKB-121 NASH trial is a randomized, double-masked, placebo-controlled study design. As there is no proven pharmacologic therapy for NASH, using a placebo for comparative purposes is justified.

9.4 Study Medication Packaging and Labeling

Study drug will be labeled in accordance with GMP and all applicable regulations.

The label will include full manufacturing details, storage instructions and caution statements. Study drug will be dispensed to subjects at each scheduled visit, or at specific pharmacy visits, according to the dispensing practices prevailing at the site pharmacy.

Figure 1 Sample Drug Bottle Label

9.4.1 Storage and Stability

Study medication must be stored according to labeled storage conditions, and in a secure place in the research facility. This study product must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply product to other

investigators or clinics, or allow the drug supplies to be used other than as directed by this protocol without prior authorization from TaiwanJ Pharmaceuticals.

9.4.2 Drug Preparation/Administration/Dispensing

Each patient will receive one bottle of study medication at each visit during the treatment period. Bottles will contain single panel label. One part of the label, containing study and patient information is attached to the carton. Directions on the bottles will indicate the following:

- JKB-121 is for oral use.
- The tablet should be swallowed whole.
- The tablet should not be divided or crushed because nalmefene may cause skin sensitivity when in direct contact with the skin Take one tablet within 12 hours of the initial dose, but no sooner than 8 hours after initial dose.

9.5 Drug Accountability

Adequate records on receipt, use, return, loss or other disposition of medication must be maintained. A specific drug accountability form supplied by TaiwanJ Pharmaceuticals or computer records used by the pharmacy at the investigational site, can be used to provide drug accountability information. Drug compliance will be based on pill count. Paper diaries will be provided to subjects, reviewed at each visit, signed by study coordinator or investigator. Other required data includes relevant dates, quantities, and patient identification (subject number and initials) for patients who receive study. Patients will be instructed to return all unused study medication to the investigator, in the original bottles.

At the end of the study, all unused products will be collected and counted by the local Investigational Drug Pharmacy monitor or designee and destroyed according to institutional pharmacy policies and procedures.

10. SCHEDULE OF EVENTS

10.1 Tests and Evaluations

No subject may be permitted to undergo any protocol-specified investigations or interventions in this study until they have provided a signed, dated, written informed consent.

All tests and evaluations must be performed prior to that day's administration of study drug treatment. Subjects should undergo all tests and evaluations specified in the protocol, regardless of whether they remain on study drug. Insofar as possible, all blood samples should be collected at approximately the same time of day in each visit, in order to minimize diurnal variation

The required procedures for patient evaluation at each study visit are outlined in the study. Schedule of Visits and Procedures are in Sections 10.2 and detail items in section 10.3 through 10.4.8. Detailed instructions for specific procedures are presented in Section 11.

10.2 Schedule of Visits and Procedures: Detailed in Table 10.2.1

10.2.1. Visit Schedule Table

	Screening Period	Enrollment	Treatment period						End of Treatment	Follow-up Visit
Visit	0	1	2	3	4	5	6	7	8	9
Week	up to 4 wks	0	2	4	8	12	16	20	24	28
Day (Visit window)	-1 to -28	1	14 (±4)	28 (±4)	56 (±4)	84 (±4)	112 (±4)	140 (±4)	168 (±4)	196 (±4)
Determination of eligibility	X	X								
Review of medical history	X	X								
Obtain informed consent	X									
Randomization		X								
Study drug dispensing		X	X	X	X	X	X			
Managing drug accountability			X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X		X	X	X	X	X	X	X
Serum hCG (if applicable)	X									
Urine hCG (if applicable)		X		X	X	X	X	X	X	X
Hematology panel	X	X	X	X	X	X	X	X	X	X
Comprehensive metabolic panel	X	X	X	X	X	X	X	X	X	X
Lipid panel		X		X	X	X	X	X	X	X
Glycosylated hemoglobin	X	X		X	X	X	X	X	X	X
HOMA-IR	X	X		X	X	X	X	X	X	X
Urinalysis	X					X			X	X
Electrocardiogram	X					X			X	
Liver biopsy (if prior biopsy > 12 mo)	X									
PK study		X*			X		X		X	
Review concomitant meds	X	X	X	X	X	X	X	X	X	X
Review adverse events**		X**	X	X	X	X**	X	X	X**	X
MRI for hepatic steatosis	X					X			X	
CK-18		X				X			X	X
Stored serum for exploratory measures and noninvasive biomarkers of fibrosis		X				X			X	X
Exit Interview										X

* Frequent sampling PK study on Day 1 for Substudy subjects only. All patients to have a pre-dose trough level drug measures (6 ml blood) at treatment Weeks 8, 16, and 24 (Visits 4, 6, and 8).
** Hospital Anxiety and Depression Scale and Columbia Suicide Severity Scale

10.3 Study Procedures

Complete study procedures to be conducted for each subject enrolled in the study are presented in tabular form in Table 10.2.1 and described in the text that follows.

Any deviation from protocol procedures should be documented.

10.3.1 Subject Enrollment and Treatment Assignment

After written informed consent has been obtained and eligibility to participate established, investigative site personnel will obtain the subject's identification number and study drug kit assignment from investigational drug pharmacy or IVRS/IWRS system. Patients will be stratified into treatment arms based diabetes status.

10.3.2 Screening Period (within 4 weeks before randomization)

Prior to performing any study procedures, an informed consent form will be reviewed and signed by each subject. Subjects will be screened within 4 weeks before starting study treatment to determine eligibility for participation in the study, unless otherwise noted below. Subjects who fail to meet eligibility due to an abnormal laboratory results (other than what is otherwise explained by NAFLD alone) may undergo retesting of the abnormal laboratory test during the screening window. This will be done at the discretion of the investigator and with prior approval from the Sponsor or Medical Monitor.

Sufficient time should be allowed for all screening laboratory studies to be resulted prior to scheduling subject for MRI testing. The following will be performed and documented at screening:

- Obtain subject screening number
- Obtain medical history and complete physical examination
- Record vital signs (blood pressure, heart rate, respiratory rate, body temperature)
- Record height, weight and body mass index
- Study pathologist to review screening liver biopsy (within 12 months of screening) for confirmation and staging of NASH as defined by NASH Clinical Research Network (NASH CRN) Grading and Staging System [28].
- MRI to be performed and study radiologist to review and confirm > 6% hepatic steatosis for inclusion
- Review concomitant medications that the subject has taken within 30 days prior to screening.
- Assess and record pre-dosing signs / symptoms
- Determine study eligibility
- Electrocardiogram
- Liver biopsy may be performed if subject has historical biopsy c/w NASH and biopsy is > 12 months of screening or subject at increased risk for NASH (increased liver enzymes with > 3 features of metabolic syndrome)

- Urinalysis
- Urine drug screen for amphetamine, cocaine and opiate drug screening
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and gamma-glutamyl transferase (GGT).
    3. Fasting glucose and insulin level for HOMA-IR
    4. Glycosylated hemoglobin
    5. Prothrombin time and International normalized ratio (INR)
    6. Serum pregnancy test (only for women of childbearing potential).
    7. HCV RNA
    8. HBsAg
    9. Serum and plasma samples for non-invasive biomarkers related to liver injury pathophysiology and/or TLR-4 pathway
- Record any adverse events occurring after signing the consent form

10.3.3 Enrollment Visit (Day 1, Visit 1)

Subjects returning to the clinic for randomization at Day 1 must be in a fasted state for at least 8 hours prior to sample collection prior dosing for this visit, which is a requirement for some of the blood test that will be performed as noted below.

Subjects will be randomized to study drug kit assignment and receive their Subject Identification Number prior to the first dose of study drug being administered. The following will be performed for all subjects who meet the inclusion criteria and not of the exclusion criteria

- Confirm that the subject continues to be eligible for participation
- Record vital signs
- Record weight
- Physical examination
- Hospital Anxiety and Depression Scal and Columbia Suicide Severity Scale Questionnaires.
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT.
    3. Fasting glucose and insulin level for HOMA-IR
    4. Lipid panel
    5. Glycosylated hemoglobin
    6. Urine pregnancy test (only for women of childbearing potential).

7. Serum and plasma samples for non-invasive biomarkers related to liver injury pathophysiology and/or TLR-4 pathway
8. ELF™ test—Subjects must be an a fasted state prior to the sample collection
9. FibroSURE/ FibroMAX/ FibroTest—Subject must be in a fasted state prior to the sample collection
10. Additional blood sample needs to be collected for pharmacokinetic study.

- Counsel subjects on weight loss, diet and exercise
- Record any adverse events occurring after signing of the informed consent form
- Record all medications that the subject has taken since prior visit
- Dispense study drug

10.3.3.1 PK Substudy Subjects Only: From volunteered subjects for intensive pharmacokinetic studies: Blood samples will be obtained *prior to 12 hour dosing* (predose and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12, and 24 (next day) hours post dose administration in three volunteered subjects from each treatment group (5 mg and 10 mg bid). Additional pre-dose blood sample for trough PK level at Day 1, weeks 8, 16, and 24 will be collected for assessment of steady state PK analysis as well as biomarker study.

Biosample collection and handling procedures will be detailed before the implementation of the study. These will be based on information obtained from a validated analytical method for nalmefene with respect to its stability, handling, and storage conditions of the biosamples.

Six mL of blood will be collected from fasting (at least 8 hours) subjects on Day 1 prior to dosing, and during Visits 4, 6, and 8 for measurements of the plasma concentrations of JKB-121. In a subset of volunteers who consent to participate in PK Substudy,blood samples will be collected on Day 1 pre-dose and at each sampling point post-dose for pharmacokinetic evaluation as described in Section 10.3.2.

10.4 Treatment Assessments

10.4.1 Visit 2 (Week 2 ± 4 days)

Subject may be in a nonfasted state for this visit. Starting with week 2, subjects will return to the investigative site every 4 weeks during the treatment phase of the protocol for 24 weeks.
During the Week 2 visit (+/- 4 days), the subject will return to the investigational site and the following will be completed:

- Verify the study drug was administered correctly
- Document drug accountability and any problems with drug administration in the source documents.
- Record vital signs
- Record weight
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, LDH magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT.
- Counsel subjects on weight loss, diet and exercise
- Record any adverse events occurring after signing of the informed consent form
- Record all medications that the subject has taken since prior visit

10.4.2 Visits 3, 4, 6 and 7 (week 4, 8, 16 and 20 ± 4 days)

Subjects must be in a fasted state for at least 8 hours prior to sample collection at these visits, which is a requirement for some of the blood tests that will be performed as noted below. The following will be performed for all subjects who meet the inclusion criteria and not of the exclusion criteria

- Verify the study drug was administered correctly
- Document drug accountability and any problems with drug administration in the source documents.
- Record vital signs
- Physical examination
- Record weight
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT.
    3. Fasting glucose and insulin level for HOMA-IR
    4. Lipid panel
    5. Glycosylated hemoglobin
    6. Urine pregnancy test (only for women of childbearing potential).
    7. Additional trough (pre-dose) blood sample needs to be collected for pharmacokinetic study at visits 4 (week 8) and 6 (week 16).
- Counsel subjects on weight loss, diet, and exercise
- Record any adverse events occurring after signing of the informed consent form
- Record all medications that the subject has taken since prior visit

10.4.3 Visit 5 (week 12 ± 4 days)

Subjects must be in a fasted state for at least 8 hours prior to sample collection at this visit, which is a requirement for some of the blood tests that will be performed as noted below:

- Verify the study medication
- Document drug accountability and any problems with drug administration in the source documents.
- Record vital signs
- Physical examination
- Record weight
- Electrocardiogram
- Hospital Anxiety and Depression Scale and Columbia Suicide Severity Scale
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT.
    3. Fasting glucose and insulin level for HOMA-IR
    4. Lipid panel
    5. Glycosylated hemoglobin
    6. Urine pregnancy test (only for women of childbearing potential)
    7. Serum and plasma samples for non-invasive biomarkers related to liver injury pathophysiology and/or TLR 4 pathway
    8. ELF test—Subjects must be an a fasted state prior to the sample collection
    9. FibroSURE/ FibroMAX/ FibroTest—Subject must be in a fasted state prior to the sample collection
    10. Urinalysis
    11. Sample collection/storage for CK-18 and exploratory measures
    12. Perform MRI for quantitation of hepatic fat
- Counsel subjects on weight loss, diet and exercise
- Record any adverse events occurring after signing of the informed consent form
- Record all medications that the subject has taken since prior visit

10.4.4 End of Treatment Visit 8 (week 24 ± 4 days)

Subjects must be in a fasted state for at least 8 hours prior to sample collection at these visits, which is a requirement for some of the blood tests that will be performed as noted below. If a subject has discontinued treatment, every attempt should be made to have the subject return to the site and complete the procedures / assessments that are required at the End of Treatment Visit (Week 24) and the Follow-up Visit (Week 28). The following will be performed for all subjects who meet the inclusion criteria and not of the exclusion criteria

- Verify the study drug was administered correctly
- Document drug accountability and any problems with drug administration in the source documents.
- Record vital signs
- Physical examination
- Record weight
- Electrocardiogram
- Urinalysis
- Hospital Anxiety and Depression Scale and Columbia Suicide Severity Scale
- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT/
    3. Fasting glucose and insulin level for HOMA-IR
    4. Lipid panel
    5. Glycosylated hemoglobin
    6. Urine pregnancy test (only for women of childbearing potential).
    7. Serum and plasma samples for non-invasive biomarkers related to liver injury pathophysiology and/or TLR-4 pathway
    8. ELF test—Subjects must be an a fasted state prior to the sample collection
    9. FibroSURE/ FibroMAX/ FibroTest—Subject must be in a fasted state prior to the sample collection
    10. Additional blood sample needs to be collected for pharmacokinetic study
- Counsel subjects on weight loss, diet and exercise
- MRI for quantification of hepatic fat
- Record any adverse events occurring after signing of the informed consent form
- Record all medications that the subject has taken since prior visit

10.4.5 Follow-up Visit 9 (week 28 ± 4 days) and Exit Interview

Subjects must be in a fasted state for at least 8 hours prior to sample collection at these visits, which is a requirement for some of the blood tests that will be performed as noted below. If a subject has discontinued treatment, every attempt should be made to have the subject return to the site and complete the procedures / assessments that are required at the End of Treatment Visit (Week 24) and the Follow-up Visit (Week 28). The following will be performed for all subjects who meet the inclusion criteria and not of the exclusion criteria

- Verify the study drug was administered correctly
- Document drug accountability and any problems with drug administration in the source documents.
- Record vital signs
- Physical examination
- Record weight

- Complete blood samples for:
    1. Complete blood count with differential
    2. Blood chemistry including: ALT, AST, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, magnesium, phosphorus, potassium, sodium, total and direct bilirubin, protein, uric acid, and GGT.
    3. Fasting glucose and insulin level for HOMA-IR
    4. Lipid panel
    5. Glycosylated hemoglobin
    6. Urinalysis
    7. Urine pregnancy test (only for women of childbearing potential).
    8. Serum and plasma samples for non-invasive biomarkers related to liver injury pathophysiology and/or TLR-4 pathway
    9. ELF test—Subjects must be an a fasted state prior to the sample collection
    10. FibroSURE/ FibroMAX/ FibroTest—Subject must be in a fasted state prior to the sample collection
- Counsel subjects on weight loss, diet and exercise
- Record any adverse events occurring after signing of the informed consent formRecord all medications that the subject has taken since prior visit

10.4.6 Assessments for Early Discontinuation from Study

- Subjects discontinuing early should have an End of Treatment Visit performed, if possible and then have a Follow-up Visit 4 weeks later

10.4.7 Criteria for Discontinuation of Study Treatment

Study medication may be discontinued in the following instances

- Intercurrent illness that would, in the judgment of the investigator, affect assessment of clinical status to a significant degree
- Any concern for drug-induced liver injury as defined by change from baseline liver aminotransferases according to the following criteria:
    - ALT or AST > 10 x ULN
    - ALT or AST > 5 x ULN for more than 2 weeks
    - ALT or AST > 3 x ULN and total bilirubin > 2 x ULN or INR > 1.5
    - ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant abdominal pain, fever, rash or eosinophilia (> 5%).
- Unacceptable toxicity, as defined in the toxicity management section of the protocol, 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
- Subject noncompliance
- Pregnancy during the study
- Sponsor discretion

- Discontinuation of the study at the request of TaiwanJ. Pharmaceuticals, any Regulatory Authorities or an IRB/IEC.

10.5 Pregnancy

An effective method of birth control must be used during the course of the study, in a manner such that the risk of birth control failure is minimized. Prior to enrollment, study candidates who are women of childbearing potential or men whose partners are women of child bearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. All women of childbearing potential must have a negative pregnancy test within 72 hours prior to receiving JKB-121 per the eligibility criteria. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of human chorionic gonadotropin (HCG). If the pregnancy test is positive, the patient must not receive JKB-121 and may not be enrolled in the study.

Women of childbearing potential is defined as females who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy), are not postmenopausal (defined as amenorrhea $\geq$12 consecutive months or women on hormone replacement therapy with a documented serum follicle stimulating hormone level > 35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products, such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides), to prevent pregnancy, who are practicing abstinence, and who have a sterile partner (e.g., vasectomy) should be considered of child bearing potential.

All study participants should be instructed to contact the investigator immediately if they suspect they or their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

In the case of pregnancy, JKB-121 will be discontinued and protocol-required procedures for study discontinuation and follow-up must be performed on the patient unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy testing and assessment as would otherwise be standard for care should follow.

In the event of a subject becoming pregnant during the study, they must immediately and permanently discontinue study drug. However, such subjects should be encouraged to continue completing all scheduled visits, evaluations and assessments as per the protocol, with the exception of radiological assessments such as MRI.

Every attempt should be made to ascertain the outcome of the pregnancy. The investigator will report the pregnancy to Medpace Clinical Safety within 24 hours of being notified. Medpace Clinical Safety will then forward the Exposure In Utero form to the investigator for completion.

In the event of a subject's partner becoming pregnant, the subject may continue to receive study drug per protocol, but all efforts should be made to ascertain the outcome of the partner's pregnancy. The pregnancy should be reported to the CRO on the appropriate form as described above.

10.6 Occurrence of Hepatocellular Carcinoma in Patients with NAFLD

Any liver tumor occurrence will, in all cases, be diagnosed at the site according to local standard of care practices. In the event of a suspected tumor, subjects should undergo any confirmatory investigations that may be required.

These confirmatory investigations should be performed as soon as possible and in all cases within 21 days of diagnosis. All confirmatory investigations, including any additional scans that may be required, will be the responsibility of the study site.

If, after performing any confirmatory investigations that may be required, the diagnosis of malignancy is confirmed, the subject should immediately and permanently cease administration of study drug. Subjects with confirmed malignancy may then receive any other therapies or treatment modalities that their attending physicians consider appropriate.

However, such subjects should be encouraged to remain in the study and complete all scheduled visits, evaluations and assessments as per the protocol. If the subject elects not to participate in remaining visits, they should be strongly encouraged to complete a 'premature withdrawal' visit or early discontinuation visit as detailed in Section 10.4.

10.7 Death

In the event of a death on study, the investigator should complete the 'death' page of the Case report form (CRF), including the date and reason of death. All attempts should be made to secure access to any autopsy findings and to retrieve the patient compliance diary. The cause of death must be immediately notified to the CRO as a serious adverse event as described in Section 12.1 and, if applicable, to institutional review boards and / or local regulatory authorities, even if the death is considered to be due liver-related mortality.

10.8 Withdrawal of Consent

If a subject voluntarily elects to discontinue participation in the study, administration of study drug must be immediately and permanently ceased. However, such subjects should be encouraged to continue completing all scheduled visits, evaluations and assessments as per the protocol. If a subject elects not to participate in remaining visits, they should be strongly encouraged to complete a 'premature withdrawal' visit, encompassing:

- Recording of adverse events
- Review of concomitant medications and therapies
- Physical examination and vital signs
  Completion of end of treatment visit at time of withdrawal

11.0 STUDY ASSESSMENTS

11.1 Clinical Efficacy Endpoints

The clinical endpoints of this study is time to remission (TTR). TTR is defined as time in weeks from randomization to improvement in hepatic steatosis as measured b MRI and liver function remission as defined as two consecutive ALT values within normal range (< 20 U/mL for woman and < 30 U/L for men) or 20% reduction from the baseline during study period.

11.2 Safety Assessments

11. 2.1 Clinical Safety Assessments

Physical examination and vital signs will be assessed monthly. Subjects must sit quietly for 5 minutes before having their pulse and blood pressure recorded.

All adverse events will be collected from the onset of study drug treatment. Serious adverse events are to be reported immediately to the CRO. Pre-dosing signs and symptoms will be recorded in the screening section of the CRF.

11.2.2 Laboratory Safety Assessments

All laboratory tests following randomization will be performed by a study approved laboratory service. Samples will be drawn at sites and forwarded under appropriate conditions to the approved laboratory. The laboratory will communicate all test results to site study personnel. The Laboratory Assessing Physician will immediately notify sites of any values which lie outside pre-specified ranges. Screening samples, prior to initiation of study drug, may be analyzed at site.

Sites will be immediately notified of any test results that lie outside of pre-specified ranges. Site personnel will not be expected to transcribe results from the laboratory into the CRF, although abnormal results that are considered clinically significant should be entered in the CRF as adverse events. Screening assessments, prior to initiation of study drug, may be performed at sites. Sites are discouraged from performing additional routine investigations locally, but site personnel are permitted to perform any investigations at any time that may be necessary to safeguard patient welfare.

11.3 Pharmacokinetics and Exposure/Response Assessments

Pharmacokinetics of JKB-121 will be assessed by measuring the plasma concentrations of JKB-121 at the steady state (Day 1) in three subjects in each treatment dose, i.e. 5 mg and 10 mg twice daily with the agreement of the patients. The exposure/response relationship of JKB-121 will be explored by measuring the plasma concentrations of JKB-121 in the sparse samples collected in all subjects and correlating them with the changes from baseline in ALT.

11.4 Communication of Laboratory Results to Sites

All laboratory results will be communicated directly to sites by fax or other means. All laboratory results which equate to an NCI CTCAE grade 3 or above toxicity will be escalated for internal medical review by the contract research organization and/or medical monitor.

11.5 Study interventions

Peripheral blood collection: Blood will be collected venipuncture. Serum and plasma will be collected for assessing non-invasive biomarkers related to NAFLD / NASH as well as liver fibrosis (i.e. CK-18, adiponectin, leptin, TNF-α, TGF-β, hyaluronic acid, and MMP-2). MRI Procedures as detailed in Section 11.3**.**

Magnetic Resonance Imaging (MRI)

The purpose of the magnetic resonance imaging (MRI) exam is to quantify the hepatic proton density fat fraction non-invasively in participants who participate in the study. The fat fraction is the proportion of mobile protons in liver tissue attributable to fat and thus is a non-invasive MR-based assessment of liver triglyceride concentration. To quantify the fat fraction, the MRI exam will use a fast spoiled gradient recalled echo sequence that uses a low flip angle to reduce T1 bias, acquires multiple echoes after a single excitation to measure and correct for T2*decay, and uses spectral modeling to address fat-water and fat-fat signal interference effects. The proposed MRI technique measures hepatic fat fraction accurately in human subjects at 1.5T or 3T and across different vendors. The technique provides high within-examination and between-examination precision. Linearity is maintained across the entire relevant biological range from <1% to >40% hepatic fat fraction. The technique is robust to minor variations in acquisition parameters, including those that may be encountered during usage in a clinical trial. The echo sequence proposed for imaging-based hepatic fat fraction quantification can be implemented on any up-to-date clinical scanner and thus can be used at any clinical centers with access to such a scanner. Moreover, the technique is imaging based and covers the whole liver, thus providing information on both the quantity and distribution of hepatic fat fraction. MRI is currently the most accurate noninvasive technique to quantify hepatic fat fraction.

Percutaneous Liver Biopsy

The purpose of the liver biopsy within 12 months of screening is to ensure the presence of NASH and the absence of cirrhosis prior to randomization. The histologic features of liver injury (ie. necroinflammation, ballooned hepatocytes) and hepatic fibrosis is best assessed by liver biopsy as this remains the “gold-standard” for grading and staging NASH. The ideal target population “at risk” for disease progression, and thus, the appropriate cohort of subjects for investigational therapies are those patients with histologic evidence of NASH with hepatic fibrosis. Due to the early phase of investigation and the interdeterminate safety of JKB-121 in patietns with cirrhosis, subjects with histologic features of cirrhosis (stage 4 fibrosis) and/or any history of hepatic decompensation attributable to cirrhosis will be excluded. The screening liver biopsy should be considered for patients with known history of NASH and for whom historical liver biopsy is in excess of 12 months. Given the dynamic changes in NASH which may occur due to life-style changes alone and the reassurance of targeting patient with histologic features of NASH, only patients with biopsy-proven NASH within 12 months of screening will be enrolled.

12. ADVERSE EVENTS: DEFINITIONS AND MANAGEMENT

At the time of written informed consent, the subject must be given the name and telephone number of personnel at the study site who can be contacted in the event of an emergency or to report any medical symptom or untoward medical occurrence that is of concern to the subject. Investigators will closely monitor the subject for adverse events.

All adverse events reported by the subject or observed by study site personnel from time of informed consent until completion of the study or premature withdrawal must be recorded in the subject's CRF as per the CRF instructions. Adverse events are to be recorded regardless of relationship to study drug. Information to be recorded will include, but not be limited to, relationship of the event to study drug and severity of the event. These terms are defined in Sections 12.4.5.5 &12.4.5.6 below. Specific instructions for management of adverse events will be reviewed with study site personnel by the Clinical Monitor prior to study start.

12.1 Definitions

12.1.1 Pre-Dosing Signs and Symptoms

For the purposes of this study, any sign (including a clinically significant laboratory result) or medical diagnosis noted by medical personnel, or symptom reported by the subject that occurs from the time the subject signs the informed consent to the start of study drug treatment is considered to be *preexisting* and should not be reported as an adverse event unless it worsens subsequent to the start of study drug treatment.

12.1.2 Adverse Events

For the purposes of this study, any sign (including a clinically significant laboratory result) or medical diagnosis noted by medical personnel, or symptom reported by the subject, regardless of relationship to investigational drug, that is treatment-emergent is considered to be an adverse event.

TaiwanJ Pharmaceuticals defines treatment-emergent are defined as follows:

1) Has onset any time after the start of investigational drug treatment OR
2) Has worsened since the event was previously reported (this includes worsening of signs, symptoms, abnormal laboratory values, or diagnoses that were present prior to the first dose of investigational drug but then worsened any time after the start of investigational drug treatment)

12.1.3 Classification of Events as Serious

As soon as a subject has given written informed consent to participate in the study, any abnormal sign (including the clinical manifestations of an abnormal laboratory result) or medical diagnosis noted by medical personnel, or symptom reported by the subject regardless of whether or not the subject has received investigational drug is to be classified by the investigator as either a serious or non-serious event using the following definition:

Events are classified as serious if they meet any of the following criteria (in accordance with 21 United States Code of Federal Regulations Part 312.32 and the recommendations of the International Conference on Harmonization (ICH) [Federal Register, October 7, 1997, Vol 62, No. 194, pp 52239-45]):

- Any death
- Any life-threatening event, i.e., an event that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death)
- Any event that requires or prolongs in-patient hospitalization
- Any event that results in persistent or significant disability/incapacity
- Any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study and received investigational drug
- Other medically important events that in the opinion of the investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above

A new diagnosis of cancer should also be considered serious and should be reported on the Serious Adverse Event (SAE) form. In the case of a death on study, the immediate cause of death should be notified as an SAE.

Overdose and pregnancy are not in and of themselves adverse events or SAEs. However, if there are subsequent adverse events or SAEs as a result of overdose or pregnancy, these events should be captured on the adverse event and SAE forms, as appropriate.

Note: A pre-scheduled or elective procedure or a routinely scheduled treatment shall not be considered an SAE, even if the subject is hospitalized, provided the site stipulates that:

a) the pre-scheduled elective procedure or routinely scheduled treatment was scheduled prior to obtaining the subject's consent to participate in the clinical trial,

b) the condition requiring the pre-scheduled elective procedure or routinely scheduled treatment was present before and did not worsen or progress between the subject's consent to participate in the clinical trial and the time of the procedure or treatment, and

c) the pre-scheduled elective procedure or routinely scheduled treatment is the sole reason for admission and intervention.

However, if complications occur during procedures or surgeries, they are considered to be adverse events and should be recorded. Any adverse event classified as serious must be reported immediately to the CRO via the electronic data capture (EDC) system. \.

In the event of a medical emergency when knowledge of the subject's treatment assignment would influence the subject's clinical care, the principal investigator must contact the Medical Monitor to describe the emergency. Only under these circumstances will unblinding of the

subject's treatment assignment be authorized. Refer to Section 12.4 for emergency decoding procedures.

12.3.1 Immediate Reporting of Serious Events

The study sponsor must immediately be made aware of events classified as SERIOUS in order to adhere to all applicable laws and regulations for reporting serious events. Therefore, it is the investigator's responsibility to ensure the following:

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 serious adverse events 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 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.

Safety Contact Information:

Medpace Clinical Safety
Medpace SAE hotline – USA:
Telephone: +1-800-730-5779, ext. 2999 **or** +1-513-579-9911, ext. 2999
Facsimile: +1-866-336-5320 **or** +1-513-579-0444
e-mail: medpace-safetynotification@medpace.com

Follow-Up Reports

The investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment) or the subject 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 (e.g., subject 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.

The investigator must also notify the local review committee, (ie. Institutional Review Board (IRB) or Ethics Committee (EC) as per local IRB/EC requirements. Documentation of these

reports must be kept in the site's study files and will be checked routinely by the Clinical Monitor.

Any serious event that has onset of informed consent that is unresolved at the time the subject permanently discontinues the study must be followed until the event resolves or until the subject's clinical course has stabilized.

12.3.2 Reporting of Serious Events to Regulatory Agencies

After receipt of a serious adverse event report, TaiwanJ Pharmaceuticals (or CRO) will code and classify the serious adverse event and inform regulatory authorities, as necessary, within the required time frames. TaiwanJ Pharmaceuticals or the CRO will complete written safety reports with the assistance of the investigator and other study site personnel, and will submit the reports to regulatory authorities, as needed.**12.3.3 Guidelines for Determining Relationship Causality of Adverse Events**

Not related Any event which does not follow a reasonable temporal sequence from administration of study drug AND that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject

Related An event that follows a reasonable temporal sequence from administration of study drug OR that follows a known response pattern to the suspected drug OR that is improved by stopping the drug or reducing the dose but recurs with re-challenge AND that cannot readily be explained by the subject's clinical state or other modes of therapy administered to the subject

12.3.4 Guidelines for Determining Severity of Adverse Events

All adverse events and serious adverse events will be graded according to the NCI CTCAE v3.0. Any event not listed in the CTCAE will be graded as follows:-

Grade 1 Mild Adverse Event: symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s) but may be given because of personality of subject.

Grade 2 Moderate Adverse Event: symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.

Grade 3 Severe Adverse Event: symptom(s) cause severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with investigational drug; treatment for symptom(s) may be given and/or subject hospitalized.

Grade 4 Life-Threatening or Disabling Adverse Event: symptom(s) symptoms pose significant risk to life or risk of permanent disability.

Grade 5 Death

12.4 Toxicity Management Guidelines

The toxicity management will be based on DAIDS AE Grading system,

Hepatic toxicity:

ALL patients will have increased liver enzymes at baseline. Liver function tests will monitored with each visit. For any elevated ALT 5-10.0 X ULN (Grade 3 or 4) on study drug, repeat testing and evaluation will be performed in 1 week. Treatment will be interrupted for drug-related Grade 3 AE until their AE Grade is less than 3. Liver function tests and assessment will be performed weekly to ensure resolution of Grade 3 AE. Study drug may be resumed following confirmation of resolution of Grade 3 AE or if investigator confirms AE is keeping with patient's underlying NASH. The investigator must assess whether or not Grade 3 AE is clinically or not clinically significant in the setting of NASH and in reference to baseline ALT levels. Increases in liver enzymes > *3 x subject's baseline* will prompt retesting and patient examination within 1 week. Increases in liver enzymes > 5 x subject's baseline will prompt immediate drug discontinuation and withdrawal from study. For non-drug related Grade 3 AE, Investigator will perform further clinical management based on subject's clinical condition and/or other biochemistry results.

DAIDS Grading	Lab Value							Clinical management	
	ALT *		AST		Bilirubin (total)		INR	Non Drug Related	Drug Related
Grade 1	1.25-2.5 x ULN	and/or	1.25-2.5 x ULN	and/or	1.1-1.5 x ULN	and/or	1.2-1.3	Non treatment modification	
Grade 2	2.6-5.0 x ULN	and/or	2.6-5.0 x ULN	and/or	1.6-2.5 x ULN	and/or	1.4-1.5	Non treatment modification	
Grade 3	5.1-10.0 x ULN	and/or	5.1-10.0 x ULN	and/or	2.6-5.0 X ULN	and/or	1.5-2.0	Further clinical management will be judged by Investigator	Treatment interruption may be suggested by Investigator
Grade 4	> 10.0 x ULN	and/or	> 10.0 x ULN	and/or	> 5.0 x ULN	and/or	> 2.0	Treatment termination and withdraw	
* Subjects with elevated ALT > 10.0 ULN will be withdrawn from this study.									

Discontinuation of treatment should be considered if:

- ALT or AST > 10 x ULN
- ALT or AST > 5 x ULN for more than 2 weeks
- ALT or AST > 3 x ULN and total bilirubin > 2 x ULN or INR > 1.5
- ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant abdominal pain, fever, rash or eosinophilia (> 5%).

Other toxicities

For other grade ≥ 3 toxicities, if they are considered drug-related adverse event, treatment discontinuation will be suggested based on investigator's judgment. If they are considered non drug-related AE, further clinical management will be conducted based on Investigator's clinical judgment.

All adverse events occurring during the study and follow-up period should be followed until fully resolution or the event is considered chronic or stable.

12.5 Emergency Procedures

In the event of a medical emergency (i.e., an event that requires Taiwan's immediate attention regarding the treatment of the subject, operation of the clinical study, and/or use of study drug), study site personnel will immediately contact the Medical Monitor.

The investigator will ensure that all study site personnel responsible for the subject's medical care are familiar with the Medical Emergency Call number, its location and have access to it.

Prior to the start of dosing at the site, the Clinical Monitor will review medical emergency call procedures with study personnel.

12.5.1 Emergency Decoding

As each subject is randomized into the study, his/her treatment assignment will be individually determined using IVRS/IWRS. This information will be kept in a secured fashion.

In the event of a medical emergency when knowledge of the subject's treatment assignment would influence the subject's clinical care, the principal investigator must contact the Medical Monitor to describe the emergency. Only under these circumstances will TaiwanJ and/or Medical Monitor authorize unbinding of the subject's treatment assignment.

If a subject's treatment assignment is unblended for a medical emergency or for regulatory purposes, that subject must immediately and permanently discontinue treatment with study drug but should remain in the study and continue the protocol specified follow-up evaluations.

If a subject's treatment assignment is accidentally unblended, that subject should continue treatment with study drug and remain in the study. The data will be included in the intent-to-treat data set.

The investigator must document the reasons for the unbinding in the subject's CRF and is strongly encouraged not to divulge the subject's treatment assignment to any individuals not directly involved in managing the medical emergency.

13. STATISTICAL CONSIDERATIONS

13.1 Sample Size Calculation

The study will need to screen approximately 100 subjects to enroll 60 patients with biopsy-proven NAFLD who meet the study inclusion / exclusion criteria. With 20 subjects per treatment group, the study will not be powered for efficacy assessment between the two doses. Based on precision analysis, the proposed sample size will provide the maximum error (defined as the half-way of the width of the 95% confidence interval for the difference (change in liver-biochemistry) between two doses (5 mg twice daily vs 10 mg twice daily dose) allowed.

Presuming the observed difference between two treatment groups is 20%, then the 95% confidence interval for the true mean is given by (2.5%, 37.5%) with 20 subjects in each group.

13.2 General Statistical Methodology

Prior to database lock, a final detailed Statistical Analysis Plan (SAP) will be available. Results, when available, will be summarized into tabulations, case listings, plots, and histograms for comparison.

Three major study populations are planned, modified intent-to-treat (met) population, per protocol (PP), and safety populations.

Baseline variables will be ascertained for conformity among all stratification factors. All baseline analysis will be based on the met population. In the met population, the subjects will be counted in the treatment group to which they were randomized, as long as they received at least one dose of study medication and have at least one return visit.

The safety analysis will be based on the safety population. In the safety population, subjects will be counted in the group in which they were treated. To assess the robustness of the study outcomes, a sensitivity analysis will be performed on all pertinent major efficacy variables. The sensitivity analysis will include and exclude patients with major study deviations and early withdrawals to assess the impact on the study endpoints.

The criteria may be amended prior to unbinding of the treatment assignment for the analysis of data. Reasons for changes may include study conduct changes, protocol amendments, or changes in the appropriateness of the criteria, which may be assessed based on review of blinded data.

For all analyses, all missing values due to patient early termination from the study will be not imputed.

For each patient, the Baseline values will be defined as those values recorded at Day 1 prior to dosing or Screening, as appropriate.

All hypotheses will be tested for statistical significance with two-tailed p-values. Results of all tests will be considered statistically significant if their p-value is less than or equal to 0.05, except for results of the tests for interaction will be considered statistically significant if the p-value is less than or equal to 0.10.

13.2.1. Analysis of Continuous Variables

Continuous variables will be summarized with descriptive statistics (i.e., sample size, mean, standard deviation, minimum, maximum, median, and quintiles). The continuous variables will be analyzed using a general linear model to test the treatment effect of the changes from pre- to post-treatment [29].

If the data are not transformable to symmetry, rank methods such as the Friedman rank test will be used. The possible transformation will be applied on the original pre- and post-treatment values only.

13.2.2. Analysis of Categorical Variables

Categorical variables will be summarized with descriptive statistics (i.e., sample size, frequency counts, percentage, and 95% confidence interval) and will be analyzed with CMH test adjusting for tumor size [30].

13.2.3. Analysis of Time-to-Event Variables

Time to event variables (such as time to treatment success) will be assessed by survival analysis. The cumulative portion of the time-to-event variables of each treatment group will be determined by Kaplan-Meier estimates. Fisher's exact or chi-square test will be used to compare the portion of patients with the event occurring at different time intervals throughout the study period.

When it is beneficial, a more precise comparison of the treatment effects will be analyzed by fitting a Cox proportional hazard model to assess the benefit-risk ratio.

13.4. Analyses

13.4.1. Efficacy Analyses

13.4.1.1. Primary Efficacy Endpoint

The primary endpoint of this study is assessment of safety and the change in hepatic steatosis as measured by MRI.

13.4.1.2. Secondary Efficacy Endpoint

The secondary endpoints of this study are:

- Time to remission (TTR) is defined as time, in weeks, from randomization to liver function remission as defined as two consecutive ALT values within normal range (< 40 IU/mL) during study period. The Cox proportional hazard model with treatment and diabetes status as factors, will be used.
    - ALT changes from baseline. The statistical analysis will the same as the one used for the primary efficacy endpoint
    - Changes in metabolic markers and NASH related biomarkers

13.4.2. Timing of Analyses

- All endpoints will be analyzed at the time point when all subjects have completed the treatment program and at the time when all subjects have completed the study.

13.4.3. Safety Analyses

13.4.3.1. Primary Safety Endpoints

The rate of patients by treatment groups who experience (1) one or more adverse events (AEs); (2) SAEs; and, (3) AEs leading to discontinuation of study medication (4) adverse events in each grade of the Common Terminology Criteria for Adverse Events (CTCAE) v3.0, published by the National Cancer Institute will be displayed. Treatment effect will be explored by inspection of observed rates for the dose groups within each treatment regimen.

Adverse events will be summarized in frequency counts by treatment group using current Medical Dictionary for Regulatory Activities (Mudra) terms. Summaries of AEs will be displayed:

1. All AEs by system organ class regardless of the relationship to the study treatment;
2. By treatment relationship ("possible, probable or definite" related vs. not related); and
3. By maximum severity (mild, moderate, or severe).

13.4.3.2. Other Safety Endpoints

Change from Baseline for each interval-valued laboratory test, vital signs, and other safety related variables will be summarized regarding each scheduled visit. Shift tables will be presented for laboratory values based on the central laboratory normal ranges.

All abnormalities deemed clinically significant by the investigator will be presented in detail and listed by patient identifier. The patients who have no laboratory results at the Screening or post-Baseline visit will be excluded from the change from Baseline and shift table analyses. Treatment effect will be explored by inspection of observed means or rates for the dose groups within each treatment regimen.

13.4.4. Sensitivity Analysis

Sensitivity analyses of the primary efficacy variable will be conducted as described below to assess the robustness of the primary analysis results. In the sensitivity analysis, decreases in p-values due to drops in power (decreasing sample sizes and increasing standard errors) will be distinguished from changes in the effect size. The criterion that defines substantial changes will be those changes that alter the effect parameter by more than one standard error.

The sensitivity analysis will use the following algorithm:

1. Subjects who are excluded from per protocol population and subjects who withdraw from the study at or after interim analysis will be considered as a responders or non-responders at the end of the study according to their response status at the time of withdrawal.
2. Subjects who drop out at the time are considered as non-responders at the time of withdrawal. Subjects who withdraw early, regardless of their final outcomes, will be considered as non-responders at the end of study
3. Remaining patients assigned to treatment will be considered as responders at the end of study if the withdrawals occur at the end of the study and they are also responders

at the time of withdrawal and at the last visit prior to the withdrawal. Remaining JKB-121 patients who drop out of the study at or after interim analysis will be considered as JKB-121 non-responders at the end of the study if they don't satisfy the described above.

4. Sensitivity analysis will be performed to assess the impact of patients with significant protocol violations possibly leading to improved efficacy, e.g. use of concomitant medications. Definitions of significant protocol violations will be described in detail in the statistical analysis plan before the study blind is broken.

13.5 Interim Analysis Plan

No interim analysis is planned for this study,

13.6 Medical Monitoring and Independent Data Review Board

A Medical Monitor, independent of TaiwanJ Pharmaceuticals, will be appointed to monitor safety and efficacy of the study. The medical monitor will be a hepatologist experienced in clinical trials in the field of NASH. The medical monitor will have statistical expertise and/or access to biostatistician with the contacted CRO to monitor safety as assess reported adverse events. An independent Data Review Board consisting of a hepatologist and biostatistician unblinded to treatment allocation will be compiled to assess safety events in treatment groups as compared to placebo.

14. QUALITY CONTROL AND QUALITY ASSURANCE

Monitoring visits to the trial site will be made periodically during the trial to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of agreement with data on case report forms. The investigator/institution guarantees direct access to source documents by TaiwanJ Pharmaceuticals and appropriate regulatory authorities.

The trial site may also be subject to review by the institutional review board (IRB)/independent ethics committee (IEC), to quality assurance audits performed by TaiwanJ Pharmaceuticals, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

15. DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms

A case report form is required and should be completed for each included subject. The completed original case report forms are the sole property of TaiwanJ Pharmaceuticals and should not be made available in any form to third parties, except for authorized representatives of appropriate regulatory authorities, without written permission from TaiwanJ Pharmaceuticals.

It is the investigator's responsibility to ensure completion and to review and approve all case report forms. Case report forms must be signed by the investigator. These signatures serve to attest that the information contained on the case report forms is true.

At all times, the investigator has final responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the case report forms. Subject source documents are the physician's subject records maintained at the study site. In most cases, the source documents will be the hospital's or the physician's chart. In cases where the source documents are the hospital or the physician's chart, the information collected on the case report forms must match those charts. In some cases, a portion of the source documents for a given subject may be the case report forms.

15.2 Record Retention

To enable evaluations and/or audits from regulatory authorities or TaiwanJ Pharmaceuticals the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, e.g., case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to TaiwanJ Pharmaceuticals. The investigator must obtain TaiwanJ’s written permission before disposing of any records.

16. ETHICS

16.1 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

It is the responsibility of the investigator to obtain approval of the trial protocol and subsequent protocol amendments, if applicable, from the IRB/IEC. All correspondence with the IRB/IEC should be retained in the Investigator File. Copies of IRB/IEC approvals should be forwarded to TaiwanJ Pharmaceuticals.

The only circumstance in which an amendment may be initiated prior to IRB/IEC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IRB/IEC and the local TaiwanJ Pharmaceutical site personnel (see protocol cover page) in writing within 5 working days after the implementation.

16.2 Ethical Conduct of the Trial

The trial will be performed in accordance with International Conference on Harmonization Good Clinical Practice guidelines and applicable regulatory requirements.

16.3 Subject Information and Consent

It is the responsibility of the investigator to give each subject (or the subject's acceptable representative) full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. This information must be provided to the subject prior to undertaking any trial-related procedure.

The subjects must be informed about their right to withdraw from the trial at any time. Written subject information (included as an appendix to the protocol) must be given to each subject before any trial-related procedure is undertaken. The written subject information must not be changed without prior approval by TaiwanJ Pharmaceuticals and the IRB/IEC. Furthermore, it is the responsibility of the investigator to obtain signed informed consent or witnessed verbal consent according to applicable regulations, from all subjects, and a signature from the persons conducting the informed consent discussion, prior to undertaking any trial-related procedure.

17. SPONSOR DISCONTINUATION CRITERIA

TaiwanJ Pharmaceuticals reserves the right to discontinue the trial prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the investigator must contact all participating subjects within 1 month. All study materials must be collected and all case report forms completed to the greatest extent possible.

18. DISSEMINATION AND PUBLICATION OF RESULTS

The conditions regulating dissemination of the information derived from this clinical study are described in the Clinical Trial Agreement.

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SIGNED AGREEMENT OF THE STUDY PROTOCOL

Protocol Number JKB-121-001

Protocol Version: 4.0 (Amendment 1)

Protocol Final Date: 2, March 2016

Title: A Randomized, Double-Blind, Placebo Controlled, Parallel-Group, Phase II trial of JKB-121 in Treating Subjects with Nonalcoholic Steatohepatitis (NASH)

I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. I will accept the Sponsor or designee overseeing of the study. I will abide by the publication plan set forth in my agreement with the Sponsor. I will promptly submit the protocol to applicable ethical review board(s). I confirm that if I or any of my staff are members of the ethical review board, we will abstain from voting on this protocol.

Investigator Name:	
Name of Facility:	
Address of Facility:	
Telephone:	
Investigator Signature:	
Investigator Title:	
Date:	

APPENDIX I

APPENDIX I: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added) 55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added) 59th WMA General Assembly, Seoul, Republic of Korea, October 2008 64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.
2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”
4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
5. Medical progress is based on research that ultimately must include studies involving human subjects.
6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.
9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
11. Medical research should be conducted in a manner that minimizes possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation. Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.
18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm. All vulnerable groups and individuals should receive specifically considered protection.
20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

    The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.
26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition

that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorized representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
32. For medical research using identifiable human material or data, such as research on material or data contained in bio banks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.
36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have

a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX II

Histological Scoring System for Nonalcoholic Fatty Liver Disease

Components of NAFLD Activity Score (NAS) and Fibrosis Staging

NAS Components (see scoring interpretation)			
Item	Score	Extent	*Definition and Comment*
Steatosis	0	<5%	Refers to amount of surface area involved by steatosis as evaluated on low to medium power examination; minimal steatosis (<5%) receives a score of 0 to avoid giving excess weight to biopsies with very little fatty change
	1	5-33%	
	2	>33-66%	
	3	>66%	
Lobular Inflammation	0	No foci	Acidophil bodies are not included in this assessment, nor is portal inflammation
	1	<2 foci/200x	
	2	2-4 foci/200x	
	3	>4 foci/200x	
Hepatocyte Ballooning	0	None	
	1	Few balloon cells	The term "few" means rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline
	2	Many cells/prominent ballooning	Most cases with prominent ballooning also had Mallory's hyalin, but Mallory's hyaline is not scored separately for the NAS
Fibrosis Stage (Evaluated separately from NAS)			
Fibrosis	0	None	
	1	Perisinusoidal or periportal	
	1A	Mild, zone 3, perisinusoidal	"delicate" fibrosis
	1B	Moderate, zone 3, perisinusoidal	"dense" fibrosis
	1C	Portal/periportal	This category is included to accommodate cases with portal and/or periportal fibrosis without accompanying pericellular/perisinusoidal fibrosis
	2	Perisinusoidal and portal/periportal	
	3	Bridging fibrosis	
	4	Cirrhosis	
Total NAS score represents the sum of scores for steatosis, lobular inflammation, and ballooning, and ranges from 0-8. Diagnosis of NASH (or, alternatively, fatty liver not diagnostic of NASH) should be made first, then NAS is used to grade activity. In the reference study, NAS scores of 0-2 occurred in cases largely considered not diagnostic of NASH, scores of 3-4 were evenly divided among those considered not diagnostic, borderline, or positive for NASH. Scores of 5-8 occurred in cases that were largely considered diagnostic of NASH			