Clinical Study of the BreathID® System to train the algorithm for the ^{13}C-Octanoate Breath Test with or without the ^{13}C-Methacetin Breath Test (OBT and MBT respectively) for correlation with histological findings of Non-Alcoholic Fatty Liver Disease (NAFLD)

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1a	06 JAN2015	Avraham Hershkowitz	Minor revisions for clarity and defining monitoring plan
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1e	25 OCT 2015	Avraham Hershkowitz	Removal of contraceptive from exclusion criteria
1f	1 MAY 2016	Avraham Hershkowitz	Allowing biopsy after BT

1 TABLE OF CONTENTS

2 ABBREVIATIONS

AE - Adverse Event

ALT - Alanine Aminotransferase

APAP - n-acetyl-p-aminophenol (acetaminophen)

AST - Aspartate Aminotransferase

AUC - Area Under the Curve

BMI - Body Mass Index

BUN - Blood Urea Nitrogen

CFR - Code of Federal Regulation

CK18 - Cytokeratin 18

CO_2 - Carbon dioxide

CoA - Coenzyme A

CRF - Case Report Form

CRP - C-Reactive Protein

CT - Computed Tomography

CTCAE - Common Terminology Criteria for Adverse Events

EC - Ethics Committee

eCRF - Electronic Case Report Form

EDC - Electronic Data Capture

EU - European Union

FDA - Food and Drug Administration

GCP - Good Clinical Practice

GI - Gastrointestinal

GGTP - Gamma-Glutamyl Transpeptidase

GRAS - Generally Recognized As Safe

HbA1c	-	Hemoglobin A1c
HCC	-	Hepatocellular Carcinoma
HCO_3	-	Bicarbonate
HCV	-	Hepatitis C Virus
HE	-	Hepatic Encephalopathy
HVPG	-	Hepatic Venous Pressure Gradient
IC	-	Informed Consent
ICF	-	Informed Consent Form
ICH	-	International Conference of Harmonization
IDE	-	Investigational Device Exemption
IL6	-	Interleukin 6
INR	-	International Normalized Ratio (for prothrombin time)
IRB	-	Institutional Review Board
K^+	-	Potassium
MBT	-	^{13}C-Methacetin Breath Test
MCS	-	Molecular Correlation Spectrometry
MELD	-	Model for End-stage Liver Disease
MRI	-	Magnetic Resonance Imaging
Na^+	-	Sodium
NAFLD	-	Non-Alcoholic Fatty Liver Disease
NAS	-	NASH Activity Score
NASH	-	Non-Alcoholic Steato-Hepatitis
NASH CRN	-	NIH NASH Clinical Research Network
NIH	-	National Institute of Health
NPV	-	Negative Predictive Value
NSBB	-	Non-Selective Beta Blocker

OUS	-	Out of the United States
O_2	-	Oxygen
OBT	-	^{13}C-Octanoate Breath Test
PHT	-	Portal Hypertension
PPV	-	Positive Predictive Value
PT	-	Prothrombin Time
PTT	-	Partial Thromboplastin Time
ROC	-	Receiver Operating Characteristic
SAE	-	Serious Adverse Event
SUSAR	-	Suspected Unexpected Serious Adverse Reactions
TNFα	-	Tumor Necrosis Factor Alpha
TIPS	-	Transjugular Intrahepatic Portosystemic Shunt
US	-	United States (of America)
US	-	Ultrasound
USA	-	United States of America
WBC	-	White Blood Cells

3 PROTOCOL SYNOPSIS

Protocol Title:	Clinical Study of the BreathID® System to train the algorithm for the ^{13}C-Octanoate Breath Test with or without the ^{13}C-Methacetin Breath Test (OBT and MBT respectively) for correlation with histological findings of Non-Alcoholic Fatty Liver Disease (NAFLD)
Protocol Number:	NASH-EX-1114
Version and Date:	v 1f, May1, 2016
Investigational subject:	Biopsy characteristics of patients suspected of Non-Alcoholic Fatty Liver Disease (NAFLD)
Investigational Product	BreathID® System together with ^{13}C-labeled Octanoate and ^{13}C-labeled Methacetin
Primary Objective:	The primary objective of the study is to develop an algorithm based on OBT with or without MBT with or without additional clinical and/or laboratory parameters, and its cut-off to correlate with histological findings of NAFLD.
Primary Endpoint:	Correlation of histological findings, as assessed by a central reader, of NAFLD compared to ^{13}C-Octanoate Breath Test with or without ^{13}C-Methactin Breath Test with or without additional clinical and/or laboratory parameters
Secondary Endpoint:	Correlation between OBT±MBT and clinical course (i.e. clinical outcome: liver decompensation events etc.) at 18 and 36 months post the last breath test performed (visit / phone). NOTE: Patients undergoing intervention for NASH / NAFLD, including both approved and experimental approaches prior to the defined time points will be terminated hence will not be evaluated for this endpoint.
Safety Endpoints:	Safety will be assessed by reviewing all adverse and serious adverse events occurring in all subjects enrolled from the time of enrollment until the subject completes the protocol.

Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

Study Design: A multicenter, non-randomized, open label, cross-sectional comparative exploratory study comparing OBT with or without MBT values to histology.

This study will be used to train an algorithm using OBT with or without MBT with or without clinical and/or laboratory parameters correlating with histological findings of NAFLD.

Study Population: At least 200 consecutive patients suspected of having NAFLD with a fresh liver biopsy (within 6 months before or 1 month after) indicated to rule-out / confirm NAFLD, meeting all inclusion/exclusion criteria will be enrolled.

Inclusion Criteria:

1. Adult men or women (≥18 years of age)
2. Liver biopsy performed indicating NAFLD/NASH performed within 6 months prior to both breath-tests

   NOTE: The samples must meet pre-defined quality criteria. (See Appendix II for biopsy quality requirements)

   OR:

   Undergoing Liver biopsy to rule-out or confirm NAFLD/NASH.
3. No other known co-existent liver disease, excluded by appropriate serologic / other testing
4. Patient able and willing to sign an Informed Consent Form
5. Can tolerate an overnight (8-hour) fast

Exclusion Criteria:

1. Positive studies for any of the following within three years prior to biopsy
   a. Anti HCV positive
   b. HBsAg positive

c. Iron saturation > 60% + gene test for hereditary hemochromatosis or iron overload as defined by presence of 3+ or 4+ stainable iron on liver biopsy

d. Antinuclear antibody at a titer > 1: 160 along with hypergammaglobulinemia and 5 times ALT normal levels

e. Alpha-1-antitrypsin level below lower limit of normal (< 150 mg/dl) or no PAS diastase resistant globules on biopsy.

f. Primary biliary cirrhosis as defined by elevation of alkaline phosphatase greater than upper limit of normal and anti-mitochondrial antibody (AMA) of greater than 1:80 and consistent liver histology

g. Low level of ceruloplasmin (< 10 ng/mL)

h. Drug-induced liver disease as defined on the basis of typical exposure and history

Note: These studies do not need to be performed if they are not available, as it is assumed that if they are not on file, the condition does not exist

2. Patients known to have chronic liver disease other than NAFLD as routinely diagnosed by the investigator
3. Concurrent acute hepatic condition other than NAFLD
4. Alcohol consumption > 20 gm/day (0.71 oz./day) for women and > 30 gm/day (1.06 oz./day) for men
5. Drugs that may interfere with octanoate metabolism or can also cause NAFLD independent of the metabolic syndrome, including: corticosteroids, amiodarone, tetracycline, valproic acid, methotrexate, stavudine, zidovudine
6. When MBT is performed subject should not have taken any of the following at least 48 hours prior to the breath test: Acyclovir , allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, (herbal) disulfiram, echinacea, enoxacin, famotidine, fluvoxamine, methoxsalen, mexiletine, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlopidine, thiabendazole, verapamil, zileuton or any medication that might interfere with Methacetin metabolism or might affect CYP 1A2
7. Patients that have had more than 10% weight change between biopsy and enrollment.

8. Hypersensitivity to any of the study substrates: Octanoate or Methacetin and their metabolites, i.e. paracetamol, acetaminophen.
9. Known extra-hepatic diseases including but not limited to: severe congestive heart failure (NIHA>2), known severe pulmonary hypertension (>35 mmHg), history of chronic obstructive pulmonary disease or uncontrolled symptomatic bronchial asthma or uncontrolled diabetes mellitus (HA1c>9.5%)
10. Previous surgical GI bypass surgery
11. Extensive small bowel resection (>100 cm)
12. Known uncontrolled malabsorption or diarrhea
13. Concurrent total parenteral nutrition
14. Any organ transplant
15. Patients receiving any anti-viral treatment or any other liver directed therapy, procedure or surgery between the time of the biopsy and the breath test
16. Pregnant or breast feeding
17. Patients unable or refusing to sign informed consent
18. Patients that, based on the opinion of the investigator, should not be enrolled into this study due to safety / adherence reasons.
19. Patients participating in other clinical trials and already receiving experimental treatments or procedures
20. Patients with suspected or documented hepatocellular carcinoma by ultra-sound or other imaging modality
21. Patients diagnosed with partial / complete portal venous occlusion, hepatic venous occlusion, previous PHT surgery, or placement of a transjugular intrahepatic portosystemic shunt (TIPS) according to initial imaging studies.

On the day of the OBT/MBT:

1. 8 hours fasting prior to OBT/MBT including all PO morning medications except for beta blockers. Diabetic patients that are driving should test their blood sugars to rule out hypoglycemia and respond accordingly.
2. No Smoking on the day of the MBT
3. No paracetamol (acetaminophen) based medications within 24 hours prior to MBT

4. No general anesthesia or sedation within 24 hours prior to OBT/MBT
5. No alcohol within 24 hours prior to OBT/MBT
6. Patient should be symptom free of any prior intervention (e.g. biopsy)

Listing of Study Procedures:

1. Informed consenting and eligibility including fresh liver biopsy confirmed to meet quality standards (see Appendix II)
2. Baseline and Physical Examination
3. Blood drawing for:
   a. Insulin and Glucose
   b. Complete Blood count (WBC, Hemoglobin, Platelets) and CRP
   c. Coagulation: PT (INR)
   d. Renal panel and electrolyte panel (BUN, creatinine, electrolytes (Na^+ and K^+),
   e. Collection of 4ml of blood for serum and freezing for storage (no preservative tube) where possible.
   f. Hepatic panel (AST, ALT, alkaline phosphatase, GGTP, total bilirubin, direct bilirubin, serum albumin and total protein) - if not available from within 3 months
   g. Additional Coagulation Panel (PTT, fibrinogen) - if not available from within 3 months
   h. Total calcium, PO_4 (phosphate corrected), magnesium - if not available from within 6 months
   i. Lipid profile and HbA1c - if not available within 6 months
   j. Thyroid Panel - if not available within 12 months
   k. Other assays where routinely practiced; (optional): Adiponectin + Leptin + IL6 + CK18, TNFα, C-peptide
4. OBT - ^{13}C-Octanoate Breath Test (within 6 months of liver biopsy or within 1 month before liver biopsy) and ensuring MBT is feasible within 30 days but not less than 48 hours). If done on same day, OBT must be performed before the biopsy.
5. MBT - ^{13}C-Methacetin Breath Test (within 30 days post OBT but not less than 48 hours). If done on the same day as the liver biopsy, the MBT must be performed before the biopsy.
6. Safety monitoring phone call 48 hours post last breath test. Any AEs reported that are possibly or probably related to the use of the product or related to the

procedure will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

7. Follow-up Visit 18 months post last breath test performed: clinical evaluation (in person or telephone)
8. Follow-up Visit 36 months post last breath test performed: clinical evaluation (in person or telephone)
9. Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

Rules of discontinuations (Subject and/or Study):

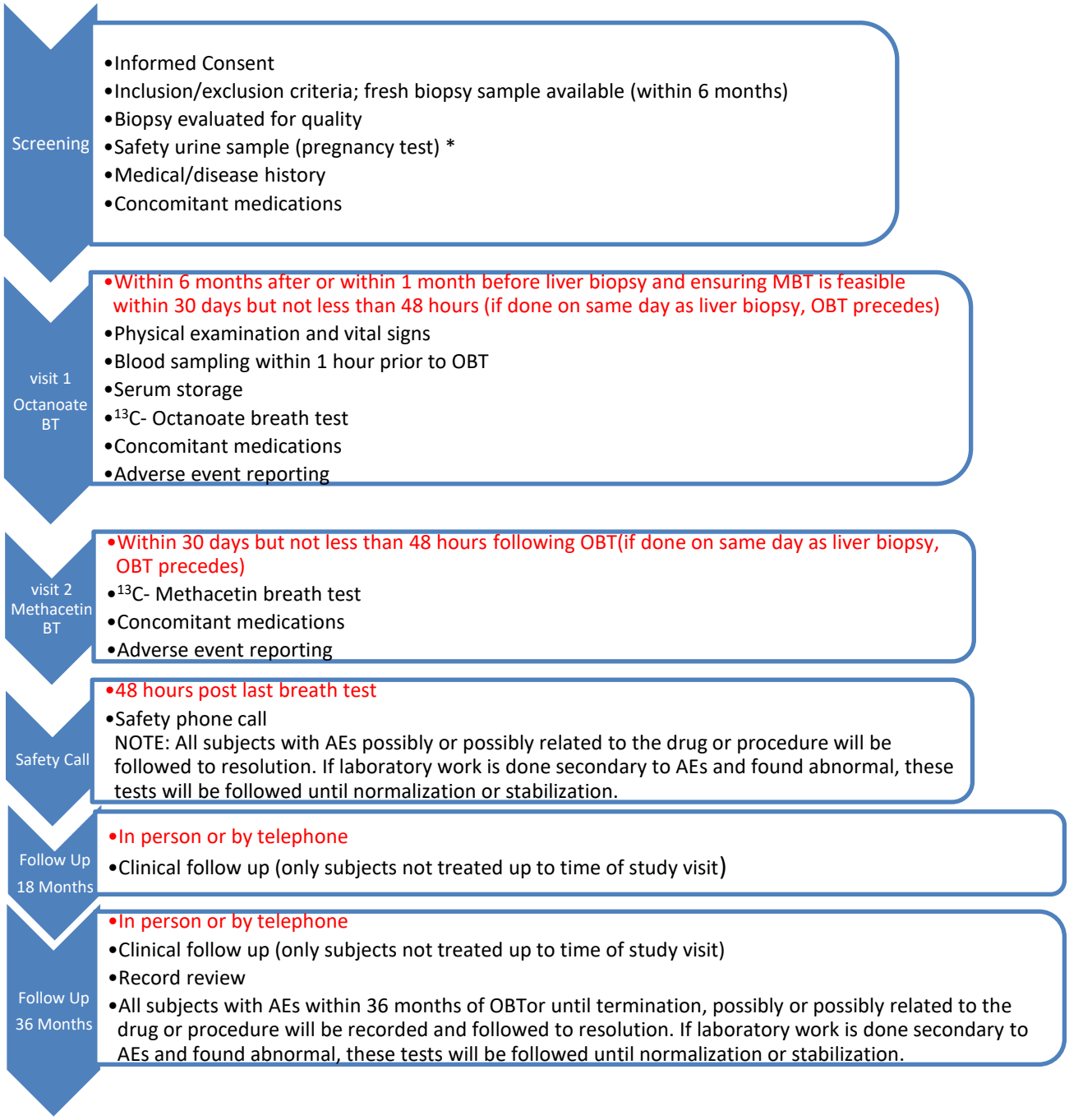
1. Withdrawal of patients from the study if they have a CTCAE (Common Terminology Criteria for Adverse Events) Grade 3 or higher regardless of whether the event is attributed to the drug unless it is caused by an accident that could not reasonably be attributable to the drug (CTCAE: Common Terminology Criteria for Adverse Events Version 4.0)
2. Discontinuance of study, if ≥ 2 patients on Octanoate/Methacetin develop the same CTCAE Grade 3 event or 1 patient develops a CTCAE Grade 4 or higher event due to one of the breath tests

Data Analysis / Statistics: An ROC analysis will be used to determine the optimal parameter and cut-offs for required performance measures sensitivity and specificity.

All efficacy analysis will include the point estimates of sensitivity, specificity, PPV and NPV with respective exact 95% confidence intervals. The respective Positive and Negative Predictive Values (PPV and NPV) will be presented as a function of possible NAFLD prevalence and will include the prevalence of the enrolled population.

This test will not replace biopsies to rule out other liver diseases such as autoimmune hepatitis, alcoholic hepatitis, alpha-1-antitrypsin deficiency, etc.

4 VISIT SCHEDULE OVERVIEW



5 BACKGROUND

Liver diseases can be caused by a variety of etiologies such as viral infection, metabolic diseases, alcohol abuse and autoimmune disorders. Liver disease can be acute or develop into chronic conditions. The conditions may vary from subtle disease to life threatening episodes, and/or may appear as mild inflammation up to significant inflammation leading to fibrosis, resulting in cirrhosis. Chronic liver disease and cirrhosis are currently the 12th leading cause of death, accounting for approximately 27,000 deaths annually (in the United States), increasing in numbers due to HCV, obesity and metabolic syndrome epidemic[(1)].

Standard biochemical and clinical tests are not capable of providing good correlation with disease staging and grading.

The unmet clinical need for a non-invasive means to evaluate liver fibrosis can be clearly reflected from the recently published NIH action plan for improving liver disease management. It positions this challenge second to improving HCV eradication rates.

The concept of a metabolic test, which could be utilized to assess the severity of liver disease, was first explored several decades ago. Such tests are performed by administering a compound either orally or intravenously, the compound is taken up by the liver and metabolized; the end-products of the metabolic process can be measured in either blood, bile, urine, saliva or exhaled breath, supplying a measurable value to the level of liver metabolic pathway. Several compounds have been utilized to evaluate hepatic metabolic function in this manner, including indocyanine green, galactose, aminopyrine, caffeine, lidocaine, phenylalanine, Methacetin and Octanoate [N-(4-Methoxy-phenyl) acetamide] and sodium-octanoate. For example, previous studies have demonstrated hepatic metabolism of lidocaine to monoethylglycinexylidide (MEGX) declines in association with liver fibrosis and cirrhosis and improves with successful treatment of the underlying liver disease. Furthermore, these studies showed the lidocaine test to accurately predict which patients with stable cirrhosis awaiting liver transplantation were at risk to develop future hepatic decompensation. Most of these methods have been abandoned due to impracticality or undesired side effects.

5.1 Non-Alcoholic Fatty Liver Disease - NAFLD

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of predominantly macrovesicular hepatic steatosis or steatohepatitis in individuals who either do not consume any alcohol or consume alcohol in quantities that are not generally considered to be harmful to the liver[(1)]. The histologic spectrum of NAFLD includes:

(1) Isolated hepatic steatosis: characterized by a fatty liver and no other histologic abnormalities. It is also referred to as nonalcoholic fatty liver (NAFL).

(2) Steatohepatitis: characterized by steatosis along with varying combinations of histologic findings, including: cytologic ballooning, Mallory's hyaline, and inflammation and pericellular fibrosis. The minimal histologic criteria for the diagnosis of non-alcoholic steatohepatitis (NASH) is the presence of steatosis, inflammation and cytologic ballooning[(2)]. Given the variable presence of these individual parameters, the presence of steatohepatitis is often made as an overall gestalt of these histologic findings. The NASH activity score (NAS) was developed by the NIH NASH Clinical Research Network (NASH CRN). It is based on individual scores for steatosis, inflammation and cytologic ballooning which have been validated[(2)]. Steatohepatitis is diagnosed when the NAS is 5 or higher.

5.1.1 Pathogenesis of NAFLD

NAFLD is considered the hepatic manifestation of the metabolic syndrome. The major risk factors associated with NAFLD are obesity, diabetes, hypertension and hypertriglyceridemia. Hepatic steatosis results from insulin resistance, which is the main pathophysiologic abnormality in the metabolic syndrome. The development of steatohepatitis requires both accumulation of fat and additional injurious processes in the liver which produce the steatohepatitis. It is believed that oxidative stress plays an important role in this process. The probability of having NAFLD rises with increasing body mass index (BMI) with over 80% of subjects with a BMI > 35 having NAFLD.

5.1.2 The importance of diagnosing and characterizing NASH

The public health priority of any disease is based on three considerations: (1) prevalence, (2) impact of disease on the affected individuals and (3) availability of effective therapy.

Since the gold-standard for diagnosis of NASH and other liver diseases is a liver biopsy, hospital-based studies with liver biopsies are subject to ascertainment bias. Population based studies have utilized imaging modalities such as ultrasound and MRI to diagnose NAFLD but are limited by the absence of histologic confirmation. Recently, specific changes on MRI have been correlated with hepatic lipid content and presence of NAFLD may be diagnosed with relative confidence. Based on MRI, it has been estimated that the overall prevalence of NAFLD in the United States is about 30%[(3)]. MRI does not distinguish between NAFL and NASH[(4)]. Approximately

4-5% of the US population is estimated to have NASH. NAFL is associated with a benign clinical course and the majority of cases of NAFL remain asymptomatic and free of fibrosis or development of steatohepatitis over a 5-10 year time frame from diagnosis[5]. On the other hand, NASH can progress to cirrhosis in about 20% of the cases[6].

About 15% of cases referred for liver transplant have either NASH or cryptogenic cirrhosis, which is believed to be the end result of NASH[7].

5.1.3 Defining the need for a non-invasive test for the diagnosis and characterization of NASH and other liver conditions

About 7.9% of the US population has persistently elevated liver enzymes with negative findings for viral hepatitis and other common causes of liver diseases[8]. Over 80% of such cases are estimated to be due to NAFLD (NAFL or NASH). In those who have concomitant features of the metabolic syndrome, the likelihood of NAFLD exceeds 90%. However, there are no noninvasive ways to distinguish between NAFL and NASH. Moreover, NASH would progress to cirrhosis in approximately 20% of the cases[6] hence requires disease surveillance..

Currently, such patients are offered a liver biopsy as standard of care to diagnose NAFLD and assess the risk of potential cirrhosis. This is the population to be studied using the ^{13}C-Octanoate Breath Test with or without ^{13}C-Methacetin Breath Test in an attempt to find a noninvasive tool to discriminate various histological findings of NAFLD, in order to distinguish between high versus low risk for deterioration and avoid unnecessary biopsies (rule-out) and eventually replacing the need for biopsy in the diagnosis of NASH. It is important to note that a liver biopsy is invasive, can be painful and carries a small but definite risk of hemorrhage and even death amongst other complications. Also, given the sheer numbers of subjects with NAFLD in the world, it is not logistically feasible to biopsy all subjects with NAFLD, thus there is a great need for a simple non-invasive method to diagnosis NASH in large populations.

5.2 ^{13}C-Breath Tests

Breath testing using ^{13}C-labeled substrates provides a safe, non-invasive means for evaluating organ function. The concept of ^{13}C-breath testing is the administration of a specific ^{13}C-labeled substrate known to undergo degradation through a specific metabolic

path through which $^{13}CO_2$ is produced. The monitoring of the $^{13}CO_2$ levels within the patient's exhaled breath is presumed to quantify the metabolic rate of the investigated metabolic path. When targeting a major metabolic path; e.g. the p450 cytochrome system within the liver, the test should supply a good estimate of the target organ function, and a decrease in function could be interpreted as impairment associated with disease. Other targeted metabolic pathways might be used for diagnosis when they are presumed to be defective in certain disease states (e.g. mitochondrial beta-oxidation in NASH). ^{13}C is a stable, non-radioactive isotope, which can be incorporated into a specific location within a test substrate in order for it to be metabolized into $^{13}CO_2$. Ideally, the ^{13}C-compound should be administered orally and rapidly absorbed and exclusively metabolized by the targeted metabolic path.

5.2.1 The BreathID System

The BreathID® System consisting of the BreathID® device and a test kit containing a breath collection cannula and a non-radioactive isotope ^{13}C-labeled substrate. It measures and computes the ratio between $^{13}CO_2$ and $^{12}CO_2$ in the patient's exhaled breath.

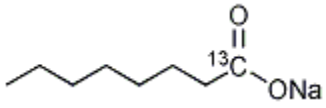
For more details please refer to the study's Investigator Brochure.

The device is based on a CE marked and FDA cleared device (510k#: K130524) for assessment of H. pylori infection in the stomach utilizing ^{13}C-Urea as a substrate to assess the bacterial urease activity. Performance and safety of the original BreathID (510k# K011668) device utilizing multiple ^{13}C- substrates (including ^{13}C-Methacetin and ^{13}C-Octanoate) for assessment of liver function, have been studied in thousands of patients worldwide, including in a large US pivotal study – under IDE G080107 – of over 400 patients with chronic liver disease from 11 participating sites (including 141 patients with biopsy proven cirrhosis),[9] which validated the safety of the device using ^{13}C-labeled Methacetin.

5.2.2 ^{13}C-Octanoate (Sodium Octanoate) Breath Test (OBT)

The sodium octanoate is metabolized through mitochondrial (beta-oxidation) in the liver and in other organ / tissues.

$C_8H_{15}NaO_2$



CAS# 1984-06-1, EC# 217-850-1, MW=166.2 amu

Synonyms: Caprylic acid sodium salt, sodium caprylate, octanoic acid sodium, salt, sodium n-Octanoate.

Octanoate is the salt form of octanoic (caprylic) acid (highly solubile in water: 50mg/ml), a medium chain fatty acid, possessing the physical and chemical properties making it a good candidate to assess hepatic mitochondrial beta-oxidation; Octanoate is absorbed promptly from the intestinal lumen and transported rapidly to the liver through the portal venous system, enters the hepatic mitochondria independently of the carnitine transport system and undergoes hepatic mitochondrial beta-oxidation which produces acetyl coenzyme A (CoA). Finally, acetyl CoA enters the Krebs cycle and is oxidized by carbon dioxide (CO_2) which in-turn is exhaled. Non-Alcoholic Fatty Liver Disease (NAFLD) is associated with changes in beta-oxidation, hence targeting this metabolic pathway might reflect the extent of the disease.

The current recommended dose (for an adult) used in the OBT, is 100mg[10].

5.2.2.1 ^{13}C-Octanoate Safety

During the past 20 years Octanoate has been used mainly to assess gastric motility and liver health function. No complications were reported by leading researchers throughout the globe (US, Europe and Japan) [11-16].

Moreover, this material is used in vitamins and food supplements such as Caprylate, butter and malted barley[1], all in higher doses than proposed under current protocol[2]. Furthermore, Octanoate (Caprylic Acid) is listed at the FDA as a Generally Recognized As Safe (GRAS) substance since 1974[3].

For more information please refer to the investigator brochure.

[1] http://www.opsi.gov.uk/SI/si1989/Uksi_19890945_en_3.htm
[2] http://www.vitaminshoppe.com/store/en/browse/sku_detail.jsp?id=AM-1098#prodInfo
[3] http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm261239.htm

5.2.2.2 Rationale for use: ^{13}C-Octanaote Breath Test

It has been previously suggested NAFLD is associated with changes in fatty acid β-oxidation which might be demonstrated in $^{13}CO_2$ production in NASH compared to NAFL or normal controls[17]. It was therefore hypothesized that by evaluating the area under the curve, peak value and time to peak for $^{13}CO_2$ after ingestion of labeled ^{13}C-Octanoate, a nontoxic medium chain fatty acid which is oxidized in the mitochondrial rather than the peroxisomes, can distinguish those with NAFL vs. NASH. The advantages of this test are that: (1) octanoate is nontoxic, (2) the small liquid dose is not affected substantially by gastric emptying, (3) ^{13}C is a stable isotope and avoid radioactivity, (4) $^{13}CO_2$ can be measured easily and accurately and (5) this test can be performed in the office environment providing immediate results.

This test will not replace biopsies to rule out other liver diseases such as autoimmune hepatitis, alcoholic hepatitis, alpha-1-antitrypsin deficiency, etc.

For more details please refer to the study's Investigator Brochure.

5.2.3 ^{13}C-Methacetin Breath Test (MBT) Overview

5.2.3.1 ^{13}C-labelled Substrate: ^{13}C-Methacetin

^{13}C-Methacetin is a white, crystalline powder bearing the chemical name [N-(4-Methoxy phenyl) acetamide]. ^{13}C-Methacetin is rapidly absorbed and metabolized exclusively by hepatic microsomal function oxidase via O-demethylation, mainly by cytochrome p450 1A2, into acetaminophen (also known as paracetamol, Tylenol™, and n-acetyl-p-aminophenol or APAP) and formaldehyde[18-23]. Formaldehyde is then transformed, through two successive oxidative steps, into $^{13}CO_2$. The rate limiting factor in this process is the O-demethylation performed by the liver cytochrome p450[24]. The general reaction is described in the following scheme:

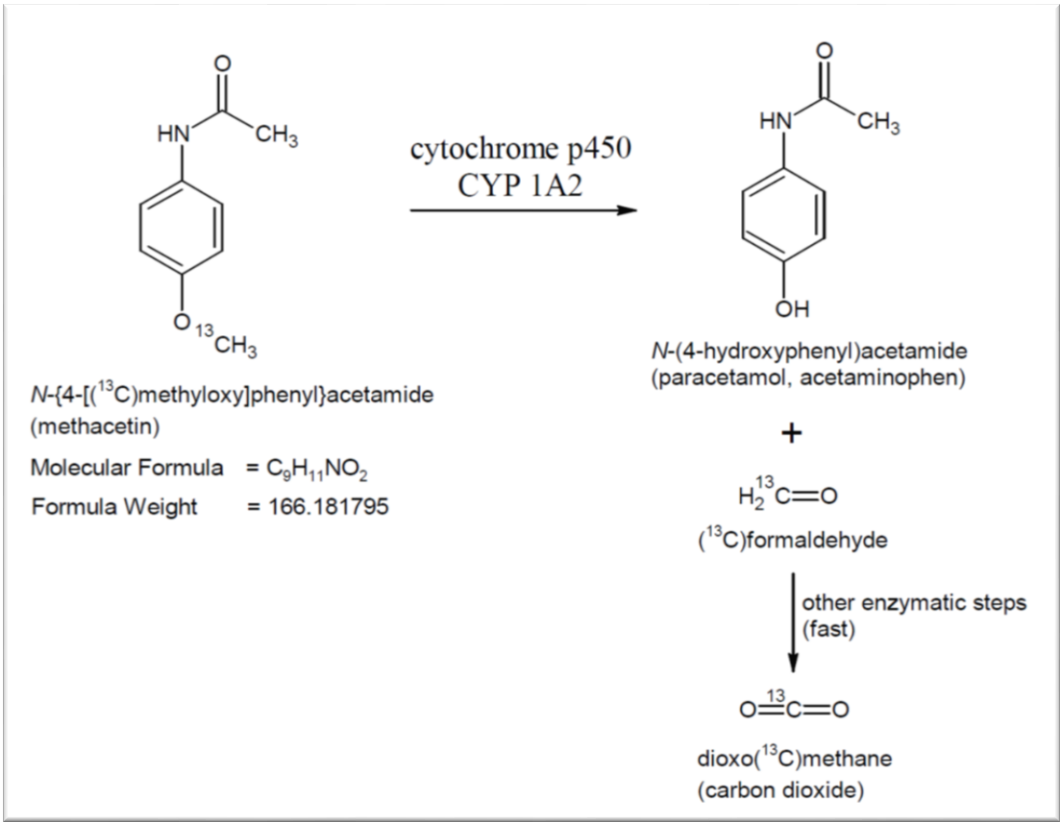


Figure 1: Oxidative Cleavage of Methacetin to Acetaminophen and Formaldehyde

The resultant $^{13}CO_2$ can be measured in the exhaled breath. The amount of metabolized Methacetin indicates the capability of the liver to accomplish one of its main physiological tasks and has been shown to correlate with liver fibrosis and cirrhosis[25-28].

5.2.3.2 ^{13}C-Methacetin Safety

Methacetin had been available and described in literature for over 100 years[29]. Within the last 40 years its use for breath testing has been described as well[30]. No risks have been reported in the literature using much higher doses of Methacetin than currently proposed to be used in this protocol. No adverse events have been reported in performing ^{13}C-Methacetin Breath Test (MBT) using the proposed device or substrate to date including in more vulnerable populations. Additional safety assessments will be made during this clinical study.

Methacetin metabolism involves an initial O-demethylation process carried out exclusively by hepatic cytochrome p450 Iso-enzyme 1A2 producing two products: O-demethylated Methacetin, similar to acetaminophen, and formaldehyde[18-23]. Formaldehyde is then transformed into $^{13}CO_2$, through additional fast enzymatic steps. The rate limiting factor in this process is the O-demethylation performed by the liver cytochrome p450[24]. More details regarding the Methacetin metabolism are presented in section 5.2.3.1.

In current literature regarding pre-clinical and clinical studies performed with Methacetin, results from a total of 2987 individuals (2021 chronic liver disease patients including critically ill patients and 966 controls) were reported in over 70 publications[25, 26, 30-98], including neonates,[55] infants,[53] healthy adults, adults with various forms of liver disease (including toxic injury), pregnant women[30] and the elderly - with no reports of adverse events. MBT has also been used in patients with Acute Liver Failure. Some of the data was recorded utilizing the Exalenz BreathID device and substrate, while the majority were performed with other similar systems.

Regarding internal unpublished data using the BreathID device and Methacetin, 1466 chronic liver patients (of these 278 cirrhotic - some before transplant) and 74 healthy controls/subjects without liver disease, have also been tested. No adverse events related to the substrate or the device were experienced by the subjects

Toxicology testing results in animals and other information support the safe use of Methacetin in humans. Based on the acute toxicity studies in mice and rats where relatively high LD50 values of 1190mg/kg were administrated,[99] the Methacetin dose administered in human breath tests in adults of 75 mg, or approximately 1mg/kg, has a safety ratio in excess of 1000-fold.

Many studies using ^{13}C-Methacetin for liver function assessment have been published. Representative references supporting the conclusion ^{13}C-Methacetin is a “safe” molecule have been cited including the use in high-risk population groups such as the elderly, neonates or pregnant women.[71, 73, 82, 100] This provides further assurance that the ^{13}C-Methacetin substrate is appropriate to use in breath tests intended for liver function assessment.

5.2.4 Breath Test Components

The following are the components of the *BreathID® System*: The *BreathID®* device, the ^{13}C-labelled substrate and the sampling kit containing breath collection cannulae.

The *BreathID®* device will be provided by Exalenz Bioscience Ltd. The cannulae, accessories and the test substrate will be supplied in proper packaging and will bear all required labeling based on applicable regulatory requirements.

For the MBT, the solution is administered orally to the patient is a 0.05% solution of ^{13}C-Methacetin in purified water, supplied in amber thermoplastic polyester (PET) bottles with a child resistant plastic stopper (75mg of ^{13}C-Methacetin in 150mL purified water). All bottles are for single use only and must be used solely for per-protocol investigational product administration.

For the OBT, 100 mg ^{13}C-Octanoate, supplied in powder form, in amber glass vials will be used. Each vial contains a single test dose and must be used solely for per-protocol investigational product administration.

All study test components must be stored in an environment according to the Investigator Brochure and product labeling guidance.

For more details please refer to the study's Investigator Brochure.

6 INTENDED USE / INDICATION FOR USE

The BreathID® System together with ^{13}C-Octanoate Breath Test (OBT) with or without ^{13}C-Methacetin Breath Test (MBT) with or without additional clinical and/or laboratory parameters are to be used for the characterization of the histological image of Non-Alcoholic Fatty Liver Disease (NAFLD).

7 PROPOSED CLINICAL ALGORITHM USING THE OBT±MBT±CLINICAL / LABORATORY PARAMETERS

The Sponsor's goal is to develop a point-of-care device for the characterization of Non-Alcoholic Fatty Liver Disease (NAFLD) compared to liver histology findings.

The Sponsor proposes to train a selected OBT±MBT-based variable to reliably correlate with non-alcoholic Fatty Liver Disease (NAFLD) histological characteristics. This will enable the use of OBT±MBT to assess NAFLD by means of rule-in or rule-out or both.

8 STUDY DESIGN

This study is a multicenter, non-randomized, open-label, cross-sectional comparative exploratory study comparing OBT values with or without MBT values to histologic findings of liver biopsy.

This study will be used to train an algorithm using OBT with or without MBT combined with other commercially available biomarkers and to select a cut-off to correlate with histological findings, as assessed by a central reader, of NALFD and determine clinical outcome at 18 and 36 months, if available.

The modeled algorithm will be validated in a separate study.

9 STUDY OBJECTIVES

9.1 Efficacy Objective

The primary objective of the study is to develop an algorithm based on OBT with or without MBT with or without additional clinical and/or laboratory parameters, and its cut-off to correlate with histological findings, as assessed by a central reader, of NAFLD.

9.2 Safety Objective

All adverse events, serious adverse events (SAE) and suspected unexpected serious adverse reactions (SUSAR) will be reported according to local regulations. The actual reporting is discussed in section 15 below. No breath-test related adverse events are expected.

For more information please refer to the Investigator Brochure.

9.3 Secondary Objectives

To assess correlation between OBT with or without MBT with or without other available biomarkers and disease course, defined by clinical outcomes, at 18 and 36 months.

9.4 Efficacy Endpoints

9.4.1 Primary efficacy endpoint

Correlation of histological findings, as assessed by a central reader, of NAFLD compared to ^{13}C-Octanoate Breath Test with or without ^{13}C-Methactin Breath Test with or without additional clinical and/or laboratory parameters.

9.4.2 Secondary efficacy endpoint

Correlation between OBT with or without MBT and clinical course (i.e. clinical outcome: liver decompensation events etc.) at 18 and 36 months post the last breath test performed (visit / phone).

NOTE: Patients undergoing intervention for NASH / NAFLD, including both approved and experimental approaches prior to the defined time points will be terminated hence will not be evaluated for this endpoint.

9.5 Safety Endpoints

Safety will be assessed by assessing all adverse and serious adverse events occurring in all subjects enrolled from the time of enrollment until the subject completes the protocol.

Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

10 SUBJECT SELECTION

The study population will be enrolled on a consecutive basis of able and willing subjects with a fresh up-to-standard liver biopsy (i.e. tissue sample biopsied within 6 months prior to the OBT and MBT and evaluated for quality prior to enrollment), indicated to rule-out / confirm NAFLD, meeting all inclusion/exclusion criteria.

Patients to be enrolled in the proposed study will be from centers in Europe and the US. It is presumed patients on both continents have similar disease phenotypes and prognosis and the produced data can be used interchangeably in this multicenter clinical trial investigating NAFLD populations.

10.1 Inclusion Criteria

1. Adult men or women (≥18 years of age)
2. Liver biopsy indicating NAFLD/NASH performed within 6 months prior to both breath-tests

   NOTE: The samples must meet pre-defined quality criteria. (See Appendix II for biopsy quality requirements)

   OR:

   Undergoing Liver biopsy to rule-out or confirm NAFLD/NASH.
3. No other known co-existent liver disease, excluded by appropriate serologic / other testing
4. Patient able and willing to sign an Informed Consent Form
5. Can tolerate an overnight (8-hour) fast

10.2 Exclusion Criteria:

1. Positive studies for any of the following within three years prior to biopsy
   a. Anti HCV positive
   b. HBsAg positive
   c. Iron saturation > 60% + gene test for hereditary hemochromatosis or iron overload as defined by presence of 3+ or 4+ stainable iron on liver biopsy
   d. Antinuclear antibody at a titer > 1: 160 along with hypergammaglobulinemia and 5 times ALT normal levels
   e. Alpha-1-antitrypsin level below lower limit of normal (< 150 mg/dl) or no PAS diastase resistant globules on biopsy.
   f. Primary biliary cirrhosis as defined by elevation of alkaline phosphatase greater than upper limit of normal and anti-mitochondrial antibody (AMA) of greater than 1:80 and consistent liver histology
   g. Low level of ceruloplasmin (< 10 ng/mL)
   h. Drug-induced liver disease as defined on the basis of typical exposure and history

   Note: these studies do not need to be performed if they are not available, as it is assumed that if they are not on file, the condition does not exist.
2. Patients known to have chronic liver disease other than NAFLD as routinely diagnosed by the investigator
3. Concurrent acute hepatic condition other than NAFLD

4. Alcohol consumption > 20 gm/day (0.71 oz./day) for women and > 30 gm/day (1.06 oz./day) for men
5. Drugs that may interfere with octanoate metabolism or that can also cause NAFLD independent of the metabolic syndrome, including: corticosteroids, amiodarone, tetracycline, valproic acid, methotrexate, stavudine, zidovudine
6. When MBT is performed, subject should not have taken any of the following at least 48 hours prior to the breath test: Acyclovir , allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, (herbal) disulfiram, echinacea, enoxacin, famotidine, fluvoxamine, methoxsalen, mexiletine, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlopidine, thiabendazole, verapamil, zileuton or any medication that might interfere with Methacetin metabolism or might affect CYP 1A2
7. Patients that have had more than 10% weight change between biopsy and enrollment.
8. Hypersensitivity to any of the study substrates: Octanoate or Methacetin and their metabolites, i.e. paracetamol, acetaminophen
9. Known extra-hepatic diseases including but not limited to: severe congestive heart failure (NIHA>2), known severe pulmonary hypertension (>35 mmHg), history of chronic obstructive pulmonary disease or uncontrolled symptomatic bronchial asthma or uncontrolled diabetes mellitus (HA1c>9.5%)
10. Previous surgical GI bypass surgery
11. Extensive small bowel resection (>100 cm)
12. Known uncontrolled malabsorption or diarrhea
13. Concurrent total parenteral nutrition
14. Any organ transplant
15. Patients receiving any anti-viral treatment or any other liver directed therapy, procedure or surgery between the time of the biopsy and the breath test
16. Pregnant or breast feeding
17. Patients unable or refusing to sign informed consent
18. Patients that, based on the opinion of the investigator, should not be enrolled into this study due to safety / adherence reasons
19. Patients participating in other clinical trials and already receiving experimental treatments or procedures

20. Patients with suspected or documented hepatocellular carcinoma by ultra-sound or other imaging modality
21. Patients diagnosed with partial / complete portal venous occlusion, hepatic venous occlusion, previous PHT surgery, or placement of a trans-jugular intrahepatic porto-systemic shunt (TIPS) according to initial imaging studies

10.3 On the day of the OBT:

1. Eight (8) hours fasting prior to OBT including all PO morning medications except for beta blockers. Diabetic patients that are driving should test their blood sugars to rule out hypoglycemia and respond accordingly.
2. No general anesthesia or sedation within 24 hours prior to OBT
3. No alcohol within 24 hours prior to OBT
4. Patient should be symptom free of any prior intervention (e.g. biopsy)

10.4 On the day of the MBT:

1. Eight (8) hours fasting prior to MBT including all PO morning medications except for beta blockers. Diabetic patients that are driving should test their blood sugars to rule out hypoglycemia and respond accordingly.
2. No smoking on the day of the MBT
3. No paracetamol (acetaminophen) based medications within 24 hours prior to MBT
4. No general anesthesia or sedation within 24 hours prior to MBT
5. No alcohol within 24 hours prior to MBT
6. Patient should be symptom free of any prior intervention (e.g. biopsy)

10.5 Consenting

Patients will sign a consent form prior to study participation.

The consent will include willingness to allow acquisition and collation of blood, clinical and imaging data obtained on entry to the study and incorporate all other data from time of enrollment until the patient's completion/termination of the study including consent to future surveillance for long term (up to 3 years) clinical outcomes.

10.6 Safety Termination of Subjects or Study

1. Withdrawal of patients from the study if they have a CTCAE Grade 3 or higher regardless of whether the event is attributed to the drug unless it is caused by an accident that could not reasonably be attributable to the drug (CTCAE: Common Terminology Criteria for Adverse Events Version 4.0).
2. Discontinuance of study, if ≥ 2 patients administered Octanoate / Methacetin develop the same CTCAE Grade 3 event or 1 patient develops a CTCAE Grade 4 or higher event due to one of the breath tests.

10.7 Early Withdrawal from the Study

Patients, their relatives, their representatives or the patients' physician may withdraw the patient from the study at any time if they feel the study is not in the patients' best interest, or they may be withdrawn by the investigator or sponsor for safety, behavioral, or administrative reasons. All withdrawals must be fully documented.

If the patient withdraws from the study and also withdraws consent for the disclosure of future information, no further evaluations should be performed and no additional data should be collected. Previously acquired data will be analyzed. This will not have any effect on the treatment that the patient receives.

10.8 Expected Duration of Recruitment

The enrollment to the study is planned for a duration of approximately 6-12 months.

11 STATISTICAL CONSIDERATIONS

11.1 Study Design and Objectives

This is a multicenter, non-randomized, open-label, cross-sectional comparative exploratory study comparing OBT with or without MBT values to histologic findings from liver biopsy associated with NAFLD.

This study will be used to train an algorithm using OBT with or without MBT with or without other commercially available biomarkers and to select a cut-off to determine correlation with histological findings of NALFD and determine clinical outcome at 18 and 36 months, if available.

The modeled algorithm will be validated in a separate study.

11.2 Primary endpoints

Correlation of histological findings of NAFLD compared to ^{13}C-Octanoate Breath Test with or without ^{13}C-Methacetin Breath Test with or without additional clinical and/or laboratory parameters.

11.3 Secondary efficacy endpoint

Correlation between OBT with or without MBT and clinical course (i.e. clinical outcome: liver decompensation events etc.) at 18 and 36 months post the last breath test performed (visit / phone). NOTE: Patients undergoing intervention for NASH / NAFLD, including both approved and experimental approaches prior to the defined time points will be terminated hence will not be evaluated for this endpoint.

11.4 Safety Endpoints

All Adverse Events recorded from the time of enrollment until the subject completes the protocol.

Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

11.5 Sample Size estimation

It is estimated that a total of 200 subjects with a fresh and evaluable liver biopsy undergoing OBT and MBT of which at least 50 will be positive for NASH and at least 50 will be positive for NAFLD will enable the estimation of a conservative AUC of 0.75 with a half width of the 95% confidence interval of 0.1.

During the study the correlation between OBT with or without MBT and histological findings of NAFLD will be assessed periodically in order to see if there is any confounding factor(s) affecting the correlation. In the case that such a factor(s) is identified showing a significant difference in correlation, the study population may be increased up to 400 patients.

11.6 Analysis sets

1. Primary analysis set (per protocol): All subjects enrolled in the study with a valid biopsy and valid OBT and MBT results together with the proposed additional biomarkers.
2. Secondary analysis set – ITT: all subjects performing at least one breath test.
3. Safety analysis set: all patients performing at least one breath test

11.7 Statistical Analysis

11.7.1 General Considerations

Statistical analyses will be performed using SAS® v9.3 or higher (SAS Institute, Cary NC, USA).

If any statistical tests are performed, they will be two-sided. The required significance level of findings will be equal to or lower than 5%. Where confidence limits are appropriate, the confidence level will be 95%.

Baseline values are defined as the last valid value prior to study treatment start.

All statistical analyses of safety and performance measures will be descriptive in nature. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage. Confidence intervals will be provided where relevant.

If multiple measurements are taken in a single patient, statistics described below will be appropriately modified to accommodate the within patient correlation.

11.7.2 Demographic and Other Baseline Characteristics

Demographic, medical and clinical history variables will be tabulated. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage.

11.7.3 Disposition of Subjects

The numbers of patients who were enrolled will be provided, as well as the reasons for all enrollment discontinuations, grouped by major reason (e.g., lost to follow-up, adverse event, poor compliance). A list of discontinued patients, protocol deviations, and patients excluded from the efficacy analysis will be provided as well

11.7.4 Safety Analysis

The events related to study drug and/or device AEs rate will be presented along with a 95% exact binomial two sided 95% confidence interval. The analysis of all adverse events will include incidence tables and will include analyses by severity, relationship to device or drug and baseline variables.

11.7.5 Performance Analysis

A model/algorithm will be developed for the correlation between histological findings of NAFLD and OBT with or without MBT. It will be developed using either logistic regression or neural network or any other known methodology. Using a cut-point on the score a binary diagnosis of NASH (Yes/No) or NAFL (Yes/No) will be obtained. The minimal criterion for sufficient efficacy is an Area under the Receiver Operating Characteristics (ROC) Curve (AUC) $\geq$0.75 in this patient population. A potential clinical utility was designated for the algorithm/model output; where this algorithm could aid the clinician in ruling-in or out NASH or NAFL. This will be translated into finding a model prediction cut-point that provides a high sensitivity (true positive rate) and at least moderate specificity (true negative rate), which in turn achieves a high negative predictive value (NPV). High NPV is considered to be near 90% (i.e. 88%) thus sensitivity should be at least 90% and specificity should be at least 50%. Cross validation methods will be used to test algorithm stability.

11.7.6 Interim Analysis

This study is an open label study for training purposes. The data may be assessed prior to achieving the full sample size. This will be done specifically in order to assess the effect

of confounding factors on the correlation between OBT with or without MBT and binary diagnosis of biopsy proven NASH or NAFL. If any confounding factor shows significantly different correlation than the rest of the population, the study population may be increased to up to 400 patients.

11.7.7 Pooling

Subgroup analysis of the primary performance endpoint by center via a regression model will be used to evaluate the poolability of the results. If the variable by center analysis is found significant, the reason for this will be further explored and rationalized. This evaluation may include demographic features, symptoms at presentation, clinical and treatment history, and center comparability in the features found to be associated with the primary efficacy variable

11.7.8 Handling of Missing Data

The study variables cannot be evaluated for patients for whom OBT or MBT results or liver biopsy results are not available and therefore these subjects will be left out of the primary efficacy analysis. Demographic clinical characteristics and safety data of patients with missing data will be compared to patients with complete data.

11.7.8.1 Procedure

After all the relevant data is entered into the database, a soft lock to the database will be performed. The study statistician will perform the assessment of the primary and safety endpoints.

11.7.8.2 Decision Rules

Stop the study in case of severe safety concern.

12 STUDY PROCEDURES

12.1 General

1. The study will be conducted in compliance with this protocol, in concordance with GCP standards, and applicable regulatory requirements.
2. The study design is: cross-sectional, enrolling patients in a consecutive manner per site, so as to prevent any bias.
3. Patients will sign a consent/assent form prior to study participation
4. An eCRF will be completed for each subject.
5. The raw data will be saved on the *BreathID*® device.
6. All patients undergoing liver biopsy to rule-out or confirm NAFLD, within 6 months prior to breath tests and within 1 month after first breath test, should be considered for enrollment. If the biopsy is done on the day of one of the breath tests, the breath test must precede the biopsy.
7. Biopsy slides of enrolled patients may undergo high-resolution scanning to simplify central reading and filing.
8. All tissue slides or their respective scans if relevant, will be read twice; once by the local (study site) pathologist and once by the central reading laboratory. In case of discrepancy the central reading results prevail. The characteristics to record for each sample are specified within Appendix II to this protocol. All parameters will be entered into the study eCRF.
9. Any adverse events will be recorded according to GCP ICH guidelines. Section 0 below15 below relates to the reportable and non-reportable SAEs.
10. All investigators will be blinded to the OBT and MBT results until the end of the study recruitment. This is to ensure that OBT and MBT results will not affect the current patient management. Hard copies of the device printouts will be stored separately from the other source data and the section within the eCRF containing the

breath tests results will remain inaccessible to the treating physician (i.e. the investigator login will keep this section hidden).

12.2 Visit 1: Screening / enrollment

1. Pre-screening for inclusion / exclusion criteria
2. As part of eligibility, biopsy slides should be "up to standard" according to the quality requirements specified within Appendix II to this protocol. (Biopsy quality requirements are as follows: Unfragmented needle core ≥ 20mm length with ≥ 10 portal tracts is recommended, according to the AASLD practice guidelines or equivalent.)
3. A minimum of Hematoxyline & Eosine (H&E) and Masson's trichome stained slides should be available. Additional optional stains include: Chromotrope Aniline blue (connective tissue), Prussian blue (iron storage), Sirius red stain (for fibrosis) and SMA will be filed if available. Additional unstained slides are recommended.
4. Histology slides or their respective scans, will be assessed by both the local pathologist and a central reader according to the parameters listed within Appendix II to this protocol. In case of significant differences in the assessment the central reader will always prevail.
5. Consenting patient.
6. Complete study eligibility and enrollment.
7. Physical or high resolution scanned slides sent to local and central reading according to Appendix II.

12.3 Visit 2: ^{13}C-Octanoate Breath Test (OBT)

1. Female patients of childbearing age meeting all criteria will undergo pregnancy test if not available from the past 7 days. Abstinence or oral contraceptives are requested until completion of MBT (second breath test).

2. OBT will be performed within 6 months post liver biopsy.
3. Medical History including the following:
    a. Demographics
    b. Detailed medical history including liver directed and other concomitant diseases (cardiovascular, respiratory, renal and neurological), presentation, interventions, etiology, habits etc.
    c. Imaging results,- (if available) Ultrasound, CT and/or MRI, (liver outline, vasculature, evidence of fat etc.), imaging of major hepatic vessels, pancreas, intestines, and liver volume
    d. Other assays: e.g. HVPG
    e. Most recent laboratory blood test results within 6 months.
    f. Concomitant medications
4. Physical examination (targeting liver related findings) including liver related clinical scoring.
5. In preparation for the OBT:
    a. Eight (8) hours fasting prior to OBT including all PO morning medications except for beta blockers. Diabetic patients that are driving should test their blood sugars to rule out hypoglycemia and respond accordingly.
    b. No general anesthesia or sedation within 24 hours prior to OBT
    c. No alcohol within 24 hours prior to OBT
    d. Patient should be symptom free of any prior intervention (e.g. biopsy)
6. Within one hour prior to the OBT blood will be drawn for the following panels (note the relevant remarks):
    a. Insulin and Glucose
    b. Complete Blood count (WBC, Hemoglobin, Platelets) and CRP
    c. Coagulation: PT (INR)

d. Renal panel and electrolyte panel (BUN, creatinine, electrolytes (Na^+ and K^+),
e. Collection of 4ml of blood for serum and freezing for storage (no preservative tube) where possible.
f. Hepatic panel (AST, ALT, alkaline phosphatase, GGTP, total bilirubin, direct bilirubin, serum albumin and total protein) - if not available from within 3 months
g. Additional Coagulation Panel (PTT, fibrinogen) - if not available from within 3 months
h. Total calcium, PO_4 (phosphate corrected), magnesium - if not available from within 6 months
i. Lipid profile and HbA1c - if not available within 6 months
j. Thyroid Panel - if not available within 12 months
k. Other assays where routinely practiced; (optional): Adiponectin + Leptin + IL6 + CK18, TNFα, C-peptide

NOTE: if possible more than just the essential blood panels (sections a. to d.) will be collected adjacent to the OBT.

7. ^{13}C-Octanoate Breath Test:

   NOTE: Expected warm-up time for the device to be ready to measure patient's breath is up to 90 minutes. Ensure the device is turned on at least 2 hours prior to scheduled patient visit.
   The breath test is performed automatically while the patient is connected to the device using a nasal cannula. The test is of 2 phases: baseline and the sampling phase separated by the administration of the test substrate. The 2 phases' durations are up to 30 and up to 60 minutes respectively.

   For more information regarding the OBT procedure please refer to Section 5 of the Investigator's Brochure.

8. Adverse events recording until the subject completes the protocol.
   NOTE: Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post OBT or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and

found abnormal, these tests will be followed until normalization or stabilization.

9. Diabetic patients will be followed throughout the entire visit and should be instructed to resume their medication immediately after the breath tests.

12.4 Visit 3: ^{13}C-Methacetin Breath Test (MBT)

1. MBT will be conducted within 6 months post liver biopsy and within 30 days of the OBT but not less than 48 hours post OBT.
2. In preparation for the MBT:
    a. Eight (8) hours fasting prior to MBT including all PO morning medications except for beta blockers. Diabetic patients that are driving should test their blood sugars to rule out hypoglycemia and respond accordingly.
    b. No smoking on the day of the MBT
    c. No paracetamol (acetaminophen) based medications within 24 hours prior to MBT
    d. No general anesthesia or sedation within 24 hours prior to MBT
    e. No alcohol within 24 hours prior to MBT
    f. Patient should be symptom free of any prior intervention (e.g. biopsy)
3. ^{13}C-Methacetin Breath Test:

    NOTE: Expected warm-up time for the device to be ready to measure patient's breath is up to 90 minutes. Ensure the device is turned on at least 2 hours prior to scheduled patient visit.

    The breath test is performed automatically while the patient is connected to the device using a nasal cannula. The test is of 2 phases: baseline and the sampling phase separated by the administration of the test substrate. The 2 phases' durations are up to 30 and up to 60 minutes respectively.

For more information regarding the OBT procedure please refer to Section 5 of the Investigator's Brochure.

4. Adverse events recording until the subject completes the protocol.
   NOTE: Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.
5. Diabetic patients will be followed throughout the entire visit and should be instructed to resume their medication immediately after the breath tests.

12.5 Visit 4: 48 safety hours phone call

1. 48 hours post the last breath-test (presumably MBT) a safety oriented phone call will be conducted.
2. All adverse events post OBT will be recorded until the subject completes the protocol.
   NOTE: Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.
3. This visit determines the last safety reporting point to be reported in to the safety endpoint.

12.6 Visit 5: Clinical Follow-Up 18 Months

18 months ± 4 weeks post the last breath test performed a phone / in-person visit will be conducted in order to assess major clinical events (i.e. Death, liver transplant, liver decompensation events including: ascites, hepatic encephalopathy, variceal bleeding, hepato-renal syndrome, spontaneous bacterial peritonitis). In addition, the patient's clinical records will be reviewed.

NOTE: Patients undergoing intervention for NASH / NAFLD, including both approved and experimental approaches prior to the defined time points will be terminated hence will not be evaluated for this endpoint.

12.7 Visit 6: Clinical Follow-Up 36 Months

36 months ± 8 weeks post the last breath test performed a phone / in-person visit will be conducted in order to assess major clinical events (i.e. Death, liver transplant, liver decompensation events including: ascites, hepatic encephalopathy, variceal bleeding, hepato-renal syndrome, spontaneous bacterial peritonitis). In addition, the patient's clinical records will be reviewed.

NOTE: Patients undergoing intervention for NASH / NAFLD, including both approved and experimental approaches prior to the defined time points will be terminated hence will not be evaluated for this endpoint.

NOTE: Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

13 ETHICS & REGULATORY CONSIDERATIONS

13.1 Ethics & Regulatory Approvals

The study will be conducted in both the United States of America and Europe. As such, regulatory bodies that are relevant for all countries will be mentioned in this section.

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements.

This protocol and related documents will be submitted for review to all relevant ethics committees delegated to approve the study at their respective sites. Any amendment to the protocol may be proposed by the principal investigator. The amendment must be submitted to the Sponsor and, when approved to the relevant institutional review board (IRB) or ethics committee (EC). When applicable, the implementation of the amendment will take place only once approved by the appropriate ethics committee.

Annual progress and safety reports and a final report at conclusion of the study will be submitted by Exalenz Bioscience (or on behalf of the Sponsor) to the applicable regulatory bodies within the timelines defined in the Regulations.

14 INVESTIGATOR RESPONSIBILITIES

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on GCP, and applicable regulatory requirements. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

The investigational devices will bear an identification number and their accountability will be filed in the Investigator's Study File. Study supplies logged in will be kept by the investigator or the delegated persons in a secure place. All supplies (device, substrate and cannulae) will be used for this study only. After completion of the study, the device, drug and all unused accessories must be returned to Exalenz Bioscience Ltd. as per their request or alternatively, destroyed according to local regulations after receiving explicit authorization by Exalenz to do so and provide Exalenz with a confirmation.

The Principal Investigator will act as custodian for the study data. Patient data will be anonymized and all the anonymized data will be stored on a password protected computer. All data will be compliant with CFR and GCP.

14.1 Investigational Product Handling

The Investigator and Research Pharmacist (if relevant) will be provided with *Investigational Product Handling Guidelines* that will provide details regarding the packaging and labeling requirements, receipt of investigational product, dispensing and accountability procedures, preparation instructions, storage and stability of Investigational product, disposition of Investigational product, with the following forms required: Proof of Receipt Form, Temperature Logs, Accountability Logs, Investigational Product and Material Transfer/Disposition Form and Pharmacy Staff Identification Log (if applicable).

Local forms may be authorized for use after being approved by the Sponsor or his assigned representative.

14.2 Investigational Product Accountability

The Investigator and Study Pharmacist (if relevant) are responsible for ensuring that all study supplies received at the site are inventoried and accounted for throughout the study. The dispensing of study substrate to the subject must be documented in the respective accountability form. The study Investigational Product must be handled in strict accordance with the protocol and the container label and will be stored in a limited access area or in a locked cabinet under appropriate environmental conditions. Unused study materials must be available for verification by the sponsor's site monitor during on-site monitoring. The destruction of unused study materials (both, expired or unexpired) will be documented on the return/disposition form. The Sponsor will authorize to destroy excess supplies on site according to local policy. In this case, before proceeding, the site must seek authorization from the Sponsor using the Return/destruction form and this must also be documented on the Study Supply Return Form.

Study substrate should be dispensed under the supervision of the investigator, a qualified member of the investigational staff, or by hospital clinical pharmacist.

15 SAFETY CONSIDERATIONS

15.1 Adverse Event Definitions

An adverse event (AE) is any undesirable or unintentional event that occurs during use of the study medical device or drug, whether or not considered related to the investigational product; this includes clinically significant changes in laboratory values related to acetaminophen exposure. As this study is not therapeutic in nature, there will be no therapeutic failures to report. Regardless of severity or relationship to the study investigational product, these are recorded in the patient's CRF, all adverse events occurring during the time period when study investigational product is being administered. Associated with the use of the investigational product is defined as a reasonable possibility that the event may have been caused by the medical device or drug.

NOTE: Any AEs reported that are possibly or probably related to the use of the product or related to the procedure within 36 months post last breath test performed or until subject termination will be recorded and followed until resolution and if laboratory work is done secondary to AEs and found abnormal, these tests will be followed until normalization or stabilization.

Serious Adverse Event (SAE) and Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse event (SAE) is an event that is either:

a) fatal
b) life-threatening
c) results in persistent or significant disability/incapacity
d) requires or prolongs inpatient hospitalization
e) is a congenital anomaly or birth defect.

A Suspected Unexpected Serious Adverse Reactions (SUSAR) is an SAE, for which the nature or severity is not consistent with the expected outcomes of the treatment being offered.

Life threatening is defined as an event in which the patient is at risk of death at the time of the event. It does not refer to an event that hypothetically might have cause death if it was more severe. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions. An unexpected adverse event is one that has not been previously observed, or one that is of a specificity or severity not consistent with the current investigator brochure.

When an adverse event occurs, record the following information and assessments in the adverse event section of the CRF:

- The signs, symptoms, or diagnosis of the event
- The date and time of onset of the event using the 24 hour clock where midnight is 00:00 and noon is 12:00
- The adverse event severity using the criteria outlined below
- The relationship of the event to the study medical device as outlined below
- Describe any action taken regarding study medical device disposition
- List any required therapy, medication, treatment, or diagnostic procedure.

15.2 Relationship to Study medical device

Use the following definitions to assess the relationship between an adverse event and the study medical device.

- Not Related:

The event is clearly related to other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.

- Remotely Related:

The event was most likely produced by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs and does not follow a known response pattern to the study medical device.

- Possibly Related:

The event has a reasonable temporal relationship to study medical device administration and follows a known response pattern to the study medical device. However, a potential alternate etiology may be responsible for the event. The effect of medical device withdrawal is unclear. Re-challenge information is unclear or lacking.

- Probably Related:

The event follows a reasonable temporal sequence from the time of medical device administration and follows a known response pattern to the study medical device and cannot be reasonably explained by other factors. There is a reasonable response to withdrawal of the medical device. Re-challenge information is not available or advisable.

- Related:

The event follows a temporal sequence from the time of medical device administration and follows a known response pattern to the study medical device and either occurs immediately following study medical device administration, or improves on stopping the medical device, or reappears on repeat exposure.

15.3 Adverse Event Severity

- Mild Adverse Events

A mild adverse event is one that the symptoms are barely noticeable to the patient. It does not influence performance, require drug treatment or prevent the patient from carrying on with normal life activities.

- Moderate Adverse Events

A moderate adverse event is one that the symptoms make the patient uncomfortable and causes some impairment to normal life activities. Treatment for symptom(s) may be required.

- Severe Adverse Events

A severe adverse event is one that the symptoms cause severe discomforts to the patient and severely limits the patient's normal daily activities. Treatment for symptom(s) is given.

Note: Serious and severe are not synonymous. A serious adverse event must fulfill the requirements listed in the definition above.

15.4 Adverse Event Reporting

All adverse events will be recorded in the case report form. The investigator is responsible for the appropriate medical management of all adverse events. Any serious and unexpected adverse event will be reported to the medical monitor immediately by telephone or fax and follow-up the call with a written report within five days. Full details of the event, any sequealae and an assessment of the relationship to the study medical device will be provided in the report. Any serious and unexpected adverse events and all deaths will also be reported to the EC or IRB within ten days and a copy of this report will be sent to the medical monitor. Exalenz Bioscience will notify all investigators of serious or unexpected adverse events when this information is of global importance to subject safety and welfare. Exalenz Bioscience or its designee will report all adverse events to the FDA, EC and other competent authorities.

A written report is required for all patients who die within 30 days after the end of their participation in the study. This report must document the events surrounding the patient's death and the cause of death. Attach a copy or summary of autopsy findings, if performed.

15.5 Study-Specific Exceptions

Disease complications or death as a result of disease progression is expected and it is not necessary to report them as SUSARs, but they should be reported in the follow-up section of the case report form (CRF).

Furthermore, the following situations and non-fatal toxicities that fulfill the definition of an AE are excluded from expedited notification on an AE form, but should be reported in the follow-up section of the CRF form:

- Elevation of bilirubin
- Elevation liver enzymes
- Nausea, vomiting , diarrhea

Lastly, the following elective procedures should be excluded from expedited notification on an SAE form and should be reported in the follow-up section of the CRF form:

- Central and arterial line insertion, intubation and ventilation, tracheostomy
- Liver transplantation

16 ACCESS TO SOURCE DATA AND DOCUMENTS

16.1 Availability of Source Data and Documents

The Investigator will permit study-related monitoring, audits, EC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents (i.e. patients' case sheets, blood test reports, histology reports etc.).

16.2 Patient Confidentiality

The patient's name and personal data will remain confidential and will not be published in any way. All data will be coded and stored in locked offices or on password protected computers.

17 MONITORING AND QUALITY ASSURANCE

Monitoring of this study will be to ensure compliance with GCP, local regulations, Exalenz Standard Operating Procedure (SOP) for Clinical Monitoring, scientific integrity and will be managed and oversight retained by Exalenz Bioscience (the Sponsor) or its assigned representative.

17.1 Data Collection and Monitoring

An eCRF will be completed for each patient. The EDC (electronic data capture) system will be 21 CRF Part 11 and Annex 11 compliant and the site staff and all involved parties will be trained on the system prior to patient enrolment.

The frequency of monitoring will be determined based on enrollment, but is estimated at a minimum of once every 3 months per site or every 10 subjects, whichever comes first.

Source data verification is deemed necessary for at least 10% of the subjects.

IRB and EC members and regulatory authorities will be permitted to review study documents at the site according to local and federal regulations.

18 PUBLICATION POLICY AND FINANCE

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. The policy regarding publications appears in the non-disclosure agreement signed by each study Investigator prior to signing of the contract.

19 FINANCIAL ASPECTS

The device and drug compounds will be provided by the Sponsor. Funding for regulatory approvals and administration will also be provided by the Sponsor. Funding will also be provided for a study support staff at local sites.

20 STUDY TERMINATION

The study may be terminated after appropriate consultation between the study sponsor and the chief investigator and co investigators. Conditions warranting termination include, but are not limited to:

- Failure of the investigator to enroll patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Failure to adhere to GCP
- Decision by the study sponsor to suspend or discontinue development of the device or kit

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APPENDIX I - PROTOCOL APPROVAL SIGNATURE PAGE

Protocol No: NASH-EX-1114

Protocol Title: Clinical Study of the BreathID® System to train the algorithm for the ^{13}C-Octanoate Breath Test with or without the ^{13}C-Methacetin Breath Test (OBT and MBT respectively) for correlation with histological features associated with findings of Non-Alcoholic Fatty Liver Disease (NAFLD).

Version: 01f

Date of Protocol: 1-MAY-2016

Site Name: _____

Principal Investigator: _____

Print name

I have read this protocol and agree to conduct the study as outlined herein and as per GCP and local regulations.

Principal/ Chief Investigator_____ Date_____

APPENDIX II - HISTOPATHOLOGICAL READING OF LIVER BIOPSY SPECIMEN

The clinical study protocol (NASH-EX-1114) calls for all liver biopsy tissue slides (or their respective scans, if relevant) to be read twice; once by the local (study site) pathologist and once by the central reading pathologist. The characteristics to record for each set of samples are specified within this appendix.

It has to be noted that there may be shrinkage after formalin fixation, and that biopsy length reported by the hepatologist at the bedside is often reported on the unfixed specimen, whereas the pathologist has to report the size of the fixed specimen.

1. Characterization of Specimen Quality:

It is essential that the resulting liver biopsy specimen be adequate so as to allow detailed interpretation. This means that the biopsy should be of large enough size to view a representative amount of parenchyma and number of portal tracts. The clinical study protocol requires samples to be of significant tissue volume to allow histological evaluation; at least 2 cm of length (after formalin fixation) with at least 10 portal tracts to be obtained with a 16-gauge cutting needle or larger are commonly considered the standard qualifications for such evaluation or the equivalent. ***The actual size and number of tracts should be noted on the pathology report.***

2. Tissue Processing

The tissue cuts should be prepared according to the standard process of the local pathology laboratory. Ideally twelve (12) cuts, with a minimum of 2 should be prepared to allow the following staining.

The following stains should be performed at minimum:

(i) Hematoxylin & Eosin (used for grading of inflammation)
(ii) Masson Trichrome (used for staging of fibrosis)

The following stains are elective, but recommended by the protocol:

(i) Sirius Red (used in computer-assisted morphometric quantification of fibrosis)
(ii) Chromotrope Aniline blue (connective tissue)
(iii) Prussian blue (iron storage)

(iv) SMA

The remaining cuts (ideally 4) should be prepared for slides suited for immunohistochemistry and should be stored unstained for further immune-histochemical analysis.

Stained slides may be scanned with a high resolution scanner at x200 to x400 magnification.

3. Histo-Morphological Assessment

The tissue will be examined by light microscopy and scored based upon the NASH CRN NAFLD/NASH scoring system (NAS) as well as the FLINT Consortium (SAF) including:

a. Adequacy of the biopsy sample for reading (including sample length, number of portal tracts or equivalent and adequacy of staining);
b. Steatosis (including grade of 0-3, location, and whether microvesicular);
    i. 0 = <5% hepatocytes involved
    ii. 1 = 5–33% hepatocytes involved
    iii. 2 = 34–66% hepatocytes involved
    iv. 3 = >66% hepatocytes involved
c. Fibrosis based upon Masson's trichrome stain as 0-4 with:
    i. 0 = none
    ii. 1a = mild zone 3 perisinusoidal
    iii. 1b = moderate zone 3 perisinusoidal
    iv. 1c = portal/periportal only
    v. 2 = zone 3 and periportal OR both perisinusoidal and periportal fibrosis
    vi. 3 = bridging
    vii. 4 = cirrhosis
d. Lobular inflammation as (including grade of 0-3 seen under 20-fold magnification) and as presence or absence of microgranulomas and lipogranulomas:
    i. 0 = none
    ii. 1 = <2 foci per x 200 field
    iii. 2 = 2–4 foci per x 200 field
    iv. 3 = >4 foci per x 200 field
e. Portal inflammation (including grade of 0-2):
    i. 0 = none
    ii. 1 = mild
    iii. 2 = greater than mild

f. Liver cell injury:
    i. Ballooning:
        - 0 = none
        - 1 = few ballooned cells
        - 2 = many cells/prominent ballooning
    ii. Presence of acidophilic bodies:
        - 0 =rare/absent
        - 1 = many
    iii. Pigmented macrophages:
        - 0 = rare/absent
        - 1 = many
    iv. Megamitochrondria:
        - 0 = rare/absent
        - 1 = many

g. Mallory bodies:
    i. 0 = rare/absent
    ii. 1 = many

h. Glycogen nuclei:
    i. 0 = rare/absent
    ii. 1 = many

i. Hepatocellular iron grade based on iron stain:
    i. 0 = absent or barely discernible, 40x
    ii. 1 = barely discernable granules, 20x
    iii. 2 = discrete granules resolved, 10x
    iv. 3 = discrete granules resolves, 4x
    v. 4 = masses visible by naked eye

j. Steatohepatitis finding
    i. Pathologists own decision (NASH present, absent, probable and Comments)

k. Resulting NAS score

l. Resulting SAF score

4. Computerized Quantitative Morphometric Analysis