Acute Liver Failure Study Group 13C-Methacetin Breath Test for the Prediction **of Outcome in Acute Liver Injury or Acute Liver Failure (ALFSG-MBT)**

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Acute Liver Failure Study Group

^{13}C-Methacetin Breath Test for the Prediction of Outcome in Acute Liver Injury or Acute Liver Failure (ALFSG-MBT)

Protocol Number:	ALFSG-MBT-003
IDE Number:	G150226
Study Chair:	Robert J. Fontana, M.D. University of Michigan Ann Arbor, MI
Study Intervention Provided by:	Exalenz Bioscience Ltd.
Sponsor of IDE:	William M. Lee, M.D. UT Southwestern Medical Center at Dallas for the Acute Liver Failure Study Group
Protocol Version:	3.0
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Investigator Statement of Agreement

I have read the ^{13}C-Methacetin Breath Test for the Prediction of Outcome in Acute Liver Injury or Acute Liver Failure (ALFSG-MBT) clinical protocol version 3.0 dated 15-March-2018 and agree to implement and conduct the protocol as written in this document.

I agree to comply with the Declaration of Helsinki/Tokyo/Venice on Experimentation in Humans as required by the United States Food and Drug Administration regulations; the Code of Federal Regulations Title 21 parts 50, 56, 312, 800, as applicable; the Code of Federal Regulations Title 45 part 46; International Council on Harmonization Good Clinical Practice Guidelines; and all other applicable laws, regulations and guidelines.

I understand this document contains confidential information of Exalenz Bioscience Ltd., the ALFSG-MBT Executive Committee and the Statistical and Data Coordinating Center at the Medical University of South Carolina that cannot be disclosed to anyone other than members of my staff conducting this trial and members of my Institutional Review Board or Ethical Committee.

I agree to ensure that this information will not be used for any purpose other than the evaluation or conduct of this clinical trial without the prior written permission of the ALFSG-MBT Executive Committee.

_____ _____

Signature of Site Principal Investigator Date

_____

Printed name of Site Principal Investigator

_____

Institution

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1 ABBREVIATIONS

AASLD	American Association for the Study of Liver Disease
AE	adverse event
ALF	acute liver failure
ALF-MBT	Acute Liver Failure ^{13}C-Methacetin Breath Test
ALFSG	Acute Liver Failure Study Group
ALFSG-PI	ALFSG Prognostic Index
ALI	acute liver injury
ALT	alanine transaminase
APACHE II	Acute Physiology and Chronic Health Evaluation II
APAP	acetaminophen (acetyl-para-aminophenol)
ARDS	Acute Respiratory Distress Syndrome
AST	aspartate aminotransferase
AUROC	area under the ROC curve
CPDR	cumulative percentage of ^{13}C dose recovered over time
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CVP	central venous pressure
DCR	Data Clarification Request
DCU	Data Coordination Unit
DIC	disseminated intravascular coagulation
DHHS	Department of Health and Human Services
DOB	delta over baseline
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
eCRF	electronic case report form
ET	endotracheal
FDA	U.S. Food and Drug Administration
FEV_1	forced expiratory volume in one second
FiO_2	fraction inspiratory oxygen
GCP	Good Clinical Practice
GI	gastro-intestinal
HE	hepatic encephalopathy
ICH/GCP	International Conference on Harmonization Good Clinical Practice Guidelines

ICU	Intensive Care Unit
IDE	Investigational Device Exemption
INR	International Normalized Ratio
IRB	Institutional Review Board
KCC	King's College Criteria
LAR	legally authorized representative
LT	liver transplant
MAP	mean arterial pressure
MBT	^{13}C-Methacetin Breath Test
MCS	molecular correlation spectrometry
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
MOP	Manual of Procedures
MSM	medical safety monitor
NAC	n-acetyl cysteine
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PaO_2	partial arterial oxygen
PDR	percentage of dose recovered over time
PDR_{Peak}	the maximum value of PDRs
PEEP	positive end expiratory pressure
PI	principal investigator
RBC	red blood cell
ROC	receiver operating characteristic
RRT	renal replacement therapy
SAE	serious adverse event
SAR	suspected adverse reaction
$ScvO_2$	central venous oxygen saturation
SDCC/MUSC	Statistical Data Coordinating Center at the Medical University of South Carolina
SOFA	Sequential Organ Failure Assessment
SS	spontaneous survivors
SSL	secure socket layer
WBC	white blood cells

2 PROTOCOL SYNOPSIS

Protocol Number/ Title	ALFSG-MBT-003: ^{13}C-Methacetin Breath Test for the Prediction of Outcome in Acute Liver Injury or Acute Liver Failure (ALFSG-MBT)
IDE #	G150226
Version and Date	Version 3.0; 15-March-2018
Study Chair	Robert J. Fontana, MD, University of Michigan, Ann Arbor, MI
Co-Principal Investigators	Valerie Durkalski, PhD, Medical University of South Carolina, Charleston, SC R. Todd Stravitz, MD, VCU Medical Center, Richmond, VA
Sponsor of IDE & Administrative PI	William M. Lee, MD, UT Southwestern Medical Center, Dallas, Texas
Investigative Sites	Approximately 11 investigative sites in the United States.
Objective & Outcomes	**Primary Objective:** To assess the relationship between Day 1 PDR_{Peak} values and Day 21 spontaneous survival in adult patients with ALI that is not related to acetaminophen overdose (non-APAP ALI) or ALF. This outcome requires only one measurement per subject and examines whether the distributions (means) of enrollment PDR_{Peak} values vary between those that spontaneously survive at Day 21 and those that either die or get a transplant by Day 21. **Secondary Outcomes:** 1. Using a continuous variable, we will determine the optimal cut point in the PDR_{Peak} that best distinguishes between outcomes. 2. There are several exploratory aims that will be conducted for future hypothesis generation. These analyses include: assessing the change in MBT measurements (CPDR20, PDR_{Peak}, PDR20 and CPDR30) over a maximum of 7 days from enrollment; assessing the relationship between single time points of MBT measurements (PDR_{Peak}, PDR20 and CPDR20) and clinical outcome (both early and late outcomes, Day 7 and Day 21 SS); and, evaluating the use of the PDR_{Peak} as a prognosis for non-APAP ALI and ALF outcomes (Day 7 and Day 21 SS) either alone or in conjunction with other clinical parameters including etiology, laboratory measurements and other available prognostic indices.
Investigational Product	BreathID® ^{13}C-Methacetin Breath Test System Supplied to the Investigator by Exalenz Bioscience Ltd.

Study Population	Approximately 200 male and female patients, aged 18 to 80 years (have not reached their 81st birthday) with severe acute liver injury that is not related to acetaminophen overdose or acute liver failure present at the time of enrollment into the ALFSG Registry will participate.
Study Design	This is a multicenter, open label, non-randomized study to determine the value of BreathID® ^{13}C-Methacetin Breath Test System (MBT) in predicting the outcome of patients diagnosed with severe acute liver injury that is not related to acetaminophen overdose and acute liver failure who meet inclusion/exclusion criteria. Informed consent will be obtained from the patient and/or patient's legally authorized representative (LAR) or family member as defined in 21CFR50.3(m). All patients will receive medical care for severe acute liver injury or acute liver failure according to the institution's standards of care. Up to 200 evaluable patients will be consecutively enrolled. The MBT (also referred to as "Breath Test") will be performed up to five times during the study period on all enrolled patients. An evaluable patient is one who has completed one or more Breath Tests measured for a minimum of 30 (and ideally 60) minutes after administration of the ^{13}C-Methacetin solution (also referred to as "test substrate"). The first Breath Test will be performed upon admission into the study (Day 1) and repeated on Days 2, 3, 5 and 7, as close to the same time of day as possible, provided no contra-indications are present. If a patient who is enrolled into the ALFSG-MBT Trial with ALI converts to ALF, breath test collection will continue until the maximum of five Breath Tests have been performed. Once the ALI patient has been enrolled into the ALFSG Registry with ALF, subsequent Breath Tests should be performed as close to the same time of day as the previous Breath Tests provided no contra-indications are present. During the initial consent process, the enrolled ALI patient should indicate consent to continue participation into the ALFSG-MBT study should the disease progress to ALF. If an enrolled ALI or ALF patient receives a liver transplant, is discharged /transferred from the hospital or dies prior to Day 7, additional Breath Tests will not be performed. Subjects will be contacted to determine Day 21 spontaneous survival, transplantation and occurrence of serious adverse events since the last Breath Test. Only trained study personnel will perform the breath test procedure and administer the test substrate. Site principal investigators (PIs) and clinical staff will be blinded to the results of the MBT so that there cannot be any reliance on the MBT for clinical decision-making.

Inclusion Criteria	1. Adult men or women (18-80 years of age) 2. Severe acute liver injury (ALI), defined as the development of coagulopathy (International normalized ratio [INR] ≥2.0) and not related to acetaminophen overdose, with no evidence of hepatic encephalopathy (HE); these patients may provide consent themselves. 3. Acute liver failure, defined as the development of coagulopathy (INR ≥1.5) with HE 4. Duration of illness <26 weeks 5. Enrolled into the ALFSG Registry 6. Written informed consent from the patient or patient's legally authorized representative or family member as defined in 21CFR50.3(m)
Exclusion Criteria	1. Evidence of pre-existing chronic liver disease 2. Pre-existing New York Heart Association stage III/IV heart failure 3. Evidence of pre-existing chronic renal failure requiring hemodialysis 4. Chronic hemodialysis prior to hospital admission 5. Evidence of cirrhosis (unless clinically acute Wilson disease or autoimmune non-APAP or ALF) 6. Severe obstructive lung disease (FEV_1 <50% of predicted on previous spirometry) 7. Severe shock, defined as MAP <70 mmHg despite >15 µg/kg/min dopamine, >0.1 µg/kg/min epinephrine, or >0.1 norepinephrine µg/kg/min 8. Extensive small bowel resection (>50 cm) 9. Any evidence of upper GI bleeding at enrollment requiring intervention (endoscopy or RBC transfusion specifically for upper GI bleeding) 10. Liver transplantation prior to enrollment (Note: Listing for LT does not preclude participation in the trial.) 11. Pregnancy or breastfeeding women (Note: Pregnancy related non-APAP ALI or ALF may be considered for entry following the delivery of the baby and assuming the mother does not wish to breastfeed or collect breast milk during the study period.) 12. Allergic to acetaminophen (such as Tylenol® or any other acetaminophen-containing medications) 13. Participation in other clinical studies evaluating other experimental treatments or procedures. (Note: Participation in observatory studies is not an exclusion.) 14. Patients in whom enteral drugs or fluids are contra-indicated or the patient either does not have an appropriately placed naso-

Exclusion Criteria Continued	enteric/orogastric tube *in situ* or cannot tolerate taking the drug preparation orally (200 ml) 15. Budd-Chiari Syndrome 16. Non-APAP ALI or ALF caused by malignancy 17. Moderate and severe ARDS, as defined by Berlin Criteria (see ALFSG-MBT Manual of Procedures (MOP)) 18. Subjects who have received amiodarone in the 30 days prior to study enrollment 19. Consumption of any food or beverage that contains caffeine in the 24 hours prior to enrollment 20. Consumption of any of the following drugs that may interfere with the metabolism of ^{13}C-Methacetin in the 48 hours prior to study enrollment including: allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, disulfiram, Echinacea, enoxacin, fluvoxamine, methoxsalen, mexilitene, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlodipine, thiabendazole, verapamil, zileuton or oral contraceptives 21. Consumption of alcohol in the 24 hours prior to enrollment 22. Smoking cigarettes in the 8 hours prior to enrollment
Study Procedures	The MBT requires administration of an oral dose of ^{13}C-Methacetin solution, followed by retrieval of expired $^{13}CO_2$ over a period of one hour following the test substrate administration, representing first pass kinetics and the ability of the liver to withdraw and metabolize ^{13}C-Methacetin, yielding the product, $^{13}CO_2$. Subjects will have up to five Breath Tests performed once enrolled into the trial and all requirements for the administration of the test substrate have been met. Subjects will be fasting for a minimum of 6 hours from solid food or a minimum of 4 hours from naso-enteric/orogastric tube feeding and tested, in most instances, in the morning hours. The test substrate may be administered in 200 ml of water by mouth or by naso-enteric/orogastric tube. Breath is collected automatically for a total of 75 minutes (up to 15 minutes for baseline measurement and 60 minutes following enteral administration of ^{13}C-Methacetin solution) using the BreathID® MCS device at the subject's bedside. Exhaled breath is collected via a nasal retrieval cannula or via connection of the BreathID® MCS device to the endotracheal tube in intubated patients. The BreathID® MCS device will record data over the 75 minute interval and then the cannula or ET tube connection is removed. During the study hour, all other care may be administered to the patient, including renal replacement therapy, other food intake as necessary; however, it is preferred that the test substrate and Breath Test are not

Study Procedures Continued	administered during dialysis treatments and subjects remain fasting for the duration of the breath test collection. Consumption of the following drugs that may interfere with (compete with or induce) the metabolism of ^{13}C-Methacetin will be recorded on the case report forms and include the following: a. HMG-CoA reductase (Statin) drugs in the 30 days prior to study enrollment; b. Acyclovir and famotidine taken in the 48 hours prior to study enrollment; and c. Any of the following taken during the study period: allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, disulfiram, Echinacea, enoxacin, fluvoxamine, methoxsalen, mexilitene, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlodipine, thiabendazole, verapamil, zileuton or oral contraceptives.
Safety Evaluations	This is not a therapy trial; however, the safety of administration of ^{13}C-Methacetin solution in this setting will be evaluated. Safety assessments will be performed by assessing for acetaminophen levels and levels of acetaminophen-Cys adducts, by collecting daily serum samples for adduct and APAP testing in the laboratory of Dr. Laura James, University of Arkansas for Medical Sciences, Little Rock, AR, on the first 20 evaluable ALF patients.
Safety Analysis	The detection of serum APAP-Cys adducts in those ALF patients not thought secondary to APAP toxicity will be considered significant if a value > 1.0 nmol/L is determined. Likewise, the detection of any level serum APAP in these patients will also be considered significant. An increase of significance in the adduct level in APAP cases or in serial serum APAP levels will also require adjudication as a possible indicator of an additional adverse effect of ^{13}C-Methacetin administration. In addition, standard laboratory parameters including serum electrolytes and Liver biochemistries will be measured in all subjects through a maximum of 7 days (See Schedule of Assessments, Section 8.5).

3 INTRODUCTION

3.1 Background and Rationale

The importance of identifying the patient with ALI or ALF who is likely to die without a liver transplant cannot be overstated and has remained a primary focus of clinical investigation for 25 years. A recent analysis also conducted by the Acute Liver Failure Study Group (ALFSG) found that poor outcomes in the ALI patients are less frequent than is observed in the ALF population. However, in cases where ALI was not related to an acetaminophen (APAP) overdose, progression to poor outcomes was similar: 41% of patients with non-APAP ALI progressed to ALF, transplant and/or death compared to 7% of patients with APAP ALI (1). Unfortunately, analyses have shown that the King's College Criteria (KCC) are inaccurate predictors of prognosis. Clinically based criteria such as the Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores offer improved but still inadequate accuracy (2-4). These traditional scoring systems and prognostic models currently used to monitor patients with ALF lack individual sensitivity and specificity and do not provide direct information about the liver's metabolic function, which is a key variable in assessing liver status and potential disease progression versus recovery in ALF patients. The Acute Liver Failure Study Group (ALFSG) completed an analysis of predictors of outcome in the largest cohort ever studied, 1,974 patients, half of whom were randomly assigned to participate in a model development cohort, and half in a validation cohort (5). The ALFSG Prognostic Index (ALFSG-PI) identified coma grade, ALF etiology, vasopressor use, and the log transformations of admission bilirubin and INR as independent clinical predictors of spontaneous survival in patients with ALF, with a c-statistic of 0.84 by AUROC curve analysis. The ALFSG-PI was also significantly better than Model for End-Stage Liver Disease (MELD) score (0.70) or KCC (0.65). The group also developed a prognosis index for ALI patients that identified etiology, admission values of bilirubin, INR, APAP level and duration of jaundice prior to enrollment as variables associated with prediction of progression to ALF, transplant or death within 21days (1). The ALI prognosis index had a c-statistic of 0.84. Despite these advances, better predictive modalities are still needed.

To more precisely assess the functional reserve of the liver and its capacity to regenerate, we propose to use an actual measure of liver metabolic function, the ^{13}C-Methacetin Breath Test (MBT; Breath ID®, Exalenz Bioscience Ltd., Modi'in, Israel). MBT is a rapid, reproducible, point-

of-care test of liver metabolic function. After oral or naso-enteric/orogastric tube administration, the ^{13}C labeled Methacetin is *O*-demethylated by cytochrome P4501A2 in the liver and further biotransformed into $^{13}CO_2$, which is expired in breath. The BreathID® MCS device captures and quantifies expired $^{13}CO_2$ and standardizes recovery against expired $^{12}CO_2$ through a nasal cannula (in conscious patients) or an adaptor connected to the ventilator line (for intubated patients). The results obtained from the device are expressed as **d**elta **o**ver **b**aseline (DOB), which expresses the change in $^{13}CO_2/^{12}CO_2$ ratio in comparison to the baseline measurement. It can be transformed into the **p**ercentage of ^{13}C **d**ose **r**ecovered over time (PDR) after the ingestion of Methacetin, and the cumulative PDR (CPRD), the rate at which ^{13}C-Methacetin solution is metabolized, derived from the breath $^{13}C/^{12}C$ ratio.

3.2 Summary of BreathID® and MBT Clinical Data

The BreathID® MBT has been used to assess the functional capacity of the liver in several clinical situations. In a study of 165 patients with cirrhosis which included two ALFSG sites, MBT predicted liver-related death and the appearance/exacerbation of ascites better than the Model for End-Stage Liver Disease (MELD) score (6, copy attached).

MBT has also been used in a pilot study of patients with ALF performed at Kings College Hospital and in Israel (7). Sixty-two patients with severe ALI/ALF of diverse etiologies were followed with the MBT during the acute phase of their illness. During their hospital course, 62% of patients spontaneously survived, 21% underwent liver transplantation, and 18% died with a mean follow-up of 7 days after baseline testing. In patients who spontaneously recovered, admission PDR and CPDR at 20 minutes after Methacetin ingestion were higher than those who died or underwent liver transplantation, with p-value of approximately 0.08 for the difference for both indices. Moreover, in the 38 patients in whom post-admission MBTs were available, PDR20 and CPDR20 were both much higher in spontaneous survivors (SS) than those who died or underwent transplant ($p=0.0002$ and 0.001, respectively), as were the changes in both parameters between Day 1 and Day 2-4. (The choice of the later day was not standardized. Values were used in the following order of preference depending upon their availability: Day 3, Day 4, or Day 2, if neither Day 3 nor 4 were available). Subgroup analyses clearly showed that patients who died or underwent transplant had similar PDR 20 and CPDR 20 on Day 1, but both were significantly lower than SS. These data strongly support the hypothesis that the MBT may assess the severity of ALF at

admission and the likelihood of hepatic recovery within 7 days of admission more accurately than laboratory tests regardless of the etiology of liver injury.

Table 1. Methacetin breath test (MBT) results in 62 patients with ALI/ALF on admission (Day 1) and in 38 patients at one time point after admission (Day 3, or Day 2 or 4 if Day 3 is missing).

MBT Parameter	Day	N	Total Population	Spontaneous Survivors	Death/ Transplant	P*
			Mean±SD/ Median[range]	Mean±SD/ Median[range]	Mean±SD/ Median[range]	
PDR 20	1	62	2.60[-1.71 - 33.02]	3.31[-1.71 - 13.52]	1.13[-1.22 - 12.80]	0.088
PDR 20	2-4	38	5.05[-1.41 - 28.68]	7.19[0.58 - 28.68]	1.63[-1.41 - 6.11]	0.0002
PDR 20 Delta	1-4	38	3.06±8.29	5.28±8.96	-1.74±3.46	0.013
CPDR 20	1	62	0.52[-0.32 - 5.72]	0.58[-0.29 - 5.72]	0.19[-0.32 - 3.23]	0.079
CPDR 20	2-4	38	1.13[-0.41 - 20.83]	1.54[0.12 - 20.83]	0.46[-0.41 - 1.31]	0.001
CPRD 20 Delta	1-4	38	0.73[-2.10 - 20.32]	0.85[-2.10 - 20.32]	0.0[-1.98 - 1.04]	0.0009

* ANOVA for normally distributed data; Wilcoxon for non-normally-distributed data. PDR 20, percent dose recovery of $^{13}CO_2$ against $^{12}CO_2$ in expired breath at 20 minutes after administration of ^{13}C-Methacetin. PDR 20 Delta refers to the change between Day 1 and post-admission. CPDR 20, cumulative percent dose recovery in expired breath over 20 minutes after administration of ^{13}C-Methacetin. CPDR 20 Delta refers to the change between Day 1 and post admission. Adapted from (7).

The currently marketed BreathID® Hp System was used in previous clinical trials related to liver applications under IDEs. This system has been modified to allow for longer test duration, resulting in the BreathID® MCS System. The Acute Liver Failure Study Group ^{13}C-Methacetin Breath Test (ALFSG-MBT) Trial using the BreathID® MCS device will allow several tests of each patient at point-of-care, in real-time, without requiring active patient participation (breath is collected passively). Each patient's baseline measurement is compared to the Breath Test conducted that day. The objective is to determine if these results reflect improvement or deterioration of liver metabolic function during the course of the disease, which can assist the physician in the management of patients with non-APAP ALI or ALF.

Since current treatments used to stabilize and care for patients with ALI and ALF may obscure the main parameters used to delineate the prognosis (i.e., INR, bilirubin, grade of hepatic encephalopathy and renal/metabolic status), the MBT may show superiority over the current prognosis models. For example, INR interpretation can be confounded by blood products administered to a patient or may be impacted upon by a variety of medical interventions, such as disseminated intravascular coagulation (DIC); in contrast, the MBT is not affected by blood product administration or development of DIC. Additionally, MBT monitoring could be potentially performed every 24 hours over the patient's course in the Intensive Care Unit (ICU), providing dynamic data that may show earlier changes than routine blood tests, since it measures directly the immediate metabolism of the test substrate, as opposed to assessing the presence of blood components that have accumulated over a greater period of time, such as total bilirubin levels.

3.3 BreathID® ^{13}C-Methacetin Breath Test (MBT) System Description

The ^{13}C-Methacetin Breath Test (MBT) is a non-invasive test for assessing liver metabolic function in order to aid in management of patients with non-APAP ALI or ALF. The BreathID® MCS System consists of the BreathID® MCS device and a test kit containing a breath collection cannula and a non-radioactive isotope ^{13}C-Methacetin solution. It measures and computes the ratio between $^{13}CO_2$ and $^{12}CO_2$ in the patient's exhaled breath. The MBT System has inherent design features and accessories for this indication including a small footprint and portability for deployment in an ICU environment.

The components of the MBT system include the (1) BreathID® MCS unit; (2) Cannula; and the (3) ^{13}C-Methacetin solution. A dedicated flash memory stick for automatic raw data download will be provided to all participating investigative sites. All components of the MBT system will be provided by Exalenz Bioscience Ltd., and a representative from Exalenz will provide the initial on-site training to the study team at each investigative site.

3.3.1 BreathID® MCS Unit

Exalenz Bioscience Ltd. has developed a molecular correlation spectrometer, based on specific optical-radiation emission and absorption by $^{13}CO_2$ and $^{12}CO_2$ gases. This device continuously senses exhaled breath and analyzes CO_2 in real-time through a breath collection cannula connected to the patient. Based on Molecular Correlation Spectrometry (MCS), the BreathID® MCS device

continuously measures $^{13}CO_2$ and $^{12}CO_2$ concentrations from the patient's breath and establishes the $^{13}CO_2/^{12}CO_2$ ratio. The cannula continuously transports the breath sample from the patient to the BreathID® MCS device. Depending upon the status of the patient, the cannula may be nasal (for conscious patients) or an adaptor may be used for connection to the respiratory line (for intubated patients). Results are displayed in real time on the device screen and are printed after the completion of the test. The BreathID® MCS device calculates the delta over baseline (DOB), which can be translated into the percent dose recovery (PDR) and the cumulative percent dose recovery (CPDR) for each time point derived from the ratio difference compared to baseline (normalized/adjusted to the patient's body weight and height as well as test substrate dose) throughout the course of the Breath Test. The maximum value of the PDR is called the PDR_{Peak}. The ^{13}C-Methacetin metabolism begins almost immediately since liquid passes through the stomach to the duodenum, where it is absorbed, without delay. The default breath collection test time is one hour after ingestion of ^{13}C-Methacetin solution.

The light sources are $^{13}CO_2$ and $^{12}CO_2$ discharging lamps. By using $^{13}CO_2$ and $^{12}CO_2$ discharging lamps as light sources, light absorption will be solely due to the existence of $^{13}CO_2$ and $^{12}CO_2$ in the gas mixtures. Furthermore, by using $^{13}CO_2$ and $^{12}CO_2$ discharging lamps as light sources, background radiation will be substantially reduced, leading to highly sensitive absorption curves. These highly sensitive absorption curves enable detection of very small variations in $^{13}CO_2$ and $^{12}CO_2$ concentrations and the $^{13}CO_2/^{12}CO_2$ ratio. The BreathID® MCS System can detect variations of less than 1/1000 in the $^{13}CO_2/^{12}CO_2$ ratio measurement.

The actual breath collection is automatically done by the device and is not operator dependent. If the patient is not connected properly (e.g. the breath does not reach the device), the BreathID® MCS device will prompt the operator to adjust the cannula.

3.3.2 Cannula

There are two test kit options included in the kit; one containing a breath collection cannula with nasal prongs to be used for breath collection in non-intubated/conscious patients, and the other containing a cannula that has an airway adapter to facilitate connection to the respiratory line in intubated patients. The cannula transports the breath sample from the patient to the BreathID® MCS device. The cannula test kit is a single-use kit and is comprised of a plastic sampling line

with an in-line hydrophobic filter (used to reduce the amount of moisture present from the patient's breath) and a Luer-lock connector for connection to the BreathID® MCS device.

Most subjects arriving at the ICU with ALF are unconscious and intubated. As patient status improves and the patient is removed from the respirator, the nasal cannula can be used to capture breath while they are spontaneously breathing. Both types of cannulae are necessary so as to enable regular monitoring and trending of results as patient's status changes during their stay in the ICU. No significant difference in measurements is expected between those captured by the intubated or the nasal cannulae, since the internal capnograph-like instrument of the device automatically sorts out the breath collected and allows through the system only the end tidal portion of the breath exhaled. The device is designed to accommodate an extended range of respiratory rates.

3.3.3 ^{13}C-Methacetin Solution

Exalenz Bioscience Ltd. will supply a ready to use, non-radioactive isotope ^{13}C-Methacetin solution for single-use oral administration (75 mg in 150 ml purified water), dispensed in thermoplastic polyester (PTE) bottles sealed with a plastic child resistant stopper. The ^{13}C-Methacetin solution can be administered through a feeding tube (used in standard ALF patient management), as well as via typical oral administration for ALI and ALF patients able to ingest the test substrate orally. ^{13}C-Methacetin meets all of the qualifications for a liver function test substrate: it is a nontoxic small molecule; is administered orally; is rapidly absorbed; and is exclusively metabolized by the liver. Furthermore, ^{13}C can be easily synthesized into a key location within this molecule. No related serious adverse events have been reported when using this substance, including in vulnerable populations, and the compound remains stable over time.

The ^{13}C-Methacetin solution is a well-known diagnostic reagent that has been described in the literature and used for over 30 years by researchers around the world (8-10). ^{13}C-Methacetin is rapidly absorbed and metabolized by the hepatic mixed function oxidase, via O-demethylation. This process is carried out by hepatic cytochrome P450 enzymes that produce two products, acetaminophen and formaldehyde, which is transformed through two successive oxidative steps to $^{13}CO_2$.

Toxicology testing results in animals and other clinical 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 administered, 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 (11). There have been no reports of any complications with the use of this substance in over 2500 patients tested worldwide (see IDE filing). The main metabolite of Methacetin is acetaminophen which has wide routine clinical use at much higher doses than orally administered dose of ^{13}C-Methacetin of 75mg used in this study. Testing of sera after exposure to 75mg ^{13}C-Methacetin in 23 ALF patients at King's College disclosed no increase in serum acetaminophen-cysteine adducts that would suggest further hepatocyte injury due to the ^{13}C-Methacetin (12).

Many studies using ^{13}C-Methacetin for liver function assessment have been published. Representative references have been cited that support the conclusion that ^{13}C-Methacetin solution is a "safe" test substrate and that it has been used on high-risk population groups such as the elderly, neonates or pregnant women, and patients with cirrhosis (13-16). This provides further assurance that ^{13}C-Methacetin is appropriate to use in breath tests intended for liver function assessment.

4 OBJECTIVES AND ENDPOINTS

4.1 Primary Objective

The primary aim of the study is to assess the relationship between Day 1 PDR_{Peak} values and Day 21 spontaneous survival in patients with ALF or non-APAP ALI. This outcome requires only one measurement per subject and examines whether the distributions (means) of enrollment PDR_{Peak} values vary between those that spontaneously survive at Day 21 and those that either die or get a transplant by Day 21.

4.2 Endpoints

4.2.1 Primary Endpoint

- The primary end-point of the study is PDR_{Peak} at Day 1 that will be treated as a continuous variable.

4.2.2 Secondary Endpoints

1. Using the Day 1 PDR_{Peak} as a continuous variable, we will determine a cut point value that best distinguishes between those that do and do not spontaneously survive at Day 21.

2. There are several exploratory aims that will be conducted for future hypothesis generation. These analyses include: assessing the change in MBT measurements (CPDR20, PDR_{Peak}, PDR20 and CPDR30) over a maximum of 7 days from enrollment; assessing the relationship between single time points of MBT measurements (PDR_{Peak}, PDR20 and CPDR20) and clinical outcome (both early and late outcomes, Day 7 and Day 21 SS); and, evaluating the use of the PDR_{Peak} as a prognosis for Day 7 and Day 21 SS either alone or in conjunction with other clinical parameters including etiology, laboratory measurements and other available prognostic indices.

4.2.3 Safety Endpoints

The administration of Methacetin (75mg/dose) results in the generation of acetaminophen (75mg/dose), and therefore presents a modest safety concern in patients with ALF, particularly in those with APAP overdose. Use of NAC in patients admitted with APAP overdose or non-APAP ALF should obviate minor adverse effect potential of this small dose. In a retrospective study, sera from ALF patients who were given the ^{13}C-Methacetin MBT at King's College were assayed for presence of acetaminophen adducts. Participants of this study received MBT daily up to 7 days. No *de novo* appearance of adducts was observed for non-APAP subjects, nor was there an increase in adduct levels in acetaminophen toxicity subjects.

To confirm the safety profile of the MBT, a prospective safety sub-study is planned for this protocol as outlined in Section 10.1. This safety sub-study will include determining APAP and APAP-adduct concentrations in sera from study participants with ALF immediately prior to each administration of the ^{13}C-Methacetin solution. A positive safety endpoint is defined as:

1. the absence of decline in APAP or APAP-adduct concentration between two consecutive determinations for patients with ALF due to APAP overdose;
2. presence of a significant (toxic quantity, $\geq$ 1 μmol/L) APAP-adducts in patients with non-APAP ALF;
3. adjudicated cases where APAP (non-adduct, parent compound) is detected in the serum of a patient with non-APAP ALF. It should be noted that in some instances, APAP appears to be detected in patients with non-APAP ALF if the total bilirubin is ≥10 mg/dl; these are considered false-positive due to the cross-reactivity between APAP reactants and bilirubin in some colorimetric APAP assays (Polson, et al.). Such cases of detectable APAP in patients with non-APAP ALF will be adjudicated on an individual basis by the study's appointed

independent Medical Safety Monitor to determine the likelihood of Methacetin toxicity. Results of adduct testing will initially be reviewed internally and forwarded in real time to the MSM for review. These data will then be reported in summary fashion to the DSMB.

5 INVESTIGATIONAL PLAN

5.1 Study Design

This is a multicenter, open label, non-randomized study of the MBT to assess functional trends of liver metabolism in patients diagnosed with ALI not related to acetaminophen overdose and patients diagnosed with ALF. The Breath Test will be performed up to five times on all enrolled subjects. The first Breath Test will be performed upon admission into the study (Day 1) and repeated on Days 2, 3, 5 and 7, as close to the same time of day as possible provided no contra-indications are present. If an enrolled ALI or ALF subject receives a liver transplant, is discharged/transferred from the hospital or dies prior to Day 7, no additional Breath Tests will be performed. The study ends after the subject has been contacted to determine Day 21 spontaneous survival, transplantation and occurrence of serious adverse events since the last Breath Test.

If a subject who is enrolled into the ALFSG-MBT Trial with ALI converts to ALF, breath test collection will continue until a maximum of five Breath Tests have been performed. Once the ALI subject has been enrolled into the ALFSG Registry with ALF, subsequent Breath Tests should be performed as close to the same time of day as the previous Breath Tests provided no contra-indications are present.

Site principal investigators (PIs) and clinical staff will be blinded to the results of the MBT so that there cannot be any reliance on the MBT for clinical decision-making.

5.2 Study Population

Up to 200 evaluable male and female patients, aged 18 to 80 years (have not reached their 81st birthday) with severe acute liver injury not related to acetaminophen overdose or acute liver failure present at the time of enrollment into the ALFSG Registry will be consecutively enrolled. An evaluable patient is one who has completed one or more Breath Tests measured for a minimum of 30 (and ideally 60) minutes after administration of the ^{13}C-Methacetin solution.

5.3 Study Sites

Study sites will include up to 11 of the clinical sites located in the United States that are involved in the ALFSG.

5.4 Investigational Product

5.4.1 Preparation of ^{13}C-Methacetin Solution

Exalenz will provide 75 mg ^{13}C-Methacetin in a 0.05% solution in 150mL purified water supplied in amber thermoplastic polyester (PET) bottles. No preparation is needed.

5.4.2 Investigational Product Handling

The Investigator and/or 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.

5.4.3 ^{13}C-Methacetin Accountability

The Investigator and/or Lead Study Pharmacist are responsible for ensuring that all test substrate supplies received at the site are inventoried and accounted for throughout the study. The dispensing of test 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 will be verified by the Sponsor's site monitor when on-site monitoring is conducted. 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.

The ^{13}C-Methacetin solution should be dispensed under the supervision of the investigator, a qualified member of the investigational staff, or by hospital clinical pharmacist.

5.4.4 Investigational Device Accountability

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, test 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 Sponsor to do so and provide the Sponsor with written confirmation.

5.5 Risk Based Monitoring

To ensure compliance with GCP, local regulations and scientific integrity, monitoring of this trial will be managed and oversight retained by the Sponsor or designee. The scope of monitoring includes on-site visits, remote and central review as described in the ALFSG-MBT Monitoring Plan.

6 ELIGIBILITY CRITERIA

6.1 Inclusion Criteria

1. Adult men or women (18-80 years of age)
2. Severe acute liver injury, (ALI) defined as the development of coagulopathy (International normalized ratio [INR] ≥2.0) and not related to acetaminophen overdose, with no evidence of hepatic encephalopathy (HE); ALI patients may provide consent themselves.
3. Acute liver failure, defined as the development of coagulopathy (INR ≥1.5) with presence of any degree of hepatic encephalopathy
4. Duration of illness <26 weeks
5. Enrolled into the ALFSG Registry.
6. Written informed consent from the patient or patient's legally authorized representative or family member as defined in 21CFR50.3(m)

6.2 Exclusion Criteria

1. Evidence of pre-existing chronic liver disease

2. Pre-existing New York Heart Association stage III/IV heart failure
3. Evidence of pre-existing chronic renal failure
4. Chronic hemodialysis prior to hospital admission
5. Evidence of cirrhosis (unless clinically acute Wilson disease or autoimmune non-APAP ALI or ALF)
6. Severe obstructive lung disease (FEV_1 <50% of predicted on previous spirometry)
7. Severe shock, defined as MAP <70 mmHg despite >15 µg/kg/min dopamine, >0.1 µg/kg/min epinephrine, or >0.1 norepinephrine µg/kg/min
8. Extensive small bowel resection (>50 cm)
9. Any evidence of upper GI bleeding at enrollment requiring intervention (endoscopy or RBC transfusion specifically for upper GI bleeding)
10. Liver transplantation prior to enrollment. (Note: Listing for LT does not preclude participation in the trial.)
11. Pregnancy or breastfeeding women (Note: Pregnancy related non-APAP ALI or ALF may be considered for entry following the delivery of the baby and assuming the mother does not wish to breastfeed or collect breast milk during the study period.)
12. Allergic to acetaminophen (such as Tylenol® or any other acetaminophen-containing medications)
13. Participation in other clinical studies evaluating other experimental treatments or procedures. (Note: Participation in observatory studies is not an exclusion.)
14. Patients in whom enteral drugs or fluids are contra-indicated or the patient either does not have an appropriately placed naso-enteric/orogastric tube *in situ* or cannot tolerate taking the drug preparation orally (200 ml)
15. Budd-Chiari Syndrome
16. Non-APAP ALI or ALF caused by malignancy
17. Moderate and severe ARDS, as defined by Berlin Criteria ALFSG-MBT MOP
18. Subjects who have received amiodarone in the 30 days prior to study enrollment
19. Consumption of any food or beverage that contains caffeine in the 24 hours prior to enrollment
20. Consumption of any of the following drugs that may interfere with the metabolism of ^{13}C-Methacetin in the 48 hours prior to study enrollment including: allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, disulfiram, Echinacea, enoxacin, fluvoxamine,

methoxsalen, mexilitene, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlodipine, thiabendazole, verapamil, zileuton or oral contraceptives

21. Consumption of alcohol in the 24 hours prior to enrollment
22. Smoking cigarettes in the 8 hours prior to enrollment.

6.3 Prohibited During Study Period

There are no specific contraindications in this protocol. However, consumption of drugs that may interfere with (competes or induces) the metabolism of ^{13}C-Methacetin will be recorded on the case report forms and include the following:

a. HMG-CoA reductase (Statin) drugs in the 30 days prior to study enrollment;
b. Acyclovir and famotidine taken in the 48 hours prior to study enrollment; and
c. Any of the following taken during the study period: allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, disulfiram, Echinacea, enoxacin, fluvoxamine, methoxsalen, mexilitene, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlodipine, thiabendazole, verapamil, zileuton or oral contraceptives.

7 SUBJECT ENROLLMENT

7.1 Eligibility Assessment

All patients with ALI not related to acetaminophen overdose and patients with ALF enrolled into the ALFSG Registry should be screened for eligibility for entry into this trial. As in all trials, the goal is to achieve a high level of compliance with protocol requirements by assuring during the eligibility assessment that the potential subject or legally authorized representative (LAR) is fully informed and agrees to the protocol requirements. Careful assessment of the patient's or LAR's understanding of the trial is required prior to enrollment.

7.2 Presentation of Informed Consent

Patients who meet criteria for ALI are, by definition, not encephalopathic and will be able to consent for enrollment without additional consents being obtained from the LAR or family member, although both consents may be obtained. During the initial consent process, ALI patients should indicate consent to continue participation into the ALFSG-MBT study should the disease progress to ALF.

In some cases, patients with ALF may have already returned to their baseline mental status and will be able to provide consent for themselves. In all other cases, the LAR of patients with ALF will sign a consent/assent form prior to study participation. Due to the critical state of many patients with ALF and the fact that patients are not always escorted by a relative or other representative, if local IRB will allow, a documented telephone consent with a patient's LAR may suffice to provide consent.

Informed consent will be obtained by either the site Principal Investigator or by individuals approved by the site Principal Investigator and whose names have been submitted to the ALFSG-MBT regulatory database. The consent should be the current IRB-approved version corresponding to the version of the protocol approved when the screening was initiated. All elements listed in the International Council on Harmonization Good Clinical Practice guidelines (ICH/GCP), to the extent that it is compatible with the US FDA and DHHS regulations, must be included in the informed consent.

In accordance with US FDA regulations (21 CFR 50) and guidelines (Federal Register, May 9, 1997, Vol. 62, Number 90 - ICH Good Clinical Practice Consolidated Guideline) it is the investigator's responsibility to ensure that informed consent is obtained from the patient or patient's LAR before participating in an investigational study, after an adequate explanation of the purpose, methods, risks, potential benefits and subject responsibilities of the study. Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent.

On the other hand, informed consent must be obtained prior to initiation of any screening procedures that are performed solely for the purpose of determining eligibility for research. For the ALFSG-MBT study, there is no activity required in the screening process that is not typically included within the reasonable scope of standard care evaluation for patients with acute liver injury and acute liver failure; patients would be approached for consent only after the clinical screening process had established eligibility.

Informed consent will be obtained from the patient or patient's LAR after the details of the protocol have been reviewed. The individual responsible for obtaining consent will assure, prior to signing

of the informed consent, that the patient has had all questions regarding study procedures and the protocol answered. During the initial consent process, ALI patients should indicate consent to continue participation into the ALFSG-MBT study should the disease progress to ALF.

Each subject/LAR must be given a copy of the informed consent. The original signed consent must be retained in the institution's records and is subject to review by the Sponsor, SDCC/MUSC, the FDA or representative from another agency that performs the same function, and the IRB responsible for the conduct of the institution.

8 STUDY PROCEDURES

8.1 Preamble

The study will be conducted in compliance with this protocol, with GCP standards, and applicable regulatory requirements. Only trained personnel on the delegation of authority log (doctors, nurses, study coordinators) will perform the breath test procedure. The ^{13}C-Methacetin solution should be administered by a medical practitioner registered on the delegation log or a research coordinator with specific training in test substrate administration. The site will contact the subject to determine Day 21 spontaneous survival, transplantation and occurrence of serious adverse events since the subject's last study procedure.

All investigators will be blinded to the MBT score results until the end of the study. The MBT results will therefore not affect the current patient management. In order to maintain the blind, the site Study Coordinator will perform the MBT and transfer the data to the DCU directly, without review by the site-PI.

8.2 Administration of the Breath Test

The Breath Test will be performed up to five times during the study period on all enrolled subjects. The first Breath Test will be performed as close to the time of study enrollment as possible upon admission into the study (Day 1). The Breath Test will be repeated on Days 2, 3, 5 and 7 as close as possible to the same time of day as the first Breath Test. If a subject who is enrolled into the ALFSG-MBT Trial with ALI converts to ALF, breath test collection will continue until a maximum of five Breath Tests have been performed. Once the ALI subject has been enrolled into the ALFSG Registry with ALF, subsequent Breath Tests should be performed as close to the same time of day as the first Breath Test. If an enrolled ALI or ALF subject receives a liver transplant,

is discharged/transferred from the hospital or dies prior to Day 7, no additional Breath Tests will be performed.

The following characteristics will be collected on each day of the MBT before administration of test substrate:

- The mechanism of ventilation (spontaneous or mechanical);
- Route of administration of test substrate (by mouth or by naso-enteric or orogastric tube);
- Location of the distal end of the naso-enteric tube (intragastric or intra-duodenal) or orogastric tube;
- Concurrent use of vasopressors, acyclovir, famotidine and statins;
- Last meal or tube feeding;
- Subject's weight;
- Concurrently administered medications, including NAC; and
- Use of renal replacement therapy during the MBT.

8.2.1 Requirements for Administration of Test Substrate

1. Fasting

   Subjects will perform the breath test once enrolled into the trial and when the following criteria have been met:

   - Protocol-specified labs are completed prior to the administration of the test substrate.
   - *Oral administration of test substrate:* The subject should be fasting from solid food for a minimum 6 hours and from oral medications for a minimum of 1 hour.
   - *Naso-enteric and orogastric tube administration of test substrate.* In subjects who are receiving tube feeding, feeding should be discontinued for a minimum of 4 hours. Gastric contents should be aspirated prior to the instillation of ^{13}C-Methacetin solution *via* the naso-enteric or orogastric tube.

   Feeding may be resumed after the MBT has been completed (*i.e.*, at 60 minutes after administration of the test substrate).
2. Subject has not ingested acetaminophen-related medications (e.g. Tylenol) within the past 24 hours (subjects with acetaminophen intoxication may be included 24 hours after ingestion).
3. Subject has not received general anesthesia in the last 24 hours. This does not include appropriate utilization of sedative and analgesic drugs for subjects within a critical care

environment, such as propofol sedation or sedation related to liver biopsy. In those instances, all enterally and parenterally administered medications will be recorded in the study CRF.

4. Evidence of ileus or presenting with contra-indication to administration of oral drugs.
5. The MBT should not be performed in cases where there is a suspicion that aspiration may occur.
6. Subjects who require RRT (intermittent and continuous) after enrollment into the study will not be excluded; however, it is preferred that the test substrate and Breath Test are not administered during dialysis treatments.

Note: Subjects with gastroparesis may be included as the delayed emptying has negligible impact on fluids (17). The presence of evidence of delayed gastric emptying and/or ileus should be noted on the CRF for further analysis to show that the impact is negligible for MBT measures. Naso-enteric and orogastric tubes should be clamped for 10 minutes after administration of the ^{13}C-Methacetin solution.

8.2.2 Performance of the Breath Collection

The breath test collection will be performed after the test substrate is administered to the subject; no waiting period is required once the test substrate has been ingested. The entire contents of the test substrate need to be ingested by the subject; if the subject is not able to ingest the entire amount, the Breath Test should not be performed.

Breath is collected automatically *via* a nasal cannula, or if the subject is intubated, by a ventilator hose adaptor at the subject's bedside for a total of 75 minutes (up to 15 minutes for baseline measurement and 60 minutes following ingestion of ^{13}C-Methacetin solution). All test-specific equipment will be supplied by Exalenz.

8.2.3 Early Termination of the Breath Test

Key personnel will be trained how to terminate the breath test early if a subject is unable to complete the full 75 minutes of breath collection. In the following situations, the breath test collection will be terminated:

- The subject vomits.
- The subject has to be disconnected from the nasal cannula/ collection device for more than 1 minute due to an urgent procedure.
- The subject is inadvertently disconnected from the BreathID® MCS device.

- The BreathID® MCS device malfunctions.

8.2.4 Interruptions and Confounders of the Breath Test

Breath Tests should be timed to anticipate interruptions and possible confounders. Recognizing that patients with ALI and ALF are critically ill, guidelines if deviations occur can be found in the ALFSG-MBT Manual of Procedures.

Should an interruption in breath collection due to removal of the cannula or its disconnection occur during the administration of the MBT and the device cannot compute the delta over baseline (DOB) results, the next MBT will not be repeated until 24-hours after the interruption time of the previous Breath Test. If the interruption occurred at the beginning of the Breath Test (during baseline breath collection) and before ingestion of the ^{13}C-Methacetin solution, the test can be repeated immediately. If the interruption occurs on Days 3 or 5, then the study team should perform the MBT on the next study day as outlined in the Schedule of Assessments. The MBT is considered 'performed' and the subject "evaluable" if it has been measuring the subject's breath for a minimum of 30 (and preferably 60) minutes after test substrate has been administered.

8.3 Performance of the APAP Adduct Assays and APAP Concentration Assays

Serum for acetaminophen (APAP) and APAP-adduct levels will be collected with routine morning labs on each day from Day 2-7, as well as Day 1 as a baseline value, from the first 20 ALF subjects enrolled. Serum samples will be reserved for the sole purpose of determining whether APAP or APAP-adducts accumulate during repeated administrations of the ^{13}C-Methacetin solution in patients with ALF. The collected samples will be shipped to and analyzed by Dr. Laura James at the University of Arkansas.

8.4 Clinical and Laboratory Assessments

Subjects will routinely undergo laboratory tests as part of the ALFSG Registry. Specific to this study, sites will be required to collect lactate, ammonia, phosphate, AST/ALT, creatinine, bilirubin and INR on a daily basis.

8.5 Schedule of Assessments

	Screening/ Subject Enrollment	Day 1	Day 2	Day 3	Day 5	Day 7	Day 21
Demographics	**X**						
Medical History	**X**						
Confirm Eligibility Criteria^	**X**						
Prior Medications	**X**						
Concomitant Medications		**X**	**X**	**X**	**X**	**X**	
Grade of HE	**X**	**X**	**X**	**X**	**X**	**X**	
Labs (lactate, bicarbonate, INR, total bilirubin, phosphate, Creatinine, WBC, Platelet count, ammonia-arterial or venous (if clinically indicated)		**X**	**X**	**X**	**X**	**X**	
RRT (record time/tx prior to MBT)		**X**	**X**	**X**	**X**	**X**	
MBT		**X**	**X**	**X**	**X**	**X**	
Serum for APAP and APAP-adduct levels*		**X**	**X**	**X**	**X**	**X**	
AE Reporting		**X**	**X**	**X**	**X**	**X**	**(SAEs only)**
End of Study							**X**
Collection of data for MELD, KCC, and ALFSG-PI	**X**	**X**	**X**	**X**	**X**	**X**	

*** Only in the first 20 consecutive ALF subjects enrolled**

^ In women of child-bearing potential; Urine pregnancy test or blood human chorionic gonadotropin (hCG) is acceptable.

9 DISCONTINUATION OF PARTICIPATION

9.1 Withdrawal of Consent

The subject or the subject's LAR has the right to voluntarily withdraw consent from the study at any time for any reason without prejudice to his/her future medical care by the physician or at the institution. The Investigator must withdraw any subject from the study if requested.

For the occasional subject who withdraws consent, the date, time and reason for consent withdrawal should be documented. Subject data will be included in the analysis up to the date of the consent withdrawal. No further study evaluations should be performed and no additional data or biological samples should be collected once consent is withdrawn by the subject or the subject's LAR.

Below is the procedure to be followed at the time a subject/LAR withdraws consent from the trial:

(1) Check for the development of adverse events.

(2) Complete the End-of-Study form and include an explanation of why the subject is withdrawing consent.

(3) Subject or subject's LAR will be required to document in writing his or her desire to withdraw consent.

9.2 Withdrawal from Study Procedures

As participation in the ALFSG-MBT Trial is voluntary, the subject or subject's LAR may request to discontinue study procedures at any time. In addition, the treating physician may stop the study procedures if there is a safety concern; reasons that subjects may have procedures discontinued include, but are not limited to the following:

- The subject has recovered or has been discharged from the hospital prior to the Day 7 MBT. The subject will be considered to have recovered if hepatic encephalopathy has resolved, INR < 1.6 and/or AST < 150 U/L and/or Bilirubin < 50% of its peak value. Alternately, if the subject has been discharged from hospital the subject will be considered to have recovered.
- Liver transplantation during the study procedure period (excluding the Day 21 visit)
- The subject's naso-enteric/orogastric tube is removed and oral administration of the drug preparation is not tolerated.
- Plasma transfusion

Subjects who had an early discontinuation of study procedure period (excluding the Day 21 visit) will continue to be followed carefully through the 21-day study period unless consent is withdrawn and all follow-up data will be included in the analysis.

9.3 Subject Lost to Follow-Up

In the event that all possible attempts to locate the subject for the Day 21 follow up interview have failed, all efforts made by the site study personnel to contact the subject will be documented and included in the subject's study binder.

10 SAFETY AND ADVERSE EVENTS

10.1 Safety Sub-study

The safety of the MBT has been demonstrated in several clinical applications. However, the repeated administration of the test substrate, which represents the equivalent of 75 mg of APAP per administration, will be closely monitored to ensure that this small dose of APAP will not result in additional liver injury (particularly in the presence of NAC). The results of the Retrospective Safety Study were examined before enrollment in the prognosis study began, as outlined in Section 4.2.3 of this protocol. A second, prospective safety study will be performed and sera collected from the first 20 ALF subjects enrolled into the ALF-MBT Trial as described herein, in whom we propose to administer MBT on Day 2, 3, 5, and 7 unless the subject is discharged/transferred, transplanted or dies. Serum for acetaminophen (APAP) and APAP-adduct levels will be collected with routine AM labs on each day from Day 2-7, as well as Day 1 as a baseline value. Serum samples will be reserved for the sole purpose of determining APAP or APAP-adducts levels in patients with ALF during repeated administrations of the ^{13}C-Methacetin solution and will be performed by Dr. James at the University of Arkansas for Medical Sciences, Little Rock, AR.

10.2 Adverse Events

Adverse event reporting and procedures are described in detail in the MOP.

In the ALFSG-MBT Trial, any medical conditions not present prior to the initial administration of the test substrate but that emerge after the initial test substrate is administered are considered AEs. Adverse Events encountered from Day 1 through Day 7 of the study period will be recorded, and Serious Adverse Events from Day 1 through the Day 21 of the study period will be recorded.

Designated study personnel at all investigative sites are responsible for entering any and all non-serious and serious adverse events, regardless of severity or relationship to the ^{13}C-Methacetin solution and device, and including updates to adverse event information (e.g., date of resolution, action taken) as needed, into the WebDCU™ database within timelines defined in the MOP and in the subject's source documents. Upon data submission of the SAE eCRF, the system will trigger an automatic e-mail notification to the independent Medical Safety Monitor (MSM) stating that an SAE has occurred. The MSM will access the information via the password protected web based system for review and report safety concerns to the DSMB.

Adverse events will be defined and severity graded according to the Common Terminology Criteria for Adverse Events (CTCAE) found in the MOP. AEs will be submitted online through WebDCU™ and coded centrally into standard terminology using AEs will be using the Medical Dictionary for Regulatory Activities (MedDRA). Guidelines for report content and structure are provided in the MOP.

10.3 Data Safety Monitoring Board (DSMB)

The Data Safety Monitoring Board (DSMB) used for this study will be that designated by NIDDK to oversee the ALFSG. Semi-annual data quality and safety reports will be provided to the DSMB. This timeline can be changed by the DSMB if requested. The analysis of all adverse events will include incidence tables and summary of AEs and SAEs by severity and relationship to device or drug.

11 DATA MANAGEMENT

11.1 Data Handling

The raw data from the individual tests on the BreathID® MCS device will be analyzed using Exalenz's trending software. This program provided by Exalenz will combine all tests for any given subject bearing the same identification number and will plot the trend of the breath test results with the percent change displayed. This analysis will be carried out only after the subject has completed his/her participation in the study and in order not to bias the treating physician, the results will not be shared until the study is completed.

For all other study data, sites will enter case report form data into the password protected study database housed in WebDCU™. The WebDCU™ is a validated web-based clinical trials

management system that provides the infrastructure for real-time data capture and data sharing and includes designated web servers and supporting database servers. This user-friendly web-based database system will be used for subject enrollment, data entry, data validation, project progress monitoring, user customizable report generation, and secure data transfer. The web-based data capturing system allows for study data to be directly entered into the study specific database by the site via a secure internet connection. Secure Socket Layer (SSL) is used for data encryption. The web system combines all study tools into one system which includes study database, online training and help desk, subject calendar, electronic data clarification request (DCR) process, data entry, case report form (CRF) and participate tracking system, audit trail, and report generation mechanisms. This is the system used for the currently ongoing ALFSG Registry. Clinical and laboratory data will be collected by the site in order to derive MELD, ALFSG-PI, ALI-PI and KCC scores on a daily basis. These clinical data and laboratories are mandatory as they will be needed for calculation of the clinical outcome predictor indices.

12 STATISTICAL CONSIDERATIONS

12.1 Sample Size

The relationship between the Day 1 PDR_{Peak} and clinical outcome (21-day spontaneous survival) will be assessed using logistic regression with a two-sided alpha of 0.10. Based on previous enrollment rates from the ALFSG Registry, we estimate an accrual rate of approximately 100 ALF or non-APAP ALI patients per year into the ALFSG-MBT Trial. The power of the logistic model predicting 'good' outcome (21 Day SS) is based on the proportion of 'good' outcome at the mean MBT value. Assuming this proportion is 0.50, we determined that approximately 200 enrolled non-APAP ALI and ALF patients are needed in order to ensure sufficient power (80%) to detect odds ratios of 1.5 or higher.

12.2 Statistical Analyses

12.2.1 Futility Analysis

A futility analysis will be conducted after 100 consecutive enrollments with 21-day outcome obtained**.** This analysis is based on the error spending function method with O'Brien and Fleming (OBF) type stopping guidelines. The goal of this analysis is to consider stopping the study early if there is no signal of a difference in the PDR_{Peak} distributions between those that SS at 21days

and those that do not SS. If the p-value of a two-sided t-test for the difference in means at an alpha level of 0.10 is 0.807 or greater, then the study may be considered stopped for futility.

12.2.2 Final Analyses

Preliminary analyses will be conducted to describe the collected variables. Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all collected variables along with two-sided 95% confidence intervals. The primary endpoint, Day 1 PDR_{Peak} will be compared between those that spontaneously survive (SS) by Day 21 and those that either die or receive a transplant by Day 21 with a logistic regression model at a two-sided alpha level of 0.10. All enrolled patients with a value at Day 1 will be included in the analysis. The primary analysis will not stratify by non-APAP ALI/ALF but exploratory analyses will be conducted that examine potential differences as described below. It is not anticipated that any enrolled patient will be missing the outcome of SS.

There are several exploratory aims that will be conducted for future hypothesis generation. In addition to the comparison of mean PDR_{Peak} values between the two cohorts, a cutpoint for the PDR_{Peak} value will be examined using a receiver operating curve. The identified cutpoint will be compared to other known prognostic indices including Kings College criteria (KCC), Model of End-Stage Liver Disease (MELD), and the ALFSG-PI (prognostic index). As a secondary analysis and where serial measurements are available, the trends in the MBT results (PDR_{Peak}, PDR20, CPDR20) will be related to outcome (Day 21 SS) using repeated measures analysis. A multivariable regression will be performed to assess if PDR_{Peak}, in combination with traditional prognostic measures provides an accurate prediction of Day 21 outcome. These pre-specified exploratory analyses will be conducted using a significance level of 0.05.

With the inclusion of non-APAP ALI patients, we will conduct exploratory subgroup analyses and examine the association with MBT results for each subgroup (non-APAP ALI and ALF). Based on current registry enrolments, we anticipate the study population to consist of 17-20% non-APAP ALI enrollments. A total of 35-40 non-APAP ALIs will not provide definitive evidence but will provide further hypothesis generating information. For the non-APAP ALI subgroup, we will also examine the PDR peak when poor outcome is defined as progression to ALF, transplant or death within 21days. Point estimates and confidence intervals will be used for interpretation of these analyses more than pvalues due to the exploratory nature of the analyses. We anticipate roughly

10% will convert to ALF. All statistical analyses will be conducted using SAS V9.2 or higher (SAS Institute, Inc., Cary, NC). The specified analyses will be repeated as an exploratory aim with the Day 7 outcome of SS.

13 OBLIGATIONS OF THE INVESTIGATOR

13.1 Compliance with Good Clinical Practice (GCP)

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. Compliance with GCP 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.

13.2 Access to Source Data and Documents

The PI and designees agree to maintain accurate CRFs and source documentation as part of the case histories. Source documents are the originals of any documents used by the PI or Sub-Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. The study CRFs will be available in printable format on the study website. The CRFs for each patient must be completed only by persons designated by the PI and who have data entry permissions for the study database. All data entered into the CRF must also be available in the source documents. Once a subject completes the study, the PI must review the completed study book and sign the End of Study Form that acknowledges this review.

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.).

13.3 Retention of Documentation

In June 2005, Federal law extended the statute of limitations to six (6) years to bring forward an allegation of research misconduct. In response to this extension, research records must be retained for a sufficient period to investigate an allegation of research misconduct--a minimum period of six (6) years.

Additionally, existing Federal regulations [56 CFR 56.115(b)] require that IRB records be retained for at least 3 years after completion of the research. All records must be accessible for inspection and copying by authorized representatives of DHHS and FDA at reasonable times and in a reasonable manner. At the end of three years, the IRB records may be boxed, labeled and sent to central storage for an additional 3-10 years. A log of stored records is maintained in the IRB office for retrieval if files are needed for audit or other purposes.

An agreement must be in place between the clinical site Principal Investigator and the Principal Investigator/Sponsor regarding records that may be destroyed. Records will be maintained in a de-identified manner in a locked location to ensure confidentiality.

13.4 Confidentiality of Material Provided by Exalenz Bioscience Ltd.

Any written materials provided by Exalenz Bioscience Ltd., as well as documentation, data, and all other information generated as part of this study will be held in strict confidence by the Investigator and participating clinical site staff. Conduct of this study will comply with all provisions of the Health Insurance Portability and Accountability Act of 1996.

13.5 Publication

Publication of the results of this trial will be governed by the policies and procedures developed by the ALFSG Executive Committee. All manuscripts pertaining to this study will be forwarded to Exalenz Bioscience Ltd. and the NIDDK staff for review before submission for publication. The Publication Policy will be fully compliant with the voluntary NIH Public Access Policy mandated by the Consolidated Appropriations Act of 2008 (Division G, Title II, Section 218 of PL 110-161). The Executive Committee will follow NIH policies on data-sharing (as described at the site: http://grants2.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm and any updates thereto).

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