Mitochondrial Cofactors for the Treatment of Hyperbilirubinemia Due to PEG-asparaginase or Inotuzumab Ozogamicin in Patients with Acute Lymphoblastic Leukemia (ALL)

1.0 Objectives

1.1 Primary Objectives:
- 1.1.1 To evaluate the efficacy of levocarnitine in combination with vitamin B complex in treating PEG-asparaginase or inotuzumab ozogamicin induced hyperbilirubinemia (total bilirubin >3xULN) in patients with ALL.

1.2 Secondary Objectives
- 1.2.1 To evaluate chemotherapy dose intensity in patients treated with PEG-asparaginase or inotuzumab ozogamicin
- 1.2.2 To characterize the safety, tolerability, and adverse event profile of levocarnitine and vitamin B for the treatment of hyperbilirubinemia.

2.0 Background

2.1 PEG-asparaginase hepatotoxicity in adult patients with acute lymphoblastic leukemia (ALL).

Despite exceptionally high rates of survival in pediatric patients with ALL of greater than 90%, adult patients continue to have markedly inferior outcomes[1,2]. As age at diagnosis increases, survival decreases to a rate of less than 20% for patients over the age of 60 treated in the United States[2]. This large disparity in outcomes is likely multifactorial and may be due to the fact that adult patients more frequently have worse genetic aberrations at diagnosis in addition to comorbidities which result in poor tolerability and dose reduction of cytotoxic chemotherapy or elimination of certain agents completely. *E. coli* L-asparaginase is an agent that is effective in the treatment of pediatric ALL, but has traditionally been omitted from adult treatment regimens due to poor tolerability. The unique side effect profile of L-asparaginase includes infusion-related reactions, hyperglycemia, hypertriglyceridemia, thrombosis and hemorrhage, hepatotoxicity, and pancreatitis[3]. A higher incidence of hepatotoxicity in addition to hyperglycemia, and hypofibrinoginemia has been reported in adult patients treated with asparaginase, compared to the pediatric population[3]. PEG-asparaginase (PEG) is the pegylated version of *E. coli* L-asparaginase and has replaced the original formulation, *E.coli* L-asparaginase, on the United States market. While pegylation of the product results in a prolonged half-life and less frequent dose administration, toxicities can be observed for a prolonged period of time after drug administration. Hepatotoxicity is a particularly difficult toxicity to manage and has played a large role in limiting the use of PEG in adult patients. Unlike other side effects such as thrombosis, bleeding and hyperglycemia that can be directly managed through supportive care treatments, hepatotoxicity lacks such treatments. Furthermore, severe hepatotoxicity itself can cause alterations in bleeding and clotting, ascites, renal dysfunction, treatment delays leading to increased morbidity and mortality especially in the setting of neutropenia[4]. Historical experience with PEG in adult patients (median 42 yo, range 22-69) at our institution resulted in 41% of patients experiencing grade $\geq$ 3 hyperbilirubinemia; and 51% of patients unable to complete their intended therapy, largely due to hepatotoxicity[5]. Even in a younger cohort of patients (median age 21), 38% of patients experienced hyperbilirubinemia when treated with PEG as part of a separate protocol[6]. Outside institutions have also reported similar rates of hepatotoxicity, ranging from 10%-37%, in adult patients

treated with PEG[4,7-9]. The high rates of hepatotoxicity limit the use of PEG in adults, therefore, assessing the efficacy of supportive therapy for its management is warranted.

Although hyperbilirubinemia is frequently reported in adult patients treated with PEG, determining risk factors associated with severe hyperbilirubinemia has been challenging due to the variations in protocols. Amongst the published protocols with PEG, there are differences in patients' age, stage of treatment, doses and frequency, and concomitant medications (e.g. anthracyclines). These variations have led to discrepancies in the reported incidence of hyperbilirubinemia. Age has traditionally been considered a risk factor for hyperbilirubinemia due to the poor tolerance among the adult patient population. Age cutoffs of both 40 and 45 years have been associated with increased induction mortality and toxicity in PEG-containing protocols[4,5]. However, Douer et al. successfully administered PEG to patients up to 55 years of age with no deaths reported as a result of PEG toxicity[9]. Furthermore, advanced age was not associated with hyperbilirubinemia in patients treated on the MOAD protocol at our institution which included patients up to 69 years of age[5]. Patel et al. identified obesity as a risk factor for grade $\geq$ 3 (Tbili > 3 – 10 x ULN) liver adverse events, with an increased risk for every 5 unit BMI increment (OR: 1.58, p=0.041)[4]. A retrospective analysis also identified patients with BMI $\geq$ 30 to be at an increased risk of grade $\geq$ 3 hepatotoxicity, and did not find age to be a risk factor[10]. Patients of Hispanic ethnicity, especially those with larger BMI, have also been reported to experience hepatotoxicity more frequently upon retrospective review[11]. Specific comorbidities including type 2 diabetes mellitus and dyslipidemia have not been evaluated as risk factors for asparaginase toxicity, however like obesity, these are risk factors for non-alcoholic fatty liver disease, and consequently are worth investigating as risk factors for PEG-induced hyperbilirubinemia[12]. Hypoalbuminemia is an indicator of nutritional status as well as the capacity of protein synthesis in the liver, and therefore may also be an indicator of poor PEG tolerance. Anecdotally, at our institution, we have observed significant hyperbilirubinemia in relapsed/refractory patients treated with PEG post allogeneic stem cell transplantation (alloSCT), potentially making transplant another risk factor. The mechanism in which PEG leads to hepatotoxicity is a result of protein derangements caused by glutamine deficiency and impairment of mitochondrial β-oxidation[13-15]. Glutamine depletion results in increased cellular stress and premature restoration of protein synthesis resulting in increased inflammation and cell death[14,15]. Impaired mitochondrial β-oxidation prevents oxidation of long-chain fatty acids which results in fatty acid and triglyceride accumulation, leading to steatosis[16]. Evidence of steatosis has been reported in both pediatric and adult patients treated with asparaginase, indicating this toxicity is not limited only to adults[17,18]. This protocol will investigate the use of levocarnitine for treatment of PEG-induced hyperbilirubinemia specified as Tbili >3xULN.

2.1.2 Inotuzumab ozogamicin hepatotoxicity

In an effort to improve efficacy as well as treatment related mortality, novel therapies, especially monoclonal antibodies, are being investigated for the treatment of adult patients with ALL. Inotuzumab ozogamicin is one such monoclonal antibody. Inotuzumab ozogamicin (INO) is an immunoconjugate consisting of humanized IgG4 anti-CD22 antibody linked via an acid-hydrolysable butanoic acid linker to a derivative of calicheamicin, N-acetyl-γ-calicheamicin dimethyl hydrazide. Calicheamicin is a natural product of *Micromonospora echinospora calichensis* and is the most potent member of the enediyne class of DNA-damaging agents[19]. INO targets the B-cell receptor CD22 and upon binding, the INO/CD-22 complex is rapidly internalized, calicheamicin is activated intracellularly, and induces double-stranded DNA breaks through DNA binding, impairing transcription and inducing

apoptosis[19,20]. INO is a desirable immunoconjugate due to the high prevalence of CD22 expression on ALL cell lines as well as its observed clinical efficacy. The toxicity profile of INO includes hepatotoxicity, hyperbilirubinemia, and veno-occlusive disease (VOD) especially in patients undergoing alloSCT after treatment with INO[21]. The mechanism by which INO leads to VOD is thought to be similar to that of gemtuzumab ozogamicin, another calicheamicin immunoconjugate[22]. VOD may be due to direct injury to endothelial cells of the hepatic arteriole by calicheamicin. This injury triggers an inflammatory cascade which results in a pro-thrombotic and hypofibrinolytic state, similar to what is reported in patients undergoing alloSCT with dual-alkylator conditioning regimens. Furthermore, CD22 expression on sinusoidal endothelial cells may also lead to the uptake of INO and consequently result in sinusoidal damage. Due to the hepatic stress induced by INO, levocarnitine may be a possible treatment in patients experiencing hyperbilirubinemia due to this agent.

2.2 Levocarnitine and Vitamin B Complex

2.2.1 Description

Levocarnitine is a naturally occurring amino acid that acts as a carrier molecule in the transport of long-chain fatty acids across the inner mitochondrial membrane. The shuttling of long-chain fatty acids across the mitochondria allows for oxidation and energy production. Levocarnitine is obtained through dietary consumption and is also biosynthesized. The biosynthesis requires lysine, methionine, niacin, vitamin B6, vitamin C, and iron.

Vitamin B complex is a dietary supplement composed of thiamine (B1) 3 mg; riboflavin (B2) 3mg; niacin (B3) 20 mg; pyridoxine HCl (B6) 0.5 mg; cyanocobalamin (B12) 1 mcg; pantothenic acid 5 mg per capsule and may be used for patients with nutritional deficiencies.

2.2.2 Rationale for the use of levocarnitine and vitamin B complex for treatment of hyperbilirubinemia

Treatment with both PEG and INO can result in significant hepatotoxicity. In animal models, levocarnitine has demonstrated efficacy in normalizing liver enzyme elevations seen in asparaginase damaged steatotic liver[23]. Furthermore, levocarnitine deficiency has been observed as a risk factor for valproic acid liver toxicity, which is thought to also be caused by impaired mitochondrial β-oxidation[24]. The combination of levocarnitine and vitamin B complex has only been reported in a retrospective manner for the treatment of PEG-induced hyperbilirubinemia[25-28], however due to the success in this setting and low side effect profile associated with this intervention, it is warranted to investigate the use of these agents to treat hyperbilirubinemia due to either PEG or INO. Levocarnitine and vitamin B complex will be initiated for Tbili > 3xULN. Initiating treatment earlier can hopefully prevent the severity of hyperbilirubinemia that has been reported with PEG. Furthermore, despite an effort to identify patients at risk of developing hyperbilirubinemia after treatment with PEG, we are unaware of any intervention that has been done to try to prevent this toxicity other than dose reduction or avoidance of the agent altogether.

3.0 Drug Information

3.1 Levocarnitine[29-31]

3.1.1 Product description

Levocarnitine is a white crystalline, hygroscopic powder. It is readily soluble in water, hot alcohol, and insoluble in acetone. The chemical name of levocarnitine is 3-carboxy-2(R)-hydroxy-N,N,N-trimethyl-1-propanaminium, inner salt.

3.1.2 Storage and handling

Levocarnitine vials should be stored at room temperature (25°C) and should be protected from light until use. Unused portions of an opened vial should be discarded, as the formulation does not contain a preservative. Once diluted, levocarnitine solution may be stored at room temperature (25°C) for up to 24 hours when mixed in NS or LR in PVC bags

Levocarnitine tablets and oral solution should be stored at room temperature (25°C). The oral solution should be protected from light.

3.1.3 How supplied

Levocarnitine injection is a sterile aqueous solution containing 1 g of levocarnitine per single-dose 5mL vial adjusted to a pH of 6.0-6.5 with hydrochloric acid or sodium hydroxide. Each carton contains 5 single-dose 5mL vials.

Levocarnitine is also available in oral dosage forms including tablets, oral solution, and sugar-free oral solution. 330 mg tablets are white, round compressed tablets debossed “cor” over “160” on one side, and the other side is plain. Tablets are supplied in blister packs of 1- tablets, in boxes of 90 tablets. Levocarnitine oral solution is supplied in 118 mL (4 FL. OZ.) multiple-unit plastic containers that are packaged 24 per case. Sugar-Free Oral Solution is supplied in 118 mL (4 FL. OZ.) multiple-unit plastic containers that are packaged 24 per case.

3.1.4 Route of administration and pharmacokinetics

Levocarnitine can be administered both intravenously (IV) and orally. IV levocarnitine will be administered as an IV push over 2-3 minutes per standard of care, in accordance with MD Anderson Cancer Center IV push guidelines. Following IV administration, approximately 76% of the dose was excreted in the urine during the first 24 hours after administration. The plasma concentration profiles following IV administration is described as a two-compartment model where the mean distribution half-life was estimated at 0.585 hours and mean apparent terminal elimination half-life was 17.4 hours. Total body clearance of levocarnitine was a mean of 4.0 L/h. Levocarnitine is not plasma protein bound.

Oral levocarnitine tablets and solution were found to be bio-equivalent with maximum plasma concentration (Cmax) approximately 80 umol/L and time to maximum plasma concentration (Tmax) occurred at 3.3 hours. The absolute bioavailability of oral levocarnitine is poor and was found to be 15.1 $\pm$ 5.3% for tablets and 15.9 $\pm$ 4.9% for the oral solution.

After treatment with oral levocarnitine, major metabolites found were trimethylamine N-oxide, primarily in urine (8% to 49% of the administered dose) and [3H]-γ-butyrobetaine, primarily in feces (0.44% to 45% of the administered dose). Urinary excretion of levocarnitine was about 4 to 8% of the dose. Fecal excretion of total carnitine was less than 1% of the administered dose.

Intravenous levocarnitine supplementation has been studied in patients with end-stage renal disease receiving hemodialysis and has been shown to increase carnitine concentrations in this population

when administered at doses ranging from 10 mg/kg to 40 mg/kg 3 times weekly following dialysis. Chronic, oral supplementation has not been evaluated in patients with severe renal impairment or ESRD. Chronic oral supplementation may result in accumulation of renally excreted metabolites trimethylamine (TMA) and trimethylamine-N-oxide (TMAO).

3.1.5 Availability
Levocarnitine is FDA approved and commercially available. Commercial supply will be used.

3.1.6 Agent destruction and return
Unused/expired drug will be disposed of onsite according to institutional guidelines

3.1.7 Warnings and precautions

3.1.7.1 Embryo-fetal toxicity: levocarnitine is pregnancy category B. Reproductive studies performed in rats and rabbits at doses up to 3.8 times the human dose on the basis of surface area have not revealed any evidence of impaired fertility or harm to the fetus due to levocarnitine. There are no adequate and well controlled studies in pregnant women, therefore should only be used during pregnancy if necessary.
Levocarnitine has not been studied in nursing mothers. Studies in cows indicate an increased concentration of levocarnitine in milk following exogenous levocarnitine administration. Nursing mothers receiving levocarnitine should weigh the risks to the child of excess carnitine intake against the potential benefits of supplementation.

Pregnant and nursing mothers will be excluded from this study.

3.1.7.2 Adverse events: Transient nausea and vomiting have been observed with oral and intravenous levocarnitine use. Various mild gastrointestinal complaints including transient nausea, vomiting, abdominal cramps, and diarrhea have been reported with long-term use of oral levocarnitine. These adverse reactions typically resolve with dose reduction.
Seizures have been reported in patients with and without prior seizure activity and in patients with pre-existing seizure activity, an increase in seizure frequency and/or severity has been reported.

Adverse events with a frequency of $\geq$ 5% regardless of causality by body system reported are listed in appendix I.

3.1.7.2 Overdosage: No reports of toxicity from levocarnitine overdose have been reported. Levocarnitine is easily removed from the plasma by dialysis. Large doses of the oral formulations may cause diarrhea.

3.2 Vitamin B Complex

3.2.1 Product description
Vitamin B complex is a softgel capsule composed of thiamine (B1) 3 mg; riboflaxin (B2) 3mg; niacin (B3) 20 mg; pyridoxine HCl (B6) 0.5 mg; cyanocobalamin (B12) 1 mcg; pantothenic acid 5 mg

3.2.2 Storage and handling
Vitamin B complex capsules should be stored between 15°C and 30°C.

3.2.3 How supplied
Vitamin B complex capsules are softgel capsules supplied in bottles of 100.

3.2.4 Availability
Vitamin B complex is FDA approved and commercially available. Commercial supply will be used.

3.2.5 Agent destruction and return
Unused/expired drug will be disposed of onsite according to institutional guidelines

4.0 Study Design
This is an open-label, two-arm, phase II clinical study.

5.0 Patient Eligibility

Inclusion Criteria:
1. Patients at least 12 years of age
2. Patients with a diagnosis of ALL who are receiving treatment with PEG-asparaginase or inotuzumab ozogamicin with Tbili >3xULN
3. Signed informed consent.

Exclusion criteria:
1. Pregnant or nursing women
2. Known hypersensitivity to levocarnitine or vitamin B complex

6.0 Treatment Plan

6.1 General

All patients will be registered with the Data Management Office PDMS/CORE system.

Treatment with levocarnitine and vitamin B complex will be initiated when total bilirubin is >3xULN. Treatment will consist of IV levocarnitine 50 mg/kg loading dose (maximum 3000 mg) followed by 50 mg/kg/day divided every 6 hours beginning 6 hours after the loading dose was administered. Levocarnitine dose may be increased by 50 mg/kg/day to a maximum of 150 mg/kg/day divided every 6 hours (maximum 3000 mg per dose) if no improvement in total bilirubin after 2 consecutive days of treatment at each dose level. See Table 1 for dosing levels. Treatment will continue until total bilirubin is $\leq$ 1.5 x the upper limit of normal (ULN) or at least a 50% reduction in peak total bilirubin is achieved. If hyperbilirubinemia recurs (Tbili >3xULN), levocarnitine and vitamin B may be reinitiated at the previous dose level and continued until Tbili $\leq$ 1.5 x ULN or at least a 50% reduction in peak total bilirubin is achieved. It is preferred that the patient continue to receive treatment as inpatient. If the treating physician deems patient appropriate for discharge prior to achieving a Tbili of $\leq$ 1.5 ULN or at least a 50% reduction in peak total bilirubin, it is recommended for patient to continue IV levocarnitine as an outpatient. It is recommended for patient to continue IV levocarnitine as an outpatient at the same inpatient dosage divided twice daily (maximum 3000mg per dose). If patient is unable to continue IV levocarnitine as an outpatient, it is recommended the patient receive levocarnitine 990 mg by mouth three times daily until Tbili of $\leq$ 1.5 ULN or at least a 50% reduction in

peak total bilirubin is achieved. Vitamin B complex 1 capsule will be administered by mouth twice daily until Tbili of ≤ 1.5 ULN or at least a 50% reduction in peak total bilirubin is achieved. Patients who do not achieve a response within the timeframe of interest may continue to receive protocol medications including levocarnitine and vitamin B complex if there is clinical benefit, and deemed in best interest of the patient by the treating physician.

Patients who are re-challenged with inotuzumab or PEG-asparaginase after receiving treatment for hyperbilirubinemia may continue levocarnitine and vitamin B capsules by mouth at the discretion of the treating physician. If hyperbilirubinemia (Tbili >3xULN) occurs, patients should receive levocarnitine and vitamin B complex starting at dose level 1 and escalate to a maximum of 150 mg/kg/day according to the recommendations above. Patients will remain on study for 30 days after their last dose of either PEG-asparaginase or inotuzumab.

Intravenous levocarnitine will be administered as an IV push over 2-3 minutes per standard of care, in accordance with MD Anderson Cancer Center IV push guidelines. Levocarnitine oral solution may be dissolved in drink or other liquid food. Oral tablets and oral solution should be administered during or following a meal. Oral solution should be consumed slowly to maximize tolerance. Missed or vomited doses of the oral tablets or solution will not be made up.

Patients will receive vitamin B complex capsules composed of: thiamine (B1) 3 mg; riboflavin (B2) 3mg; niacin (B3) 20 mg; pyridoxine HCl (B6) 0.5 mg; cyanocobalamin (B12) 1 mcg; pantothenic acid 5 mg per capsule while inpatient. Patients may receive any commercially available vitamin B complex product while outpatient even if the composition differs from what is described above. Missed or vomited doses will not be made up.

Table 1. Levocarnitine dose levels:

Dose level	IV levocarnitine dose
1	50 mg/kg/day divided every 6 hours
2	100 mg/kg/day divided every 6 hours
3	150 mg/kg/day divided every 6 hours

6.1.1 Compatibility:

Levocarnitine injection solution is compatible and stable when mixed in parenteral solutions of Sodium Chloride 0.9% or Lactated Ringer's in concentrations ranging from 250 mg/500 mL (0.5 mg/mL) to 4200 mg/500 mL (8.0 mg/mL)

7.0 Pretreatment evaluation

7.1 Informed consent
7.2 Baseline comprehensive metabolic panel (CMP)
7.3 Concomitant medications within 14 days of enrollment
7.4 Urine or serum pregnancy test for women of childbearing potential within 7 days of initiating chemotherapy

8.0 Evaluation During Study

8.1 Comprehensive metabolic panel (CMP) at least weekly
8.2 Concomitant medications
8.3 Additional monitoring procedures including but not limited to CMP, fractionated bilirubin, serum albumin, diagnostic imaging, to be completed per institutional guidelines. Additional monitoring not required for study. Failure to perform this will not be considered a protocol deviation or violation.
8.4 Fractionated bilirubin daily while inpatient, then per treating physician discretion while outpatient.

9.0 Response

9.1. Normalization of hyperbilirubinemia (Tbili $\leq$ 1.5 x ULN) or at least 50% reduction in peak total bilirubin within 11 days in patients who received PEG-asparaginase
9.2. Normalization of hyperbilirubinemia (Tbili $\leq$ 1.5 x ULN) or at least 50% reduction in peak total bilirubin within 7 days in patients who received inotuzumab ozogamicin

10.0 OUTSIDE PHYSICIAN PARTICIPATION DURING TREATMENT

10.1 MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician. This will be documented in the patient record.
10.2 A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care.
10.3 Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it.
10.4 Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.
10.5
10.6 Documentation to be provided by the local physician will include drug administration records, progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.
10.7 The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.
10.8

11.0 STATISTICAL CONSIDERATIONS

This is an open label phase II study to evaluate the efficacy of levocarnitine and vitamin B complex for the treatment of hyperbilirubinemia (Tbili >3xULN) in adult patients with ALL treated with PEG-asparaginase or inotuzumab ozogamicin.

Two cohorts of patients will be enrolled in the study, patients who were previously treated with PEG-asparaginase and patients who were previously treated with inotuzumab. Evaluation for two cohorts of patients will be separated. Up to 39 patients will be enrolled for each cohort. Total sample size is up to 78.

The primary efficacy endpoint is response, which is defined as bilirubin normalization (TBili $\leq$ 1.5 x ULN) or at least a 50% reduction in peak bilirubin level in 11 days for patients previously treated with PEG-asparaginase and bilirubin normalization (TBili $\leq$ 1.5 x ULN) or at least a 50% reduction in peak bilirubin level in 7 days for patients previously treated with inotuzumab after initiation of the study treatment. All patients who receive at least 1 dose of levocarnitine will be assessed for safety. Patients must receive at least 5 consecutive days of levocarnitine to be considered evaluable for response.

Our retrospective data suggested that without any treatment the median time to bilirubin normalization (TBili $\leq$ 1.5 x ULN) or at least a 50% reduction in peak bilirubin level was 21 days for patients previously treated with PEG-asparaginase, and the median time to achieve hyperbilirubinemia normalization (TBili $\leq$ 1.5 x ULN) or at least a 50% reduction in peak bilirubin level was 14 days for patients previously treated with inotuzumab. Assuming that the time to normalization follows exponential distributions, then estimated failure rate (no normalization of grade $\geq$ 3 hyperbilirubinemia or less than 50% reduction in peak total bilirubin) would be 70% at day 11 for patients previously treated with PEG-asparaginase and at day 7 for patients previously treated with inotuzumab, respectively. This corresponding to a response rate of 30%.

With the proposed study treatment, it is expected that the median time to achieve hyperbilirubinemia normalization or at least a 50% reduction in peak total bilirubin will be shortened to 11 days for patients previously treated with PEG-asparaginase, and to 7 days for patients previously treated with inotuzumab. That is corresponding to a 20% increase in response rate (i.e. an increase from 30% to 50%) for each of the cohort of patients.

Simon's two-stage Minimax design[32] will be implemented for each cohort of patients. The null hypothesis that the true response rate is 30% will be tested against a one-sided alternative 50%. In the first stage, 19 patients will be accrued. If there are 6 or fewer responses in these 19 patients, the study will be stopped. Otherwise, 20 additional patients will be accrued for a total of 39. The null hypothesis will be rejected if 17 or more responses are observed in 39 patients. This design yields a one-sided type I error rate of 0.05 and power of 80% when the true response rate is 50%. The expected sample size is 25.7 and the probability of early stop is 66.6% under the null hypothesis.

Analysis Plan:

The primary endpoint is bilirubin normalization or at least a 50% reduction in peak total bilirubinin patients treated with levocarnitine and vitamin B complex. Response rates will be estimated along with the 95% confidence interval. Secondary endpoints include tolerability of levocarnitine and vitamin B complex and chemotherapy dose intensity. Descriptive statistics will be used to summarize secondary endpoints. The incidence rates of binary secondary endpoints will be estimated, along with the 95% confidence intervals. The duration of time to hyperbilirubinemia normalization or at least a 50% reduction in peak total bilirubin will be estimated using the Kaplan-Meier method. For exploratory purposes, the time to hyperbilirubinemia normalization or achieving at least a 50% reduction in peak total bilirubin will be compared to historical controls using the Log rank test. Competing risk analysis will be considered in the case that patients died before bilirubin normalization or at least a 50% reduction in peak total bilirubin is achieved.

Data from all subjects who receive any study drug will be included in the safety analyses. Subjects who entered the study but did not take any of the study drug and had this confirmed will not be evaluated for safety. The severity of the toxicities will be graded according to the NCI CTCAE v4.0 whenever possible. We will follow standard reporting guidelines for adverse events. Safety data will be summarized by AE category, severity and frequency. The proportion of patients with AEs will be estimated.

11.0 Reporting Requirements

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The investigator (or physician designee) is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for all adverse events for subjects enrolled.

Adverse event reporting will be as per the NCI criteria and the MDACC Leukemia Specific Adverse Event Recording and Reporting Guidelines (Appendix C).

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html).

Protocol specific data will be entered into PDMS/CORe. PDMS/CORe will be used as the electronic case report form for this protocol All AEs, regardless of expectedness will be collected and recorded on the Adverse Event Record and scanned into the patient's medical record. However, only unexpected AEs will be recorded in the Case Report Form (CRF). The Principal Investigator will sign and date the PDMS Case Report Form toxicity pages per each patient at the completion of each course. Following signature, the Case Report Form will be used as source documentation for the adverse events for attribution.

Serious Adverse Event Reporting (SAE) for M. D. Anderson-sponsored IND Protocols

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research teams to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

12.0 References

1. Hunger SP, Loh ML, Whitlock JA, et al. . Children's Oncology Group's 2013 blueprint for research: acute lymphoblastic leukemia. Pediatr Blood Cancer 2013;60:957-963.
2. Pulte D, Jansen L, Gondos A, et al. . Survival of adults with acute lymphoblastic leukemia in Germany and the United States. PLoS One 2014;9:e85554.
3. Stock W, Douer D, DeAngelo DJ, et al. . Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. Leukemia & lymphoma 2011;52:2237-2253.
4. Patel B, Kirkwood AA, Dey A, et al. . Pegylated-asparaginase during induction therapy for adult acute lymphoblastic leukaemia: toxicity data from the UKALL14 trial. Leukemia 2017;31:58-64.
5. Kadia TM, Kantarjian HM, Thomas DA, et al. . Phase II study of methotrexate, vincristine, pegylated-asparaginase, and dexamethasone (MOpAD) in patients with relapsed/refractory acute lymphoblastic leukemia. American Journal of Hematology 2015;90:120-124.
6. Rytting ME, Thomas DA, O'Brien SM, et al. . Augmented Berlin-Frankfurt-Münster therapy in adolescents and young adults (AYAs) with acute lymphoblastic leukemia (ALL). Cancer 2014;120:3660-3668.
7. Huguet F, Leguay T, Raffoux E, et al. . Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol 2009;27:911-918.
8. Douer D, Yampolsky H, Cohen LJ, et al. . Pharmacodynamics and safety of intravenous pegaspargase during remission induction in adults aged 55 years or younger with newly diagnosed acute lymphoblastic leukemia. Blood 2007;109:2744-2750.
9. Douer D, Aldoss I, Lunning MA, et al. . Pharmacokinetics-based integration of multiple doses of intravenous pegaspargase in a pediatric regimen for adults with newly diagnosed acute lymphoblastic leukemia. J Clin Oncol 2014;32:905-911.
10. Christ TN, Stock W, Knoebel RW. Incidence of asparaginase-related hepatotoxicity, pancreatitis, and thrombotic events in adults with acute lymphoblastic leukemia treated with a pediatric-inspired regimen. J Oncol Pharm Pract 2017:1078155217701291.
11. Law JY, Dao BD, Le P, et al. . Impact of body mass index and ethnicity on asparaginase toxicity in adult acute lymphoblastic leukemia patients. Journal of Clinical Oncology 2015;33:e18051-e18051.
12. Weiss J, Rau M, Geier A. Non-alcoholic fatty liver disease: epidemiology, clinical course, investigation, and treatment. Deutsches Arzteblatt international 2014;111:447-452.
13. Alshiekh-Nasany R, Douer D. L-Carnitine for Treatment of Pegasparaginase-Induced Hepatotoxicity. Acta Haematol 2016;135:208-210.
14. Wilson GJ, Bunpo P, Cundiff JK, Wek RC, Anthony TG. The eukaryotic initiation factor 2 kinase GCN2 protects against hepatotoxicity during asparaginase treatment. American journal of physiology.Endocrinology and metabolism 2013;305:E1124-1133.
15. Bunpo P, Dudley A, Cundiff JK, Cavener DR, Wek RC, Anthony TG. GCN2 protein kinase is required to activate amino acid deprivation responses in mice treated with the anti-cancer agent L-asparaginase. The Journal of biological chemistry 2009;284:32742-32749.
16. Walter P, Schaffhauser AO. L-carnitine, a ‘vitamin-like substance’ for functional food. Proceedings of the symposium on L-carnitine, April 28 to May 1, 2000, Zermatt, Switzerland. Ann Nutr Metab 2000;44(2):75-96.
17. Sahoo S, Hart J. Histopathological features of L-asparaginase-induced liver disease. Seminars in liver disease 2003;23:295-299.

18. Bodmer M, Sulz M, Stadlmann S, Droll A, Terracciano L, Krahenbuhl S. Fatal liver failure in an adult patient with acute lymphoblastic leukemia following treatment with L-asparaginase. Digestion 2006;74:28-32.
19. de Vries JF, Zwaan CM, De Bie M *et al.* The novel calicheamicin-conjugated CD22 antibody inotuzumab ozogamicin (CMC-544) effectively kills primary pediatric acute lymphoblastic leukemia cells. *Leukemia*, 26(2), 255-264 (2012).
20. Shor B, Gerber HP, Sapra P. Preclinical and clinical development of inotuzumab-ozogamicin in hematological malignancies. *Mol Immunol*, 67(2 Pt A), 107-116 (2015).
21. Kantarjian HM, DeAngelo DJ, Stelljes M *et al.* Inotuzumab Ozogamicin versus Standard Therapy for Acute Lymphoblastic Leukemia. *N Engl J Med*, 375(8), 740-753 (2016).
22. Giles FJ, Kantarjian HM, Kornblau SM *et al.* Mylotarg (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation. *Cancer*, 92(2), 406-413 (2001).
23. Roesmann A, Afify M, Panse J, Eisert A, Steitz J, Tolba RH. L-carnitine ameliorates L-asparaginase-induced acute liver toxicity in steatotic rat livers. Chemotherapy 2013;59:167-175.
24. K napp A C, T odesco L , B eier K , e t a l. T oxicity of valproic acid in mice with decreased plasma and tissue carnitine stores . J Pharmacol Exp Th er 2008 ; 324 : 568 – 575 .
25. Al-Nawakil C, Willems L, Mauprivez C, et al. . Successful treatment of l-asparaginase-induced severe acute hepatotoxicity using mitochondrial cofactors. Leuk Lymphoma 2014;55:1670-1674.
26. Alshiekh-Nasany R, Douer D. L-Carnitine for Treatment of Pegasparaginase-Induced Hepatotoxicity. Acta Haematol 2016;135:208-210.
27. Lu G, Karur V, Herrington JD, Walker MG. Successful treatment of pegaspargase-induced acute hepatotoxicity with vitamin B complex and L-carnitine. Proc (Bayl Univ Med Cent) 2016;29:46-47.
28. Blackman A, Boutin A, Shimanovsky A, et al. Levocarnitine and vitamin B complex for the treatment of pegaspargase-induced hepatotoxicity: a case report and review of the literature. JOPP 2017 Jan 1; DOI: 10.1177/1078155217710714 [Epub ahead of print]
29. Levocarnitine injection solution [package insert]. Gaithersburg, MD: Sigma-tau Pharmaceuticals Inc; 2016.
30. Levocarnitine tablet [package insert]. Middlesex, NJ: CorePharma, LLC; 2015.
31. Levocarnitine oral solution [package insert]. Brockton, MA: Lyne Laboratories, Inc; 2014.
32. Simon R (1989). Controlled Clinical Trials 10: 1-10

Appendix I

Adverse Events with a Frequency ≥5% Regardless of Causality by Body System

	Placebo (n=63)	Levocarnitine 10 mg (n=34)	Levocarnitine 20 mg (n=62)	Levocarnitine 40 mg (n=34)	Levocarnitine 10, 20 & 40 mg (n=130)
Body as Whole					
Abdominal pain	17	21	5	6	9
Accidental injury	10	12	8	12	10
Allergic reaction	5	6			2
Asthenia	8	9	8	12	9
Back pain	10	9	8	6	8
Chest pain	14	6	15	12	12
Fever	5	6	5	12	7
Flu syndrome	40	15	27	29	25
Headache	16	12	37	3	22
Infection	17	15	10	24	15
Injection site reaction	59	38	27	38	33
Pain	49	21	32	35	30
Cardiovascular					
Arrhythmia	5	3		3	2
Atrial fibrillation			2	6	2
Cardiovascular disorder	6	3	5	6	5
Electrocardiogram abnormal		3		6	2
Hemorrhage	6	9	2	3	4
Hypertension	14	18	21	21	20
Hypotension	19	15	19	3	14
Palpitations		3	8		5
Tachycardia	5	6	5	9	6
Vascular disorder	2		2	6	2
Digestive					
Anorexia	3	3	5	6	5
Constipation	6	3	3	3	3
Diarrhea	19	9	10	35	16
Dyspepsia	10	9	6		5
Gastrointestinal disorder	2	3		6	2
Melena	3	6			2
Nausea	10	9	5	12	8
Stomach atony	5				
Vomiting	16	9	16	21	15
Endocrine System					
Parathyroid disorder	2	6	2	6	4
Hemic/Lymphatic					

Anemia	3	3	5	12	6
Metabolic/Nutritional					
Hypercalcemia	3	15	8	6	9
Hyperkalemia	6	6	6	6	6
Hypervolemia	17	3	3	12	5
Peripheral edema	3	6	5	3	5
Weight decrease	3	3	8	3	5
Weight increase	2	3		6	2
Musculo-Skeletal					
Leg cramps	13		8		4
Myalgia	6				
Nervous					
Anxiety	5		2		1
Depression	3	6	5	6	5
Dizziness	11	18	10	15	13
Drug dependence	2	6			2
Hypertonia	5	3			1
Insomnia	6	3	6		4
Paresthesia	3	3	3	12	5
Vertigo		6			2
Respiratory					
Bronchitis			5	3	3
Cough increase	16		10	18	9
Dyspnea	19	3	11	3	7
Pharyngitis	33	24	27	15	23
Respiratory disorder	5				
Rhinitis	10	6	11	6	9
Sinusitis	5		2	3	2
Skin And Appendages					
Pruritus	13		8	3	5
Rash	3		5	3	3
Special Senses					
Amblyopia	2		6		3
Eye disorder	3	6	3		3
Taste perversion			2	9	3
Urogenital					
Urinary tract infect	6	3	3		2
Kidney failure	5	6	6	6	6