A Phase 2 Trial of N-Acetylcysteine in Biliary Atresia after Kasai Portoenterostomy

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V1.10	07/07/2023	Removed Sub-Investigator
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V 1.8	11/06/2020	Added clarification for subject replacement
V1.7	12/12/2019	Added Sub-Investigator
V1.6	06/28/2019	Added Sub-Investigator
V1.5	01/11/2019	Protocol Amendment
v1.4	7/21/17	Reflects changes to consent from BCM for Protocol H-40962
v1.3	7/19/17	Reflects changes from 6/30/17 FDA call for IND #135796
v1.2	4/28/17	Reflects changes from 1st DSMB meeting
v1.1	-	-

Precis

Biliary atresia (**BA**) is a devastating liver disease of infancy, characterized by bile duct obstruction leading to liver fibrosis, cirrhosis, and eventual need for transplantation in most cases. BA is treated with Kasai portoenterostomy (**KP**). KPs can achieve bile drainage and improve outcomes. However, even with standard evidence of "good bile flow," bile flow rarely normalizes completely and liver disease continues to progress.

In this study, we test whether intravenous N-acetylcysteine (**NAC**) can improve bile flow after KP. Our rationale is that NAC leads to synthesis of glutathione, which is a powerful stimulator of bile flow. Our primary objective is to determine whether NAC normalizes total serum bile acid (**TSBA**) concentrations within 24 weeks of KP. Achieving normal TSBAs is uncommon with current standard-of-care, and is predicted to be associated with better long-term outcomes. Our secondary objectives are to describe how other parameters commonly followed in BA change with NAC therapy, as well as report adverse events occurring with therapy and in the first two years of life. This study follows the "minimax" Phase 2 clinical trial design.

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A. Objectives

1) To determine if N-acetylcysteine (**NAC**) therapy normalizes total serum bile acids (**TSBAs**) within 24 weeks after Kasai portoenterostomy (**KP**) for biliary atresia (**BA**) (primary outcome)
2) To describe changes in laboratory markers for disease progression in the first two years of life (secondary outcome)
3) To describe changes in weight in the first two years of life (secondary outcome)
4) To describe occurrence of sentinel events related to worsening liver disease (cholangitis, development of ascites, variceal bleed, liver transplant listing, liver transplant, death) in the first two years of life (secondary outcome)
5) To document adverse events which may be associated with NAC therapy (secondary outcome)

B. Background and Rationale

Biliary atresia (**BA**) is a disease characterized by fibro-obliteration of extrahepatic bile ducts leading to impaired bile flow.[1] BA is treated with the Kasai portoenterostomy (**KP**), an operation which connects the liver directly to the intestine in attempt to relieve bile back-up and promote bile flow. KPs have variable success. KPs occasionally normalize bile flow and stop disease progression.[2] More commonly, however, bile flow never completely normalizes after KP. This can be detected by elevated total bilirubin (**TB**) or conjugated bilirubin (**Bc**) serum concentrations, or, when TB and Bc are normal, elevated total serum bile acids (**TSBA**) concentrations.[3–5] Impaired flow leads to fibrosis, cirrhosis, and eventual need for liver transplantation. Given these uneven results, therapies are urgently needed to enhance the KP's success.

We hypothesize that N-acetylcysteine (**NAC**) will improve outcomes after KP, because NAC is a precursor for the powerful choleretic molecule glutathione.[6–9] Our hypothesis assumes that better bile flow will lead to better outcomes. Two lines of evidence support this assumption. First, good bile flow correlates with slower disease progression in BA. For example, a recent study showed infants with good bile flow after KP were significantly less likely to develop failure-to-thrive, ascites, hypoalbuminemia, or coagulopathy in the first two years of life.[3] Furthermore, these infants had significantly higher transplant-free survival in the same time period. In this study, TB <2.0 mg/dL within three months of KP was used as the marker for good bile flow.

Second, poor bile flow accelerates disease progression after KP. For example, poor bile flow stimulates bile duct proliferation through bile acid-induced TGR5 signaling.[10] Proliferating bile ducts in turn may lead to two changes: (i) biliary cirrhosis, as fibrotic tissue is created around the new ducts; and (ii) further impaired flow, as bile must travel through the convoluted proliferating ducts. In addition, poor bile flow prevents hepatocytes from properly localizing MRP2 (the Bc efflux transporter) and BSEP (the bile acid efflux transporter) to the canalicular membrane.[11–13] As a result, Bc and bile acids accumulate in hepatocytes. Their intracellular concentrations eventually increase enough to drive their exit through basolateral membrane channels, which ultimately results in elevated Bc and TSBAs in the systemic circulation.

Table: Other Studies Using Intravenous NAC in Neonates

Liver-related
• Parenteral nutrition associated cholestasis[16,17]
• Nonacetaminophen acute liver failure[18,19]
• Neonatal hemochromatosis (gestational alloimmune liver disease)[20]
Liver-unrelated
• Bronchopulmonary dysplasia[21]
• Chorioamnionitis[22,23]

NAC has a number of properties that make it an especially attractive potential therapeutic agent. First, glutathione creates an osmotic gradient in the bile duct lumen which drives one-third of total bile flow in humans (the other drivers are bile acids and secretin/bicarbonate).[7–9,14] Second, NAC is a Food and Drug Administration-approved therapy for another serious liver condition in neonates and children (acetaminophen overdose).[15] It has also been used for other liver and non-liver indications in neonates, with few reported adverse events (Table).[16–23] Third, glutathione is an anti-oxidant, which could

scavenge the free radicals contributing to cirrhosis. Fourth, glutathione is catalyzed into glycine, glutamate, and cysteine by gamma-glutamyl transferase (**GGT**), thereby potentially fulfilling a nutritive role for cholangiocytes.[14,24,25] Preclinical studies are also promising, with glutathione's strong choleretic properties best established in rat flow studies and NAC's hepatoprotective effects documented in rescuing different mouse models of cholestasis.[14,26–28]

To test our hypotheses, we will administer intravenous NAC continuously for seven days and determine the number of subjects with normal TSBAs (0-10 umol/L) within 24 weeks of KP. In addition, markers of BA progression, such as abnormal laboratory results, failure-to-thrive, and occurrence of complications related to chronic liver disease, will be described over the first two years of life. Finally, all adverse events occurring during NAC infusion and in the 21 days after its completion will be recorded. Our study employs the two-stage "minimax" Phase 2 clinical trial design, a design commonly used in oncological trials to determine whether a particularly therapy has sufficient activity to warrant a larger Phase 3 trial.[29] The two-stage "minimax" design offers two distinct advantages compared to other designs: (i) early termination if the drug is not efficacious; and (ii) small sample sizes, because historical controls rather than a separate control arm are used.

C. Enrollment Procedures

i. Inclusion Criteria (all must be met)

a) Age less than or equal to 90 days at time of KP (standard age range in which KPs are performed)
b) BA diagnosis made by intraoperative cholangiography and KP performed at Texas Children's Hospital, Texas Medical Center Campus
c) Legal guardian(s) sign consent after understanding risks and investigational nature of study

ii. Exclusion Criteria

a) Decompensated liver disease (INR >1.3) despite parenteral Vitamin K administration
b) KP not performed for any reason (i.e., normal intraoperative cholangiography, or liver found to be too diseased intraoperatively to proceed with KP)
c) Active respiratory infection
d) Renal impairment, as defined by having an eGFR < 60 mL/min/1.73m^2 or creatinine clearance < 60 mL/min (https://www.niddk.nih.gov/health-information/communication-programs/nkdep/laboratory-evaluation/glomerular-filtration-rate-calculators/children-conventional-units)
e) Presence of severe concurrent illnesses, such as pulmonary (i.e., bronchopulmonary dysplasia), neurological, cardiovascular, metabolic, endocrine, and renal disorders, which may be congenital or acquired, that would interfere with the conduct and results of the study

iii. Total Planned Enrollment – 12 subjects (minimum), 16 subjects (maximum); see below for discussion of sample size.

iv. Inclusion of Women – Male and female infants will be enrolled in this study. There may be more females than males enrolled, given that BA is slightly more common in females.

v. Inclusion of Minorities – Enrollment will be open to infants of all racial and ethnic backgrounds. Minority representation will reflect the racial/ethnic diversity of patients seen at the study hospital.

vi. Inclusion of Children – This trial will only enroll infants ≤90 days old.

D. Study Implementation

i. Study Design – This is an open label, Phase 2 study of seven days of intravenous NAC treatment after KP. Subjects will be followed for the first two years of life, and no additional clinic visits or testing beyond those routinely performed for routine BA clinical care at Texas Children's Hospital will be performed.

ii. Study Procedure

a) Before KP
   (1) Identify cholestatic infants likely to have BA who fulfill inclusion criteria
   (2) Approach parent(s)/guardian(s) and explain study

b) After KP until hospital discharge (typical length-of-stay 10-14 days)
   (1) Consent parent(s)/guardian(s) and answer any remaining questions
   (2) Initiate continuous intravenous NAC therapy (6.25 mg/kg/hour of 10 mg/ml solution, or 0.625 ml/kg/hour, to give 150 mg/kg/day) within 24 hours of completion of KP (initiation to occur in pediatric intensive care unit, where all patients are admitted for observation after KP)
   (3) Continue therapy for a total of seven days
   (4) Collect results from laboratory tests drawn as part of standard clinical care
   (5) Monitor for adverse events (including serious adverse events)

c) After hospital discharge until 28 days post-KP
   (1) Monitor for adverse events

d) After hospital discharge until two years of life
   (1) Collect laboratory test results and weights measured as part of standard clinical care
   (2) Monitor for sentinel events related to progression of liver disease (cholangitis, development of ascites, variceal bleed, liver transplant listing, liver transplant, death)
   (3) Monitor for serious adverse events

iii. Consent Procedures – Parent(s)/guardian(s) will be approached before intraoperative cholangiogram/KP, and hence before the BA diagnosis is definitively made (information given but no signatures no obtained). While the KP is being performed, the study team will obtain formal consent.

iv. Drug Administration

a) Dosage:

- **NAC 150 mg/kg/day intravenous infusion run continuously over 24 hours (administered through dedicated intravenous line).** If the IV has to be interrupted due to common expected events during IV treatment (e.g. IV leak, etc), interruption will be noted in the subject chart with stop/re-start time and reason but not considered a protocol deviation unless the interruption lasts longer than 30 minutes. Interruptions > 30 minutes will be considered a protocol deviation. Similar dosing was used in infants/children in at least five studies:
  - Pharmacokinetic study delivering 100 mg/kg/day NAC intravenously over 21-24 hours for 1 day immediately after birth in premature infants gestational age 24-31 weeks.[30] No adverse events associated with NAC treatment were reported.
  - Case series delivering 100 mg/kg/day NAC intravenously over 24 hours for multiple weeks in infants ages 0-3 weeks with neonatal hemochromatosis/gestational alloimmune liver disease.[31] No adverse events associated with treatment were reported.
  - Case series delivering 70-135 mg/kg/day NAC intravenously over 12 hours for multiple months in children ages 4 months-13 years with TPN-dependent short bowel syndrome.[17] No adverse events associated with NAC treatment were reported.
  - Retrospective review of administering 100 mg/kg/day NAC for 1-77 days (median 5 days) in children median age 3.51 years (range 0.005-17.4 years) with non-acetaminophen-induced acute liver failure.[18] Three subjects had a rash (NAC therapy continued), two subjects had bradycardia (NAC therapy continued), one subject had tachycardia (NAC therapy continued), one subject had dizziness and peripheral edema (NAC dose reduced), and one child had bronchospasm from presumed allergy (NAC therapy stopped).
  - Double-blind randomized placebo-controlled trial delivering 150 mg/kg/day NAC intravenously over 24 hours for 1-7 days in children mean age 3.7 years (25%-75% 0.8-10.5 years) with non-acetaminophen-induced acute liver failure.[19] No adverse events associated with NAC treatment were reported.

b) Duration:

- **First seven days (168 hours +/-6 hours) after KP (post-operative day 0-7).** NAC was used post-operatively in numerous studies including:
  - Double-blind randomized placebo-controlled trial delivering 432 mg/kg/day NAC intravenously during abdominal aortic aneurysm repair and 288 mg/kg/day NAC intravenously for 24 hours post-operatively in adults age 72±11 years.[32] No adverse events associated with NAC treatment were reported.
  - Double-blind randomized placebo-controlled trial delivering 600 mg oral NAC the day before coronary artery bypass grafting surgery requiring cardiopulmonary bypass, followed by 150 mg/kg NAC intravenous over 15 minutes immediately before surgery, followed by 300 mg/kg/day NAC intravenously for 24 hours post-operatively in adults age 59.8±7.8 years.[33] No adverse events associated with NAC treatment were reported.
  - Double-blind randomized placebo-controlled trial delivering a total of 300 mg/kg/day NAC intravenously for 24 hours in adults undergoing cardiac surgery with cardiopulmonary bypass age 68.9±9.7 years (150 mg/kg for 15 minutes, followed by 50 mg/kg for 4 hours, followed by 100 mg/kg for 20 hrs).[34] No adverse events associated with NAC treatment were reported.

c) Formulation:
- Dilute of 200 mg/ml stock solution (2600 mOsm) stock to 10 mg/ml with 5% dextrose in water (382 mOsm), per standard pharmacy protocol
- Deliver 0.625 ml/kg/hour (equivalent to 6.25 mg/kg/hour or 150 mg/kg/day)
- Resulting final rate (>1 ml/hour, depending on weight) is sufficient to keep intravenous line open

v. Observations for this study, performed as part of standard clinical care

	Pre-KPa	Days Post-KP			Weeks Post-KP						Months of Life		
		3[b,c]	7	10	2	4	8	12	18	24	12	18	24
Demographic data	□												
TSBAs	□				□	□	□	□	□	□	□	□	□
Bc	□	□	□	□	□	□	□	□	□	□	□	□	□
AST/ALT	□	□	□	□	□	□	□	□	□	□	□	□	□
GGT	□	□	□	□	□	□	□	□	□	□	□	□	□
Albumin	□	□	□	□	□	□	□	□	□	□	□	□	□
Sodium	□	□	□	□	□	□	□	□	□	□	□	□	□
Total Bilirubin	□	□	□	□	□	□	□	□	□	□	□	□	□
Platelets	□		□		□	□	□	□	□	□	□	□	□
INR	□		□		□	□	□	□	□	□	□	□	□
25-hydroxy Vitamin D	□					□		□		□	□	□	□
Vitamin A	□					□		□		□	□	□	□
Vitamin E	□					□		□		□	□	□	□
Weight	□	□	□	□	□	□	□	□	□	□	□	□	□
Sentinel/Adverse events		□	□	□	□	□	□	□	□	□	□	□	□

[a]Drawn within seven days of KP
[b]If laboratory adverse event occurs (see Table 4), will perform an additional measurement 5 days post-KP
[c]Any "days post-KP labs (3,7,10) drawn outside of the outlined visit point will not be considered a deviation if +/- 2 days. For "weeks post-KP" labs (2,4,8,12,18,24) +/- 7 days window and "months of life" labs (12,18,24) +/-2 weeks window.

a) Primary outcomes:
- Any normal TSBA (0-10 umol/L) in the first 24 weeks after KP

b) Secondary outcomes:
- Trajectory of TSBAs changes over study period
- Trajectory of Bc changes over study period (drawn as part of liver panel)
- Trajectory of AST and ALT changes over study period (drawn as part of liver panel)
- Trajectory of GGT changes over study period (drawn as part of liver panel)
- Trajectory of albumin changes over study period (drawn as part of liver panel)

- Trajectory of sodium changes over study period (drawn as part of chemistry panel)
- Trajectory of Total Bilirubin changes over study period
- Trajectory of platelets changes over study period (drawn as part of complete blood count)
- Trajectory of INR changes over study period
- Trajectory of 25-hydroxy Vitamin D concentrations over study period and correlate with fat-soluble vitamin supplementation
- Trajectory of Vitamin A concentrations over study period and correlate with fat-soluble vitamin supplementation
- Trajectory of Vitamin E concentrations over study period and correlate with fat-soluble vitamin supplementation
- Trajectory of weight z-score changes over study period (to detect fluid overload during the infusion period and growth effects at later times)
- Sentinel events during the study period related to liver disease, such as cholangitis, ascites, liver transplant listing, liver transplant, and/or death (see Data and Safety Monitoring Plan (**DSMP**) in Appendix)
- Adverse events in the first 28 days after KP possibly related to NAC, including rash, urticaria, pruritus, tachycardia, hypotension, vomiting, edema, anaphylaxis, and intravenous line issues (see DSMP in Appendix)

vi. Dose Modifications – Rules for modifying NAC infusion are as follows, based in part on prior recommendations for NAC therapy in acetaminophen toxicity (also see DSMP in Appendix):[35]

a) Flushing alone, without pruritus, urticaria, or other serious signs
   - No intervention
   - Continue infusion, unless more severe signs develop

b) Urticaria
   - Stop infusion
   - Treat immediately with intravenous diphenhydramine (1.25 mg/kg/dose)
   - Restart infusion if symptoms resolve within 1 hour

c) Angioedema, hypotension, wheezing
   - Stop infusion
   - Treat with intramuscular epinephrine (0.01 mg/kg/dose), intravenous diphenhydramine (1.25 mg/kg/dose), intravenous glucocorticoid (1 mg/kg/dose), and/or inhaled albuterol , at the discretion of the clinical team
   - Do not restart infusion
   - Continue to follow patients for first two years of life, per study protocol

d) Elevation of ALT >700 U/L above baseline, Bc 3x above baseline, TB 3x above baseline, and/or Bc >5 mg/dL above baseline
   - Stop infusion
   - Do not restart infusion
   - Follow patients clinically until elevated ALT/Bc stabilizes/resolves
   - Evaluate for drug-induced liver injury if deemed appropriate by the clinical team, based on FDA recommendations (https://www.fda.gov/downloads/Drugs/.../guidances/UCM174090.pdf)

e) If a severe adverse event related to or possibly related to the study drug (see DSMP in Appendix for definition) occurs during the first 7 days of the trial, i.e., during NAC infusion, the infusion will be paused, and an emergency Data Safety Monitoring Board (**DSMB**) meeting will be convened. If the DSMB deems that causality is unrelated to NAC use, then the trial can be continued.

vii. Concurrent Treatments

a) Standard therapies: All standard therapies will be given during the study period, including ursodeoxycholic acid (20 mg/kg/day), fat-soluble vitamins (AquADEK, 1 ml once or twice daily), and up to one year of post-KP antibiotics (trimethoprim/sulfamethoxazole 5 mg/kg/day). In infants found

to have low Vitamin A levels (<20 mcg/dL), intramuscular Vitamin A injections of 50,000 IU will be given two days before KP and 28 days after KP. Steroids will not be used as standard post-KP care.

b) Experimental therapies: No other investigational therapies will be given during the study period.

viii. Removal of Patients from Study

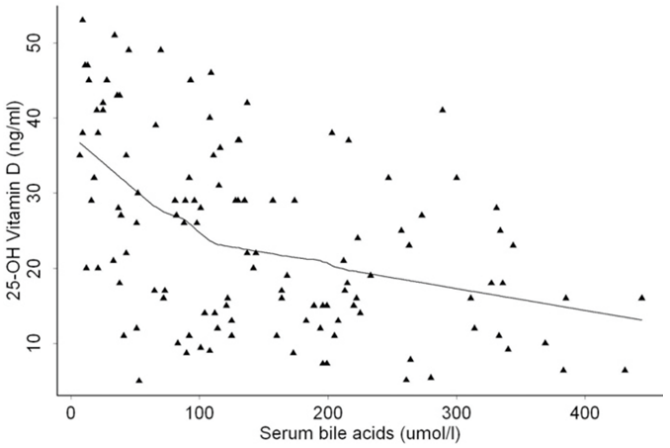
a) Parents may voluntarily exclude their infant from the study at any time
b) The investigators may remove a patient if the study team decides that continuation is not in the best interest of the participant of the study (i.e., patient receives another experimental therapy during the two-year study period that will confound the results).

E. Data Collection

i. Clinical Data – All clinical data (laboratory results, weights, occurrence of sentinel events) will be stored in the patient's electronic medical record. In addition, a comprehensive study form containing all data for each subject will be updated as new results become available.

ii. Adverse Events – All adverse events (observed by the study team, reported by parents, and/or detected by laboratory findings) will be recorded in the patient's medical record as well as separate form specifically for the study. The form will be submitted to the DSMB following a pre-determined schedule (see DSMP in Appendix).

Figure: Vitamin D concentrations vs. TSBA in START subjects 1-, 3-, and 6-months after KP.[5] Very few TSBAs were ≤10 umol/L.



F. Statistical Considerations

i. Overview

This is a Phase 2 clinical trial using the two-stage "minimax" design described by Simon.[29] As a Phase 2 trial, the trial's objective is to determine whether NAC has sufficient biological activity as adjunctive therapy for BA to warrant further study. By choosing the two-stage "minimax" design, we gain two advantages: (i) early termination if the drug is not efficacious, and (ii) using historical controls and therefore an overall smaller sample size to test the hypothesis, i.e., no randomization or control arm. This study design only identifies large effects (response >20%). For BA this is appropriate, because the field is in need of a robust therapy that can substantially limit liver damage and delay/prevent need for liver transplantation.

Historical controls from the START clinical trial will be used for the primary outcome, as this is the only systematic examination of TSBAs after KP.[4] In the START trial, subjects receiving a KP between 2005-2011 were enrolled and TSBAs drawn as many as eight times (pre-KP; 1, 2, 3, and 6-months post-KP; and 12, 18, and 24 months post-KP). We will examine these results to determine the number of subjects with normal TSBAs after six months. We expect a maximum of 5% of subjects to have normalized TSBAs, based on a recent publication of correlating TSBAs from the START trial with fat-soluble vitamin levels (118 data points taken at 1, 3, or 6 months post-KP) (Figure).[5]

For secondary outcomes, results will be compared to previously published findings and results from our institution. For example, laboratory and clinical two-year outcomes for BA have been described previously, for all infants receiving KP and those with total bilirubin <2 mg/dL vs. ≥2 mg/dL within 3 months of KP.[3,36] In addition,

of the patients receiving a KP at our institution between 2007-2014, only 1/48 (2.1%) had a normal Bc recorded in the first two weeks post-KP.

One limitation of this approach is that using historical controls can lead to a type 1 error, because BA management has evolved over time. For example, newborn screening now allows for earlier diagnosis and treatment with the KPs. In addition, at our center intramuscular Vitamin A is now used to correct Vitamin A levels quickly, alongside the oral fat-soluble vitamin preparations used in the past. Finally, nutritional approaches have changed, including use of early parenteral nutrition immediately after KP. These changes may alter outcomes independent of intravenous NAC treatment.

ii. Sample-Size Calculations

Simon's two-stage "minimax" design will be used to calculate sample sizes for the primary outcome.[29] The null hypothesis will be tested against a one-sided alternative. Assuming a normalization rate of approximately 5% (see above), the null hypothesis is that the true response rate is 5% for normalization of TSBAs 24 weeks after KP. In the first stage, 12 patients will be accrued. If no patient normalizes TSBA within 24 weeks of KP, the study will be stopped. Otherwise, 4 additional patients will be accrued for a total of 16. The null hypothesis will be rejected if ≥3 of 16 patients normalize TSBA within 24 weeks of KP. This design yields a type I error rate of 5% and power of 80% when the true response rate is 25%. Subjects that do not complete the 7 day NAC infusion after the Kasai operation and 24 weeks of follow up may be replaced at the discretion of the PI/IND Sponsor.

G. Ethical Issues

i. Informed Consent

Participants will only be enrolled after their parent(s)/guardian(s) understand the nature and objectives of the trial, as well as the attendant risks and possible discomforts. All families will be given a consent form describing NAC and possible side-effects (see Consent in appendix).

ii. Children as Research Subjects

NAC may benefit patients with BA by improving bile flow after KP. This study does have risks. Intravenous NAC has been studied extensively in pediatric populations. NAC side effects include anaphylaxis reactions, generalized urticaria, hives, rash, stomatitis, rhinorrhea, and vomiting. These side effects are most often reported at the beginning of NAC infusion for acetaminophen toxicity, when higher doses than used in this study are administered.

This study also involves an intravenous line for NAC administration and blood draws. The intravenous line is routinely placed pre-operatively to deliver fluids when enteral feeds must stop. The same intravenous line will be used for NAC administration. The only instance in which an additional peripheral intravenous line will be placed is if intravenous access is lost during the seven-day period of NAC treatment. All blood draws for this study will be part of routine clinical care, and would be performed independent of participation in this trial.

H. Pharmaceutical Information

i. **Formulation** – Stock 200 mg/ml (2600 mOsm) packaged as Acetadote, dispensed after diluting in 5% dextrose to 10 mg/ml (382 mOsm) per standard pharmacy protocol (no new formulations used for this protocol).

ii. **Storage** – Stock stored at room temperature. Following reconstitution, stable for 24 hours at room temperature.

iii. **Administration** – Days 0-7 after KP: Intravenously at 0.625 ml/kg/hour, initially through intravenous line already present for routine clinical care and additional intravenous line if needed.

iv. Toxicities

NAC has been well studied at higher doses used in this study, for treatment of acetaminophen-induced liver toxicity. In adults, the most common side effects were cutaneous hypersensitivity reactions, including rash and/or urticaria on the face, neck, or arms.[37] These symptoms resolved with antihistamine use, without requiring treatment changes in most cases. Respiratory hypersensitivity reactions have also been reported, including one case of bronchospasm in a patients with asthma resulting in death.[15] In a pediatric center, NAC treatment for acetaminophen-induced liver toxicity was associated with adverse events in 46 of 89 patients. Adverse events were present in 40 of the 72 subjects receiving oral NAC, and included vomiting (n=29), nausea alone (n=10), and abdominal pain (n=2). Adverse events were present in six of the 17 subjects receiving intravenous NAC, and included vomiting (n=2), nausea alone (n=2), and rash (n=2).[38] No serious adverse events were recorded.

NAC is well-tolerated post-operatively after surgeries not requiring or requiring cardiopulmonary bypass.[32–34] Previous in vitro results have suggested that NAC may impair platelet aggregation and coagulation parameters. One human study delivered 150 mg/kg NAC intravenously to patients undergoing abdominal aortic aneurysm repair followed by 150 mg/kg NAC intravenously for 24 hours.[39] All patients in this study received heparin during the operation and protamine post-operatively. Compared to controls, intravenous NAC treatment was associated with decreased prothrombin time Quick values, prolonged coagulation times in thromboelastometry tracings, and impaired platelet aggregation. However, NAC did not affect blood loss. While the clinical significance of these findings is unclear, the authors caution against NAC use in patients with pre-existing coagulopathies (patients with INR > 1.3 unresponsive to parenteral Vitamin K are excluded from this study).

Two potentially more serious events have been associated with NAC treatment. The first is severe hyponatremia leading to seizures, after excessive free water was given during intravenous NAC administration in infants.[40] This occurred partly because NAC therapy for acetaminophen toxicity requires three dose changes in the first five hours, with different NAC concentrations and dilution regimens. To minimize the chances of this occurring, special neonatal dilution instructions are included in the product insert and have been approved by the Food and Drug Administration.[15] The single dilution strategy used in this protocol conforms to these recommendations.

Another potentially serious event that has been reported with oral NAC is paradoxical respiratory adverse drug reactions (ADRs).[41] These events are described as worsening of respiratory disease in patients taking oral over-the-counter NAC (approximately 20-30 mg/kg/day children ages 1 month to 2 years) as a mucolytic agent in the setting of bronchiolitis or pneumonia. NAC is proposed to increase bronchial mucus flow which can overwhelm an infant's ability to spontaneously clear secretions. Fifty-nine cases were identified over a 20 year period in European country. However, in studies of intravenous doses similar to the dose used in this investigation, no paradoxical respiratory ADRs were reported.

A summary of the side-effect profile of intravenous NAC listed on formularies is as follows:

Cardiovascular:	Edema, flushing, tachycardia
Dermatologic:	Rash, pruritus, urticaria
Gastrointestinal:	Nausea, vomiting
Immunologic:	Autoimmune disease
Miscellaneous:	Anaphylactoid reaction
Respiratory:	Pharyngitis, rhinorrhea, rhonchi, throat tightness
Rare but important or life-threatening:	Anaphylaxis, angioedema, bronchospasm, chest tightness, cough, dizziness, dyspnea, hypotension, respiratory distress, stridor, wheezing

I. References

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Appendix

i) Data and Safety Monitoring Plan

ii) Data and Safety Monitoring Board Charter

Data and Safety Monitoring Plan

- **Roles and Responsibilities:**

The purpose of the Data and Safety Monitoring Plan (**DSMP**) is to ensure the safety of research participants and protect the validity and integrity of study data. The roles of individuals are (see Data Safety Monitoring Board (**DSMB**) Charter in the Appendix for additional information):

a) Principal Investigator:
   - Identify eligible patients
   - Help consent parent(s)/guardian(s)
   - Help monitor patients during N-acetylcysteine (**NAC**) infusion
   - Help collect clinical and laboratory data during the study period
   - Recruit members for the DSMB
   - Help complete Adverse Event (**AE**) Clinical Report Forms (**CRF**)
   - Help complete Data CRFs for each subject
   - Report all serious AEs to the Chairperson of the DSMB within seven days of the event's occurrence
   - Attend DSMB meetings
   - Respond to recommendations from the DSMB

b) Study staff
   - Help consent parent(s)/guardian(s)
   - Help monitor patients during NAC infusion
   - Help collect clinical and laboratory data during the study period
   - Help complete AE CRFs
   - Help complete Data CRFs for each subject
   - Help arrange logistics of DSMB meetings
   - Help prepare materials for DSMB meetings, including minutes of previous meeting, updated AE CRFs, and updated Data CRFs
   - Attend DSMB meetings

c) Data Safety Monitoring Board
   - Convene via conference call before trial starts, to review protocol and decide on any amendments
   - Meet twice yearly via conference call to review trial progress, examine AE CRFs, examine Data CRFs, determine whether changes in the study are warranted, and determine whether the study should continue
   - Meet on emergency basis as determined by Chairperson
   - (Chairperson) Respond with recommendations within seven days to serious AEs reported by the Principal Investigator

- **Adverse Events:**

AEs as specified below occurring in the first 28 days after KP will be documented. An AE is defined as any unfavorable and unintended sign, symptom, laboratory finding, or disease temporally associated with the study, whether or not the AE is related to NAC therapy. These include events observed by the study team, reported by parents, and/or detected by laboratory findings. The following targeted AEs are reportable events in this study:

a) Clinical AEs that are caused by NAC or likely caused by NAC (typically related to hypersensitivity and occurring at start of infusion):
   - Acute flushing
   - Skin erythema
   - Other rash
   - Urticaria

- Hypotension
- Tachycardia
- Edema
- Vomiting
- Wheezing
- Shortness-of-breath
- Bronchospasm
- Anapyhlaxis
- Redness/swelling/pain/bruising at intravenous line site
- Fluid infiltration at intravenous line site
- Vessel thrombosis at intravenous line site
- Infection at intravenous line site
- Loss of intravenous line

b) Clinical AEs which could occur with BA in the first 28 days after KP, independent of therapy:

- Increase in AST, ALT, and/or GGT >400 U/L above baseline (pre-KP) values (Note: in BA, baseline liver enzymes are already National Cancer Institute-Common Terminology Criteria for Adverse Events (**CTCAE**) Grade 3)
- Increase in Bc >3 mg/dL above baseline (pre-KP) values (Note: in BA, baseline liver enzymes are already National Cancer Institute **CTCAE** Grade 3)
- Increase in TB >5 mg/dL above baseline (pre-KP) values (Note: in BA, baseline liver enzymes are already National Cancer Institute **CTCAE** Grade 3)
- Vomiting with feeds, requiring acid reduction therapy
- Cholangitis, requiring antibiotic therapy
- Infection of other etiology requiring treatment
- Ascites
- Diarrhea
- Complications related to surgery
- Death

- **Serious AEs**

Serious AEs, which are a subset of AEs as specified below, occurring at any time during the study period will be documented. In addition, serious AEs will be reported to the DSMB Chairperson within seven days of occurrence with the exception of events related to the management of the natural course of BA (i.e., hospitalizations for cholangitis, poor growth, worsening liver disease after failed KP, etc). After reporting a serious AE, the Principal Investigator and/or study team will receive recommendations about how to proceed with the study within seven days. Serious AEs are those AEs which:

a) Result in death;
b) Place the subject at immediate risk of death (does not include AEs which, if more severe, might have caused death);
c) Require inpatient hospitalization or prolongation of existing hospitalization (with the exception of hospitalizations related to the natural course of BA, such as hospitalizations for cholangitis, poor growth, worsening liver disease after failed KP, etc)
d) Lead to persistent disability which prevents a subject from conducting normal life functions; or
e) In the first 28 days after KP, elevation of ALT >700 U/L above baseline, Bc 3x or >5 mg/dL above baseline, and/or TB 3x above baseline.

- **Dose Modifications for AEs (including serious AEs) related to NAC infusion (based in part on a previously published protocol)**[35]

a) Flushing alone, without pruritus, urticaria, or other serious signs
    - No intervention
    - Continue infusion, unless more severe signs develop

b) Urticaria
    - Stop infusion
    - Treat immediately with intravenous diphenhydramine (1.25 mg/kg/dose)
    - Restart infusion if symptoms resolve within 1 hour

c) Angioedema, hypotension, wheezing (serious AE)
    - Stop infusion
    - Treat with intramuscular epinephrine (0.01 mg/kg/dose), diphenhydramine (1.25 mg/kg/dose), intravenous glucocorticoid (1 mg/kg/dose), and/or inhaled albuterol, at the discretion of the intensive care unit team
    - Do not restart infusion
    - Continue to follow patients for first two years of life, per study protocol

d) Elevation of ALT >700 U/L above baseline, Bc 3x or Bc >5 mg/dL above baseline, and/or TB 3x above baseline (serious AE)
    - Stop infusion
    - Do not restart infusion
    - Follow patients clinically until elevated ALT/Bc stabilizes/resolves
    - Evaluate for drug-induced liver injury if deemed appropriate by the clinical team, based on FDA recommendations (https://www.fda.gov/downloads/Drugs/.../guidances/UCM174090.pdf)

- **Reporting**

AEs (serious and non-serious) occurring in the first 28 days will be recorded on CRFs and presented to the DSMB at each meeting. In addition, serious AEs occurring at any time during the study period will be reported directly to the Chairperson within seven days of occurrence with the exception of events related to the management of the natural course of BA (i.e., hospitalizations for cholangitis, poor growth, worsening liver disease after failed KP, etc). This reporting schedule is designed to help the DSMB decide whether the trials meets sufficient safety standards to continue. AE CRFs will have describe each AE using the following categories (see sample AE ORF):

a) Category of AE
    - AEs will be categorized into pre-determined groups, as specified by the CTCAE.
    - These groups include Anemia, Cardiovascular, Congenital, Death, Dermatological, Febrile events, Gastrointestinal, Gynecological, Hematological, Hepatic, Injury, Immunological, Infectious viral, Infectious cholangitis, Infectious surgical, Infectious, Investigation (laboratory results), Metabolic, Miscellaneous, Musculoskeletal, Neoplastic, Neurological, Nutritional, Orthopedic, Pulmonary, Surgical, Urinary, Wound non-infectious, IV Issues

b) Severity of AE – Serious or non-serious

c) Description of event – Concise description of AE, including date occurred

d) Grading of AE
    - CTCAE grade will be used, in accord with CTCAE Version 5.0
    - If the CTCAE does not have a grading for a particular AE, the severity of the event should be reported based on the following (this is independent of an AE designated as serious, which requires prompt reporting to the DSMB Chairperson):
        - Grade 1: Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated.
        - Grade 2: Moderate; minimal, local, or noninvasive intervention indicated
        - Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling. (As mentioned above, liver enzymes will be

Grade 3 at baseline, so only AST, ALT, and/or GGT >400 U/L above baseline and/or Bc >3 mg/dL above baseline will be reported as an AE. If this were to occur, the AE would be Grade 3)
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

e) Relationship to NAC
- Related: There is clear evidence that the event is related to the use of study drug (i.e., rash with NAC infusion)
- Possible: The event cannot be explained by BA, other therapies, or other causes, and these is a plausible temporal relationship between the event and NAC administration
- Unlikely/Remote: An event for which an alternative explanation is more likely or the temporal relationship to NAC administration does not support a causal relationship
- Not related: The event can readily be explained by BA, other therapies, or other causes, and therefore the Principal Investigator believes no relationship exists between the event and NAC therapy

f) Action Taken with Study Drug
- None: No changes made to NAC administration
- Discontinued: Study drug was discontinued

g) Treatment Given for AE – A list of any treatments or procedures given for the event.

h) Outcome of the AE
- Ongoing: AE persists despite intervention
- Recovered: Subject recovers completely from AE
- Change in severity: AE became serious from non-serious or vice versa
- Unknown
- Death

- Review of AEs

The DSMB will review all AE CRFs at biannual meetings and review serious AEs if an emergency meeting is called by the DSMB Chair. The DSMB will then decide if the study should be stopped for safety reasons.

- **Data Management, Analysis, and Quality Assurance**

The DMSB will review enrollment, enrollment targets, and findings recorded for each subject to ensure the validity and integrity of the data. At each DSMB meeting, updated Data CRFs for each subject will also be presented (see sample Data CRF). The DSMB will assure quality assurance, defined as “All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s).

Sample Adverse Event (**AE**) Clinical Report Form (**CRF**)

Participant ID:

#	Category[1]	Description	SAE?	Grade (CTCAE)[2]	Start Date	Outcome[3]	End Date	Relationship to NAC[4]	Action Taken with NAC[5]	Treatment Given	Investigator Initials/Date

[1] Category of AE:	[2] Grading of AE (**Only If NOT in CTCAE**)	[3] Outcome of the AE:	[4] Relationship to NAC:	[5] Action Taken with NAC:
Anemia, Cardiovascular, Congenital, Death, Dermatological, Febrile events, GI, Gynecological, Hematological, Hepatic, Injury, Immunological, Infectious Viral, Infectious Cholangitis, Infectious Surgical, Infectious, Investigation (lab results), Metabolic, Miscellaneous, Musculoskeletal, Neoplastic, Neurological, Nutritional, Orthopedic, Pulmonary, Surgical, Urinary, Wound Non-Infectious, IV Issues	**Grade 1** – Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. **Grade 2** – Moderate; minimal, local, or noninvasive intervention indicated. **Grade 3** – Severe or medical significant but not immediately life-threatening;, hospitalization or prolongation of hospitalization indicated; disabling. **Grade 4** – Life-threatening consequences; urgent intervention indicated. **Grade 5** – Death related to AE.	**1** - Ongoing; AE continues despite intervention **2** - Recovered; subject recovers completely from AE **3** - Change in severity; AE became serious from non-serious or vice versa **4** - Unknown **5** - Death	**1** - Related **2** - Possible **3** - Unlikely/Remote **4** - Not Related	**N** - none; no changes made to NAC administration **D** - Study drug discontinued

Sample Data Clinical Report Form (**CRF**)

Participant ID:

Gender:
Race:
Ethnicity:
Gestation Age:
Age at KP:

Episodes of cholangitis (and days after KP)**:**
Episodes of ascites (and days after KP)**:**
Episodes of variceal bleed (and days after KP)**:**
Liver transplant listing (days after KP)**:**
Liver transplant (days after KP)**:**

	Pre-KP	Days Post-KP			Weeks Post-KP						Months of Life		
		3	7	10	2	4	8	12	18	24	12	18	24
TSBA													
Bc													
AST													
ALT													
GGT													
Alb													
Na													
T Bili													
Plt													
INR													
Vit D													
Vit A													
Vit E													
Wt													
Wt Z													

Data and Safety Monitoring Board Charter

Study: A Phase 2 Trial of N-Acetylcysteine in Biliary Atresia after Kasai Portoenterostomy
PI: Sanjiv Harpavat, MD PhD
Institution: Baylor College of Medicine and Texas Children's Hospital

The Data and Safety Monitoring Board (**DSMB**) will act in an advisory capacity to monitor participant safety, data quality and evaluate the progress of the study.

DSMB Responsibilities

- Review the research protocol, informed consent documents and plans for data safety and monitoring prior to enrollment of the first study participant;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- Protect the safety of the study participants;
- Make recommendations concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- Review interim analyses in accordance with stopping rules, as defined in the protocol for the two-stage "minimax" trial design;
- Ensure the confidentiality of the study data and the results of monitoring; and,
- Comment on any problems with study conduct, enrollment, and/or data collection.

The DSMB will discharge itself from its duties when the last participant completes the study.

Membership

The DSMB consists of three members (two members constitute a quorum). The members will include two clinicians with expertise in pediatric hepatology and one pediatric hospital-based pharmacist, each from an institution unaffiliated with the study site. Members will have no financial, scientific, or other conflict of interest with the trial, including no ongoing collaborations with the Principal Investigator. Written documentation attesting to absence of conflict of interest is required (see below).

The Chairperson of the DSMB is responsible for overseeing the meetings and developing the agenda in consultation with the Principal Investigator. The Chair is the contact person for the DSMB. The Chair is also the safety officer, and will be the contact person for serious adverse event reporting.

In the event that a member or Chairperson has to leave, the Principal Investigator will be responsible for finding a new member within 28 days. During the vacancy of a Chairperson, the other clinician member of the DSMB will serve as Chairperson until a new one is recruited.

Meeting Format

All meetings will be performed by conference calls organized by the study team. At the first meeting, the DSMB will review the protocol and guidelines for how the board will monitor the study. Subsequently, DSMB meetings will be held at least two times per year, starting three months after the first subject is enrolled. In addition, the Chair can call an emergency meeting at any time should participant safety questions or other unanticipated problems arise.

Meetings will consist of two sessions. The first session will be attended by the Principal Investigator and key members of the study team. The second session will occur without the Principal Investigator and key members of the study team. Discussion in both sessions will focus on the conduct and progress of the study, including participant accrual, protocol compliance, and problems encountered. Each meeting must include a recommendation to continue or to terminate the study (made by a majority vote) and whether the DSMB has any concerns about participant safety.

Communication

Immediately after each DSMB meeting, the Chair will call the Principal Investigator and study team to discuss the DSMB's recommendations. The study team will record the DSMB's recommendations in a "Minutes" document, to be approved before starting the subsequent meeting.

At any time during the study, the Principal Investigator will inform the Chair about serious adverse events via email no later than seven days after the event. The Chair, at his/her discretion, may choose to share the email with the other members. The Principal Investigator is responsible for soliciting a response from the Chair within seven days after the initial email is sent, to determine if any further action is necessary. The study staff will save these emails in the study file, and present them at the next DSMB meeting.

Meeting Materials

Materials will be prepared by the study staff, and provided in electronic form to the DSMB members at least seven days before the meeting. They will include:

- Approving Minutes from previous meeting
- Summary of interim events, including updates on (i) safety data, (ii) problems encountered, (iii) total enrollment, and (iv) primary outcome results
- All Adverse Event Case Report Forms (interim updates highlighted in a different color)
- Any interim email communications between Chair and Principal Investigator regarding serious adverse events
- Copy of the study protocol, including any new updates
- Copy of informed consent, including any new updates

Confidentiality
All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality unless otherwise specified by a majority vote of the board.

I attest that I have no conflict of interest with this study.

DSMB Member Name: _____ DSMB Position: _____

Signature: _____ Date: _____