Childhood Liver Disease Research and Education Network (ChiLDREN)

A Randomized, Double-Blinded, Placebo-Controlled Trial of Corticosteroid Therapy Following Portoenterostomy in Infants with Biliary Atresia

IND# 71,411

Version 7, Amendment 5, May 4, 2010
Version 6, Amendment 4: March 7, 2008
Version 5, Amendment 3: January 16, 2007
Version 4, Amendment 2: January 26, 2006
Version 3, Amendment 1: September 21, 2005
Version 2: February 20, 2005

Approved by the ChiLDREN Steering Committee: 09/29/2004

Approved by the ChiLDREN DSMB: 12/17/2004

Working Group:
Jorge Bezerra, Cincinnati Children's Hospital Medical Center, Cincinnati
Benjamin Shneider, Children's Hospital, Pittsburgh
Frederick J. Suchy, Mount Sinai Medical Center, NYC (as of 12/31/06)
Patricia DeRusso, Johns Hopkins School of Medicine, Baltimore (end 07/17/2006)
Barbara Haber, Children's Hospital of Philadelphia, Philadelphia
Philip Rosenthal, University of California, San Francisco
Ross Shepherd, Washington University School of Medicine (end as of 6/30/2009)
Yumrile Turmelle, Washington University School of Medicine, St. Louis
Kathleen Schwarz, Johns Hopkins School of Medicine, Baltimore
Peter Whitington, Children's Memorial Hospital, Chicago
Jean Molleston, Riley Hospital for Children, Indianapolis
Kasper Wang, Children's Hospital Los Angeles, LA
Karen Murray, Seattle Children's Hospital, Seattle
Rene Romero, Children's Healthcare of Atlanta, Atlanta
Jessi Erlichman, Children's Hospital of Philadelphia, Philadelphia
Melissa Young, Seattle Children's Hospital, Seattle
Morton Brown, University of Michigan, Ann Arbor (end 06/30/2006)
John Magee, University of Michigan, Ann Arbor
Trivellore Raghunathan, University of Michigan, Ann Arbor
Ronald Sokol, The Children's Hospital, Aurora, Colorado
Patricia Robuck, Program Director, NIDDK, NIH, Bethesda

TABLE OF CONTENTS

Amendment 5: May 4, 2010

1. **Network Name Change:**
Biliary Atresia Consortium (BARC) has been replaced with The Childhood Liver Disease Research and Education Network (ChiLDREN). This name change will be reflected throughout Protocol Amendment 5, Version 07.

2. Study Name Update:
P004 was replaced with the acronym START. This change is reflected throughout Protocol Amendment 5, Version 07.

3. Change in the Title Page:
Changes will only reflect the protocol title page.

4. All references to patient replaced with subject throughout Protocol Amendment 5, Version 07.

Working Group:
The following are new members of the PROBE Working Group:
Jean Molleston, Riley Hospital for Children, Indianapolis
Kasper Wang, Children's Hospital Los Angeles, LA
Karen Murray, Seattle Children's Hospital, Seattle (as of 09/15/2009)
Yumrile Turmelle, Washington University School of Medicine, St. Louis
Rene Romero, Children's Healthcare of Atlanta, Atlanta

5. Increased the number of study sites from 10 to 14 and changes reflected throughout Protocol Amendment 5, Version 7

Rationale:
Additional study sites were added to increase enrollment to meet the expected 140 subjects per the protocol.

6. Administrative changes and typographical changes/corrections were made throughout the Protocol Amendment 5, Version 7.

7. Replaced "patient" to "subject" throughout the Protocol Amendment 5, Version 7 for consistency purposes.

8. The name "Fisher Bioservices Repository" was replaced with "NIDDK Repository" throughout the Protocol Amendment 5, Version 7.

Rationale:
The NIDDK has a contract with Fisher to be the NIDDK central Repository for the ChiLDREN studies.

9. 4.03 Comprised Immune Function

Old Text:
Impaired response to childhood vaccines
Subjects will receive all routine childhood vaccines according to the schedule recommended by the American Academy of Pediatrics (AAP), with the exception of up to a 4-week delay of vaccines that would have been given during the first 4 weeks of the study drug/placebo. Vaccinations will be administered by the subject's primary care provider. At 18 months of age or at the time of liver transplantation (if younger than 18 months of age), the vaccination records of each subject will be obtained from the primary care provider for review. The number and size of doses, dates of administration, and vaccine manufacturer will be recorded. At the age of 18 months, 6-9 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. The antibody titers will be measured by the study sites local laboratory.

Amended Text:
Impaired response to childhood vaccines
Subjects will receive all routine childhood vaccines according to the schedule recommended by the American Academy of Pediatrics (AAP), with the exception of up to a 4-week delay of vaccines that would have been given during the first 4 weeks of the study drug/placebo. Vaccinations will be administered by the subject's primary care provider. At 18 months of age or at the time of liver transplantation (if younger than 18 months of age), the vaccination records of each subject will be obtained from the primary care provider for review. The number and size of doses, dates of administration, and vaccine manufacturer will be recorded. At the age of 18 months, 6-9 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. The antibody titers will be measured by the study sites local laboratory.

Rationale:
To clarify wherethe antibody titers are measured.

10. 5.C Data Management

Removed Text:
One of the forms in the database contains a schedule of visits with both scheduled and actual times; study coordinators are prompted by email messages when visits are not scheduled within the expected windows or visits do not occur when scheduled; the emails use only the study identifier.

Rational:
The study coordinators are not prompted by an e-mail message for missed visits or visits outside of the expected visit window; therefore removed from Protocol Amendment 5, Version 07.

11. 7.C AE and SAE Reporting Plan

Original Text:
All SAEs as defined previously require expedited event notification within 24 hours of occurrence or identification to the DCC. Expedited event notification is completed through web entry of the Adverse Events Form. Web entry generates an immediate email to the Medical Safety Officer with only the subject identification number in the message. The Medical Safety Officer then logs into the secure website to see the report of the event. In addition to web entry of the SAE, the Medical Safety Officer may be contacted for any questions or clarifications regarding classification of the event. Paging the Medical Safety Officer is not required as part of the expedited notification process, but may be used if any difficulty is encountered in the web-based notification system. Completing the web entry of the AE form will automatically send e-mail notification to the Medical Safety Officer, NIDDK Program Director, DCC PI, and Project Manager. Fax cover sheets are provided if there is a need to fax supporting information per request of the Medical Safety Officer or NIDDK Program Director. Once the Medical Safety Officer reviews the information submitted on the AE form, the clinical site will be contacted if additional information is required in order to draft a preliminary report of the SAE. Once completed, the preliminary report will be sent to the clinical site PI to review for accuracy and completeness. Following review by the clinical site PI, the report will then be sent to the NIDDK Program Director, clinical site PI, DCC PI, Project Manager, and Chair of the DSMB.

Once the SAE is resolved, a final report is generated by the Medical Safety Officer. The final report will be sent to the clinical site PI to review for accuracy and completeness of the event. Following review by the clinical site PI, The Medical Safety Officer will send the final report to the NIDDK Program Director, clinical site PI, and the DCC PI and Project Manager. All SAE reports will go the DSMB for review. The NIDDK Program Director and the Chair of the DSMB will decide if any individual SAE warrants notification to the FDA and to the IRB’s of all participating BARC clinical sites.

*Amended Text:*All SAEs as defined previously require expedited event notification within 24 hours of occurrence or identification to the DCC. Expedited event notification is completed through web entry of the Adverse Events Form. Web entry generates an immediate email to the Medical Safety Officer with only the subject identification number in the message. The Medical Safety Officer then logs into the secure website to see the report of the event. In addition to web entry of the SAE, the Medical Safety Officer may be contacted for any questions or clarifications regarding classification of the event. Paging the Medical Safety Officer is not required as part of the expedited notification process, however may be used if any difficulty is encountered in the web-based notification system. Completing the web entry of the AE form will automatically send e-mail notification to the Medical Safety Officer, NIDDK Program Director, DCC PI, and Project Manager. Fax cover sheets are provided if there is a need to fax supporting information per request of the Medical Safety Officer or NIDDK Program Director. Once the Medical Safety Officer reviews the information submitted on the AE form, the clinical site will be contacted if additional information is required in order to draft a preliminary report of the SAE. Once completed, the preliminary

report will be sent to the NIDDK Program Director, clinical site PI, DCC PI, Project Manager, and Chair of the DSMB.

Once the SAE is resolved, a final report is generated by the Medical Safety Officer., The Medical Safety Officer will send the final report to the NIDDK Program Director, clinical site PI, and the DCC PI and Project Manager. All SAE reports will go the DSMB for review. The NIDDK Program Director and the Chair of the DSMB will decide if any individual SAE warrants notification to the FDA and to the IRB's of all participating ChiLDREN clinical sites.

Rationale:
The SAE reporting system was modified. The PI receives all reports from Preliminary to Final via the e-mail notification system. At any time the PI may provide requests for modifications on the reports.

12. APPENDIX 1: DSMB Charter
Removed Text:
APPENDIX 1: DSMB Charter

DSMB Charter
Biliary Atresia Research Consortium (BARC)

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the National Institute of Diabetes and Digestive and Kidney diseases (NIDDK) to monitor safety and evaluate the ability of the BARC to conduct studies to identify etiology, development of diagnostic criteria, natural history, risk factors for progression of disease, and therapy of biliary atresia and other rare, pediatric liver diseases. Ten pediatric clinical research centers and a data coordinating center (DCC) are conducting the studies and setting up a database of children with biliary atresia and other pediatric cholestatic disorders through a cooperative agreement mechanism with the NIDDK. The BARC is governed by the Steering Committee, composed of the principal investigators of the DCC and the ten pediatric clinical research centers, and the NIDDK Program Director. An Executive Committee, consisting of the chair and co-chair of the Steering Committee, the principal investigator of the DCC, and NIDDK Program Director, monitors the day-to-day progress of the study and plans Steering Committee meetings.

DSMB RESPONSIBILITIES

The initial responsibility of the DSMB will be to approve the initiation of proposed clinical studies. They will also review the prospective database. At periodic intervals during the course of each study, the DSMB responsibilities are to:

- Review the research protocols, informed consent documents and plans for data safety and monitoring, including all proposed revisions;

- Evaluate the progress of the database and the clinical studies, including periodic assessments of data quality and timeliness, participant recruitment, accrual and

retention, participant risk versus benefit, performance of the trial site, 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;

- Protect the safety of the study participants;

- Report on the safety and progress of the trial;

- Make recommendations to the NIDDK, the Steering Committee, and, if required, to the FDA and IRBs concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;

- If appropriate, review interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis and have the approval of the DSMB;

- Ensure the confidentiality of the trial data and the results of monitoring;

- Assist the NIDDK by commenting on any problems with study conduct, enrollment, sample size, and/or data collection.

MEMBERSHIP

The Data and Safety Monitoring Board will consist of at least 8 members. Five members will constitute a quorum. The members have been appointed by the NIDDK. Members of the DSMB shall have no financial, scientific, or other conflict of interest with the study. Collaborators or associates of the investigators in this trial are not eligible to serve on the DSMB. Written documentation attesting to absence of conflict of interest is required.

Dr. Joel Lavine, University of California, San Diego, has been selected by the NIDDK to serve as the DSMB Chairperson. He is responsible for overseeing the meetings, developing the agenda in consultation with the NIDDK Program Director. The Chair is the contact person for the DSMB. An NIDDK Representative will serve as the DSMB Executive Secretary (ES). Other NIDDK official(s) may serve as an ex-officio member(s) of the DSMB. The DCC shall provide the logistical management and support of the DSMB.

The safety officer, John Magee, MD, will be the contact person for adverse event reporting. Procedures for notifying the Chair of the DSMB and the NIDDK Program Director will be discussed and approved by the DSMB. Those procedures will be part of the DSMB written plan.

BOARD PROCESS

The DSMB will meet at least two times a year at the call of the Chair, with advance approval of the NIDDK Program Director. An NIDDK representative(s) will be present at every meeting.

Meetings shall be closed to the public because discussions may address confidential patient data. Members of the Steering Committee attend meetings, when appropriate. Meetings may be convened as conference calls as well as in person. An emergency meeting of the DSMB may be called at any time by the Chair of the DSMB or by the NIDDK Program Director should questions of patient safety arise.

MEETING FORMAT

An appropriate format for DSMB meetings consists of an open, closed, and executive session. This format may be modified as needed.

Open Session:
The voting members of the DSMB, the Executive Committee, the NIDDK staff and ES, will attend the open session. Other members of the Steering Committee may also attend.

Issues discussed will include conduct and progress of the study, including patient recruitment, data quality, general adherence and toxicity issues, compliance with protocol, and any other logistical matters that may affect either the conduct or outcome of the study. Proposed protocol amendments will also be presented in this session. Patient-specific and subgroup-specific data may not be presented in the open session.

Closed Session:
The closed session will be attended only by voting DSMB members, representatives from NIDDK, the principal investigator of the DCC or statistician, and the NIDDK ES.

Primary and secondary outcomes data will be reviewed, as well as adverse events, adherence and dropouts, and examination of any relevant subgroups. The discussion at the closed session is completely confidential.

Executive Session:
The executive session will be attended by voting DSMB members and the NIDDK Program Director and the NIDDK ES.

The DSMB will discuss information presented to it during the closed and open sessions and decide whether to recommend continuation or termination, protocol modification or other changes to the conduct of the study.

Should the DSMB decide to issue a termination recommendation, a full vote of the DSMB will be required. In the event of a split vote on continuation, majority vote will rule and a minority report should be appended. Reasons for early termination include:

- An exceedingly large number of serious and unexpected adverse events.
- Logistical or data quality problems so severe that correction is not feasible.
- Results of interim analysis.

Final Open Session (optional):

The final session will be attended by voting DSMB members, the Executive Committee, statistician (if in attendance), and NIDDK staff.

The Chair of the DSMB shall report its recommendations regarding study continuation and concerns regarding the conduct of the study. Requests regarding data presentation for subsequent meetings will be made. Scheduling of the next DSMB meeting will also be done.

REPORTS

Interim Reports: Interim reports are generally prepared by the study statistician(s) and distributed to the DSMB, the NIDDK Program Director and the NIDDK ES at least 10 days prior to a scheduled meeting. These interim reports are numbered and provided in sealed envelopes within an express mailing package or by email if the DSMB prefers. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be directed by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts. Part 1 (Open Session Report) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status. Part 2 (Closed Session Report) may contain data on study outcomes, including safety data and, perhaps efficacy data. The Closed Session Report is considered confidential and should be destroyed at the conclusion of the meeting. Data files to be used for interim analyses should have undergone established editing procedures to the extent possible.

Reports from the DSMB: A summary report prepared by the ES will be sent to the full DSMB within 4 weeks of the meeting. Once approved by the DSMB, the NIDDK will forward the DSMB report to the Steering Committee along with a letter from the NIDDK Program Director concurring or refuting the findings of the DSMB. It is the responsibility of the Steering Committee members to distribute the DSMB summary and the accompanying NIDDK letter to all co-investigators and to assure that copies are submitted to all of their respective IRBs as appropriate.

Each report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by formal majority vote. A termination recommendation may be made by the DSMB at any time by majority vote. The NIDDK is responsible for notifying the PIs of a decision to terminate the study. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report.

Mailings to the DSMB: On a scheduled basis (as agreed upon by the DSMB) safety data should be communicated to all DSMB members. Adverse events that are severe and unexpected will be reported to the DSMB Chair and the NIDDK Program Director as they occur. Any concerns noted by the DSMB Chair should be brought to the attention of the NIDDK Program Director.

Access to Interim Data: Access to the accumulating endpoint data should be limited to as small a group as possible. Limiting the access to interim data to the DSMB members helps protect the study from bias in patient entry and/or evaluation.

CONFIDENTIALITY
All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

Rationale:
The DSMB Charter was removed from this protocol amendment via NIDDK directive.

INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES
REVISION OF INFORMED CONSENT REQUIRED	YES

AMENDMENT 4: March 13, 2008

1. **CHANGE Orapred® to prednisolone [Rationale: A critical shortage of Orapred® has required a change to the generic equivalent, prednisolone, in order to maintain a supply of study drug.]**

Delete:
~~4.J3. Oral formulation~~
~~We will use Orapred® as a standard oral formulation of prednisolone (15 mg/5 mL). The choice of Orapred® was based on its commercial availability, concentration of the formulation, and the demonstrated ability of the research pharmacist to formulate a placebo that matches the medication for color and taste. Liquid forms of prednisolone and placebo will be provided to the clinical site research pharmacies by the central research pharmacist, while the randomization schedule will be provided by the DCC~~

Replace with:
"4.J3. Oral formulation
We will use an oral formulation of prednisolone (15 mg/5 mL). The choice of a prednisolone preparation is based on its commercial availability, concentration of the formulation, and the demonstrated ability of the research pharmacist to formulate a placebo that matches the medication for color and taste. Liquid forms of prednisolone and placebo will be provided to

the clinical site research pharmacies by the central research pharmacist, while the randomization schedule will be provided by the DCC."

2. <u>ADD Information and procedures for infants who have received Rotavirus vaccine prior to enrolllment</u> [Rationale: Since initiation of the protocol, a live attenuated Rotavirus vaccine has become available. It is possible that some children may receive the vaccine in the week prior to presenting with biliary atresia. According to the vaccine manufacturer's package insert, administration of the vaccine is contraindicated to infants with "history of gastrointestinal disorders including… a history of congenital abdominal disorders" and "abdominal surgery". Thus no child with biliary atresia, regardless of participation in a clinical trial, would be given the vaccine after the diagnosis of biliary atresia.]

Add:
4.D. Exclusion criteria

Infants who have received the live attenuated rotavirus vaccine (e.g. Rotateq) within 5 days prior to proposed administration of study drug.

Add:
<u>4.F1. Administration of study drug or placebo</u>
Infants who have received the live attenuated rotavirus vaccine (e.g. Rotateq) within 5 days prior to proposed administration of study drug are excluded from the study. Infants who have received the vaccine prior to this interval should not receive their first dose of steroids until a total of 5 days post vaccination has elapsed.

3. <u>ADEK replaced with AquADEK ®</u> [Rationale: Production of ADEK has ceased. AquaADEK is the replacement vitamin supplement.]

When sites run out of ADEK supply, it will be replaced with AquADEK® In March 2008, ADEK supply will be replaced with AquADEK® at all sites.

Thus, the term "ADEK" was replaced with the term "AquADEK®" in the following sections of the protocol: 1 (Specific Aim 4, Hypothesis 4a), 4.J4, 4.M4, 4.N6, 4.N7, and 4.N8.

<u>4. Allow Zantac liquid</u>

<u>4.M7. Ranitidine</u>
Add:

- If Zantac tablets are not available due to supply shortage from the manufacturer, Zantac liquid can be dispensed.

5. CHANGE handling of PIVKA specimens: [Rationale: Administrative logistics and ability to keep personal identification materials from research study laboratory site.]

Delete:
4.N9. Monitoring of vitamin levels
~~Two central labs will be measuring vitamin levels for this study. All samples will be shipped to the Pediatric CTRC Core Laboratory at The Children's Hospital in Denver which will perform all the tests except the PIVKA-II. Denver will ship the sample for PIVKA-II to the second central lab at Cincinnati Children's Hospital Medical Center. The samples that are sent to Denver will be identified with the following personal health information: child's name, medical record number, and date of birth. The child's unique study code will not be shared. It is necessary for this information to be collected and shared because these are clinically indicated tests. These tests will be paid by the study.~~

~~PIVKA-II samples will be barcoded using label 10 from manifest form 51 for the visit it is collected. Sites will send this barcoded sample to the Fisher Bioservices Repository along with other collected samples. Fisher will keep these PIVKA-II samples together until they accumulate approximately 50. Fisher will then batch ship these 50 samples to Cincinnati Children's Hospital Medical Center for processing. Fisher will verify which samples were sent to Cincinnati, and Cincnnati will send the results to the DCC. These tests will be paid by the study.~~

Replace with:
4.N9. Monitoring of vitamin levels
Two central labs will be measuring vitamin levels for this study. A sample will be sent to the Pediatric CTRC Core Laboratory at The Children's Hospital in Denver which will perform all the tests except the PIVKA-II. The samples that are sent to Denver will be identified with the following personal health information: child's name, medical record number, and date of birth. The child's unique study code will not be shared. It is necessary for this information to be collected and shared because these are clinically indicated tests that will be reported to the site. These tests will be paid by the study.

PIVKA-II samples will be barcoded and sent to the Fisher Bioservices Repository. Fisher will then batch ship these samples to Cincinnati Children's Hospital Medical Center for processing. Fisher will verify which samples were sent to Cincinnati, and Cincinnati will send the results to the DCC. PIIVKA-II is not being used clinically by the sites and these results will not be reported back to the site. These tests will be paid by the study.

6. CHANGE details regarding procedures and assessment of vaccine titers
[Rationale: Clinical sites have reported their laboratories cannot obtain all the requested titers with 3 mls of blood. We have increased the volume of blood for this test to be consistent with what would be required and routinely obtained at the sites. Additionally, a prioritization of specific titers has been provided in the event that it is necessary. Given standard vaccination schedules, data regarding titers drawn before 18 months or post transplant will not be interpretable, thus we will not collect these data in children transplanted before 18 months of age. Finally, the reporting of

protective antibody responses is not consistent across sites. Both the reporting of the titers, as well as the threshold for "protective", varies by site. The protocol has been changed to allow sites to conform with their specific clinical standards of care.]

Delete:

4.O3. Compromised immune function

~~At the age of 18 months, 3 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. In those infants with progressive liver disease who require liver transplantation before age 18 months, a blood sample for antibody titers will be obtained at the time of transplantation. If serum antibody titers do not achieve protective levels (Table 9), the information will be provided to the primary care provider so that booster/re-immunization of appropriate antigen(s) is given. Absent titers will also be communicated to the primary care provider, who will treat with re-immunization as directed by the AAP guidelines for catch-up immunization.~~

~~Table 9. Measurements of protective response to routine childhood immunizations (3 ml of blood).~~

~~Vaccine antigen~~	~~Protective titer~~
~~Hepatitis B virus~~	~~≥ 10 mIU/ml~~
~~Diphtheria~~	~~≥ 0.1 IU/ml~~
~~Tetanus~~	~~≥ 0.01 IU/ml~~
~~Pertussis~~	~~No recognized single immunologic correlate of protection. Thus, pertussis-specific antibodies will not be measured.~~
~~H. influenzae type B~~	~~1 ug/ml~~
~~Poliovirus 1~~	~~1:100~~
~~Poliovirus 2~~	~~1:100~~
~~Poliovirus 3~~	~~1:100~~

Replace with:

At the age of 18 months, 6-9 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. If a subject has received a transplant before the 18th month visit, titers should not be collected. Priority titers to be collected relate to vaccinations for Hepatitis B, Tetanus, and Polio. If serum antibody titers do not achieve protective levels as determined by the local laboratory standard and the investigator, the information will be provided to the primary care provider so that booster/re-immunization of appropriate antigen(s) is given. Absent titers will also be communicated to the primary care provider, who will treat with re-immunization as directed by the AAP guidelines for catch-up immunization.

Given the deletion of Table 9, subsequent tables (Tables 10, 11 and 12) have been re-numbered (to Tables 9, 10 and 11, respectively).

7. CHANGE patient incentive language
[Rationale: Given changes in the price of gasoline, as well as geographic differences, and to provide greater site autonomy, we have made the patient incentive language less proscriptive.]

Delete:
~~6.C2. Description of any incentives or rewards offered for participation~~
~~Parents or guardians of infants enrolled in the study will receive $15-20 per outpatient visit to reimburse them for meals and/or parking fees according to costs at each clinical site.~~

Replace with:
6.C2. Description of any incentives or rewards offered for participation
Parents or guardians of infants enrolled in the study will receive an appropriate fee per outpatient visit to reimburse them for meals and/or parking fees according to costs at each clinical site.

INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES
REVISION OF INFORMED CONSENT REQUIRED	YES

AMENDMENT 3: January 16, 2007

CHANGE IN THE TITLE PAGE: CHANGE IN INVERTIGATORSHIP. JOHN MAGEE WILL BE THE DATA COORDINATING CENTER INVESTIGATOR AT THE UNIVERSITY OF MICHIGAN, ANN ARBOR. TRIVELLORE RAGHUNATHAN, AT THE UNIVERSITY OF MICHIGAN, ANN ARBOR HAS BEEN ADDED TO THE WORKING GROUP. BENJAMIN SHNEIDER WILL BE INVOLVED WITH THE BARC STUDIES AT CHILDREN'S HOSPIAL IN PITTSBURGH AND FREDERICK J. SUCHY WILL BE THE NEW PI AT MOUNT SINAI IN NYC. THESE CHANGES WILL ONLY REFLECT THE PROTOCOL TITLE PAGE.

SECTION: TITLE PAGE

Change:
Morton Brown, Data Coordinating Center, University of Michigan, Ann Arbor (end 06/30/2006)
Benjamin Shneider, ~~Mount Sinai Medical Center, NYC~~ Children's Hospital, Pittsburgh

ADD:
John Magee, Data Coordinating Center, University of Michigan, Ann Arbor (as of 06/30/2006)
Trivellore Raghunathan, Data Coordinating Center, University of Michigan, Ann Arbor (as of 06/30/06)
Frederick J. Suchy, Mount Sinai Medical Center, NYC (as of 12/31/06)

1. CHANGE THE FOLLOWING SECTIONS: Clarification of the duration of the supportive study medications.

SECTIONS:

4.G. Other perioperative and medical management

Once they resume oral/enteric feedings, they will receive 12.5 mg BID oral ranitidine (2-6 mg/kg/day, see Section 4.M.7) during administration of study drug/placebo ~~for 3 months after the portoenterostomy~~; ranitidine is approved by the FDA for use in children (1 month-16 years of age).

4.J4. Other medications

In addition to the masked study drug, the following medications will be dispensed as needed per protocol free of charge by the clinical site research pharmacy: ~~while the child is in the duration of the study:~~

- ADEK and vitamin K (Mephyton®) (up to two years)
- Ursodeoxycholic acid (Urso 250® or Actigall®) (up to two years)
- TMP/SMZ (up to six months)
- Ranitidine (Zantac®) (up to three months while the masked study medication is being prescribed)

2. CHANGE THE FOLLOWING SECTIONS: Clarification that Vitamin K is supplied by the study.

4.N8. Administration of vitamins

The study will provide all cholestatic patients with total bilirubin ≥1.5 mg/dL vitamin K tablets. The families will be given instructions to give 2.5 mg by mouth three times a week as part of standard care for infants with Cholestasis. ~~They will be asked to fill a prescription for vitamin K tablets and to give 2.5 mg by mouth three times a week as part of standard care for infants with cholestasis; vitamin K is to be **paid by parents/guardians** or third party payers.~~

3. ADD THE FOLLOWING: Describe the location of where the vitamin levels for this study will be shipped and processed.

4.N9. Monitoring of vitamin levels

Two central labs will be measuring vitamin levels for this study. All samples will be shipped to the Pediatric CTRC Core Laboratory at The Children's Hospital in Denver which will perform all the tests except the PIVKA-II. Denver will ship the sample for PIVKA-II to the second central lab at Cincinnati Children's Hospital Medical Center. The samples that are sent to Denver will be identified with the following personal health information: child's name, medical record number, and date of birth. The child's unique study code will not be shared. It is necessary for this information to be collected and shared because these are clinically indicated tests. These tests will be paid by the study.

INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES
REVISION OF INFORMED CONSENT REQUIRED	YES

AMENDMENT 2: January 26, 2006

<u>CHANGE THE FOLLOWING SECTIONS</u>: TO EXTEND THE WINDOW TO BEGIN TREATMENT OF STEROID/PLACEBO FROM 24 HOURS TO 72 HOURS AFTER PORTOENTEROSTOMY AND TO PERMIT THE INCLUSION OF SUBJECTS WHO UNDERGO A GALL BLADDER KASAI (PORTOCHOLECYSTOSTOMY) INSTEAD OF A PORTOENTEROSTOMY.

After discussion at the 1/17/2006 Steering committee meeting, the members voted to extend the window to begin treatment from 24 to 72 hours; therefore, allowing additional time for the family to consider consenting into the study.

On 2/1/2006 the Steering Committee voted to permit the inclusion of subjects who undergo a gall bladder Kasai (portocholecystostomy) instead of a portoenterostomy.

Sections:

4.B. Study population

All infants enrolled in the BARC prospective database study who undergo portoenterostomy or the gall bladder Kasai operation (portocholecystostomy) for biliary atresia will be eligible for the trial. *For purposes of this protocol the term portoenterostomy will include the gall bladder Kasai procedure.* Parents/guardians will be approached about participation in this clinical trial after a decision is made by the attending physician at the BARC site that the infant will undergo an exploratory laparotomy with possible portoenterostomy, or within ~~24~~ 72 hours after portoenterostomy.

4.C. Inclusion criteria

- Portoenterostomy or gall bladder Kasai operation for biliary atresia within the previous ~~24~~ 72 hours
- Written informed consent to participate in the study obtained prior to or within ~~24~~ 72 hours of completion of portoenterostomy. *(Note: Families of potential subjects may be approached prior to the portoenterostomy.)*

4.F. Study design and intervention

<u>4.F1. Administration of study drug or placebo</u>

Written informed consent will be obtained either after a decision is made by the attending physician at the BARC site that the infant will undergo an exploratory laparotomy with possible portoenterostomy, or within ~~24~~ 72 hours after portoenterostomy.

Randomization will be performed by the DCC and either corticosteroid or placebo therapy will be initiated within ~~24~~ 72 hours after portoenterostomy; doses will be adjusted according to the dosing schedule outlined in Table 4.

Table 4. Schedule and dosing of corticosteroids or placebo following portoenterostomy in infants with biliary atresia.

~~**Time after Portoenterostomy**~~ **Day/week of dosing**	**Corticosteroids***	**Placebo**
Days 1-7		
days 1-3	Methylprednisolone, IV – 4 mg/kg/day, divided BID	IV – Normal saline (same volume, BID)
days 4-7	Prednisolone, PO – 4 mg/kg/day, divided BID	PO – Placebo (same volume, BID)

6. HUMAN SUBJECTS RESEARCH CONSIDERATIONS

6.A. Risks to study subjects

6.A1. Involvement of subjects

Subjects will be recruited into this study at the time that biliary atresia is suspected and exploratory surgery is scheduled or within ~~24~~ 72 hours following portoenterostomy.

6.A3. Duration of participation for each subject

Each subject will remain in the clinical trial until 24 months of age; i.e, for between 18 and 24 months. The use of corticosteroids will be limited to ~~the first~~ 13 weeks ~~following portoenterostomy~~.

6.B2. Description of recruitment plan

If parent(s) or guardian(s) are not approached about the clinical trial before surgery, recruitment may also occur within ~~24~~ 72 hours of the portoenterostomy.

INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES
REVISION OF INFORMED CONSENT REQUIRED	YES

AMENDMENT 1: September 21, 2005

Change: Administration of study drug or placebo (Section 4.F1): [Improved wording]

- ~~Poor height growth~~ Impaired linear growth

CHANGE: STEROID PULSES (SECTION 4.M9): [TO INDICATE THAT STEROID PULSES SHOULD NOT BE USED ROUTINELY.]

4.M9. Steroid pulses

The use of a steroid pulse (4-5 days of IV steroids at any time following the portoenterostomy) during this trial will be treated as a protocol violation.

~~Some investigators use pulses of corticosteroids when there is delayed cessation of bile flow after portoenterostomy. Although there is no scientific evidence that this is an effective treatment, it is part of the clinical care provided by those investigators. The size of the pulse is also not standardized. In order to avoid providing too much steroids to subjects randomized to active treatment, as well as to avoid unblinding the treatment group whenever the investigator chooses to give a steroid pulse, the following policy will be adhered to:~~

1. ~~Irrespective of blinded randomization to treatment or non-treatment groups, at the discretion of the attending physician, a pulse of corticosteroids may be used to try to enhance bile flow in a child with evidence of cessation of bile drainage during follow-up portoenterostomy. There will be no need to break the code.~~

2. ~~This pulse of corticosteroids may be indicated in a child with biliary atresia who had evidence of good bile flow following portoenterostomy, which has suddenly worsened (e.g. initial drop in bilirubin and return of pigmentation to stool color following portoenterostomy, with a subsequent reoccurrence of acholic stools and a rise in serum bilirubin, with or without clinical evidence of cholangitis).~~

3. ~~The dosage protocol for I.V. methyl prednisolone (Solumedrol) given as a single daily I.V. dose in the morning (with or without antibiotics as necessary) is as follows:~~
   ~~5.0 mg/kg dose on day 1~~
   ~~2.5 mg/kg dose on day 2~~
   ~~1.0 mg/kg dose on day 3~~
   ~~0.5 mg/kg dose on day 4~~
   ~~then stop.~~

~~Note: The inclusion of this policy as a modification of clinical care is not meant to change clinical care at the clinical site; each investigator will determine if and when to use steroid pulsing. However, when a steroid pulse is given, it will be given at the dose described in this policy in order to avoid an excessive dose of steroids being given to infants randomized~~

~~to corticosteroids and, therefore, to eliminate the need to unmask the treatment prior to the administration of the steroid pulse.~~

Change: Amount of blood (Section 6.A5a): [Corrections in the amount of blood.]

Blood drawn for the BARC prospective observational database:

Table 11. Amount of blood drawn from infants *to be sent to the repositories as part of the prospective observational database.*

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
4 weeks post op or post diagnosis	4	8	8
3 months post op or post diagnosis	4	8	12*
6 months post op or post diagnosis	4**	8 or 9.2 ~~11~~	8 or 9.2 ~~11~~
12 months of age	9.2 ~~10~~	9.2 ~~10~~	9.2 ~~10~~
18 months of age	4	4	4
Annually from age 2	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.
** When the 6 month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.

NOTE: Blood volume for clinically indicated tests: Approximately, 6.5 ml of blood may be removed from the child at each visit to evaluate hepatic function, electrolytes and differential. More may be withdrawn to perform additional clinically indicated lab tests.

Additional blood drawn to monitor for vitamin levels and adverse events for this clinical trial:

At each follow up visit, 1 ml of blood will be drawn to monitor serum electrolytes and glucose which may not be drawn as part of standard clinical care.

At follow-up visits (4 weeks post surgery, 3 and 6 months post, and at 12 mo, 18 mo, and 24 mo of age, 3 ml of blood will be drawn to monitor vitamin levels, total bile acids and PIVKA-II. ~~as follows:~~

1. ~~2.5 ml whole blood for serum for retinol, retinol binding protein, 25-OH vitamin D, vitamin E, and total lipids.~~
2. ~~0.5 ml whole blood for serum for total bile acids and PIVKA-II.~~

*Note: If the infant is not receiving vitamin supplementation at the 18 mo of age visit, no additional blood will be drawn for vitamin monitoring. If changes in the vitamin supplementation plan is required, additional testing will be done outside of the visits listed below.

Table 12. Total amount of blood drawn from infants *in the clinical trial (including that for the prospective observational database).*

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
2 weeks post op	1	5	5
4 weeks post op	8	13	13
2 months post op	1	14	14
3 months post op	8	17	22*
6 months post op	8**	8 or 13.2	13.2 or 14.2
12 months of age	13.2	13.2	13.2
18 months of age	8 or 11***	8 or 11	8 or 11
24 months of age	8	8	8
Annually from age 3	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.
** When the 6 month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.
***At 18 months of age, 3 ml of blood will be drawn to assess immunization antibody titer levels; ~~research~~ blood for vitamin levels, total bile acids and PIVKA-II (3 ml) will be drawn only if the infant is receiving vitamin supplementation at this visit.

Addition: 6.C3 Tests performed as part of research: [To clarify which tests are paid for by the research grant.]

Lab tests to evaluate side effects of the corticosteroids (e.g., glucose and electrolytes), vitamin levels, total bile acids, PIVKA-II and immunization antibody titer levels will be paid for by the research grant. The ophthalmologic examination at one year will also be paid for by the research grant.

Addition: 7.F Adrenal insufficiency: [Blind may be broken when adrenal insufficiency is suspected even after the taper is completed.]

During the taper: In the event of a life-threatening complication and when the physician treating the patient determines that knowing whether the subject was taking corticosteroid is essential to implement his/her treatment plan (e.g., need for stress dose of steroid in the

setting of bacteremia and hypotension or invasive tissue sepsis with systemic symptoms), the study code will be broken to reveal if the patient was receiving placebo or corticosteroids. [The Safety Monitor and the DCC will be informed immediately by the filing of a SAE report.] In this setting, if the patient had been receiving corticosteroids, the tapering protocol will typically use a stress dose of hydrocortisone (50-62.5 mg/m^2/24h) for 2-3 days, then physiologic replacement (20-25 mg/m^2/24h) for 7 days, then half of the replacement dose for 7 days; the use of hydrocortisone instead of prednisolone or methylprednisolone in the setting of infection will minimize additional immunosuppression that would result from the glucocorticoid action of prednisolone during the taper schedule. Alternatively, replacement doses may be in the form of prednisolone (using the doses outlined for weeks 2-8 of Table 5) if the subject tolerates oral/enteral feedings.

After the taper is completed: Although very unlikely, subjects may have some degree of adrenal suppression in the first year after the end of the steroid taper. Therefore, stress doses of hydrocortisone may also be used during the first year after the steroid taper in any subject that may show signs of adrenal insufficiency during a severe infectious illness or a significant stress event (such as surgery). The study code may only be broken to reveal if the patient was receiving placebo or corticosteroids if the physician treating the patient determines that knowing the information is necessary to implement his/her treatment plan. The Safety Monitor and the DCC will be informed immediately by the filling of a SAE report.

INSTITUTIONAL REVIEW BOARD APPROVAL REQUIRED	YES
REVISION OF INFORMED CONSENT REQUIRED	YES

ABBREVIATIONS USED IN THE PROTOCOL

AAP: American Academy of Pediatrics
AquADEK®: Formulation containing Vitamins A, D, E, and K
AE: Adverse Event
ChiLDREN: Childhood Liver Disease Research and Education Network
CRF: Case Report Form
DCC: Data Coordinating Center
DSMB: Data and Safety Monitoring Board
DSMP: Data and Safety Monitoring Plan
HRQOL: Health-Related Quality of Life
IRB: Institutional Review Board
NIDDK: National Institutes of Diabetes and Digestive and Kidney Diseases
PCP: *Pneumocyst carinii* pneumonia
PELD: Pediatric End-Stage Liver Disease
PI: Principal Investigator
SAE: Serious Adverse Event
TPGS: Tocopheryl polyethylene glycol-1000 succinate

1. SPECIFIC AIMS

Portoenterostomy (including the gall bladder Kasai operation) is the only operative procedure used currently to improve bile drainage in infants with biliary atresia. Although prompt diagnosis and surgical intervention may induce bile flow, progression to end-stage liver disease occurs in over 50% of the patients by 2 years of age. The biological basis for the progression of liver disease after portoenterostomy is not fully understood, but the presence of hepatobiliary inflammation and proinflammatory cytokines at the time of diagnosis suggests that inflammatory forces mediate, at least in part, the progressive injury. Consistent with this concept, prior clinical reports of corticosteroids as an adjuvant treatment following portoenterostomy for biliary atresia suggest that high doses of corticosteroids may lead to prolonged jaundice-free survival without liver transplantation. Based on these reports, we propose a multi-center randomized, double-blinded, placebo-controlled trial to prospectively determine the efficacy of corticosteroids on the outcome of infants with biliary atresia. The trial will be conducted by the NIDDK-funded network of 14 clinical centers comprising the Childhood Liver Disease Research and Education Network (ChiLDREN), whose goal is to study the etiology, pathogenesis, diagnosis, and treatment of infants with biliary atresia. For the trial, our *overall hypothesis is that therapy with corticosteroids following portoenterostomy (including gall bladder Kasai procedure will improve bile drainage and long-term outcome in infants with biliary atresia*. This hypothesis will be tested through the following specific aims and hypotheses:

Aim 1: To determine whether corticosteroid therapy decreases serum bilirubin concentration after portoenterostomy.
Hypothesis 1a: The probability of an infant having good bile drainage at 6 months after portoenterostomy (as defined by a serum total bilirubin level <1.5 ml/dL) will be greater in infants who are treated with corticosteroids than those treated with placebo.
Hypothesis 1b: Improved bile drainage (as defined by a serum total bilirubin level <1.5 mg/dL) will remain for a longer period in infants treated with corticosteroids than those treated with placebo.

Aim 2: To determine whether corticosteroid treatment after portoenterostomy will improve outcome as defined by survival without transplantation at 24 months of age.
Hypothesis 2: Survival without transplantation will be greater at 24 months of age in infants treated with corticosteroids than those treated with placebo.

Aim 3: To determine whether corticosteroid treatment after portoenterostomy will improve growth of infants with biliary atresia.
Hypothesis 3: Weight and height Z-scores at 12 and 24 months of age will be greater in the infants treated with corticosteroids than those treated with placebo.

Aim 4: To determine whether corticosteroid treatment improves biochemical indicators of each of the fat-soluble vitamins after supplementation with standard doses.
Hypothesis 4a: Treatment with corticosteroids leads to improved bile drainage (hypothesis 1) which, in turn, increases the probability that an infant will achieve

vitamin sufficiency of each of the fat-soluble vitamins following supplementation of AquADEK®.
Hypothesis 4b: Treatment with corticosteroids leads to improved bile drainage (hypothesis 1) which, in turn, reduces the dose required for supplementation and the length of time of supplementation for each of the fat-soluble vitamins.

Aim 5: To determine whether corticosteroid treatment after portoenterostomy will decrease the incidence of persistent ascites or ascites that requires medical treatment.
Hypothesis 5: The incidence of ascites will be decreased at 12 and 24 months of age in infants treated with corticosteroids than those treated with placebo.

2. BACKGROUND AND SIGNIFICANCE

Biliary atresia is a progressive fibro-inflammatory cholangiopathy of infancy that results in complete obliteration of the entire or portions of the extrahepatic biliary tree within 12 weeks of birth. This obstruction results in impaired bile drainage, reactive proliferation of intrahepatic bile ducts, cholestasis, and ongoing hepatocellular injury. If no therapy is implemented, ongoing injury leads to biliary cirrhosis, portal hypertension, and end-stage liver disease, leaving liver transplantation as the only therapeutic option for long-term survival.

2.A. Clinical Significance of Biliary Atresia

Biliary atresia is the most common cause of prolonged conjugated hyperbilirubinemia in neonates and is the most frequent indication for liver transplantation in children, accounting for 40-50% of all pediatric liver transplants[1]. The disease respects no geographic boundaries; it occurs worldwide and affects approximately 1 in 8,000 to 1 in 15,000 live births. The health care costs associated with medical/surgical intervention and transplantation for infants with biliary atresia are significant, estimated to reach $65 million/year in the United States. From a total of $77 million spent annually on pediatric liver transplantation in the United States[2], about half relates to biliary atresia. Notably, this sum of money covers 0.2% of total health care expenditures for children, even though these children represent only 0.0006% of the total pediatric population. This disproportionate expenditure could be decreased by half if improved medical therapies were developed that reduce the need for liver transplantation.

2.B. Clinical Phenotypes

There are two well-recognized clinical forms of biliary atresia: embryonic and perinatal. These forms share the cardinal features of jaundice, acholic stools, and hepatomegaly but differ in the presence of associated anomalies, the timing of onset of jaundice, and perhaps clinical outcome. The embryonic form of biliary atresia (also referred to as “congenital” or “fetal”) accounts for 10 to 20% of cases and is defined by the presence of congenital nonhepatic anomalies and earlier onset of disease[3,4]. Among congenital nonhepatic anomalies, patients may have defects in laterality (or asymmetric left-right determination of visceral organs), poly- or asplenia, and gastrointestinal or cardiovascular anomalies. Infants

present with pathologic jaundice at birth or shortly thereafter, frequently overlapping with physiologic jaundice such that there is no jaundice-free interval. There is some evidence that s with this clinical form have worse outcome following portoenterostomy[3,5].

The perinatal forms of biliary atresia (also referred to as “acquired” or “postnatal”) accounts for the majority of the cases (80-90%) and occurs in the absence of other congenital anomalies. Most of the infants are born at term with an appropriate weight for gestational age. They have a variable jaundice-free interval after birth but develop jaundice, acholic stools, and dark-colored urine within the first few weeks of life. In contrast to the clinical setting observed in infants with the embryonic form, the presence of a jaundice-free period is more consistent with a biliary injury that results from a perinatal or early postnatal insult. Efforts to determine the biologic relationship of potential pathogenic mechanisms are particularly important to understand the molecular basis of the clinical forms and to develop new diagnostic/therapeutic modalities for infants with biliary atresia.

2.C. Pathogenic Mechanisms of Disease

Theoretical considerations of the pathogenesis of biliary atresia are based largely on epidemiologic and clinical features, reported predisposing genetic factors, and pace of disease progression[1]. Based on these observations, five mechanisms have been proposed to play important roles in the pathogenesis of biliary atresia: 1) defect in morphogenesis of the biliary tract, 2) defect in fetal/prenatal circulation, 3) immunologic dysregulation, 4) viral infection, and 5) environmental toxin exposure[1,6,7].

Table 1. Potential Mechanisms Involved in the Pathogenesis of Biliary Atresia

Mechanism	Supporting Data
Defect in morphogenesis	Development of jaundice soon after birth Coexistence of other embryologic abnormalities Abnormal remodeling of the “ductal plate” Mutations in *laterality* genes *(CFC1, ZIC3)* in patients with biliary atresia and laterality defects *Inv* mouse: model of biliary obstruction and situs inversus
Defect in prenatal circulation	Intrauterine devascularization results in abnormal extrahepatic bile ducts
Immunologic dysregulation	Increased expression of intercellular adhesion molecules Genetic polymorphism of cytokines (such as TNF□) Increased frequency of the HLA-B12, B8 or DR3 alleles Hepatic profile displaying a predominant Th1-like phenotype
Viral infection	CMV, HPV, reovirus, and rotavirus detected in infants with biliary atresia Biliary obstruction in newborn mice infected with rotavirus
Toxin exposure	Time-space clustering of cases

2.C1. Evidence for Genetic Susceptibility to Biliary Atresia

The earlier onset of disease (3-4 weeks of life) and non-hepatic malformations in the embryonic form of biliary atresia suggest a prenatal onset and a pathogenesis that differs, at least in part, from that of infants with the acquired form. The main associated malformations, poly- or asplenia, cardiovascular defects, abdominal situs inversus, intestinal malrotation, and anomalies of the portal vein and hepatic artery point to potential defects in embryogenesis and asymmetric left-right determination of visceral organs[4]. In support for this concept, abnormalities in organ asymmetry and biliary drainage have been identified in the *inv* mouse. In these mice, a recessive insertional mutation of the *inversin* gene results in complete abdominal situs inversus, severe jaundice, and death within the first week of life[8]. In a detailed morphological analysis of the hepatobiliary system in the *inv* mouse, a primary defect in patency of the extrahepatic ductular system with intrahepatic ductular proliferation was described[9]; however, the absence of inflammation or necrosis within the hepatic parenchyma is not consistent with the histologic features in infants with biliary atresia. More recently, genetic inactivation of the homeoproteins hepatocyte nuclear factor (HNF)-1□and HNF6 produced morphologic abnormalities of the intrahepatic bile ducts and gall bladder[10,11]. Taken together, these data suggest that abnormalities in genes regulating development may play a role in obliteration of the extrahepatic bile duct, but the extent to which specific genes may regulate disease pathogenesis in humans remains largely unknown.

2.C2. Evidence for Inflammatory/Immunologic Dysfunction in Biliary Atresia

Several clinical reports suggest that inflammatory cells may play a role in the destruction of bile ducts in infants with biliary atresia. For example, epithelial cell pyknosis and necrosis were associated with intramural infiltration of mononuclear cells in bile ducts, as well as with lymphocytes in intrahepatic portal tracts, porta hepatis, and common hepatic duct remnants of infants with biliary atresia[12-14]. Phenotypic characterization of lymphocytes revealed a small population of $CD8^+$ T cells (without expression of cytotoxic markers) in the vicinity of bile duct cells[15]. More frequently, $CD4^+$ T cells predominated in portal tracts of affected livers and expressed markers of activation of T helper (T_H) lymphocytes, such as interleukin-2 receptor[16-18]. T_H cells are broadly divided into the T_H1 and T_H2 types, which regulate cellular inflammatory and humoral immunity, respectively. In order for T-cells to effectively mediate inflammation, they must encounter pathogen-associated antigens from a competent antigen-presenting cell. Both bile duct epithelial cells and Kupffer cells (hepatic macrophages) have been proposed to function as antigen-presenting cell. In this context, bile duct cells, which normally express MHC class I but not class II antigens, have been shown to aberrantly express HLA-DR (MHC class II) in infants with biliary atresia[16,19,20]. A potential role for Kupffer cells as another APC was inferred from increased numbers and size of these cells in the livers of infants with biliary atresia[18,21,22]. Similar analyses also point to a potential interplay of Kupffer cells and lymphocytes in pathogenesis of biliary atresia, such as the infiltration of portal tracts by Kupffer cells expressing the B7 costimulatory molecule (CD86) and of IL-18, a macrophage-derived cytokine that promotes T lymphocyte differentiation[18,21,22]. More recently, a large scale gene expression analysis of infants with biliary atresia revealed a profile of differential lymphocyte activation, with an increased expression of proinflammatory genes at the time of diagnosis when compared to age-matched infants with intrahepatic cholestasis[23].

Although circumstantial, these findings point to a potential functional synergism of inflammatory effectors in the development of bile duct injury in infants with biliary atresia.

2.C3. Evidence for Viral Infection in the Etiology of Biliary Atresia

Different viruses have been detected in livers of infants with biliary atresia sporadically. Despite a report of HBV antigens in biliary atresia in Japan, the findings were not reproducible in the U.S.[24,25]. Based on the presence of non-A, non-B hepatotropic viruses identified in affected livers[26], subsequent reports revealed cytomegalovirus, retroviral antigens, human papilloma virus, reovirus, and rotavirus in specific groups of patients[27-33]. The etiologic role for one single agent, however, was not supported by follow-up studies which attempted to confirm these associations in other patient populations[34-37]. Notably, inoculation of specific strains of reovirus or rotavirus into newborn mice has been reported to produce portal tract inflammation, necrosis of biliary epithelium, and variable degrees of obliteration of bile ducts[38,39].

Evidence for a disease-specific interplay between a viral insult and a development-regulated immunologic response can be inferred from the onset of hepatobiliary destruction in neonatal mice infected with rhesus rotavirus (RRV)[39,40]. In these experiments, oral or intraperitoneal inoculation of RRV into 2-3 day old Balb/c mice induced inflammation and edema of extra-hepatic bile ducts, which progressed to concentric infiltration and complete obstruction; infection of older mice did not result in biliary tract inflammation or obstruction[41].

2.D. Treatment for Biliary Atresia

2.D1. Surgical Treatment

No medical therapy has been developed to date that effectively halts or reverses cholestasis and hepatic injury in children with biliary atresia. The only therapeutic choice to increase bile flow and improve jaundice is the surgical portoenterostomy, in which the fibrotic extrahepatic bile ducts are completely excised and an intestinal conduit is anastomosed to the transected surface of the porta hepatis in a Roux-en-Y fashion. Occasionally the gall bladder is anastomosed to the porta if the distal biliary tree is still patent, the so-called gall bladder Kasai.If performed within the first 60 days of life by experienced surgeons, the portoenterostomy should yield bile drainage from the liver into the intestinal tract in 67-82% of patients[7,42,43], resulting in increased pigmentation of the stools and resolution of jaundice. Improvement in bile drainage decreases to 45-62% of infants when surgery is performed between 60-90 days of life, and to 10-44% if beyond 90 days of life. Therefore, prompt diagnosis and timely execution of portoenterostomy are critical to improved success of the surgical treatment.

Despite the potential for improved bile drainage, portoenterostomy does not stop the inflammatory intrahepatic injury; cholestasis and ongoing cellular injury lead to cirrhosis in most patients. In the absence of other proven therapeutic options, current clinical care is primarily supportive, and includes optimizing nutrition, promoting choleresis, and preventing/treating ascending cholangitis. Ultimately, >70% of all children with biliary atresia will require liver transplantation because of failure of the portoenterostomy or

complications of end-stage liver disease. Therefore, the development of novel effective therapies to improve post-operative bile drainage and reduce or prevent progressive hepatic fibrosis in biliary atresia is a high priority, with the potential to dramatically improve long-term outcome and reduce/delay the need for liver transplantation. One such potential therapy is high-dose corticosteroids following portoenterostomy.

2.D2. Corticosteroid Treatment

Corticosteroid treatment may improve bile drainage following portoenterostomy by at least two proposed mechanisms. In the first, at pharmacologic doses, corticosteroids may stimulate bile salt-independent bile flow by inducing expression of hepatic Na-K adenosine triphosphatase, a sinusoidal transporter that helps maintain the osmotic and electrical forces necessary for bile formation44. In the second, corticosteroids have well-described anti-inflammatory and immunomodulatory properties (reviewed in reference 45). For example, administration of corticosteroids decreases the number and immune response of lymphocytes, with suppression of interleukin (IL)-1, IL2, IL3, IL6, TNF□, and interferon gamma. The anti-inflammatory properties of corticosteroids also result from a much broader effect in a variety of cells, such as the decrease in production of cytokines, adhesion molecules, and metabolic signals by macrophages, monocytes, basophils, fibroblasts, and endothelial cells. These anti-inflammatory functions modulate both humoral and cellular immunity, and have assured a central role for corticosteroids in the treatment of allergic and immunologic disorders. With the increasing body of evidence that the livers of infants with biliary atresia produce inflammatory mediators (reviewed in Section 2.C2), treatment of corticosteroids has the potential to decrease inflammation, edema, and progression of fibrosis.

The effectiveness of corticosteroid therapy to improve bile drainage and clinical outcome in infants with biliary atresia has not been evaluated prospectively to date. However, four retrospective reports suggest that corticosteroids may improve bile drainage and outcome of patients with biliary atresia[46-49]. In an initial clinical report, a short course of high doses of methylprednisolone in infants/children with reduced bile flow at variable lengths of time from the initial portoenterostomy for biliary atresia increased daily bilirubin excretion and decreased serum levels of bilirubin, alkaline phosphatase, and aminotransferases[46-49]. Serum levels of bilirubin and aminotransferases also improved in another group of infants with biliary atresia receiving prednisolone 6-8 days after portoenterostomy[47], but the duration of corticosteroid use was not clearly stated. More recently, two reports described high doses of corticosteroids soon after portoenterostomy followed by a taper over 6-12 weeks in infants with biliary atresia[48,49]. Both reports suggested improved bile drainage and increased survival with native liver in over 70% of patientsubjects.

2.D3. Safety of Corticosteroids in Children with Biliary Atresia

In addition to an overall trend toward biochemical and clinical improvement, the reports discussed above did not list any adverse events in s treated with corticosteroids, or obvious differences in height or weight over the study time, as summarized below (Table 2).

Table 2. Safety Data from Clinical Reports of the Use of Corticosteroids as Treatment of Infants with Biliary Atresia.

TIMING OF TX[@] NUMBER OF SUBJECTS (N)	Drug, dose, and duration	Safety data
Ref. [46] -Revision of PE*; N=7 -Not described; N=7 -Not described; 30 courses	Methylprednisolone 10mg/kg/d; reducing by 50% daily thru 5th day	No adverse events reported No significant differences in body weight
Ref. [47] -6-8 days after PE*; N=13	Prednisolone 4mg/kg/day x 2 weeks, then taper (duration not explicit)	No adverse events reported No significant differences in body weight One death from subdural hematoma One liver transplantation for intractable pruritus
Ref. [48] -After PE*; N=14	Prednisone 4mg/kg/day, then taper over at least 6 weeks	No complications reported One death (unrelated to tx) Cholangitis in 32%
Ref. [49] -At the time of PE*; N=14	Methylprednisolone 10mg/kg/day, tapered to 2mg/kg/day by 7 days, then prednisone at 2mg/kg/day for 8-12 weeks	No adverse events reported

*PE=portoenterostomy
[@]Tx=corticosteroid treatment

Unfortunately, these clinical reports were limited by the use of varying dosing regimens, different indications and timing of treatment, lack of randomization and of an appropriate control group, and inclusion of small cohorts of patients. Moreover, the safety of corticosteroids in this group of patients with biliary atresia was not adequately addressed. Infants treated with corticosteroids may have general side effects, such as hyperglycemia, hypertension, and gastrointestinal bleeding, and also age-specific side effects, such as impaired neurological outcome in preterm infants treated with dexamethasone. Therefore, we will closely monitor for these potential side effects in the clinical trial.

The corticosteroid dexamethasone has been used extensively to treat or prevent chronic lung disease of prematurity. Despite its efficacy minimizing the pulmonary consequences of prematurity, there is a significant concern that dexamethasone treatment of preterm infants may result in impaired neuromotor and cognitive function at school age. For example, long-term neurodevelopmental outcome was evaluated prospectively in a randomized, placebo-

controlled study of 262 preterm infants weighing 500-1999 grams; subjects in the treatment group received 0.25 mg dexamethasone/kg every 12 hours initiated at the onset of respiratory distress syndrome shortly after birth[50]. From 146 children that survived to school age, 72 were treated with dexamethasone and were shown to have lower neuromotor skills, visual integration, IQ scores, and clinically significant disabilities when compared to controls.

At least three factors may contribute to the impaired neurodevelopmental outcome of preterm infants treated with dexamethasone: 1) choice of corticosteroids, 2) patient population, and 3) timing of corticosteroid treatment[51]. Dexamethasone has been associated with an increase in periventricular leukomalacia in preterm infants, whereas betamethasone has not, perhaps based on different pharmacological properties of each type of corticosteroid in the cytoplasm, before binding to the nuclear receptor[52]. Prednisolone, which has 13-20% of the glucocorticoid potency of dexamethasone, was not tested in the same clinical setting.

In regards to the patient population, preterm infants (especially very-low birth weight infants) may be more susceptible to the undesired effects of high doses of dexamethasone in view of the immature state of the central nervous system at the time of birth. This susceptibility may be further amplified by the initiation of dexamethasone treatment in the first 24 hours of life. A delay in steroid treatment to beyond the immediate postnatal period may minimize or prevent the undesired long-term effects of dexamethasone. In a Cochrane meta-analysis of nine clinical trials involving a total of 562 preterm infants treated with dexamethasone after three weeks of age, the incidence of neurosensory disability and the combined rate of death or major neurosensory disability were not significantly different between steroid and control groups[53].

Although the data from preterm infants cannot be automatically applied to the patient population proposed for this clinical trial, we recognize the need to monitor for potential neurodevelopmental effects closely. In addition, we intend to further minimize the occurrence of these potential effects by excluding infants with combined gestational and postnatal age <36 weeks or weighing less than 2000 grams at enrollment. Postnatal age will not be an inclusion or exclusion criteria because the vast majority of infants with biliary atresia are diagnosed after 2 weeks of age. However, in the infrequent event that an infant with the embryonic form of biliary atresia is diagnosed in the first 2 weeks of age, he/she will be eligible for the study, subject to the above age and weight limitations. Although such a subject may be susceptible to adverse neurodevelopmental effects, the inclusion of this population at potential risk is important for us to objectively assess whether corticosteroid poses any significant risk to this age group.

While dexamethasone has not been used in biliary atresia, prednisolone was used in earlier clinical reports as discussed above, and has a shorter half life in plasma (2.1-3.5 hours versus 3-4.5 hours for dexamethasone) and in tissue (18-36 hours versus 36-54 hours for dexamethasone) which may decrease the cumulative exposure time to high levels of glucocorticoid function during the study period (a comparative analysis of the different corticosteroid formulations is shown in Table 3 below)[45]. For this trial, we will use

prednisolone as the corticosteroid formulation, with the intravenous form (i.e. methylprednisolone) to be administered in the immediate postoperative period.

Table 3. Glucocorticoid Comparison (From the website of the American Society of Health System Pharmacists - http://www.ashp.org/)*.

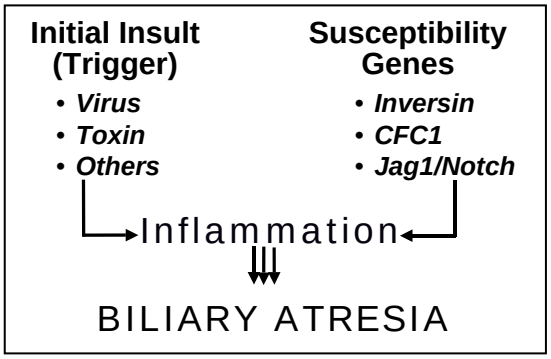
AGENT	Equivalent Dose (mg)	Route of Administration	Relative Anti-inflammatory Potency	Relative Mineralo-corticoid Potency	Biological Half-Life (hours)
Betamethasone[a]	0.6–0.75	IM, PO	20–30	0	36–54
Dexamethasone	0.75	IM, IV, PO	25–30	0	36–54
Hydrocortisone[a,b]	20	IM, IV, PO	1	2	8–12
Methylprednisolone[a]	4	IM, IV, PO	5	0	18–36
Prednisolone	5	PO	4	1	18–36
Prednisone	5	PO	4	1	18–36

*Data on biological half-life for children and adults; values for infants are not yet defined.

[a] Betamethasone suspension for injection, methylprednisolone injection, and hydrocortisone injection are currently in short supply.
[b] Some patients may not be able to tolerate the higher mineralocorticoid activity of hydrocortisone.

2.E. Summary

Biliary atresia is the most common cause of cholestasis in infants and the most frequent indication for pediatric liver transplantation. The disease results from a destructive inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract. Although little is known about the etiology or pathogenesis of biliary atresia, epidemiologic and virologic studies point to a complex trait disorder, in which environmental factors trigger an inflammatory process that recognizes and abnormally targets the biliary system during a specific phase of postnatal development. Based on these data, the following unifying pathogenesis model can be preliminarily proposed:



Biliary atresia results from the interplay of environmental factors and susceptibility genes. An insult by viruses, toxins or other environmental agents in a genetically susceptible subject (e.g.: genetic abnormality in genes regulating laterality or cell fate) may trigger a fibro-inflammatory injury of the extrahepatic bile ducts.

Regardless of initiating (environmental) and modifying (genetic) factors for disease development, the inflammatory and fibrosing destruction of the biliary epithelium is common to all clinical forms of biliary atresia. In this setting, the potential decrease of this inflammatory component by corticosteroid treatment may result in improved bile flow and better outcome after portoenterostomy. Therefore, in this clinical trial we propose to objectively determine whether corticosteroid treatment improves bile flow in infants with biliary atresia. *The significance of the proposed trial is that it will determine whether corticosteroids are an effective medical treatment to improve bile drainage and long-term outcome, and whether its use reduces the need for liver transplantation in infants with biliary atresia.*

The trial will be performed by the NIH-supported Childhood Liver Disease Research and Education Network (ChiLDREN). ChiLDREN has the infrastructure to prospectively follow a sufficiently large number of subjects and to collect samples necessary for clinical research studies addressing etiology, pathogenesis, diagnosis, and treatment of children with biliary atresia.

3. PRELIMINARY DATA

ChiLDREN is a clinical network funded by the NIDDK with goals to: (1) establish a database of clinical information and a repository of blood and tissue samples from children with biliary atresia and other forms of neonatal liver diseases; (2) conduct clinical studies on the etiology, diagnosis and pathogenesis of infants with biliary atresia; and (3) conduct multi-center clinical trials to determine the impact of novel treatment modalities on long-term outcome of affected children. ChiLDREN is governed by a Steering Committee comprised of the principal investigators (PI) from each of the participating clinical centers and the Data Coordinating Center (DCC), and the NIDDK Program Director.

3.A. The ChiLDREN Prospective Database

ChiLDREN has developed a prospective observational database of infants with biliary atresia and other causes of cholestasis in infants. In this prospective protocol, infants with cholestasis who are ≤180 days old are screened and enrolled at presentation to the participating clinical sites. Clinical data collection, laboratory investigations, liver biopsy specimens, and long-term follow-up of these children will be performed in accordance with the normal standard of care for diagnosis and treatment of infants with liver disorders. In order to collect data from all clinical centers uniformly, ChiLDREN has established guidelines for gathering diagnostic, clinical and outcomes data and for collecting specimens, codified to protect subject confidentiality. The data are stored in a secure research database at the DCC and the specimens are shipped to repositories under contract to NIDDK.

Since only infants with biliary atresia can be entered into the proposed clinical trial, we describe the data and specimens collected from subjects with biliary atresia who are enrolled into the prospective database.

3.A1. Recruitment
Following diagnosis of cholestasis in an infant ≤180 days old, the family will be approached for recruitment into the study. Parent(s) or guardian(s) must sign written informed consent before data collection can begin.

3.A2. Baseline
Once informed consent is obtained, the coordinator will abstract information from the subject's medical chart, including the physical examination at intake. The coordinator or delegated designee will interview the parents/guardians to review the child's medical history and the relevant medical history of the immediate family.

3.A3. Surgery/Diagnosis
The timeline for follow-up is triggered by the date of the portoenterostomy for subjects with biliary atresia. The surgeon will complete a case report form that describes in detail the surgical findings. The pathologist will detail the pathological findings that are relevant to the diagnosis.

3.A4. In-patient / Discharge
Data will be collected from the time of surgery to the time of discharge.

3.A5. Follow-up
Subjects with biliary atresia will be followed closely during the first year post-portoenterostomy, at 18 and 24 months of age and then annually to 10 years of age or to the time of transplant or death, whichever comes first.

3.A6. Specimen Collection
At baseline and follow up visits, serum, plasma and urine will be collected for the repository. At the time of a liver biopsy, the portoenterostomy and transplant, specimens from the liver, gall bladder, biliary remnant and lymph node may be collected for the repository when the specimens are clinically indicated to be excised and they are not needed for diagnosis.

3.B. Current Outcome of Children with Biliary Atresia Treated at ChiLDREN Centers

One unique feature that is critical to the implementation of controlled clinical studies is the standardization of surgical and medical approaches, which will enable investigators to determine the impact of novel therapeutic interventions (such as this proposed corticosteroid clinical trial) on clinical outcomes across all participating clinical centers. In preparation for this multicenter trial, the DCC carried out a survey among the ChiLDREN clinical centers to determine the outcome of infants with biliary atresia following portoenterostomy during the periods of 1998-2001. This retrospective survey collected data on serum bilirubin levels at 6 months after portoenterostomy and the number of children requiring liver transplantation or dying from a complication of biliary atresia at or before 24 months of age. In total, portoenterostomies were performed on 118 subjects in the 4-year period. Of these, 46% had good bile flow at 6 months after portoenterostomy, and 51% underwent liver transplantation by 24 months of age.

4. RESEARCH DESIGN AND METHODS

4.A. Overview

The overall hypothesis is that therapy with corticosteroids following portoenterostomy will improve bile drainage and long-term outcome in infants with biliary atresia. This hypothesis will be tested by a multicenter prospective, randomized, double-blinded, placebo-controlled trial. Subjects will be recruited from subjects enrolled in the ChiLDREN prospective observational database study who undergo portoenterostomy for biliary atresia. The investigator or clinical research coordinator will approach the subject's parent(s) or guardian(s) and discuss the study design, benefits and possible risks with the family. After IRB-approved written consent is obtained from the parent or guardian, the clinical research coordinator will:

- Complete the Eligibility Form
- Randomize the subject to 13 weeks of treatment with placebo or corticosteroids. (The research team will be blinded to the treatment. The randomization scheme will be prepared by the DCC and implemented by the research pharmacist.)
- Collect clinical and biochemical data at 2 weeks after portoenterostomy, at 1, 2, 3, and 6 months after portoenterostomy, and at 12, 18, and 24 months of age.

All subjects will receive standard clinical care that is routinely used for all infants with biliary atresia, which will include nutritional support and medications unrelated to this trial. This routine clinical care will not be modified due to participation in this study.

4.B. Study Population

All infants enrolled in the ChiLDREN prospective database study who undergo portoenterostomy or the gall bladder Kasai operation (portocholecystostomy) for biliary atresia will be eligible for the trial. *For purposes of this protocol the term portoenterostomy will include the gall bladder Kasai procedure.* Parents/guardians will be approached about participation in this clinical trial after a decision is made by the attending physician at the ChiLDREN site that the infant will undergo an exploratory laparotomy with possible portoenterostomy, or within 72 hours after portoenterostomy. Biliary atresia is defined as a fibroinflammatory obliteration of the lumen of one or more segments of the extrahepatic biliary tree within the first 3-6 months of life. Rarely, post-operative examination of the excised biliary remnant will demonstrate that a disease process other than biliary atresia caused the biliary obstruction. If this is found in a subject already enrolled in this trial, the study drug/placebo will be discontinued and the subject will be removed from the study.

The total number of subjects to be entered in the study at all clinical sites will be 140.

4.C. Inclusion Criteria

- Portoenterostomy or gall bladder Kasai operation for biliary atresia within the previous 72 hours
- Post-conception age ≥ 36 weeks
- Weight at enrolment ≥ 2000 gm
- Written informed consent to participate in the study obtained prior to or within 72 hours of completion of portoenterostomy. *(Note: Families of potential subjects may be approached prior to the portoenterostomy.)*

4.D. Exclusion Criteria

- Known immunodeficiency
- Diabetes mellitus
- Presence of significant systemic hypertension for age (persistent systolic blood pressure ≥112 mmHg)
- A serum indirect (unconjugated) bilirubin ≥ 5 mg/dL for infants under 4 weeks of age or ≥ 7 mg/dL for infants between 4 and 8 weeks of age
- Known sensitivity to corticosteroids
- Documented bacteremia or other tissue infection which is felt to be clinically relevant
- Known congenital infection or disease with herpes simplex virus, toxoplasmosis, or cytomegalovirus inclusion disease of the liver
- Infants whose mother is known to have human immunodeficiency virus infection
- Infants whose mother is known to be HBsAg or hepatitis C virus positive
- Infants with other severe concurrent illnesses such as neurological, cardiovascular, pulmonary, metabolic, endocrine, and renal disorders that would interfere with the conduct and results of the study
- Any other clinical condition that is a contraindication to the use of corticosteroid (e.g., bowel perforation)
- Infants who have received the live attenuated rotavirus vaccine (e.g. Rotateq) within 5 days prior to proposed administration of study drug.

In the unlikely event that the diagnosis of biliary atresia is established during exploratory laparotomy, but the surgeon did not perform a portoenterostomy (as may occur in cases of advanced cirrhosis), the subject will not be eligible for this study.

4.E. Primary and Secondary Outcome Measures

- Primary outcome measure:
  Percentage of subjects with serum total bilirubin <1.5 mg/dL and with native liver at 6 months after portoenterostomy.

- Secondary outcome measures:
  All measurements will be made at 12 and 24 months of age (unless noted otherwise):
  1. Serum total bilirubin concentration (and also at 3 months after portoenterostomy)
  2. Survival with native liver at 24 months of age

3. Growth
   a. Weight for age Z-score (in subjects without ascites)
   b. Height for age Z score
4. Serum biomarkers of sufficiency of fat-soluble vitamins
   a. Vitamin A: molar ratio of serum retinol/retinol binding protein
   b. Vitamin D: serum level of 25-hydroxy vitamin D
   c. Vitamin E: ratio of serum vitamin E/total lipids
   d. Vitamin K: International Normalized Ratio (INR)
5. Presence of ascites

- Tertiary outcome measures:
  All measurements will be made at 12 and 24 months of age:
  1. International Normalized Ratio (INR)
  2. Serum albumin concentration
  3. Pediatric End-Stage Liver Disease (PELD) score
  4. Platelet count
  5. Serum sodium concentration
  6. Serum bile acid concentrations
  7. Serum concentration of undercarboxylated prothrombin (PIVKA-II)
  8. Head circumference Z-score
  9. Mid-arm circumference and triceps skinfold thickness
  10. Health-related quality of life (HRQOL)
  11. Neurodevelopmental assessment (Bayley score)
  12. Immunologic response to routine childhood vaccination (at 18 months of age)

In addition, we will monitor study subjects for any potential adverse events that may result from the use of corticosteroids. Although no significant complications were reported in the uncontrolled clinical reports of corticosteroids as adjuvant treatment of biliary atresia[46-49], we will be particularly cognizant of the need to carefully monitor the subjects to determine the safety of the proposed treatment.

4.F. Study Design and Intervention

4.F1. Administration of Study Drug or Placebo

Written informed consent will be obtained either after a decision is made by the attending physician at the ChiLDREN site that the infant will undergo an exploratory laparotomy with possible portoenterostomy, or within 72 hours after portoenterostomy. As part of the informed consent, parents/legal guardians will be informed of potential side effects of corticosteroids, as well as of potential adverse consequences of the sudden discontinuation of high doses of corticosteroids (after use for more than 2 weeks). Some of the potential side effects of corticosteroid medications are listed below. This information will be shared with the subject's parents at the time of enrollment and are included in the "ChiLDREN corticosteroid information sheet", which will also be given to the subject's parents. The strategy to carefully monitor for the side effects is outlined in the Section 7.D.

- Hypertension
- Hyperglycemia
- Increased appetite, fluid retention, and weight gain

- Increased crying and decreased sleeping
- Increased risk of infection
- Impaired linear growth
- Cataracts
- Decreased response to immunizations
- Gastrointestinal bleeding
- Pancreatitis
- Hypokalemia
- Impaired wound healing

Randomization will be performed by the DCC and either corticosteroid or placebo therapy will be initiated within 72 hours after portoenterostomy; doses will be adjusted according to the dosing schedule outlined in Table 4. The dose and regimen for corticosteroids were based on prior uncontrolled clinical reports in infants with biliary atresia[46-49]. The choice of the corticosteroid prednisolone is consistent with these reports, and may represent an additional safety feature in view of the lower plasma and biological tissue half-life that is typical for prednisolone when compared to dexamethasone. The decrease in half life of prednisolone may decrease the cumulative exposure to high levels of glucocorticoid function, thus decreasing the potential risk of toxicity to subjects randomized to receive corticosteroids. Corticosteroids or placebo will be given intravenously for at least 2 post-operative days or until the infant resumes oral or enteral feedings. When the infant is tolerating oral or enteral feedings, prednisolone or placebo will be given orally for the remainder of the course of study drug/placebo. Compounding of placebo by a registered pharmacist (see below) will match the corticosteroid product for appearance and taste.

Table 4. Schedule and Dosing of Corticosteroids or Placebo Following Portoenterostomy in Infants with Biliary Atresia.

Day/week of dosing	Corticosteroids*	Placebo
Days 1-7		
days 1-3	Methylprednisolone, IV – 4 mg/kg/day, divided BID	IV – Normal saline (same volume, BID)
days 4-7	Prednisolone, PO – 4 mg/kg/day, divided BID	PO – Placebo (same volume, BID)
Week 2	4 mg/kg/day, divided BID	PO same volume BID
Week 3	2 mg/kg/day, divided BID	PO same volume BID
Week 4	2 mg/kg/day, divided BID	PO same volume BID
Week 5	1 mg/kg/day, once a day	PO once a day
Week 6	1 mg/kg/day, once a day	PO once a day
Week 7	0.8 mg/kg/day, once a day	PO once a day
Week 8	0.6 mg/kg/day, once a day	PO once a day
Week 9	0.4 mg/kg/day, once a day	PO once a day
Week 10	0.2 mg/kg/day, once a day	PO once a day
Week 11	0.1 mg/kg/day, once a day	PO once a day
Week 12-13	0.1 mg/kg/every other day	PO every other day
Week 14	Stop	Stop

*Initial dosage will be based on subject's weight. Subsequent doses will be adjusted based on subject's weight measured monthly at each scheduled outpatient visit.

Infants who have received the live attenuated rotavirus vaccine (e.g. Rotateq) within 5 days prior to proposed administration of study drug are excluded from the study. Infants who have received the vaccine prior to this interval should not receive their first dose of steroids until a total of 5 days post vaccination has elapsed.

4.F2. Dose Reduction

In the event that a subject has a potential expected or unanticipated side effect of the study drug/placebo (such as irritability) but there is no indication to stop the study drug/placebo, the clinical site PI will have the option to reduce the dose of the study drug/placebo by 50%. The subject will then be monitored for improvement in these symptoms. If the symptoms improve within 48 hours, the subject will be maintained at the reduced dose for the remainder of the days planned for the original dose, as well as for the duration of treatment with this dose according to the normal taper schedule (Table 4). If symptoms do not improve/resolve, a further reduction by 50% of the new dose will be considered by the clinical site PI. Any reduction in the dosage of study drug/placebo will be reported to the DCC by the clinical site PI, along with the notification of the adverse event. If the symptoms persist beyond 48 hours and the clinical site PI judges it necessary that the study drug/placebo be discontinued, the tapering and discontinuation protocol outlined below will be followed.

4.F3. Tapering and Discontinuation of Study Drug or Placebo in the Event of a Serious Adverse Event (SAE) or Early Withdrawal

Blinded taper: If a SAE (see definition in Section 7.B3) is documented and thought by the clinical site PI to be possibly related to the study drug/placebo or if the subject withdraws from the study before the completion of the study drug/placebo, the clinical site PI will initiate a blinded taper of the study drug/placebo following the schedule outlined in Table 5 (below). The taper will be implemented in a blinded fashion in order to maintain objectivity in subject care and data collection during follow-up visits for the duration of the study.

Table 5. Tapering Protocol for Discontinuation of Study Drug or Placebo

Duration of treatment	Plan
Week 1	No need to taper; discontinue study drug or placebo
Weeks 2-8	Decrease dose to 0.6 mg/kg/day study drug/placebo orally x 7 days Then decrease the dose by 50% x 7 days and stop
Weeks 9-10	Decrease the dose by 50% for 7 days and stop
Weeks 11-13	Stop without taper

Unblinded taper: In the event of a life-threatening complication and when the physician treating the subject determines that knowing whether the subject was taking corticosteroid is essential to implement his/her treatment plan (e.g., need for stress dose of steroid in the setting of bacteremia and hypotension or invasive tissue sepsis with systemic symptoms), the study code will be broken to reveal if the subject was receiving placebo or corticosteroids. [The Safety Monitor and the DCC will be informed immediately by the filing of a SAE report.] In this setting, if the subject had been receiving corticosteroids, the tapering protocol will use a stress dose of hydrocortisone (50-62.5 mg/m^2/24h) for 2-3 days, then physiologic replacement (20-25 mg/m^2/24h) for 7 days, then half of the replacement dose for 7 days; the use of hydrocortisone instead of prednisolone or methylprednisolone in the setting of infection will minimize additional immunosuppression that would result from the glucocorticoid action of prednisolone during the taper schedule. Alternatively, replacement doses may be in the form of prednisolone (using the doses outlined for weeks 2-8 of Table 5) if the subject tolerates oral/enteral feedings.

4.G. Other Perioperative and Medical Management

All subjects with biliary atresia will undergo standard surgical portoenterostomy following the technical guidelines agreed upon by the ChiLDREN Surgical Committee (see Appendix). Postoperatively, subjects will receive intravenous medication to suppress gastric acid production (H_2 blocker or proton pump inhibitor, according to the local standard of care) until they resume oral/enteric feedings. Once they resume oral/enteric feedings, they will receive 12.5 mg BID oral ranitidine (2-6 mg/kg/day, see Section 4.M.7) during administration of study drug/placebo; ranitidine is approved by the FDA for use in children (1 month-16 years of age).

All subjects will receive antibiotics intravenously for at least 2 days postoperative or until they are able to tolerate oral/enteric feedings. Intravenous antibiotics are routinely used postoperatively at ChiLDREN centers as prophylaxis against ascending cholangitis. The choice of antibiotics will be according to the local standard of care. Once oral/enteric feedings are tolerated, oral trimethoprim-sulfamethoxazole (TMP/SMZ, 4-5 mg TMP/kg/day) will be initiated and continued for 6 months. In the event of the occurrence of an episode of cholangitis despite antimicrobial prophylaxis, subjects will be treated with parenteral antibiotics according to the standard of care at the clinical center where the subject is enrolled. Thereafter, prophylaxis with oral TMP/SMZ will be used for 6 months. In addition to serving as prophylaxis against ascending cholangitis for all subjects[54], this regimen will also provide prophylaxis for pneumocystis carinii pneumonia (PCP) in subjects receiving corticosteroids. In the unlikely event that the subject develops a hypersensitivity reaction to TMP/SMZ manifested as a skin rash (see section 7.D8), the medication will be discontinued promptly and the subject will undergo a blinded taper and discontinuation of the study drug/placebo as outlined in Section 4.F3.

4.H. Follow-up Study Visits for this Clinical Trial

Following portoenterostomy and discharge from the hospital, subjects will be evaluated in the outpatient clinic 2 weeks after portoenterostomy , at 1, 2, 3, and 6 months after portoenterostomy, and then at 12, 18, and 24 months of age. The inclusion of the 2-week time point will allow for careful monitoring for side effects of corticosteroid treatment. During each outpatient visit, information will be recorded on ChiLDREN Case Report Forms (CRFs), which are the same forms used in the ChiLDREN prospective observational database protocol, and on additional forms that collect information that is specific for this trial. These forms will capture data on the subject's physical findings, diet and medication records, laboratory findings, interval medical history, interval sentinel events, illnesses or hospital admissions, surgery, imaging follow-up, pathology, HRQOL, and antibody titers to vaccines according to the schedule in Table 6 (below).

Table 6. Schedule of Evaluations for the Trial of Corticosteroid Therapy in Infants with Biliary Atresia.

Evaluation	Recruitment or Baseline	Diagnosis/ Surgery/ Discharge	2 wk post	1,2,3 & 6 m post Kasai	12 m age	18 m age	24 m age	At transplant
Windows for visits			±1 wk	±2wks; ±4 wks for 6 m	±1 mo	±2 mo	±2 mo	
Inf. Consent & eligibility	X							
Intake/Medical History	X		X	X	X	X	X	X
Diagnosis and surgery		X						
Discharge Assessment		X						
Medication Record	X	X	X	X	X	X	X	X
Physical Exam, Growth Measures	X	X	X	X	X	X	X	X
Blood tests -Complete blood count -LFTs, PT/INR -Electrolytes, creatinine, BUN, glucose	X	X	X	X	X	X	X	X
Blood tests -Retinol, RBP, 25OH-VitD -Vitamin E, total lipids -Bile acid, PIVKA-II				X*	X	X**	X	
Interval Medical History			X	X	X	X	X	X
Interval Sentinel Events			X	X	X	X	X	X
Neurodevelopmental Assessment (Bayley)					X		X	
Health-Related Quality of Life Inventory (HRQOL)					X		X	
Ophthalmological exam					X			
Antibody titers to vaccines						X		

*Will not be done at the 2 month follow-up, unless a dose adjustment was performed at the 4 week follow-up.
**Will be done only if infant still requires AquADEK® to maintain adequate serum levels of fat-soluble vitamins.

4.I. Surgical procedures and Laboratory Tests

Results of surgical procedure(s) and laboratory tests performed for the initial diagnosis of biliary atresia will be obtained from the medical records. These tests will include hematologic and biochemical analysis (such as complete blood count, liver function tests, prothrombin time/INR, urinalysis, etc), imaging (such as ultrasound examination and hepatobiliary scan), and results of hepatobiliary pathology. Results for tests done at the time of hospital discharge following portoenterostomy will also be obtained from the medical records. Following discharge, the following laboratory tests will be obtained in all subjects, at the time points shown in Table 6, in order to assess study outcomes: liver function tests, complete blood count, vitamin levels and prothrombin time. Serum electrolytes and glucose will also be measured to monitor for potential adverse effects of corticosteroid treatment. These laboratory tests will be performed using standard automated assays in certified clinical laboratories located at each ChiLDREN clinical center.

4.J. Preparation and Administration of Study Medication and Placebo

4.J1. Preparation, Packaging, and Labeling

The central research pharmacy located at Cincinnati Children's Hospital Medical Center will acquire the study drug or placebo, prepare and code packages for treatment and control groups, and supply each ChiLDREN site research pharmacy with coded packages to be used for each subject. The DCC will provide each clinical site research pharmacist with a set of randomization numbers that will be assigned sequentially; each randomization number will identify a coded package to be used for the subject. The package will contain the study drug/placebo, supplies, and an information sheet for the subject's family. The central research pharmacist will also provide information support, inventory control, and drug accountability checks for all clinical site research pharmacies. Study medication/placebo will be dispensed by the clinical site research pharmacist every month. At each monthly follow-up visit, dosing will be adjusted by the site research pharmacist according to the infant's weight. Any changes in dosage will be communicated to the DCC on the CRFs, and to the family by the research coordinator.

4.J2. Intravenous Formulation

A standard intravenous formulation of methylprednisolone (Solu-Medrol®) will be used in the trial. The central study pharmacy will obtain methylprednisolone for the study, and provide it to the clinical site pharmacies for use at the time of subject enrollment. Placebo (normal saline) or methylprednisolone will be prepared by the clinical site research pharmacist according to the randomization schedule provided by the DCC.

4.J3. Oral Formulation

We will use an oral formulation of prednisolone (15 mg/5 mL). The choice of prednisolone preparation is based on its commercial availability, concentration of the formulation, and the demonstrated ability of the research pharmacist to formulate a placebo that matches the medication for color and taste. Liquid forms of prednisolone and placebo will be

provided to the clinical site research pharmacies by the central research pharmacist, while the randomization schedule will be provided by the DCC.

4.J4. Other Medications

- After discharge from the hospital, the investigator will prescribe fat-soluble vitamins and other medications routinely used in the post-operative care of children with biliary atresia. (see sections 4.M4-M7 below). In addition to the masked study drug, the following medications will be dispensed as needed per protocol free of charge by the clinical site research pharmacy:
- AquADEK® and vitamin K (Mephyton®) (up to two years)
- Ursodeoxycholic acid (Urso 250® or Actigall®) (up to two years)
- TMP/SMZ (up to six months)
- Ranitidine (Zantac®) (up to three months while the masked study medication is being prescribed)

4.K. Adherence to protocol and withdrawal from the study

4.K1. Adherence to Protocol

Every effort will be made by study personnel to ensure adherence to the protocol by study subjects and their families. This includes enrollment of all eligible subjects, prompt randomization to the study drug or placebo, and initiation of data collection in a timely manner. All interactions with the study subjects and their caregivers will be performed by the same personnel utilizing supportive and positive reinforcement communication skills, and will include direct feedback regarding amount of unused medication. Compliance will be assessed by the site research coordinator by reviewing the medication bottles. To this end, parents will be asked to bring the previously dispensed study drug/placebo to each follow-up visit so that compliance can be estimated by the difference between the actual and the expected volumes remaining in the drug/placebo container.

4.K2. Subject Withdrawal from Study

It is the right of the subject's parents, or guardians, or the primary care providers caring for each subject to withdraw the subject from the study and wean (taper) the study drug/placebo at any time during the study. Subjects will be removed from the study for any of the following reasons:

- Withdrawal of consent.
- Subject is lost to follow-up. If a subject fails to appear for scheduled appointments, the research coordinator will call the parents/guardians by phone. If the family does not respond or cannot be located, a certified letter will be mailed to the parents/guardians asking them to immediately contact the clinical center.

Any subject who withdraws from the study will undergo the drug/placebo taper protocol described in Section 4.F3, and will receive standard medical care.

If the attrition rate is greater than 20%, the sample size may be adjusted.

4.K3. Discontinuation of Study Drug/Placebo

Study drug/placebo will be discontinued (see Section 4.F3), however subjects will continue to be followed for the duration of this study, if:

- The investigator believes it is no longer in the best interest of the subject to remain in the study.
- There is a serious adverse event, as outlined in the Data and Safety Monitoring Plan (Section 7).

Any subject who is withdrawn from the study drug/placebo will undergo the drug/placebo taper protocol described in Section 4.F3, and will receive standard medical care.

All subjects will continue to be followed per protocol even if the study drug/placebo is tapered and/or discontinued.

4.L. Retention of Participants

Special effort will be made to keep attrition of study subjects below 20% using the following methods:

- Some of the costs associated with provision of care (such as laboratory tests) will be covered by this study. Thus, we expect minimal attrition due to financial constraints.
- The study coordinator will provide assistance to the parents/legal guardians of study participants, such as scheduling of appointments. Thus, we would make an effort to minimize attrition due to logistical considerations.

4.M. Routine Clinical Care Guidelines for ChiLDREN

Routine clinical care guidelines were established for infants enrolled in the ChiLDREN prospective database and will be followed in this clinical trial. These guidelines were developed based on a consensus of the ChiLDREN clinical investigators in regard to optimal medical and nutritional management and the frequency of follow-up visits for infants with biliary atresia.

4.M1. Frequency of Medical Encounters/Visits

- Enrollment and initial evaluation
- Discharge from the hospital
- 2 weeks postoperative, 1, 2, 3 and 6 months post-operative, and 12, 18 and 24 months of age. The visit at 12 months of age may be combined with the 6-month postoperative visit when the latter is at 10 months of age or greater (which can happen if the portoenterostomy is performed at 4 months of age or later)
- Annually thereafter

4.M2. Physical Exam

At each medical encounter/visit, the following will be obtained:

- Height or recumbent length, weight, head circumference, triceps skinfold thickness, mid-arm circumference.
- Vital signs.
- Skin examination for jaundice, xanthomas, palmar erythema, and vascular pattern.
- Abdomen examination to determine liver size and texture, spleen size, ascites.
- Examination of extremities for edema and clubbing.

4.M3. Diet

When the total bilirubin is ≥1.5 mg/dL and the child is less than 12 months of age, MCT-containing formula or breast milk should be used, as long as the child's growth is "adequate". When the total bilirubin is <1.5 mg/dL, the child can be transitioned to standard infant formula (if <1year of age) and whole milk (if ≥1 year of age). MCT-containing formula will be continued until 24 months of age if the total bilirubin is ≥1.5 mg/dL and the child is over 12 months of age. When growth is inadequate, measures will be taken for nutritional rehabilitation according to medical management used at each ChiLDREN clinical center.

4.M4. Vitamin Supplementation

All cholestatic subjects with total bilirubin ≥1.5 mg/dL will receive:

- AquADEK® vitamin drops: 2 ml orally per day until 2 years of age
- Vitamin K : 2.5 mg orally coadministered with AquADEK® vitamin drops on Mondays, Wednesdays and Fridays

AquADEK® and Vitamin K may be stopped when the total bilirubin is <1.5 mg/dL. Serum vitamin levels and prothrombin time/INR will be measured during the follow-up visit at one month after portoenterostomy. When an abnormal value is obtained, the dosage of the specific vitamin will be augmented or reduced, as appropriate, and the level will be rechecked in 4 weeks (see Section 4N, below).

4.M5. Ursodeoxycholic Acid

- Ursodeoxycholic acid (Urso 250® or Actigall®): 20 mg/kg/day divided BID orally until 2 years of age.

Ursodeoxycholic acid will be discontinued if serum total bilirubin is >15 mg/dL to avoid potential toxicity.

4.M6. Antibiotics for Prophylaxis Against Ascending Cholangitis

- TMP/SMZ: 4-5 mg TMP/kg/day orally for 6 months

If the subject has evidence of decreased renal function as indicated by an elevated serum level of creatinine, we will adjust the dose of TMP/SMZ for renal insufficiency. For serum creatinine > 1.0 to 1.5 mg/dL, the dose will be reduced by 25%; for creatinine > 1.5 to 2.5 mg/dL, the dose will be reduced by 50%; and for serum creatinine > 2.5, the subject will be withdrawn from the study. These adjustments are based on a normal serum creatinine of <0.6 mg/dL in the first year of life.

In the unlikely event that the subject develops a hypersensitivity reaction to TMP/SMZ, the medication will be discontinued promptly and oral neomycin (25/mg/kg twice a day orally) will be used for prophylaxis against ascending cholangitis. Because neomycin provides no prophylaxis against PCP, the subject will also undergo a blinded taper and discontinuation of the study drug/placebo as outlined in Section 4.F3. Our choice to discontinue the study drug/placebo is based on our concerns that the subjects would be

exposed to additional risks that would derive from adding another antimicrobial for PCP prophylaxis.

4.M7. Ranitidine

- Ranitidine (Zantac®): 12.5 mg orally twice daily (2-6 mg/kg/day) during administration of study drug/placebo
- If Zantac tablets are not available due to supply shortage from the manufacturer, Zantac liquid can be dispensed.

Because of the association between corticosteroid use and gastritis/peptic ulcer, all study subjects will receive 12.5 mg orally twice daily (2-6 mg/kg/day) throughout the duration of the administration of study drug/placebo. Monthly supplies of Ranitidine will be dispensed by the research pharmacist to the study subject's parent/guardian.

4.M8. Routine Childhood Immunizations

It is expected that routine primary immunizations will be given to all children by their primary care provider as recommended by the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP), except that immunizations will not be given for the first 4 weeks after portoenterostomy (a period of time when the corticosteroid dose is 2-4 mg/kg/day). The normal immunization schedule will then be resumed, with immunizations to be given prior to one year of age being delayed by up to 4 weeks. If there is a need to catch-up with routine immunization schedule, it is anticipated that the catch-up schedule recommended by the Committee on Infectious Diseases of the AAP will be used by the primary care provider. Additional vaccines that are recommended for children with chronic liver disease are Hepatitis A and annual influenza vaccine, and will be administered as recommended by AAP guidelines.

4.M9. Steroid Pulses

The use of a steroid pulse (4-5 days of IV steroids at any time following the portoenterostomy) during this trial will be treated as a protocol violation.

4.N. Corticosteroid Treatment and Absorption of Fat-Soluble Vitamins

Infants with biliary atresia who have cholestasis are at risk for significant nutritional problems. Impaired bile acid secretion and bile flow resulting in decreased concentrations of bile acids in the intestinal lumen and ineffective micellar concentration cause malabsorption of dietary fat and fat-soluble vitamins. Therefore, standard clinical practice is to supplement infants with biliary atresia and cholestasis with vitamins A,D,E,K to prevent vitamin deficiencies. Although corticosteroid therapy has not been shown to directly modify absorption of fat-soluble vitamins, this treatment may improve vitamin absorption indirectly through its potential effect of improving bile flow. Since efficient bile flow is critical for the proper absorption of fat-soluble vitamins, it is likely that by improving bile flow, corticosteroids may increase absorption of fat-soluble vitamins. Therefore, it is important to carefully assess the impact that improved bile flow may have on the routine

supplementation of fat-soluble vitamins in infants with biliary atresia. In addition, fat-soluble vitamin nutriture should reflect overall fat absorption and be a marker for improved fat absorption if corticosteroid therapy is successful.

In view of the lack of published controlled studies assessing dosing regimens in this subject population and the potential impact of corticosteroid treatment on improving bile flow, we will determine prospectively whether oral supplementation with commonly used doses of fat-soluble vitamins achieves fat-soluble vitamin sufficiency. Since the solubility and absorption differs for each of the fat-soluble vitamins, each vitamin will be analyzed separately for sufficiency after supplementation.

4.N1. Malabsorption of Fat and Fat-Soluble Vitamins:

Fat-soluble vitamins are absorbed when dietary fat, which consists primarily of long chain triglycerides, is absorbed. Absorption of triglycerides requires hydrolysis within the intestinal lumen and micellar solubilization for transport across the enterocyte where triglycerides are reformed. Triglycerides are then transported into lymphatics from the intestinal cell in chylomicrons. In the intestinal lumen, pancreatic enzymes and bile acids are critical for intralumenal digestion. Pancreatic enzymes break down triglycerides into free fatty acids and glycerol by emulsification and hydrolysis. Solubilization of the free fatty acids for transport across the brush border membrane of the cell is accomplished by the formation of micelles. Bile acids from the liver are essential for the formation of micelles and a critical micellar concentration is necessary for solubilizing fat into micelles. When bile acids are not present or are reduced in amount within the intestinal lumen, micellar formation is disrupted and steatorrhea and fat-soluble vitamin malabsorption occur.

4.N2. Vitamin A

Vitamin A consists of a group of compounds that are essential for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system[55]. Clinical signs of vitamin A deficiency are primarily ocular, but also include loss of appetite, increased risk for infection, and epithelial changes. In foods of animal origin, vitamin A is present mainly as retinyl ester. In the intestine, retinyl ester is hydrolyzed and then associated with bile salt containing mixed micelles. In fat malabsorption, retinol absorption is impaired and deficient serum and hepatic vitamin A concentrations have been reported in infants with cholestasis. In 19 biliary atresia subjects who had been prescribed 5000 IU of oral vitamin A daily after portoenterostomy, the plasma vitamin A levels were significantly lower in a jaundiced group compared to a non-jaundiced group[56]. An investigation of hepatic vitamin A concentrations in infants less than 6 months revealed deficient hepatic vitamin A levels in 14/17 cholestatic infants but not in 3 non-cholestatic infants[57]. This study reported a poor correlation between hepatic and serum vitamin A levels except when serum retinol levels were less than 10 ug/dL. Because hepatic vitamin A levels are not feasible in clinical practice, a non-invasive method of monitoring for vitamin A deficiency has been proposed. Since retinol is released from the liver in combination with retinol binding protein (RBP) in a 1:1 molar ratio and circulating RBP-bound retinol is the form transported to tissues, it has been proposed that

measuring the molar ratio of retinol:RBP would be a non-invasive method of assessing hepatic vitamin A stores. In a group of subjects with cholestasis, the molar ratio of retinol:RBP was 0.62 $\pm$ 0.15 in vitamin A-deficient subjects and 1.04 $\pm$ 0.06 in vitamin A-repleted cholestatic subjects[58].

There are toxic manifestations of excessive vitamin A intake. Hepatotoxicity, bone abnormalities, dermatitis, and increased intracranial pressure can occur[55]. For these reasons, careful monitoring during vitamin A supplementation is necessary.

4.N3. Vitamin D

Vitamin D is essential for mineral homeostasis and proper formation of bones[55]. Deficiency of vitamin D is characterized by inadequate mineralization of bone and can present as rickets or osteomalacia. In addition, because the vitamin stimulates intestinal absorption of calcium and phosphorus, abnormalities in mineral homeostasis can occur. Vitamin D consists of several forms including vitamin D_2, vitamin D_3, 25 hydroxy-vitamin D, and 1,25 dihydroxy-vitamin D. Exposure of the skin to ultraviolet rays of the sun catalyzes the synthesis of vitamin D_3 from 7-dehydrocholesterol. Vitamin D_2 is synthesized from ergosterol in plants and is added to vitamin D supplemented milk. Vitamin D absorption occurs in the small intestine where it is then incorporated into chylomicrons and absorbed primarily via the lymphatics while a smaller amount is absorbed directly into the portal circulation. The liver catalyzes the hydroxylation of vitamin D_3 to 25 OH-vitamin D. In the kidney, 25-OH vitamin D is converted to 1,25 dihydroxy-vitamin D, which is the biologically active form of the vitamin.

There are many studies indicating that vitamin D malabsorption occurs in cholestasis[59-62]. In a study with a small number of biliary atresia infants, baseline serum 25-OH vitamin D was deficient in 5/6 biliary atresia subjects after portoenterostomy with poor bile flow compared to 1/5 similar subjects with good bile flow. The peak change and area under the absorption curve for serum 25-OH vitamin D after administration of a one- time oral dose of 10 ug/kg 25-OH vitamin D3 was significantly lower in all 11 biliary atresia subjects compared to 5 pediatric controls[62]. The 25-OH vitamin D levels in 4 infants with biliary atresia did not increase after supplementation with 2000 IU by mouth of vitamin D_2 for 2 weeks compared to 7 infants with neonatal hepatitis or 9 normal controls[60]. In an oral 25-OH vitamin D_3 tolerance test, the mean increase in serum 25-OH vitamin D for 5 non-jaundiced biliary atresia subjects after portoenterostomy was significantly greater (48.9 $\pm$ 30.6 ng/ml versus 23.7 $\pm$ 9.5 ng/ ml) in jaundiced subjects[63]. The absorption of vitamin D_3 1000 IU/kg was enhanced in 8 cholestatic children when given with the water miscible form of vitamin E (TPGS, 25 IU/kg) compared to when vitamin D was given alone[64].

Vitamin D nutriture is assessed by serum concentration of 25-OH vitamin D and monitoring of 25 OH-vitamin D during supplementation is necessary. The effects of vitamin D toxicity include hypercalcemia and hypercalciuria leading to renal and cardiovascular damage[55].

4.N4. Vitamin E

Vitamin E refers to a group of naturally occurring compounds that include the members of the tocopherol family and tocotrienols. Tocopherols are the most important and there are four major forms, alpha, beta, gamma, and delta. The most abundant form and the one with the highest biologic activity is alpha-tocopherol. Tocopherols are antioxidants that prevent propagation of unsaturated fatty acid oxidation by trapping peroxyl free radicals[55]. Tocopherol is found in cellular membranes associated with polyunsaturated fatty acids (PUFA) in phospholipids. In vitamin E deficiency, the oxidation of PUFA occurs more readily along the cell membrane leading to cellular damage and neurological symptoms. Chronic vitamin E deficiency is associated with progressive neuromuscular syndrome that can cause cerebellar ataxia, areflexia and peripheral neuropathy[55].

The intestinal absorption of alpha-tocopherol as assessed by an oral tolerance test was found to be significantly impaired in cholestatic infants who had clinical evidence of neurologic dysfunction[65]. Low intraluminal bile acid concentrations in severe cholestasis results in malabsorption and deficiency of vitamin E since solubilization by bile acids into micelles before absorption is critical for proper absorption. Eighteen out of 37 children with cholestasis due to biliary atresia who had undergone a portoenterostomy had low serum vitamin E levels and two had hypoactive deep tendon reflexes and ataxia. Oral supplements in doses of 5 to 10 mg/kg/day were needed to maintain normal serum vitamin levels in postoperative infants[66]. Vitamin E malabsorption was much greater than vitamin D malabsorption, although both were impaired, in a study of intestinal absorption during cholestasis[61]. Vitamin E is the most hydrophobic of all the fat-soluble vitamins and therefore its absorption is greatly affected by inadequate intraluminal bile acids. The use of a solubilized form of vitamin E (TPGS) has been studied to determine its safety and efficacy in children with cholestasis. In a multi-center trial a dose of 20-25 IU/kg/day of TPGS for treatment of children with cholestasis who were vitamin E deficient and unresponsive to other forms of oral vitamin E was reportedly a safe and effective form of vitamin E for preventing or reversing vitamin E deficiency[67].

Vitamin E deficiency can occur with normal serum vitamin E levels in children with cholestasis[68], and the ratio of serum vitamin E to total serum lipids is necessary to accurately assess vitamin E status. Vitamin E can partition into plasma lipoproteins and therefore in the presence of elevated lipids (as commonly seen in biliary atresia), a normal serum vitamin E concentration may be reported, yet the subject can have profound vitamin E deficiency manifested by characteristic neurological abnormalities. A serum vitamin E to total lipid ratio of less than 0.6 mg/g in children under 1 year of age and a ratio of less than 0.8 mg/g for older children indicates biochemical vitamin E deficiency[68].

Excessively high serum vitamin E levels should be avoided as it may lead to vitamin K-deficient coagulopathy[69].

4.N5. Vitamin K

Vitamin K exists in three forms, phylloquinone (vitamin K_1), menaquinone (vitamin K_2), and the chemically synthesized form, menadione (vitamin K_3). Vitamin K is essential in

the posttranslational carboxylation of glutamic acid to gamma-carboxyglutamyl residues in factors II (prothrombin), VII, IX, and X and proteins C and S. The major sign of vitamin K deficiency is coagulopathy. The dietary form of vitamin K is vitamin K_1 and as with the other fat soluble vitamins, vitamin K absorption requires the normal flow of bile into the intestinal lumen. Malabsorption of vitamin K in cholestasis as well as antibiotic use that impairs intestinal bacterial production (vitamin K_2), can lead to vitamin K deficiency. Vitamin K status is measured clinically by prothrombin time (PT) which is an index of vitamin K dependent clotting factors. Vitamin K deficiency is suggested when the PT is prolonged yet the partial thromboplastin time (PTT) is within normal range. A diagnosis of vitamin K malabsorption and deficiency is established when the prothrombin time normalizes after parenteral vitamin K supplementation. Subclinical vitamin K deficiency states have been identified by measuring serum undercarboxylated prothrombin (PIVKA-II). This protein is considered a sensitive functional indicator of vitamin K status[70]. Since the liver synthesizes some coagulation factors, coagulopathies in cholestasis can be due to vitamin K deficiency, poor liver synthetic function, or a combination of both.

Toxic manifestations of vitamin K supplementation have not been observed[55].

4.N6. Fat-soluble Vitamin Supplementation

The fat-soluble vitamin requirements in infants with cholestasis remain unclear. The findings in most studies on fat-soluble vitamin absorption have been limited by small groups of infants. Furthermore, the requirements may vary depending on the degree of cholestasis and the primary disease. We propose to closely assess the efficacy of a formulation of fat-soluble vitamins commonly used in clinical practice by following levels of fat-soluble vitamins in both corticosteroid-treated and placebo groups. The preparation contains vitamin E in the water miscible form (TPGS), which has also been shown to improve the absorption of vitamin D[64] and theoretically enhances the absorption of the other fat-soluble vitamins. We will use the dose of 2 milliliters of vitamin solution as the starting dose for this study. This dose is based on higher requirements for infants with cholestasis than the Recommended Daily Allowances (RDA) (see Table 7 below). In addition, based on clinical experience in dosing fat-soluble vitamins in this population, additional vitamin K will be necessary, and will consist of 2.5 mg by mouth to be given three times a week. In addition to vitamins A, D, E and K, the AquADEK® vitamin formulation includes water-soluble vitamins and minerals. There is a paucity of information regarding water-soluble vitamin status of infants with cholestasis. Data from adult patients with chronic liver disease suggested that B vitamin deficiency may exist in patients with cholestasis[71]. In addition, low plasma zinc levels have been reported in infants awaiting liver transplantation[72]; an adequate zinc status is necessary for the synthesis and secretion of RBP.

Table 7. The Pediatric Liquid Formulation of AquADEK® and the RDA for Infants.

Vitamin	RDA	2 ml AquADEK®	Amount x RDA
	0-0.5 yr 0.5-1yr		0-0.5 yr 0.5-1 yr
Vitamin A (palmitate and beta carotene)	375 ug 400 ug	*1902 ug	5.1 x 4.9 x
Vitamin D (cholecalciferol)	7.5 ug 10 ug	*20 ug	2.7 x 2.0 x
Vitamin E (TPGS)	3 mg 4 mg	*54 mg	18 x 13.5 x
Vitamin K (phytonadione)	5 ug 10 ug	200 ug	40 x 20.0 x
Vitamin C (ascorbic acid)	30 mg 35 mg	90 mg	3.0 x 2.6 x
Vitamin	**RDA**	**2 ml AquADEK®**	**Amount x RDA**
	0-0.5 yr 0.5-1yr		0-0.5 yr 0.5-1 yr
Thiamine (Vitamin B_1)	0.3 mg 0.4 mg	1 mg	3.3 x 2.5 x
Riboflavin (Vitamin B_2)	0.4 mg 0.5 mg	1.2 mg	3.0 x 2.4 x
Niacin (niacinamide)	5 mg 6 mg	12 mg	2.4 x 2.0 x
Vitamin B_6 (pyridoxine)	0.3 mg 0.6 mg	1.2 mg	4.0 x 2.0 x
Vitamin B_{12}	0.3 ug 0.4 ug	8 ug	26.7 x 20.0 x

4.N7. Composition of Vitamin Supplementation

The vitamin supplement will provide vitamins A, D, E and K in amounts predicted to achieve adequate serum concentrations of each vitamin with the exception of vitamin K. The liquid AquADEK® preparation was chosen because of the feasibility of parents/guardians giving the subject one formulation instead of multiple individual vitamins. In addition, the form of vitamin E is in a water miscible form that is thought to have improved absorption in cholestatic conditions. This water miscible preparation has also been shown to enhance the absorption of other fat-soluble vitamins when given simultaneously. The vitamin supplementation will consist of 2 ml of AquADEK®, which contain the following:

- 6340 IU vitamin A (palmitate and beta carotene)
- 800 IU vitamin D (cholecalciferol)
- 80 IU vitamin E (TPGS)
- 0.2 mg vitamin K (phytonadione)

Clinical experience in supplementing infants with biliary atresia with AquADEK® has led to the recognition that additional vitamin K is needed. In addition, we will prescribe 2.5 mg of vitamin K orally (as a crushed tablet), three times per week.

4.N8. Administration of Vitamins

The families will be given a supply of AquADEK® vitamins. They will be given instructions on the amount of vitamins to administer daily. The study will provide all cholestatic subjects with total bilirubin ≥1.5 mg/dL vitamin K tablets. The families will be given instructions to give 2.5 mg by mouth three times a week as part of standard care for infants with Cholestasis. They will be advised to give the vitamins altogether and not

added to a large volume of formula. If the infant spits out the entire dose, half the dose will be given again. If the infant refuses the vitamin preparation or repeatedly spits it out, then the vitamins could be given in a small volume of formula (e.g. 15-30 ml) to help ensure full ingestion of the prescribed amount. If the infant continues to refuse the vitamin preparation or repeatedly spits it out, parents/guardians will be asked to fill prescriptions for individual vitamins (A, D, E, and K; equivalent doses of AquADEK®); the individual vitamins are to be paid by them or by third party payers. The family will be instructed to not use any other vitamin preparations. The families will be asked to return bottles of the vitamin preparation and amount of solution remaining in the bottle will be recorded. In addition, the number of vitamin K tablets remaining will be recorded. A new supply of vitamins will be dispensed to provide enough supplementation until the next scheduled visit. Blood will be obtained at the study visits before the daily dose of vitamins is given and preferably at least 4 hours after feeding. The study coordinator will call the family prior to the visit to remind them to not give the morning dose of vitamin supplements and that we would like to obtain blood when the infant has not had formula or food for approximately 4 hours.

4.N9. Monitoring of Vitamin Levels

Vitamin status will be assessed 1 month after entry into the study and then at the 3 and 6 month follow-up visits and at 12, 18, and 24 months of age while the subject is receiving vitamin supplementation. If a subject no longer receives the vitamin supplementation because the total bilirubin concentration is < 1.5 mg/dl, the vitamin concentrations will be checked at all the above times except at 18 months of age. Blood will be obtained at the study visits before the daily dose of vitamins is given. Why coordinate with feeding 4 hours after feeding. The study coordinator will call the family prior to the visit to remind them to not give the morning dose of vitamin supplements and that we would like to obtain blood when the infant has not had formula or food for approximately 4 hours.

Two central laboratories will be measuring vitamin levels for this study. A sample will be sent to the Pediatric CTRC Core Laboratory at The Children's Hospital in Denver which will perform all the tests except the PIVKA-II. The samples that are sent to Denver will be identified with the following personal health information: child's name, medical record number, and date of birth. The child's unique study code will not be shared. It is necessary for this information to be collected and shared because these are clinically indicated tests that will be reported to the site. These tests will be paid by the study.

PIVKA-II samples will be barccoded and sent to the NIDDK Repository . The NIDDK Repository will then batch ship these samples to Cincinnati Children's Hospital Medical Center for processing. The NIDDK Repository will verify which samples were sent to Cincinnati, and Cincinnati will send the results to the DCC. PIVKA-II is not being used clinically by the sites and these results will not be reported back to the site. These tests will be paid by the study.

Target vitamin levels:

- Vitamin A: A molar ratio of retinol:RBP will be used to assess vitamin A nutriture. A cut-off of 0.8 mol/mol will be used to define vitamin A deficiency. If a retinol:RBP ratio is below 0.8 and serum retinol is less than 20 ug/dl, the subject will be considered vitamin A deficient and supplementation will be recommended (see below). If the serum retinol is <20 ug/dl and the retinol:RBP ratio is >0.8, vitamin A status will be reassessed in one month. If the serum retinol is > 20 ug/dl and the ratio is < 0.8, vitamin A status will be reassessed in one month. If the serum retinol is greater than the upper limit of normal range, the vitamin A dose will be decreased by 50% and vitamin A status reassessed in one month.
- Vitamin D: The target serum 25-OH vitamin D concentration range is 15-45 ng/ml.
- Vitamin E: The target serum vitamin E:lipid ratio will be >0.6 mg/g in children less than 12 months of age and >0.8 mg/g in children older than 12 months of age. The upper limit of vitamin E:total lipids ratio will be 3.5 mg/g.
- Vitamin K: The INR will be used as surrogate marker of vitamin K status with a target INR<1.2

Target vitamin concentration within normal range: When vitamin concentrations are within target range no changes will be made and vitamin levels will be retested at the 3 and 6 month follow-up visit and at 12, 18 and 24 months of age while a subject is receiving vitamin supplementation.

Target vitamin concentrations below normal range: When a vitamin result is below target range, supplementation of the specific vitamin(s) that is deficient will be prescribed and re-checked in one month (see Section 4.N9 for supplementation plan). If the vitamin level is within the target range there will be no further adjustments. When the vitamin level is still below target range, additional supplementation will be given as outlined below (Section 4.N9).

Target vitamin concentrations above normal range: When a subject is receiving the standard vitamin supplementation and one or more vitamin concentrations is above target range, the vitamin supplementation may be reduced to 1 ml per day and the vitamins that were within target range will be supplemented individually to have the same concentration as in the formulation of 2 ml of AquADEK®. All vitamins will be rechecked within one month and adjustments made as outlined in the protocol.

4.N10.Adjustment of Individual Vitamin Doses

When individual vitamins are found to be deficient, the vitamin will be prescribed, whenever possible, at the amount described below:

- Vitamin A: When a subject is vitamin A deficient, liquid vitamin A (intravenous preparation) may be given at 5000 IU by mouth with the AquADEK® each day and vitamin A status rechecked within one month. Doses orally of up to 25,000 – 50,000 IU vitamin A per day can be given for 1-4 weeks but will require weekly monitoring of vitamin status to prevent toxicity. Alternatively, when a subject is vitamin A deficient, 50,000 IU IM may be given once a month for two months. Vitamin A status is rechecked monthly with this regimen.

- Vitamin D: If a subject is vitamin D deficient, cholecalciferol or ergocalciferol will be given at 1200-4000 IU by mouth with the liquid formulation each day. Vitamin D status will be reassessed in one month. If the subject remains vitamin D deficient, the oral dose will be increased to 4000-8000 IU by mouth to be given each day. Alternatively, 1,25 OH_2 D may be prescribed at a dose of 0.05 – 0.2 μg/kg/day. Vitamin D status will be reassessed in one month. If 1,25 OH_2D is administered, then serum 1,25 OH_2D levels need to be monitored by the clinical site physician. If the serum concentration of the appropriate vitamin D metabolite is still deficient, the vitamin D dose will be increased to 12,000 IU by mouth and vitamin D status reassessed in one month. If the 25 OH-vitamin D level still remains below normal, the clinical site physician will decide to prescribe either 1,25 OH_2 vitamin D supplementation or give intramuscular vitamin D and monitor as necessary. If 25-OH vitamin D blood levels exceed 60 ng/ml, then serum calcium and phosphate should be checked, and the vitamin D dose should be reduced by 50% and levels repeated in 2 to 4 weeks.

- Vitamin E: If a subject is vitamin E deficient, additional TPGS will be prescribed at 25 IU/kg/day and vitamin E status will be reassessed in one month. If the subject remains deficient, 50 IU/kg/day of TPGS will be prescribed and the vitamin E status will be reassessed in one month. Rarely will a subject not respond to this dose of TPGS. However, if unresponsive, TPGS dose can be increased up to 100 IU/kg/day given in 2 or 3 divided doses to prevent diarrhea, with monitoring of vitamin E status in 1 month.

- Vitamin K: If a subject is vitamin K deficient with an INR > 1.2 but $\leq$1.5, 2.5 mg of vitamin K by mouth every day will be prescribed. The PT/INR will be rechecked within 7-10 days. If the INR is rechecked and it is not less than target ratio of <1.2, 5 mg vitamin K by mouth every day will be prescribed. The PT/INR will be rechecked within 7 days-10 days.

  If the INR is > 1.5 and $\leq$1.8, 5 mg vitamin K by mouth every day will be prescribed and/or 2-5 mg vitamin K may be administered intramuscularly and the PT/INR will be rechecked within 7-10 days. If there is any clinically significant bleeding the use of parenteral vitamin K and FFP will be decided by the site PI according to local standard of care.

  When the INR is > 1.8, 2-5 mg vitamin K may be administered intramuscularly and/or 5 mg of vitamin K by mouth daily may be prescribed at the discretion of the clinical site physician. If there is any clinically significant bleeding, the use of parenteral vitamin K and FFP will be decided by the site PI according to local standard of care. The PT/INR will be rechecked within 7-10 days and again 1 month later. Additional doses of parenteral vitamin K will be given at the discretion of the clinical site physician, as clinically indicated.

When the subject is vitamin K deficient with any abnormal INR listed above, a repeat PT/INR is obtained 7-10 days after vitamin K supplementation. PIVKA-II levels will be obtained at the same time as the PT/INR.

4.N11. Monitoring of Serum Levels of Bile Acids

The intraluminal concentration of bile acids plays an important role in regulating absorption of fat-soluble vitamins. In biliary atresia, the excretion of bile acids into the duodenum is significantly impaired by variable degrees of biliary flow. This is associated with a rise in serum levels of bile acids. Therefore, we will measure serum bile acids prospectively in all study subjects. Serum levels will be used in future analysis to determine their correlation with serum vitamin levels, and whether they have predictive value in vitamin nutriture status.

4.O. Potential impact of Biliary Atresia and Corticosteroids on Growth, Neurodevelopmental Outcome, and Immune Function

Progression of liver disease in infants with biliary atresia after portoenterostomy often leads to malnutrition and growth failure. These subjects may also have recurrent infections, such as ascending cholangitis, suboptimal immunologic response to routine childhood immunization, and impaired neurodevelopmental outcome. Because some of these undesirable outcomes may also be associated with prolonged corticosteroid use in children without biliary atresia, we will carefully monitor them prospectively in all infants receiving study drug or placebo. The data will be analyzed to determine whether corticosteroid treatment improves (decreases) or exacerbates (increases) their incidence when compared to placebo-treated infants.

4.O1. Growth

Anthropometric measurements will be obtained at the time of hospital discharge and at follow-up visits (Table 6) throughout the duration of the study. Body weight will be determined with an electronic scale accurate to 0.1 kg. Length and head circumference will be obtained in triplicate using a measuring tape and reported in centimeters. The average of three results will be used for analysis. Weight, length, and head circumference will be compared with the CDC 2000 growth charts[73], and Z-scores will be derived.

Short-term impairment in linear growth may occur in subjects randomized to receive corticosteroid treatment; however, long-term growth impairment is not anticipated. It is also possible that infants treated with corticosteroids will have better liver function and actually improve growth. Given the degree of variability in growth in each infant with biliary atresia in the control group (infants without corticosteroid treatment), it is not possible a priori to define a threshold for an adverse event related to growth failure.

4.O2. Neurodevelopmental Outcome

There are no prospective, case-controlled studies assessing long-term neurodevelopmental outcome in children with biliary atresia. Suboptimal neurodevelopment, however, may result as a consequence of the chronic, debilitating disease that results from progressive liver dysfunction and recurrent hospitalizations.

Impaired neurodevelopmental outcome has also been reported in association with the use of corticosteroids in clinical trials of premature neonates with lung disease (discussed in Section 2.D3). In regard to infants with biliary atresia randomized to receive corticosteroid, it is possible that an improved liver function in these infants may improve long-term outcome and health-related quality of life, which may actually result in improved neurodevelopment. Therefore, to carefully monitor the long-term neurodevelopmental outcome, we will assess development by the Bayley Test for all subjects at 1 and 2 years of age. Testing will be performed by an appropriately trained child psychologist at each ChiLDREN clinical site. Since the effect of cholestasis and ongoing liver disease on neurodevelopment during the first two years of life has not been well characterized, data from this study will be used to determine both the effects of chronic liver disease and corticosteroid treatment on neurodevelopment. Since subjects participating in this clinical trial will also participate in the ChiLDREN prospective database study, they will also undergo neurodevelopmental testing yearly from 3-6 years, then at 8 and 10 years of age. This testing will enable additional scrutiny of potential long-term adverse effects in subjects receiving corticosteroids.

4.O3. Compromised Immune Function

Subjects with biliary atresia have increased incidence of ascending cholangitis, bacteremia, and spontaneous bacterial peritonitis. The immunologic response to routine childhood immunizations may also be variable, with failure to reach adequate antibody geometric mean titers to vaccine agents in some subjects[74-76]. Recognizing that treatment with corticosteroids also places infants at risk of compromised immune function, we will monitor all subjects participating in this study for clinically significant immunologic dysfunction (Table 8).

Table 8. Summary of Monitoring Strategy to Detect Potential Compromise in Immune Function.

Impaired responses to childhood vaccines	Assess antibody titers to vaccine antigens at 18 months of age. Recommend to the primary health care provider to administer booster vaccine if serum titer is less than the minimal protective titer. No live viral vaccines should be administered until 3 months after completion of study drug/placebo
Development of infectious diseases and vaccine-preventable diseases	All emergency department visits and hospitalizations will be reported as an adverse event. If the subject is hospitalized with lower respiratory tract symptoms of unknown etiology, nasal wash will be obtained for pertussis PCR. If S. *pneumoniae* is isolated from the blood or a sterile body site (such as cerebrospinal fluid), isolates will be serotyped.
Development of septicemia and opportunistic infections	All events with bacteremia, fungemia, or other opportunistic infections will be reported as an adverse event.

Impaired response to childhood vaccines
Subjects will receive all routine childhood vaccines according to the schedule recommended by the American Academy of Pediatrics (AAP), with the exception of up to a 4-week delay of vaccines that would have been given during the first 4 weeks of the study drug/placebo. Vaccinations will be administered by the subject's primary care provider. At 18 months of age or at the time of liver transplantation (if younger than 18 months of age), the vaccination records of each subject will be obtained from the primary care provider for review. The number and size of doses, dates of administration, and vaccine manufacturer will be recorded. At the age of 18 months, 6-9 ml of blood will be obtained to measure serum antibody titers against individual vaccine antigens. The antibody titers will be measured by the study sites local laboratory. If a subject has received a transplant before the 18th month visit, titers should not be collected. Priority titers to be collected relate to vaccinations for Hepatitis B, Tetanus, and Polio. If serum antibody titers do not achieve protective levels as determined by the local laboratory standard and the investigator, the information will be provided to the primary care provider so that booster/re-immunization of appropriate antigen(s) is given. Absent titers will also be communicated to the primary care provider, who will treat with re-immunization as directed by the AAP guidelines for catch-up immunization.

Development of infectious diseases and vaccine-preventable diseases
Subjects will be prospectively monitored for the development of infectious diseases and vaccine-preventable diseases. Treatment with corticosteroids often induces a leukocytosis (with or without mild bandemia). This leukocytosis is usually benign and expected to decrease as the dose of corticosteroid decreases. If leukocytosis is associated with any other signs or symptoms that may indicate infection (e.g.: fever, lethargy, poor feeding), the primary care provider will further evaluate and treat as clinically indicated, according to local standard of care.

Parents and primary care providers of all enrolled subjects will be instructed to contact the study coordinator promptly at the time of any infectious acute illness that leads to visit to the primary care provider, to an emergency department, or if the subject requires hospitalization. An Adverse Event Form will be completed within 24 hrs of each report. If a child is hospitalized because of a respiratory tract illness of unknown etiology, microbiology studies will be pursued according to the local standard of care, but an additional nasal aspirate will be obtained for rapid screen for *B. pertussis*. If *S. pneumoniae* is isolated from a sterile body site (e.g.: blood, cerebrospinal fluid) or lower respiratory tract by bronchial lavage, the isolate will be serotyped to determine if it is a vaccine-related strain. These diagnostic studies will be performed, when possible, by the admitting hospital's clinical microbiology laboratory; alternatively, samples may be performed by an outside laboratory, such as Quest Diagnostic Laboratories.

Development of septicemia and opportunistic infections

Subjects will be monitored for the development of infections or serious opportunistic infections (bacterial, viral, fungal). Evaluation of the subject with a febrile illness will proceed as per the local standard of care and antimicrobials will be initiated accordingly. completed. If the infection is associated with hypotension, acidosis, or tissue necrosis, a Serious Adverse Event Form will be completed. The decision to break the study medication code and institute stress dose replacement with corticosteroid for subjects in the corticosteroid arm of the study will be made by the clinical center PI based on the clinical status of the subject. If a serious opportunistic infection is diagnosed, appropriate antimicrobial therapy will be initiated and a Serious Adverse Event Form will be completed. If the subject is still receiving study drug/placebo, the drug/placebo will be tapered and discontinued according to the protocol outlined in Table 5.

4.P. Research Versus Standard Clinical Care

One important feature of this clinical trial is that subjects will receive standard clinical care at ChiLDREN centers in addition to the care related to enrollment in the trial. As outlined in Table 9, participation in this trial will specifically require: 1) randomization to receive either corticosteroids or placebo, 2) treatment with ranitidine during the duration of study drug/placebo, 3) outpatient visit at 2 weeks after portoenterostomy, 4) serum concentration of electrolytes, glucose, bile acids, and PIVKA-II, 5) developmental assessment, 6) HRQOL inventory, and 7) evaluation of serum antibody titers to vaccines. All other outpatient visits and laboratory studies are routinely performed as part of standard clinical care of infants with biliary atresia, as summarized in Table 9 below.

Table 9. Outline of Care Related to this Clinical Trial Versus Standard Clinical Care

Health Care Item	Collected by*
Medical encounters/visits	
• 2 weeks after portoenterostomy	T
• 1,2,3,6 months after portoenterostomy	DB,CC
• 12,18,24 months after portoenterostomy	DB,CC
Standard diet and nutritional support	DB,CC
Vitamin supplementation	T
Ursodeoxycholic acid	T
Antibiotic prophylaxis for ascending cholangitis	DB,CC
Administration of study drug/placebo	T
Administration of ranitidine	T
Childhood immunizations	CC
Laboratory monitoring	
• Complete blood count	DB,CC
• LFTs, PT/INR	DB,CC
• Serum retinol, RBP, 25OH-Vitamin D	CC,T
• Serum vitamin E, total lipid ratio	CC,T
• Serum bile acids, PIVKA-II	T
• Serum electrolytes, glucose	T
• Antibody titer against routine immunizations	T
Neurodevelopmental assessment	DB
HRQOL	DB

* T=Collected by this clinical Trial;
DB=Collected as part of ChiLDREN prospective Data Base; informed consent obtained to share the information with the clinical trial.
CC=Collected as part of standard Clinical Care.

5. STATISTICAL ANALYSIS AND DATA MANAGEMENT

5.A. Sample Size

5.A1. Primary Endpoint

The primary endpoint (Hypothesis 1a) of this clinical trial is whether or not the subject has a serum total bilirubin <1.5 mg/dL with his/her native liver at 6 months after portoenterostomy. To determine the sample size for the clinical trial, we conducted a retrospective study of the level of serum total bilirubin and transplant-free survival in children with biliary atresia treated with portoenterostomy at the 14 ChiLDREN pediatric liver care centers. From the study, 54 of 118 infants (46%) had good bile drainage at 6 months, as determined by a serum total bilirubin level <1 mg/dL. (The results were tabulated at both 1 mg/dL and 2 mg/dL and the results were similar.) Our goal in this trial will be to improve the rate of good bile drainage at 6 months post-surgery from 50% to

75%. Using East-2000® (Cytel Software Company, Cambridge), a total of 110 subjects would need to be randomized to the two treatment groups in order to have 80% power to identify this difference at a 2-tailed 5% level of significance. To allow interim looks at the data after 50% and 75% of the subjects complete the trial using the O'Brien-Fleming spending function, the number of subjects that would be necessary is 114. To compensate for up to 20% attrition; we increased the sample size to a total of 140 subjects; e.g., 70 per group. Attrition will be defined as withdrawal by parents or guardians (which includes subjects who are lost to follow up] occurring within the first 6 months following portoenterostomy (prior to the time of evaluation of the primary outcome measure); however, data from all randomized subjects will be included in an intent-to-treat analysis.

5.A2. Secondary Endpoints

Hypothesis 1b is that improved bile drainage, as determined by serum total bilirubin with native liver, will be improved in the infants treated with corticosteroids compared to those treated with placebo. Serum total bilirubin concentrations are measured at each clinical visit, including 3 and 6 months after portoenterostomy, and at 12, 18 and 24 months of age. (The time point of 6 months after portoenterostomy is the primary endpoint). A serum total bilirubin value >1.5 mg/dL, transplant or death, is indicative of failure of the portoenterostomy. The time to the first value >1.5 mg/dL (when the value is initially >1.5 and then declines to ≤1.5, the time to the first value >1.5 mg/dL after the decline will be used), to transplant or to death will be considered time to failure of the procedure. The two groups will then be compared by a Cox regression model fitted to the time to failure. Since the test of the primary endpoint is a special case of this analysis, there is greater power for this comparison than for the primary endpoint.

Hypothesis 2 is that survival with native liver at 24 months of age is improved in infants treated with corticosteroids compared to those treated with placebo. In the 118 subjects in our survey of the ChiLDREN centers, 60 (51%) were transplanted by 24 months of age. Similar to the primary endpoint, we will have 80% power to show a 50% improvement in this measure (lowering the transplant rate from 50% to 25%).

Hypothesis 3 is that growth will be improved in infants treated with corticosteroids compared to those treated with placebo. These secondary endpoints, weight for age Z-score (in subjects without ascites) and height for age Z-score, are continuous. Children at the time of portoenterostomy are expected to have normal or near normal growth (mean Z-score near zero). Changes in weight and height Z-scores will be compared between treatment groups. For these endpoints there will be 80% power to identify a difference of 0.55 standard deviations (SD) between groups when 110 subjects are randomized. Since a mean Z-score of -1.0 or less would represent impaired growth and a mean > -0.5 would represent satisfactory growth, there will be almost 80% power to differentiate between these two outcomes.

We do not have data to present power calculations for the other secondary endpoints (sufficiency of fat-soluble vitamins and incidence of ascites). We do not anticipate sufficient deaths by age 2 to compare survival between treatment groups.

5.A3. Tertiary Endpoints

Tertiary endpoints are those that may be affected by improved liver function. The hypothesis is that infants treated with corticosteroids will have improved liver function at 12 and 24 months of age, which will result in lower INR, higher serum albumin, lower PELD score, higher platelet count, higher serum sodium concentration, lower serum bile acid concentration, lower PIVKA-II, higher mid-arm circumference and triceps skinfold thickness, better HRQOL, improved neurodevelopmental outcome, and adequate immunologic response to childhood immunizations. However, the study is not powered to detect differences in these variables. Therefore, analyses of these data will serve to generate specific hypothesis for future studies.

5.A.4. Interim Analysis

There are both short-term (e.g., bilirubin at 6 months post-portoenterostomy) and long-term outcomes (e.g., survival with a native liver and developmental assessments at 12 and 24 months of age). Although there is a relationship between bilirubin at 6 months and survival with a native liver, the intervention may impact these endpoints differentially and, especially, may impact development and growth in a manner independent of the level of bilirubin at 6 months. Therefore, the Steering Committee recommends that the trial should not be terminated early based on a test for either efficacy or futility. The Steering Committee recognizes that the DSMB will monitor adverse events and may recommend ending the study early if there is a significant imbalance in adverse events (risk to the participants) between the two treatment arms.

5.B. Data Analysis

General

Prior to unmasking the study and the start of any formal analyses, the data for each variable will be examined to identify unusual values that need to be queried, patterns of missing values and whether their distributions are non-Gaussian (i.e., have significant skewness and kurtosis). These analyses are, in general, performed conditional on one or more covariates known to affect the variable being studied; for example, values may be examined conditioned on age of the infant or age at time of portoenterostomy. Data from variables with non-Gaussian distributions will be transformed, if necessary, before building statistical models. Missing data patterns will be examined and, when necessary for the data analysis, missing data may be imputed by appropriate methods depending on the pattern and mechanism of the missing data. Baseline variables will be compared between groups. If a baseline variable is identified as significantly different between groups and it may affect the outcome measure, the analysis of the outcome measure will be repeated with this baseline variable included as a covariate. All models will include clinical site as a random effect.

Aim 1: To determine whether corticosteroid therapy decreases serum bilirubin concentration after portoenterostomy.

Hypothesis 1a: The probability of an infant having good bile drainage at 6 month after portoenterostomy (as defined by a serum total bilirubin level <1.5 ml/dL) will be greater in subjects treated with corticosteroids than in those treated with placebo.

This is the primary hypothesis of this study, upon which the sample size was calculated. A generalized linear model (e.g., a logistic regression model) with random effects will be used to test this hypothesis. The dependent variable is dichotomous: whether the infant has good bile drainage or not; transplantation or death is considered a failure in all analyses. The covariates that will be included in the model are the treatment group, baseline total bilirubin level, age of the infant at portoenterostomy, complications before the surgery, gender and race of the infant, presence of major congenital anomalies, as well as site as a random effect. The test of the primary hypothesis is a test of whether the coefficient of treatment group is zero.

Hypothesis 1b: Improved bile drainage (as defined by a serum total bilirubin level <1.5 mg/dL) will remain for a longer period in infants treated with corticosteroids than in those treated with placebo.

Using the same definition of good bile drainage as in the primary hypothesis, the duration of time with good bile drainage (time to onset of poor bile drainage, transplant or death, whichever comes first) will be modeled by Cox proportional hazards model using the same model as above. If there is a statistically significant difference, the profiles of the bilirubin for the two treatment groups will be compared and 95% confidence limits will be computed.

Aim 2: To determine whether corticosteroid treatment after portoenterostomy will improve outcome as defined by survival without transplantation at 24 months of age.

Hypothesis 2: Survival without liver transplantation will be greater at 24 months of age in infants treated with corticosteroids than in those treated with placebo.

A Cox proportional hazards model will be fitted to the time to transplant or death. The same covariates will be included in the model as for the previous hypothesis.

Aim 3: To determine whether corticosteroid treatment after portoenterostomy will improve growth of infants with biliary atresia.

Hypothesis 3: Weight and height Z-scores at 12 and 24 months of age will be higher in the infants treated with corticosteroids than in those treated with placebo.

The weight and height Z-scores at 12 and 24 months of age will each be modeled by a random effects model with the same covariates as above.

Aim 4: To determine whether corticosteroid treatment improves biochemical indicators of each of the fat-soluble vitamins after supplementation with standard doses.

Hypothesis 4a: Treatment with corticosteroids leads to improved bile drainage (hypothesis 1) which, in turn, increases the probability that an infant will achieve vitamin sufficiency of each of the fat-soluble vitamins following supplementation of AquADEK®.

Each vitamin will be analyzed separately. For hypothesis 4a, each subject will be classified as having achieved or not achieved vitamin sufficiency, first with supplementation and then without supplementation. Vitamin sufficiency will be defined as having achieved the target concentration ranges specified in Section 4.N8. Although we can model the outcome as a function of treatment group, the underlying hypothesis is that the outcome is a function of bilirubin level. Therefore, we will model the outcome by logistic regression using bilirubin as a surrogate for bile drainage; bilirubin may be included in the model either as a continuous measure or as a dichotomy where <1.5 mg/dL is defined as good bile flow and ≥1.5 mg/dL as inadequate bile flow. Gender and race of the infant, presence of major congenital anomalies, as well as site as a random effect will be included as covariates.

Hypothesis 4b: Treatment with corticosteroids leads to improved bile drainage (hypothesis 1) which, in turn, reduces the dose required for supplementation and the length of time of supplementation for each of the fat-soluble vitamins.

As in hypothesis 4a, each vitamin will be analyzed separately. There are two components to this hypothesis. The first is the total amount of supplementation and the second is the length of supplementation. We will compute the total amount of supplementation up to 6 months of age as the first dependent variable and the time to achieving target concentrations without supplementation as the second dependent variable. The first variable (after transformation) will be analyzed by a random effects model with bilirubin as the independent variable and the same covariates as in hypothesis 4a. The second variable will be modeled by Cox regression with the same independent variable and covariates.

Aim 5: To determine whether corticosteroid treatment after portoenterostomy will decrease the incidence of persistent ascites or ascites that requires medical treatment.

Hypothesis 5: The incidence of ascites will be decreased at 12 and 24 months of age in infants treated with corticosteroids than in those treated with placebo.

Similar to hypothesis 2, a Cox proportional hazards model will be fitted to the time to diagnosis with ascites.

Other endpoints will be analyzed similarly depending on whether they are continuous, dichotomous or time-to-event.

5.C. Data Management

5.C1. Case Report Forms

The Biometrics and Outcomes Research Core in the Department of Biostatistics at the University of Michigan will serve as the data coordinating center (DCC) and is responsible for data management and analysis. The DCC maintains a password-protected website for the ChiLDREN study. All transmissions to and from the website are encrypted using SSL. In addition to providing access to the study database, the website contains the protocol, manual of operations for ChiLDREN, current versions of the case report forms that are used at the time of subject enrollment and at follow-up visits, and other information related to the study. The case report forms do not contain any personal subject identifiers, except dates, such as date of birth, which are necessary for research purposes. Either the DCC will provide the case report forms or the study coordinator will print blank case report forms from the website. An investigator and/or study coordinator can only view data in the study database from his/her clinical site.
The case report forms are completed and countersigned by the investigator or study coordinator at each study visit. On the case report forms, each subject is identified by a subject identification number that consists of two-digit site number and a five-digit identification number. The last digit of the number is a check digit that is used to identify errors in transcription of the identification number. No other personal identification (except dates) is included on case report forms that are submitted to the DCC.

The original case report forms should be securely maintained in locked file drawers at the clinical study sites. Clean completed copies of the case report forms are sent monthly to the DCC where they are entered into the study database using double entry (entry and verification). Other forms are entered by the coordinator directly into the study website; these forms are also sent monthly to the DCC and entered a second time for verification.

5.C2. Quality Assurance

The DCCProject Manager/Clinical Monitor and/or Data Manager will review data submitted to the DCC for accuracy and completeness. The DCC Data Manager and/or Project Manager/Clinical Monitor will transmit to the study coordinators at each site any queries generated by the DCC and address all questions and concerns regarding the study protocol and problems with data entry. Site visits by the Project Manager/Clinical Monitor will be made annually to review case report forms for this study and for the ChiLDREN prospective database study. During these visits the Project Manager/Clinical Monitor will review the regulatory file, check all informed consent documents, and review study procedures with the study coordinators. Interim site visits may be made to centers with low compliance or high error rates. Performance reports will be generated quarterly to investigators and study coordinators at each center, as well as to Data Safety Monitoring Board (DSMB).

5.C3. Training

The DCC has developed general and project-specific manuals of operations to assist investigators and study coordinators at each center in following the ChiLDREN-approved projects, clinical trial protocols, entering and transferring data, and collecting, processing and shipping samples. The Project Manager/Clinical Monitor will be responsible for training the study coordinators at each center about this study protocol, the completion of source documents, and the use of the web-based entry system. The Project Manager/Clinical Monitor will also review with the central research pharmacist the proper procedures for shipping samples to the site research pharmacies. Similar training sessions of study coordinators have been held for two ChiLDREN retrospective studies and for the ChiLDREN prospective database study during face-to-face meetings, which were held in parallel with meetings of the ChiLDREN Steering Committee, as well as through conference calls. Test runs of data entry into the web-based data entry system will occur prior to site initiation. The Project Manager/Clinical Monitor will review the study protocol and data entry system, and check all regulatory documents prior to site initiation. In-service training for all study coordinators will be held quarterly via conference calls to review frequently encountered questions regarding the protocol, data entry, or sample processing.

6. HUMAN SUBJECTS RESEARCH CONSIDERATIONS

6.A. Risks to Study Subjects

6.A1. Involvement of Subjects

Subjects will be recruited into this study at the time that biliary atresia is suspected and exploratory surgery is scheduled or within 72 hours following portoenterostomy. Subjects will be randomized to treatment with corticosteroids or placebo treatment for 13 weeks and followed as outpatients until age 24 months.

6.A2. Planned Duration of the Entire Study

Based on the anticipated enrollment of 40-50 subjects/year by all ChiLDREN centers, we estimate completing enrollment within 3-4 years, with an additional 6 months to gather follow-up data on subjects enrolled in the final year. However, the testing of secondary outcome measures (such as survival with native liver at 24 months of age) and the careful monitoring of potential long-term adverse effects of corticosteroids (such as administration of developmental tests to screen for impaired neurodevelopmental outcome at 1 and 2 years of age) will extend the overall duration of the study to 6 years.

6.A3. Duration of Participation for Each Subject

Each subject will remain in the clinical trial until 24 months of age; i.e, for between 18 and 24 months. The use of corticosteroids will be limited to 13 weeks. This will be followed by very close follow-up in outpatient clinics until 12 months of age, followed by outpatient visits at 18 and 24 months of age. Subjects will be seen clinically as needed (by either their primary care provider or by a gastroenterologist at the study site) for evaluation and management of medical problems that may arise because of the natural progression of liver disease in affected children.

6.A4. Sources of Research Material

Subjects will be recruited from the pool of infants with jaundice referred to or evaluated by the outpatient and inpatient services at the ChiLDREN clinical centers. Research material will include clinical and laboratory data, physical findings, and data collected as part of the prospective observational database.

6.A5. Potential Risks to Subjects

There are two potential sets of risks associated with this clinical trial. The first set derives from the time of blood draws, and includes amount of blood, as well as pain, bruising, or superficial phlebitis. Most of the scheduled venipunctures, except that at the two-week follow up visit, are part of routine tests necessary for the proper care of infants following portoenterostomy; serum levels of electrolytes and glucose will be included to monitor for possible adverse effects of corticosteroid treatment. Only the blood draw at the two-week follow up visit to monitor for side effects is added by this study.

6.A5a. Amount of Blood

Blood is drawn as part of the routine clinical care of the subject, to be sent the repositories as part of the prospective database, and to monitor for vitamin levels and adverse events in this clinical trial. The cumulative amount of blood to be drawn is outlined in Tables 10 and 11 below.

Blood drawn for the ChiLDREN Prospective Observational Database:

Table 10. Amount of blood drawn from infants *to be sent to the repositories as part of the prospective observational database.*

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
4 weeks post op or post diagnosis	4	8	8
3 months post op or post diagnosis	4	8	12*
6 months post op or post diagnosis	4**	8 or 9.2	8 or 9.2
12 months of age	9.2	9.2	9.2
18 months of age	4	4	4
Annually from age 2	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.
** When the 6 month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.

NOTE: Blood volume for clinically indicated tests: Approximately, 6.5 ml of blood may be removed from the child at each visit to evaluate hepatic function, electrolytes and differential. More may be withdrawn to perform additional clinically indicated lab tests.

Additional blood drawn to monitor for vitamin levels and adverse events for this clinical trial:

At each follow up visit, 1 ml of blood will be drawn to monitor serum electrolytes and glucose which may not be drawn as part of standard clinical care.

At follow-up visits (4 weeks post surgery, 3 and 6 months post, and at 12 mo, 18 mo, and 24 mo of age, 6-9 ml of blood will be drawn to monitor vitamin levels, total bile acids and PIVKA-II.

*Note: If the infant is not receiving vitamin supplementation at the 18 mo of age visit, no additional blood will be drawn for vitamin monitoring. If changes in the vitamin supplementation plan is required, additional testing will be done outside of the visits listed below.

Table 11. Total Amount of Blood Drawn from Infants *in the Clinical Trial (including that for the prospective observational database).*

Visit	Amount in ml drawn for research at the visit	Maximum research blood draw in ml within 2-month period	Maximum research blood draw in ml within 3-month period
Initial	4	4	4
2 weeks post op	1	5	5
4 weeks post op	8	13	13
2 months post op	1	14	14
3 months post op	8	17	22*
6 months post op	8**	8 or 13.2	13.2 or 14.2
12 months of age	13.2	13.2	13.2
18 months of age	8 or 11***	8 or 11	8 or 11
24 months of age	8	8	8
Annually from age 3	4	4	4

* This would only happen if the 3-month post-op or post-diagnosis visit was scheduled before the 3-month anniversary of the operation or diagnosis.
** When the 6 month post-op or post-diagnosis visit is at 10 months of age or greater, the blood draw at that visit will be that for 12 months of age and there will be no blood draw at 12 months of age.
***At 18 months of age, 6-9 ml of blood will be drawn to assess immunization antibody titer levels; ~~research~~ blood for vitamin levels, total bile acids and PIVKA-II (3 ml) will be drawn only if the infant is receiving vitamin supplementation at this visit.

The IRB guidance at the University of Michigan (site of the Data Coordinating Center) for the amount of blood that may be removed from an infant:

1. In human subjects in good health, and a hematocrit of not less than 38%, the following volumes of blood may be removed over a 3-month period, on a single occasion or in divided portions:

Body Weight (lb) - Blood Volume (ml)	
0-10	20
11-20	30
21-30	50

2. Additional volumes of blood may be removed in subsequent 3-month periods using the same criteria, as long as the subject remains in good health and maintains a hematocrit level of not less than 38 volume%.

3. If a subject may not be in good health, and in particular may have a cardiovascular, pulmonary or hematopoietic problem, the volume of blood to be removed should be reduced appropriately.

6.A5b. Side Effects

The second set of potential risks derive from reported side effects of corticosteroids, which include hypertension, hyperglycemia, hypokalemia, impaired wound healing, gastrointestinal bleeding, pancreatitis, and irritability. Despite these potential adverse events, this clinical trial is justified by the devastating nature of biliary atresia on the well-being of affected children and on the progression to end-stage liver disease in most subjects. In this context, corticosteroid treatment may result in improved bile flow, allow for better growth, and long-term survival with the native liver. To minimize potential adverse events to subjects, the investigators will monitor closely for side effects in all subjects, and promptly adhere to the action plan outlined in the Data Safety Monitoring Plan (DSMP) if side effects are identified.

Subjects will have a potential risk of adrenal insufficiency if administration of corticosteroids is suddenly discontinued after 2 weeks of treatment. This potential adverse effect will be reviewed at enrollment and at discharge from the hospital with the subject's parents or guardians. In subjects needing discontinuation of study drug/placebo for medical reasons (such as in the setting of a serious adverse event) or exit from the trial due to parent's request, a tapering and discontinuation protocol (see Section 4.F3) will be used to prevent/minimize occurrence of adrenal insufficiency.

Although fat-soluble vitamin supplementation is recommended for cholestatic infants with biliary atresia, there is a potential risk of toxicity from supplementation. Careful monitoring of vitamin concentrations/ratios is planned to reduce this risk. In addition, whenever a vitamin dose adjustment is made, follow-up testing will be performed. If there is a major or rapid change in the severity of cholestasis (e.g., rapidly decreasing serum bilirubin level), vitamin levels will be monitored more frequently.

6.A6. Alternative Treatments Considered

Corticosteroid treatment is being considered in this study as an adjuvant treatment for children with biliary atresia. At present, other adjuvant therapies may be under development, such as anti-oxidant cocktails, anti-inflammatory agents that target specific components of the immune system, and others. However, we are not aware of any studies actively enrolling subjects at this time. Preventive approaches may also become

available in the future as new discoveries are made on pathogenic agents causing biliary atresia.

6.B. Adequacy of Protection Against Risks

6.B1. Plan to protect subjects/mitigate risks

Subject confidentiality will be maintained by entering the data on the subject into a research database through a password-protected website (using SSL) maintained by the DCC. In the research database, subjects will not be identified by name, but by study numbers only. All consent documents will adhere to the HIPAA regulations, and will use the accepted terminology mandated by each site.

Careful attention to entry and exclusion criteria will also protect subjects against potential adverse effects of corticosteroid treatment. For example, screening for hyperglycemia, hypertension or infection will minimize any potential worsening of the clinical status of pre-existing diseases by corticosteroid treatment.

Blood will be drawn whenever possible at times of venipuncture for routine care or when intravenous lines are in place to reduce the risks of venipuncture. Only the blood draw at the two-week follow up visit is added by this study.

6.B2. Description of Recruitment Plan

Subjects will be recruited from subjects evaluated at, referred to, and followed at the ChiLDREN clinical centers, whom have been consented and enrolled into the ChiLDREN prospective database study and found to have biliary atresia. The investigator or study coordinator will recruit the parent(s) or guardian(s) during clinic visits or during an inpatient admission to the hospital when exploratory laparotomy and portoenterostomy are being planned for diagnosis and treatment of biliary atresia. If parent(s) or guardian(s) are not approached about the clinical trial before surgery, recruitment may also occur within 72 hours of the portoenterostomy. The investigator will discuss the study design, benefits and possible risks with the family. Printed information about the study and the consent form will be given to the family; questions about the study will be answered. The IRB-approved consent will include the purpose of the trial, the responsible parties and investigators, potential benefits, risks of participation, the right to refuse to be in the study, the right to withdraw from the study under no penalty, contact numbers and information about the responsibility for injury and payment for medical care.

6.B3. Informed Consent Process and Plan

A common template for the informed consent form will be used at each clinical site with modifications in the format as necessary to meet the requirements of the respective institutional human subjects committees. The parents/guardians will retain a copy of the signed consent form; one copy will be retained for the subject's chart and one will be included in the research records. No collection of data related to the study or to procedures will be done prior to completion of the informed consenting process.

6.B4. Assent

Assent will not be sought from study subjects because they will be infants at entry into this study.

6.C. Potential Benefits

6.C1. Description of Possible Health Benefits to Subjects

The use of corticosteroid as an adjuvant therapy following portoenterostomy has the potential to increase bile drainage and decrease hepatobiliary inflammation in infants with biliary atresia. We do not know whether improved bile drainage 6 months after portoenterostomy will lead to better long-term outcome. However, the design of this clinical trial within ChiLDREN will allow us to generate data on long-term clinical outcome by: 1) assessing clinical outcome without liver transplantation at 2 years of age, and 2) continued follow-up up to 10 years through participation of the subject in the ChiLDREN prospective database study.

Study subjects will undergo formal developmental evaluation and their parents/guardians will assess the impact of chronic liver disease on HRQOL. These evaluations have not been previously done in children with biliary atresia, and have the potential to uncover opportunities for targeted therapeutic interventions to improve neurodevelopmental outcome and HRQOL to affected children and their families. These potential benefits will extend to all subjects, regardless of their randomization to treatment or placebo groups.

There is a potential benefit of monitoring for vaccine response and identification of the need for booster immunization.

If the trial demonstrates that corticosteroid therapy does not produce positive outcomes, the data may guide the medical community at large regarding the lack of efficacy of this approach, which should discourage the incorporation of corticosteroid therapy into routine clinical care.

6.C2. Description of any incentives or rewards offered for participation

Parents or guardians of infants enrolled in the study will receive an appropriate fee per outpatient visit to reimburse them for meals and/or parking fees according to costs at each clinical site. The supplies of placebo or corticosteroid will be provided to study subjects. In addition, the clinical site research pharmacy will provide a one to two year supply of MCT-enriched infant formula for subjects that are not breastfed, prescribed doses of a liquid preparation of supplemental vitamins, and ursodeoxycholic acid. The research pharmacy will also provide monthly supplies of Ranitidine to be taken by the study subject while he/she is taking the study drug/placebo. Although part of routine clinical care of infants with biliary atresia, the cost for treatment with ursodeoxycholic acid is usually covered by the family or subject's insurance, but coverage for MCT-enriched formula and supplementation with fat-soluble vitamins is highly variable with different health insurance plans. Therefore, the provision at no cost of these items to the family will serve as an incentive to study participation, and will also help minimize variations in routine clinical care among ChiLDREN clinical sites. The clinical site research pharmacy

will also provide liquid preparation of ranitidine to be taken for 13 weeks, while the subject is taking the study drug/placebo.

6.C3 Tests Performed as Part of Research

Lab tests to evaluate side effects of the corticosteroids (e.g., glucose and electrolytes), vitamin levels, total bile acids, PIVKA-II and immunization antibody titer levels will be paid for by the research grant. The ophthalmologic examination at one year will also be paid for by the research grant.

6.D. Summary of Importance of Knowledge to be Gained from this Research

Knowledge gained from this clinical trial may directly impact the standard of medical care offered to infants with biliary atresia throughout the United States. In addition to objectively assessing whether corticosteroid therapy improves bile flow and long-term survival with native livers in an adequately powered subject population, the experimental design for this trial will also monitor for potential short- and long-term adverse effects from corticosteroids. This is especially important in view of the concerns about impaired neurodevelopmental outcome associated with corticosteroid treatment, as well as the potential incorporation of corticosteroids as part of routine care of infants with biliary atresia by the community of physicians at large. Collectively, these data will enable analysis of the risk-benefit ratio of corticosteroid treatment on clinical outcome of children with biliary atresia.

7. DATA AND SAFETY MONITORING PLAN (DSMP)

7.A. Summary of DSMP

The overall organization of the DSMP aims to monitor this research protocol with emphasis on subject safety and data integrity. To this end, the DSMP outlined below will: 1) monitor the progress of the trial and the safety of participants, 2) assure compliance with requirements regarding the reporting of adverse events, 3) assure that any action resulting in a temporary or permanent suspension of the trial is reported to the DCC, ChiLDREN Steering Committee and NIDDK, and 4) assure data accuracy and protocol compliances. General monitoring guidelines have been developed by ChiLDREN to aid implementation of the DSMP, which will systematically monitor for:

- Verification that subject consent for study has been properly obtained and documented ensuring compliance with standards for protection of human subjects
- Verification that research subjects entered into the study meet inclusion and exclusion criteria
- Verification that the study is conducted in compliance with the protocol
- Verification of accuracy of the data collected
- Verification that all essential documentation required for good clinical practice guidelines are present, current, and appropriately filed.
- Verification of subjects completing the study

Particular attention will be given to monitoring subject safety issues. To this end, a Study Medical Safety Officer will be assigned for this clinical trial, and notification forms have been developed to expedite and facilitate the reports of serious adverse events. The DSMP will be overseen by the NIDDK and the Data and Safety Monitoring Board (described below).

7.B. Adverse events (AE) and Serious Adverse Event (SAE)

7.B1. Adverse events (AE) – Overview

Uncontrolled clinical trials of corticosteroid therapy following portoenterostomy in infants with biliary atresia reported collectively over 75 subjects with biliary atresia and over 100 episodes of corticosteroid treatment[46-49]. Regimens have included varying doses, durations, and times following portoenterostomy; no serious complications were reported. Adverse events in these studies, however, may not have been identified because of the small number of subjects included in individual studies or because adverse events may not have been systematically documented. Therefore, to monitor for potential adverse effects appropriately, we will systematically monitor all subjects at each scheduled visit. Subjects will be followed until complete/adequate resolution of an adverse event is documented. We will continue the evaluation of adverse events if a subject withdraws or is prematurely discontinued from participation in the study for any reason. The list of potential expected adverse effects was developed based on information from a pharmacology textbook and reviews of the published results clinical trials of dexamethasone to prevent chronic lung disease in over 1000 very-low-birth weight premature infants [45]. Notably, some of the potential adverse effects of corticosteroids may also be present in infants with biliary atresia due to the natural progression of disease to biliary cirrhosis, such as gastrointestinal bleeding, growth impairment, and impaired response to routine immunizations. Therefore, it is important in this study that the frequency, severity and types of adverse effects, AEs and SAEs be compared between infants receiving study drug and those receiving placebo.

7.B2. Grading of AEs

An AE is any unfavorable or unintentional sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Grading of AEs will be based on the National Cancer Institute-Common Terminology Criteria for Adverse Events[77-79]. Severity of AEs is outlined below:

Grade 1: Mild adverse event
Grade 2: Moderate adverse event
Grade 3: Severe adverse event
Grade 4: Life-threatening or disabling adverse event
Grade 5: Death related to adverse event

7.B3. Serious Adverse Events (SAE)

In this study, an SAE is defined as any clinical adverse event or abnormal laboratory test value that is associated with events that could threaten a subject's life or functioning,

irrespective of the study arm (corticosteroids or placebo) to which the subject was assigned. The term SAE is not intended as a measure of severity or intensity[77-79]. Thus, an AE should be considered a SAE if it results in any of the following:

- Death
- Is life-threatening (subject was at risk of death as a result of the event; it does not refer to hypothetical risk of death if the event had been more severe)
- Requires insubject hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above

7.C. AE and SAE Reporting Plan

An investigator from the DCC will serve as the Medical Safety Officer. He/she will carry a pager so that all SAEs may be reported promptly. The DSMB will review all AEs and SAEs during their regularly scheduled meetings, or on an expedited basis as determined by the NIDDK Program Director or Chair of the DSMB, according to the ChiLDREN policy manual.

All unexpected adverse events (UAEs) are reported to the DCC by completion of the Adverse Events Form by the study site into the secure ChiLDREN website. This triggers an email message to the Medical Safety Officer to check the website for the report; no personal identifying or medical information is contained in the email message.

All SAEs as defined previously require expedited event notification within 24 hours of occurrence or identification to the DCC. Expedited event notification is completed through web entry of the Adverse Events Form. Web entry generates an immediate email to the Medical Safety Officer with only the subject identification number in the message. The Medical Safety Officer then logs into the secure website to see the report of the event. In addition to web entry of the SAE, the Medical Safety Officer may be contacted for any questions or clarifications regarding classification of the event. Paging the Medical Safety Officer is not required as part of the expedited notification process, however may be used if any difficulty is encountered in the web-based notification system. Completing the web entry of the AE form will automatically send e-mail notification to the Medical Safety Officer, NIDDK Program Director, DCC PI, and Project Manager. Fax cover sheets are provided if there is a need to fax supporting information per request of the Medical Safety Officer or NIDDK Program Director. Once the Medical Safety Officer reviews the information submitted on the AE form, the clinical site will be contacted if additional information is required in order to draft a preliminary report of the SAE. Once completed, the preliminary report will be sent to the, the report will then be sent to the NIDDK Program Director, clinical site PI, DCC PI, Project Manager, and Chair of the DSMB.

Once the SAE is resolved, a final report is generated by the Medical Safety Officer. The Medical Safety Officer will send the final report to the NIDDK Program Director, clinical site PI, and the DCC PI and Project Manager. All SAE reports will go the DSMB for review. The NIDDK Program Director and the Chair of the DSMB will decide if any individual SAE

warrants notification to the FDA and to the IRB's of all participating ChiLDREN clinical sites.

The clinical site at which the SAE occurred is responsible for expedited reporting of the SAE to their respective IRB. Each site is responsible to report all AE's to their IRB according to its AE reporting policy and procedures.

Updates to SAEs are submitted to the DCC through web entry of AE form. An update report is generated through the same process that the original SAE report was entered. Updates to the AE form should be made until an outcome and end date for the event are known or if study participation ends.

The Medical Safety Officer will submit an expedited report to the FDA of an SAE when the SAE is unexpected and may be related (even remotely) to the study drug.

7.D. Monitoring and Management for Specific AEs

Several side effects of corticosteroids are anticipated in the subjects randomized to corticosteroid treatment. The following section outlines the plan for monitoring and management of these AEs. This monitoring will be conducted after portoenterostomy however before the first dose of study medication is given (to provide baseline data) and then at hospital discharge and at each scheduled follow up visit.

7.D1. Hypertension:

If a significant hypertension (systolic blood pressure ≥112 mmHg for infants <12 months of age) [80a,b,c] is measured at least two times, and the subject is asymptomatic, a repeat blood pressure will be obtained within 24-48 hours. If the elevated blood pressure is resolved, no intervention will be necessary. If the repeat systolic blood pressure is ≥112 mmHg and the subject is asymptomatic, the study medication dose-reduction protocol (Section 4.F2) will be followed and a repeat blood pressure will be obtained within 48 hours; a mild AE will be reported[77]. If the repeat systolic blood pressure remains ≥112 mmHg but the subject remains asymptomatic, anti-hypertensive monotherapy will be initiated (see below). If the hypertension persists beyond 48 hours, a second dose-reduction will be initiated and a second anti-hypertensive medication may be used (see below). If hypertension does not resolve within 48 hours or if the subject develops symptoms due to hypertension (e.g.: severe irritability, seizure) or as other life-threatening consequences, such as end-organ damage, drug or placebo will be tapered and discontinued (Section 4.F3), the subject will be referred to an emergency department, and additional anti-hypertensive treatment will be initiated. This will be reported as a SAE.

a. Treatment choices for hypertension with BP ≥112 mmHg without symptoms
   - Hydrochlorothiazide: 2 mg/kg/day given once or twice a day, or
   - Amlodipine: 0.2-0.4 mg/kg/day given once or twice a day, or
   - Captopril: 0.3-1 mg/kg/day given twice a day
   - Other site-specific treatment choice

b. Treatment choices for hypertension with BP ≥112 mmHg with symptoms
   - Nifedipine 0.25-0.5 mg/kg/ sublingual
   - Labetolol: 1-3 mg/kg/hour IV
   - Other site-specific treatment of choice

7.D2. Hyperglycemia

A random plasma glucose level will be obtained at the time of discharge and at follow-up visits (Table 6). If the random plasma glucose level is 200 mg/dL or higher the study subject will return within 24 hours for a repeat level. If the repeat glucose level is 200 mg/dL or higher[81], a moderate AE will be reported and the study medication dose-reduction protocol (Section 4.F2) will be followed if the subject is asymptomatic. Persistent hyperglycemia above 200 mg/dL beyond 48 hours of dose reduction and associated with acidosis or change in mental status due to elevated serum osmolality will be considered a SAE, and the drug or placebo will be tapered and discontinued (Section 4.F3). If blood glucose is 150-200 mg/dL, treatment or observation will be directed by the local standard of care, including the option for study medication dose reduction (Section 4.F2).

7.D3. Hypokalemia

A random serum potassium level will be obtained at the time of hospital discharge and at follow-up visits (Table 6). If the potassium level is less than the lower limit of normal but above 3.0 mmol/L, a mild AE will be reported. A new serum K level will be obtained in 48 hours. If the potassium level is less than 3 mmol/L but ≥2.5 mmol/L, a moderate AE will be reported and appropriate replacement therapy will be initiated. Repeat serum potassium levels will be obtained at 24 and 48 hours after start of replacement. If the levels do not normalize and the subject is asymptomatic, increased replacement will be initiated and the subject will be monitored closely. The clinical site PI will have the option to initiate study medication dose reduction (Section 4.F2). If the potassium is less than 2.5 mmol/L, a SAE will be reported, replacement therapy will be given immediately, and the study drug or placebo will be tapered and discontinued (Section 4.F3), unless another cause is identified as the cause for the hypokalemia (such as use of furosemide or another diuretic that may be used because of progression of liver disease and development of ascites).

7.D4. Impaired Wound Healing

Impaired wound healing is defined as a situation in which any of the following conditions are met:

- Readmission due to wound healing complications after discharge following portoenterostomy,
- Delay in discharge due to wound healing complications after portoenterostomy,
- Wound infection after portoenterostomy that requires specific treatment with antibiotics
- Return to the operating room in less than 30 days for wound management.
- A late complication in wound healing following portoenterostomy, such as, ventral hernia, ascitic leak, etc

Wound healing will be assessed daily in the hospital after portoenterostomy by the clinical team. A formal evaluation by the clinical site PI and study coordinator will occur on the day of discharge and at weeks 2 and 4 after portoenterostomy. Delay in discharge or readmission due to would healing complications after surgery will be reported as an SAE. Wound infection that requires specific treatment with intravenous antibiotics will also be reported as a SAE. At outpatient study visits, an assessment will be made as to whether there is adequate wound healing. If there is a wound infection requiring surgical intervention in the operating room (i.e. reoperation) or complete wound dehiscence, it will be reported as a SAE. In the event of any SAE, drug or placebo will be tapered and discontinued (Section 4.F3). Care of the subject for impaired wound healing will be done according to local standard of care.

7.D5. Gastrointestinal Bleeding

If hematochezia, melena, hematemesis, or visible blood through nasogastric or gastrostomy tube is reported, an evaluation of gastrointestinal bleeding will be performed (e.g., to rule out anal fissure, infectious colitis, allergic colitis). If the bleeding is not associated with a drop in hematocrit and no further evaluation is indicated, a mild AE will be reported. If the presence of hematemesis, hematochezia or melena is associated with a drop in hematocrit of >5%, a moderate AE will be reported and further evaluation will be considered as clinically indicated; the clinical site PI will have the option to initiate study medication dose reduction (Section 4.F2). If a packed red blood cell transfusion is required and/or endoscopic intervention (such as sclerotherapy) is necessary, this will be reported as a SAE. The presence of a hemoglobin level < 6.5 g/dl or systemic hypotension caused by acute gastrointestinal bleeding will also be reported as a SAE, and the study drug or placebo will be tapered and discontinued (Section 4.F3).

7.D6. Pancreatitis

Because a single diagnostic test for pancreatitis is not available, a combination of tests and the best clinical judgment of the local PI will be used when evaluating the subject for possible pancreatitis. If there is new onset irritability presumed due to abdominal discomfort, progressive abdominal distension, inability to feed and/or vomiting, a serum lipase and/or pancreatic ultrasound will be ordered. If serum lipase is elevated (three times above the upper limit of normal) or ultrasound findings of pancreatitis are present[82], this will be reported as a moderate AE; the clinical site PI will have the option to initiate study medication dose reduction (Section 4.F2). If the symptoms persist or require narcotic pain medication, or are associated with a pancreatic pseudocyst, hypocalcemia or anemia felt to be secondary to hemorrhagic pancreatitis, a SAE will be reported, and drug or placebo will be tapered and discontinued (Section 4.F3).

7.D7. Irritability

The development of severe irritability in the subject without an obvious cause will be considered a moderate AE, defined as the presence of inconsolable, persistent crying without an apparent cause, which requires evaluation and confirmation by the primary care provider or a visit to an emergency department if the subject is being followed as an outsubject, or by the clinical insubject team if the subject is in hospital at the time of the

event. Following evaluation for irritability and if no obvious cause is identified, a 50% dose reduction of study drug or placebo (Section 4.F2) will be considered by the clinical site PI. If the irritability resolves on this reduced dose, the duration of reduced dose will be the same as for the original dose, and the subject will continue to follow the remainder of the scheduled study drug/placebo regimen. The Medical Safety Officer will monitor all decreases in dose by clinical centers. If severe irritability persists despite the decrease in dosage, the study drug/placebo will be reduced by an additional 50%. If severe irritability persists despite this new reduction in dosage, the subject will undergo the tapering protocol and discontinuation of the study drug/placebo (Section 4.F3).

7.D8. Hypersensitivity Reaction to Trimethoprim-Sulfamethoxazole

If the subject develops a hypersensitivity reaction to TMP/SMZ manifested by a skin rash, the medication will be discontinued promptly and oral neomycin (25 mg/kg PO twice daily) will be used as prophylaxis against ascending cholangitis. Because neomycin provides no prophylaxis against PCP, the subject will also undergo a blinded taper. The study medication will be discontinued as outlined in (Section 4.F3) and the subject will continue to be followed per protocol even when off medication. Our choice to discontinue the study drug/placebo is based on concerns of the risk of PCP in the immunocompromised infant without TMP/SMZ prophylaxis.

7.D9. Cataracts

Cataracts are a well-recognized complication of long-term corticosteroid therapy, and are related both to dosage and duration of therapy. Children appear to be particularly at risk for steroid-induced cataracts. Despite the relative short duration of corticosteroid treatment in this clinical trial, all subjects will undergo an ophthalmologic examination at 12 months of age to monitor for the development of corticosteroid-induced posterior subcapsular cataracts. The examination will be performed by a pediatric ophthalmologist. The incidence of cataracts in corticosteroid treated-subjects and in placebo controls will be reported to the DSMB.

7.E. Vitamin Toxicity Monitoring

There is a potential risk of vitamin toxicity from supplementation. However, specific toxic concentrations of each of the fat-soluble vitamins are not available in the literature. Therefore, close monitoring of vitamin levels to confirm that they are not above the normal ranges reported by the laboratory is planned and outlined in section 4.N8 and 4.N9. Fat-soluble vitamin status will be checked within one month after starting supplementation through routine testing. Whenever a vitamin dose adjustment is made, follow-up testing will be performed within one month. If there is a major or rapid change in the severity of cholestasis (e.g., rapidly decreasing levels of serum bilirubin), vitamin levels will be monitored more frequently.

7.F. Adrenal Insufficiency:

During the taper: In the event of a life-threatening complication and when the physician treating the subject determines that knowing whether the subject was taking

corticosteroid is essential to implement his/her treatment plan (e.g., need for stress dose of steroid in the setting of bacteremia and hypotension or invasive tissue sepsis with systemic symptoms), the study code will be broken to reveal if the subject was receiving placebo or corticosteroids. [The Safety Monitor and the DCC will be informed immediately by the filing of a SAE report.] In this setting, if the subject had been receiving corticosteroids, the tapering protocol will typically use a stress dose of hydrocortisone (50-62.5 mg/m^2/24h) for 2-3 days, then physiologic replacement (20-25 mg/m^2/24h) for 7 days, then half of the replacement dose for 7 days; the use of hydrocortisone instead of prednisolone or methylprednisolone in the setting of infection will minimize additional immunosuppression that would result from the glucocorticoid action of prednisolone during the taper schedule. Alternatively, replacement doses may be in the form of prednisolone (using the doses outlined for weeks 2-8 of Table 5) if the subject tolerates oral/enteral feedings.

After the taper is completed: Although very unlikely, subjects may have some degree of adrenal suppression in the first year after the end of the steroid taper. Therefore, stress doses of hydrocortisone may also be used during the first year after the steroid taper in any subject that may show signs of adrenal insufficiency during a severe infectious illness or a significant stress event (such as surgery). The study code may only be broken to reveal if the subject was receiving placebo or corticosteroids if the physician treating the subject determines that knowing the information is necessary to implement his/her treatment plan. The Safety Monitor and the DCC will be informed immediately by the filling of a SAE report.

8. DATA AND SAFETY MONITORING BOARD (DSMB)

A DSMB consisting of at least seven individuals who are independent of the institutions and investigators participating in the clinical study, and who have no financial ties to the outcome of the trial, has been appointed by the NIDDK in consultation with the ChiLDREN Steering Committee. Written documentation attesting to the absence of conflict of interest will be required of all DSMB members on an annual basis. The ongoing review of the data by this independent committee assures the investigators and the NIDDK that the trial can continue without jeopardizing subject safety. The roster and charter of the DSMB members will be given to each investigator participating in the study for submission to their IRB.

The Medical Safety Officer will serve as the contact person for AE reporting. An NIDDK executive secretary will be present for all DSMB meetings.

The DSMB serves in an advisory capacity to the NIDDK. They will review the study protocol, recommend recruitment initiation, monitor all aspects of the study (e.g.: recruitment, protocol deviations, adherence, adverse events, site visit summaries, data quality, attrition, descriptive characteristics, efficacy), and recommend protocol modifications, including early study termination. All proposed changes to the study protocol by the steering committee will be reviewed by the DSMB.

Quarterly reports will be prepared by the DCC and will be sent to the DSMB and the NIDDK. Tables showing study progress will be presented by clinical center and overall. These will minimally include recruitment, protocol deviations, adherence, attrition, adverse events, data quality, descriptive characteristics of the study sample, and efficacy. All AEs will be reported as described above. The DCC in conjunction with the Medical Safety Officer and the ChiLDREN Steering Committee will maintain a cumulative summary of AEs, overall and stratified by serious/non-serious status, which will be forwarded to the DSMB and the NIDDK Program Director every three months in the quarterly report.

The DSMB will routinely meet twice yearly, once in person and once via teleconference to review the cumulative data. The NIDDK Program Director or the chairman of the DSMB in consultation with the NIDDK Program Director may convene additional meetings. A closed session will be held to review safety and data quality. Based on the data presented, the DSMB will make recommendations including continuation or termination of the study. A summary of the DSMB findings will be forwarded to all investigators for submission to their respective IRBs. The DSMB summary will be accompanied by a letter from the NIDDK Program Director concurring or refuting the findings of the DSMB along with any other requirements of the NIDDK. This letter will also be submitted to the respective IRBs.

9. STATEMENT REGARDING CONSIDERATION OF SPECIFIC SUBJECT CATEGORIES

9.A. Inclusion of Women

Male and female children will be enrolled in the study. Although there might be a slight female predominance (1.25:1) among affected infants, this reflects the sex ratio of the incidence of biliary atresia.

9.B. Inclusion of Minorities

This study is being conducted in clinical centers across the United States in an attempt to have the greatest ethnic and minority diversity possible. The minority representation will reflect that of the local and regional population of the participating centers. Existing outreach programs at specific clinical sites, coverage of costs of laboratory tests, and provision of ursodeoxycholic acid and vitamin supplements will improve access of socially and economically diverse population to this clinical trial. The planned enrollment based on the anticipated ethnic and racial distribution at the ChiLDREN clinical centers are summarized below.

Total Planned Enrollment:

TARGETED/PLANNED ENROLLMENT: Number of Subjects The Entire Study (140)			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	11	7	18
Not Hispanic or Latino	67	55	122
Ethnic Category Total of All Subjects*	77	63	140
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	4	3	7
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	13	15	28
White	60	45	105
Racial Categories: Total of All Subjects *	77	63	140

9.C. Inclusion of Children

The proposed clinical trial will focus on young infants because biliary atresia does not occur in older children or adults.

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APPENDIX 1: Biliary Atresia Standardized Operating Technique

Prepared by the ChiLDREN Surgery Committee
Revised 10.01.2004

1. Diagnostic Phase

i. The abdomen is prepared and draped.

ii. Incision is made transversely 1-2 fingerbreadths below right costal margin or just below level of palpable liver edge. The incision is only big enough for a cholangiogram.

iii. The gallbladder is visualized. If necessary, the fundus is mobilized a short distance; a cholangiocatheter is inserted and secured.

iv. Any gall bladder content is aspirated and preserved for later study by ChiLDREN.

v. 1-2 cc's of contrast are injected under fluoroscopic control.

vi. If the typical features of biliary atresia are present (no bile in gall bladder, gall bladder is atretic with a tiny lumen, and contrast goes nowhere), then the incision is lengthened and the Kasai operation starts.

vii. If a variant of biliary atresia is found, further maneuvers may be necessary to further delineate the anatomy.

viii. If contrast flows into duodenum, but not up to liver, the surgeon must ensure that the common hepatic duct is truly absent by positioning the subject in a Trendelenberg position, and/or occluding the distal duct to force contrast up toward the liver.

ix. If small cystic structures are present along the path of the bile duct, or if contrast flows up into liver but not down, the surgeon must make sure that biliary atresia is truly present before doing anything irreversible.

x. Direct puncture of a common bile or hepatic duct may be necessary in some cases.

xi. Once the surgeon has determined the anatomy, the type of atresia will be classified according to the Ohi classification and noted in the Surgery Case Report Form.

xii. If possible, cholangiograms should be included in the ChiLDREN data base. If so, check the appropriate item in the Surgery Case Report Form.

2. Hilar Dissection

i. The gallbladder remnant is dissected off the gallbladder bed down to where the cystic duct remnant joins the common duct.

ii. The distal common duct remnant is encircled and divided just above the duodenum, dissecting it away from the hepatic artery and its branches and the main trunk of the portal vein.

iii. The duct remnants are separated from the hepatic arteries up to where the arteries branch at the hilar plate.

iv. The bile duct remnant is lifted en bloc off the portal vein. Small branches of arteries and veins to the duct remnant are divided.

v. The bile duct remnant as it approaches the liver plate widens and extends from the right to the left hepatic arteries, and overlies the bifurcation of the portal vein. This tissue is transected sharply at the liver plate with knife or scissors.

vi. Fibrous tissue that extends through the liver plate and capsule and is present next to the hepatic arteries may be further dissected deep to the capsule since duct remnants may be located there. Dissection is continued at the discretion of the surgeon.

vii. The piece of tissue comprising the gall bladder remnant in continuity with the distal and proximal duct remnants and hilar plate are handed off fresh to the pathologist or study coordinator.

viii. Bleeding is controlled with pressure or precisely placed absorbable sutures.

ix. If during the portal dissection, a hilar lymph node is resected, it should be inserted in a separate container and given fresh to the pathologist or study coordinator.

x. A digital photograph will be taken of the final operative dissection prior to placing a bowel loop over the operative site for storage in the database.

3. Drainage procedure

i. A 40 centimeter Roux-en-Y jejunal limb is fashioned. The proximal end of the loop is brought to the porta hepatis. A retrocolic position is

favored. The open end of the loop is sutured to the liver capsule circumferentially around the hilar dissection taking care to stay far enough away so that sutures don't occlude any small ductules around the portal plate.

A closed suction drain is placed under the portoenterostomy.

4. Liver biopsy

i. A wedge and needle biopsy of the liver is taken and handed off in saline to the pathologist.

5. Variables

a. Gall bladder Kasai's

i. This will be done at the discretion of the operating surgeon if it is felt that the ducts are sufficiently large in caliber to support bile flow.

ii. This option will be noted in the operative report and Surgery Case Report Form.

iii. The results of this technique will be tracked by the ChiLDREN group and compared to the results obtained in subjects with no patent bile ducts. It may also permit comparison to subjects who have patent ducts but who get a portoenterostomy.

6. Intestinal drainage

i. 40 cm loop as a Roux-en-y limb is the standard

ii. No valves

7. Perioperative antibiotics:

Ampicillin and gentamicin are peri-operative antibiotics of choice. The peri-operative antibiotic should continue for at least 48 hours post op or until the baby is able to be started on PO antibiotics.

Bactrim is started for cholangitis prophylaxis between the time that the baby starts to drink after surgery and the day of discharge.

8. Drains:

Operative drains are removed when there is no evidence of bile leak after the baby has started eating. Ascitic drainage is not a reason to keep the drain in.

9. Laparoscopic procedure

Centers performing any portion of the procedure laparoscopically should adhere to the general surgical procedures outlined, carefully noting any deviations from the open procedure on the case report forms.

Attending Surgeon should:

- Fill out surgery data collection form
- Provide proper orientation of biliary remnant
- Accurately document the presence of other intraabdominal congenital abnormalities as listed on the surgery data form (i.e. malrotation, preduodenal portal vein, etc.