Childhood Liver Disease Research and Education Network (ChiLDREN)

A Phase I/IIa Trial of Intravenous Immunoglobulin (IVIG) Therapy Following Portoenterostomy in Infants with Biliary Atresia (PRIME)

Protocol P007 PRIME

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1. STUDY OBJECTIVES

Biliary atresia (BA) is a progressive fibro-inflammatory cholangiopathy of infancy that results in complete obstruction of the entire or portions of the extrahepatic biliary tree within 3-4 months of age. The resulting impaired bile drainage from the liver causes reactive proliferation of intrahepatic bile ducts, cholestasis, and ongoing hepatocellular injury that culminates in biliary cirrhosis. If no therapy is implemented, portal hypertension and end-stage liver disease ensues within 1- 2 years, leaving liver transplantation as the only therapeutic option for long-term survival. Hepatic portoenterostomy (HPE) is the operative procedure used currently to improve bile drainage in infants with biliary atresia. Although prompt diagnosis and surgical intervention may restore bile flow, progression to end-stage liver disease still occurs in almost 80% of patients by age 20 years, with over 50% of the patients requiring liver transplantation by 2 years of age. The biological basis for the progression of liver disease after HPE is not fully understood, but the presence of inflammation and pro-inflammatory cytokines in the liver and bile ducts at the time of diagnosis suggests that the host immune response mediates, at least in part, the progressive injury. Both T-cell and B-cell immunity have been implicated in the pathogenesis of bile duct injury in BA. Recent reports suggest that cellular and humoral autoimmunity may also play a role in the progressive bile duct injury following HPE. Intravenous immunoglobulin (IVIG) has been used in a variety of immune deficiency states to restore humoral immunity. Recently, IVIG has also been used in a number of immune-mediated and autoimmune diseases to attenuate the inflammatory response and reduce symptoms or pathology. IVIG has several potential immunomodulatory actions that might play a role in reducing bile duct and liver injury in biliary atresia, although it has never been tested in this disease. Based on its beneficial effects in other immune-mediated diseases, we propose a multi-center open label phase I/IIa clinical trial of high dose IVIG in infants with BA to determine if the administration of IVIG in these infants is feasible, well tolerated and safe, to determine if there is a trend towards improved clinical outcomes, and to examine mechanisms that might explain the effects of IVIG in this disease. The trial will be conducted by the NIDDK-funded network of 16 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 BA and other rare cholestatic liver diseases of childhood. For the trial, our *overall hypothesis is that therapy with IVIG following HPE will be feasible, well tolerated and safe and will improve bile drainage and short-term outcome in infants with biliary atresia*. The overall hypothesis will be tested through the following specific aims and hypotheses:

1.1. Primary Objective:

Aim 1: To determine the feasibility, acceptability, tolerability and safety profile of IVIG treatment after hepatic portoenterostomy (HPE) for biliary atresia.

> ***Hypothesis 1*:** Administering IVIG will be feasible, acceptable and well tolerated in infants with BA without significant toxicity.

1.2. Secondary Objectives:

Aim 2: To obtain preliminary evidence whether IVIG therapy is associated with lower serum bilirubin concentration after HPE.

> ***Hypothesis 2a*:** The probability of an infant having good bile drainage at 90 and 180 days after HPE (as defined by survival 90 or 180 days after HPE with both their native liver and serum total bilirubin level <1.5 mg/dL at 90 or 180 days after HPE) will be greater in infants who are treated with IVIG compared to historical controls.
>
> ***Hypothesis 2b*:** The probability of an infant having good bile drainage at 360 days after HPE (as defined by survival 360 days after HPE with both their native liver and serum total bilirubin level <1.5 mg/dL at 360 days after HPE) will be greater in infants who are treated with IVIG compared to historical controls.

Aim 3: To obtain preliminary evidence whether IVIG treatment after HPE will improve survival without liver transplantation at 360 days after HPE.

> ***Hypothesis 3:*** Survival without transplantation will be greater at 360 days after HPE in infants treated with IVIG compared to historical controls.

Aim 4: **To explore mechanisms of action of IVIG treatment in biliary atresia.**

> ***Hypothesis 4****:* IVIG treatment in BA is associated with an increase in circulating regulatory T-Cells and reduction of inflammatory cytokines and specific autoantibodies.

2. BACKGROUND AND SIGNIFICANCE

2.1. Pathogenesis of Biliary Atresia

BA is a progressive fibro-inflammatory cholangiopathy of infancy occurring in 1 in 13,000 live births in the United States, that results in complete obstruction of the entire or portions of the extrahepatic biliary tree within the first 3-4 months of life (1). The resulting impaired bile drainage from the liver causes reactive proliferation of intrahepatic bile ducts, cholestasis, and ongoing hepatocellular injury that culminates in biliary cirrhosis. If no therapy is implemented, portal hypertension and end-stage liver disease ensues within 1- 2 years, leaving liver transplantation as the only therapeutic option for long-term survival. This makes BA the leading indication in children for liver transplantation. Currently, no therapies have been proven to improve the outcome following HPE. Because of the progression of disease and the necessity for liver transplantation in 80% of affected patients, there is an urgent need for the development of new treatments that would delay or avoid the progressive nature of biliary cirrhosis in BA.

One theory regarding the pathogenesis of BA is that the bile duct injury is initiated by a viral infection, followed by progressive, autoimmune-mediated inflammation targeting bile duct epithelia[1](Figure 1). Many investigators employ the Rhesus rotavirus-induced mouse model

of BA (murine BA) as a tool to study the role of the immune system in this bile duct injury.[2-7] Evidence exists for involvement of both adaptive and innate immunity in the bile duct damage found in BA. Adaptive immunity includes cellular (T cell) immunity with the production of cytokines and cytotoxic T-cells and humoral (B cell) immunity with generation of antibodies that can trigger antibody-dependent cytotoxicity or activate complement, resulting in cellular damage. BA is characterized by an inflammatory infiltrate of both $CD4^+$ T cells[8-10] and $CD8^+$ T cells[9,11-12] that surround and invade intrahepatic bile ducts. The portal tract T cells are highly activated, with increased expression of lymphocyte activation (LFA-1, IL-2 receptor) and proliferation (transferrin receptor) markers.[10,13] Murine and human studies have shown that these cells secrete pro-inflammatory cytokines from the Th1 pathway, including IFN-γ, IL-2, and TNF-α.[9,14] IFN-γ is essential for bile duct obstruction based on significantly increased survival associated with patency of the extrahepatic bile duct in IFN-γ-knockout mice.[7] The bile duct injury in murine BA is associated with dramatic increases in autoreactive, bile duct epithelial-specific T cells[15] and adoptive transfer of these T cells into immunodeficient recipient mice results in bile duct-targeted inflammation.[15,16] There are limited data regarding cellular autoimmunity in human BA, with the exception of one study identifying oligoclonal expansions of T cells within BA livers, suggesting antigen-driven T cell differentiation.[17] To date, published work on the role of B cells and humoral immunity in BA has been limited to characterization studies, including findings of periductal immunoglobulin deposits in both the murine model and in human BA.[7,15,18]

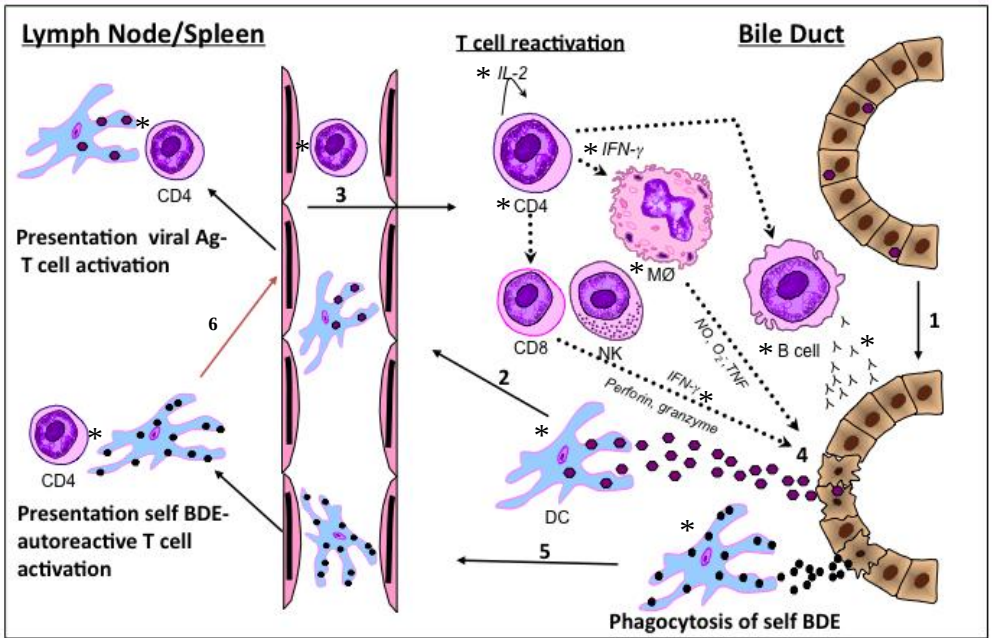


Figure 1. Theory of virus-induced autoimmunity in pathogenesis of BA. Virus infection of bile duct epithelia (1) results in initial injury of bile ducts and presentation of virus particles (2), activating virus-specific T cells (3). $CD4^+$ T cells activate many cell types including: cytotoxic $CD8^+$ T cells (perforin, granzyme and IFN-γ, also produced by NK cells); macrophages (nitric oxide, free radicals and TNF-α); B cells (antibodies activating complement). These pro-inflammatory pathways lead to ongoing bile duct injury (4). With persistent damage, bile duct epithelial proteins may be seen as foreign, resulting in uptake of self proteins (5) and activation of autoreactive T cells that travel back to the site of injury (6), continuing the inflammatory-mediated bile duct damage. *Potential targets of IVIG therapy.

of α-enolase autoantibody as a biomarker present in BA.[18] The pathogenicity of these autoantibodies however is not known and requires further investigation.

The key players in innate immunity include macrophages, dendritic cells, natural killer (NK) cells and neutrophils. Immunohistochemistry studies have shown that the portal tracts of

BA patients display a dramatic increase in macrophages and the greater the intensity of macrophage staining, the worse the prognosis. [9,10,20,21] Activated macrophages produce IL-12 and IL-18 that function together to promote Th1 cellular differentiation. Increased mRNA expression of both IL-12 and IL-18 has been reported in livers and serum, respectively, of BA patients at diagnosis. [9,22] In addition, macrophages secrete TNF-α, which is markedly upregulated in both human and murine BA.[6,9,23] Another important cholangiocyte death pathway is triggered by the interaction of Fas ligand (FasL) with Fas-bearing cells. Strong expression of Fas was identified in ~25% of human BA liver samples and cholangiocyte death may be triggered by ligation with FasL on activated T cells and macrophages.[24] NK cells also contribute to bile duct injury in BA. This was demonstrated in the murine model of BA, where NK cell depletion was associated with protection from biliary obstruction and significantly increased survival.[25]

Adaptive and innate immune responses are controlled by regulatory T cells (Tregs). Loss of Treg function would result in unchecked inflammation and autoreactivity. In BA, it has been shown that there is a significant decrease in circulating Tregs at the time of diagnosis.[26] In addition, the mouse model of BA has shown similar Treg deficits within the liver and adoptive transfer of adult Tregs into RRV-infected neonatal mice prevents biliary obstruction. [27,28] Therefore, Treg deficits may play a critical role in allowing for chronic T and B cell activation in BA.

In summary, activation of adaptive and innate immune pathways and suppression of regulatory pathways are associated with bile duct damage in BA. Inhibition of the inflammatory attack on bile duct epithelia with immune modulators, such as the high dose intravenous immunoglobulin proposed in this study, may help preserve the integrity of the bile ducts, preventing or delaying the progression to biliary cirrhosis.

2.2. IVIG as Therapeutic Agent in Immune/Autoimmune-mediated Diseases

Intravenous immunoglobulin (IVIG) was initially used to treat immunodeficient states associated with low serum IgG levels. Today, more than 70% of the IVIG used in the United States is for the immune modulation of patients with autoimmune and inflammatory disorders. [29,30] High dose IVIG has numerous mechanisms of action that result in suppression of inflammatory and autoimmune-mediated injury (Table 1). Inflammatory cells and cytokines from both the innate and adaptive immune pathways are inhibited by IVIG. High dose IVIG regulates cellular immunity by inhibiting effector T cell functions and promoting regulatory T cell capabilities. The stimulatory FcγR receptor is blocked and the inhibitory FcγRIIB receptors are enhanced on B cells, macrophages and neutrophils in the presence of high dose IVIG. Importantly, many of the known IVIG targets of the immune system have been implicated in bile duct injury in murine and human BA (Figure 1*). IVIG could also potentially reduce the frequency or severity of post-HPE ascending cholangitis episodes, inasmuch as IgG is secreted into bile and because episodes of cholangitis may further damage intrahepatic bile ducts and lead to worse overall outcomes in human BA.

Table 1. Mechanisms of Action of High Dose IVIG [29,30]

Inhibition of Adaptive Immunity
Cellular Immunity
Inhibition of T cell differentiation & proliferation and IL-2 production
Neutralization of pro-inflammatory cytokines and enhancement of anti-inflammatory cytokines
Expansion and activation of regulatory T cells
Blocking of leukocyte adhesion molecules to vascular endothelium
Inhibition of dendritic cell differentiation/maturation
Humoral Immunity
Induction of B cell apoptosis[31]
B cell growth factor neutralization
Increased inhibitory FcγRIIB receptors and blockade of activating FcγR receptor
Clearance of autoantibodies through saturation of FcRn receptors or binding with anti-idiotypic antibodies
Inhibition of complement deposition on target tissues
Inhibition of Innate Immunity
Induces changes in NK cell trafficking from blood to target organ
Increased NK cell activation
Inhibition of dendritic cell differentiation/maturation
Increased inhibitory FcγRIIB receptors and blockade of activating FcγR receptors on macrophages
Neutralization of pro-inflammatory cytokines and enhancement of anti-inflammatory cytokines
Increased neutrophil apoptosis (anti-Siglec-9 Abs)
Decreased neutrophil adhesion to endothelium and activation (blockade of FcγR)

A variety of inflammatory diseases have been treated with high dose IVIG. In the treatment of immune thrombocytopenic purpura, IVIG blocks the FcγR on macrophages in the spleen, resulting in reduced phagocytosis of platelets and rapid increases in platelet counts. [30,32] Anti-idiotypic antibodies within IVIG preparations can neutralize pathogenic autoantibodies, leading to disease remission in chronic inflammatory demyelinating polyradiculoneuropathy and Guillain-Barre syndrome. [30] IVIG-mediated inhibition of the complement cascade alleviates disease severity in autoimmune dermatomyositis. [30] In patients with Kawasaki disease, high dose IVIG inhibits IL-1, TNF-α and IFN-γ and decreases expression of adhesion molecules, resulting in protection from inflammatory-mediated vascular injury.[30,33] Furthermore, clinical improvement in Kawasaki disease after IVIG administration was associated with increased number and function of $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs). [34] Similarly, in a murine model of multiple sclerosis, IVIG treatment resulted in decreased disease severity and expanded populations of circulating Tregs.[35] Dramatic increases in survival with preservation of liver function have been shown after IVIG administration to infants with neonatal hemochromatosis (NH), however the specific IVIG

mechanism of action in this disease has not been elucidated. Infants with NH have been reported to tolerate IV infusion doses of 1g/kg of IVIG[36].

2.3. IVIG in this Trial

The feasibility, tolerability and safety of intravenous IVIG (Gamunex-C®)treatment will be tested in this clinical trial as an agent to decrease post-HPE inflammation in the liver and intrahepatic bile ducts and to improve clinical outcomes following HPE. The size of the dose of IVIG to be used in this trial (1 gm/kg body weight/dose) was chosen because that is the typical dose used for administration of IVIG in other immune, autoimmune or inflammatory diseases in children and adults [30-34,36]. The dose and regimen for IVIG is based on the typical dose used in treatment trials of autoimmune diseases, from 1 to 2 gm/kg/dose, which is generally repeated monthly [30-36].

The decision to administer 3 doses of IVIG over a 60 day period in this clinical trial protocol was based on the following justification. Since there are no reports of using IVIG in treating infants with biliary atresia, there was no published experience upon which to guide the treatment regimen. It was decided to use a duration of 60 days treatment because our understanding of the pharmacokinetics and pharmacodynamics of IVIG suggests it would still be present and active in the infant's circulation up to 90 days post-HPE and its effects could persist up to 180 or 360 days post-HPE if early suppression of the immune response had long-lasting effects. Biomarkers of bile flow at these time points (e.g., serum itotal bilirubin concentration at 90 days) have also been shown in other studies to be predictive of long term outcomes in BA. (39).

The IVIG preparation (Gamunex-C®) to be used in this study will be from a single manufacturer and used only for purposes of this study.

The only other anti-inflammatory agent tested in BA for this purpose has been the use of intravenous or oral corticosteroids. ChiLDREN is conducting a randomized, placebo controlled trial of a 13 week course of corticosteroids for treatment of post-HPE BA, which is fully enrolled as of January 2011, and awaiting 2 year follow up for all subjects before the blind will be broken and the data analyzed.

2.4. Justification for this Clinical Trial

Knowledge gained from this phase I/IIa clinical trial may increase the generalizable knowledge about the mechanisms involved in the pathogenesis of biliary atresia and its potential response to IVIG as a therapeutic agent. The risks of this study represent a minor increase above minimum risk. Currently there is no direct evidence of a benefit of IVIG in biliary atresia (it has not been reported to have been used in this disease), however many other immune and autoimmune-mediated inflammatory diseases have been shown to respond to IVIG, justifying the plausibility of IVIG as a therapy for biliary atresia. Mechanistic studies in this protocol may provide insight into immunologic processes involved in the pathogenesis of BA that may be altered by IVIG therapy. It should be emphasized that biliary atresia currently has very poor treatment options but has very serious clinical consequences. The results of this study may provide the justification for further testing of a readily available, safe therapy for treatment of biliary atresia, i.e., IVIG.

The experimental design of this trial will provide important information about short term feasibility, tolerability and safety of IVIG administration in this population of young patients. The results of this study could also provide some initial evidence of potential benefit of IVIG in biliary atresia. This combined new knowledge gained from this study should be of value in determining if a larger phase 2 or 3 clinical trial of IVIG should be entertained in infants with BA.

3. PRELIMINARY DATA

There are no published reports or clinical experience using IVIG in infants with BA following HPE, although IVIG has been shown to be well tolerated in infants with cirrhosis caused by Neonatal Hemochromatosis[36] and preterm infants in IVIG clinical trials to prevent sepsis. [38-39] Thus, there is a paucity of data in human infants with BA. To gain some preliminary data to support the justification for this trial, the laboratory of Cara Mack, MD has tested the use of high dose IgG therapy in the murine model of BA. Numerous murine models of other inflammatory or autoimmune diseases have demonstrated the therapeutic benefits from the administration of human preparations of IVIG, which have led to subsequent trials in humans. [40-43] As mentioned above, the Rhesus rotavirus (RRV)-induced murine model of BA (unique to BALB/c strain) has been employed by many investigators to study the immune responses associated with bile duct injury.[5-7,15,16,25, 27,28] In murine BA, jaundice develops by 1 week of age, followed by a Th1 inflammatory-mediated injury to bile ducts leading to biliary obstruction, despite viral clearance. In the laboratory of Cara Mack, MD at the University of Colorado School of Medicine, her research team sought to determine if therapy with high dose IgG could alter the inflammatory environment and alleviate the bile duct damage in these mice. In these studies, newborn BALB/c mice received a single injection of Rhesus rotavirus (RRV; $1.5x10^6$ pfu/ml) or control balanced salt solution (BSS). At one week of age all RRV-infected, jaundiced mice received intraperitoneal (ip) injections of either human polyclonal IgG (Gamunex, Talecris Biotherapeutics) or albumin as a control (2 g/kg body weight every 2-3 days x 6 doses. Outcomes measured included survival, serum direct bilirubin, liver immune profiles and liver and extrahepatic bile duct histology. No significant differences were found in survival between groups (Figure 2.). However, the IgG-treated mice had significantly decreased levels of serum direct bilirubin compared to controls (Figure 3) (Figure 2B) and markedly reduced extrahepatic bile duct and portal tract inflammation (Figure 2C).

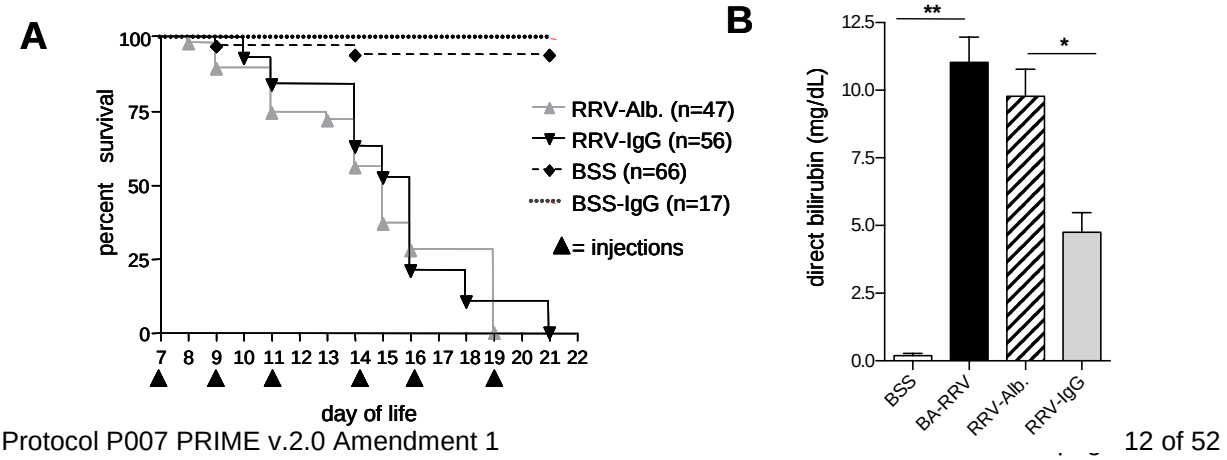
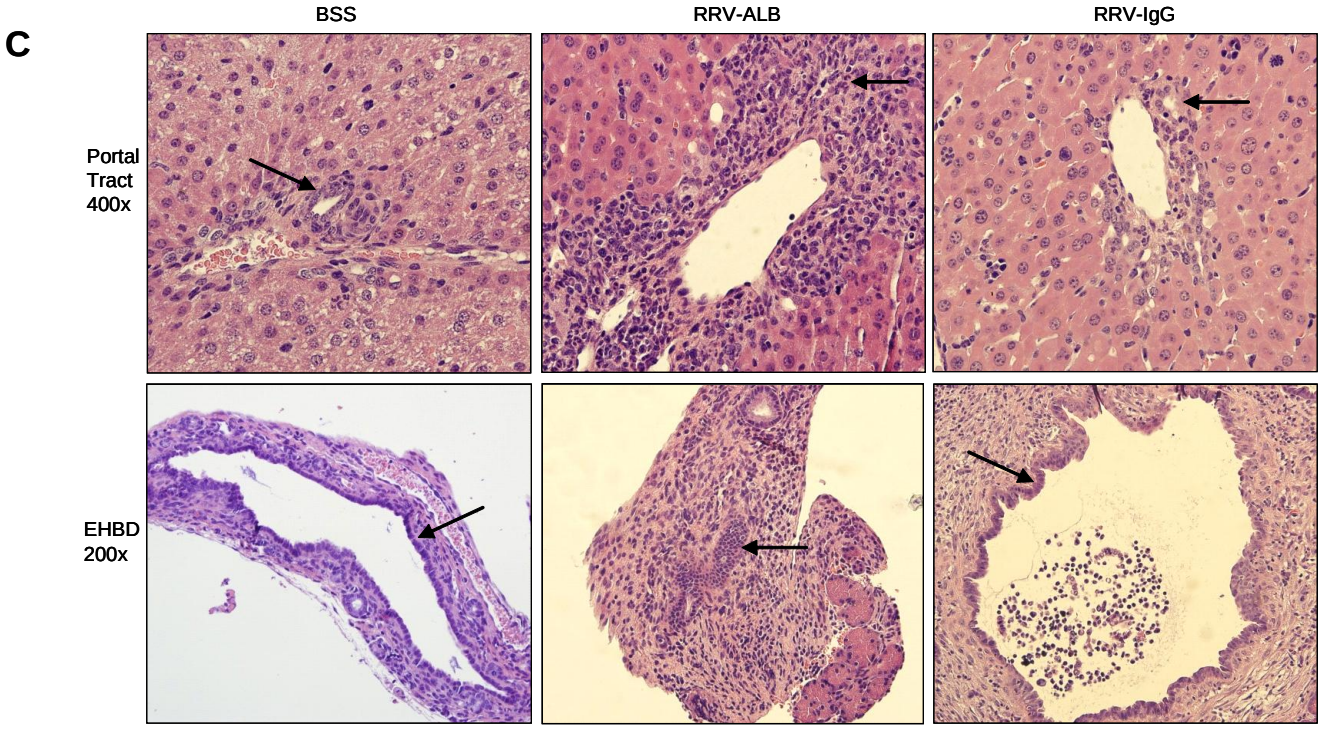


Figure 2. Survival and assessment of bile duct injury. A. Survival.



On day 7 of life, RRV-infected, jaundiced mice received albumin (RRV-alb) or IgG (RRV-IgG). Additional control groups included BSS injected mice (BSS) and BSS-injected mice that received IgG (BSS-IgG). There was no significant difference in survival in the RRV-infected groups. **B. Serum direct bilirubin.** Shown is the mean±SEM of serum direct bilirubin levels obtained at 14 days of life, demonstrating significant bilirubin reduction in RRV-IgG mice (*P<0.001;** P<0.0001). **C**. Liver and extrahepatic bile duct histology. H&E histology from intrahepatic (portal tract) and extrahepatic bile ducts (EHBD) (arrows indicate bile ducts). Note diminished portal and extrahepatic duct inflammation and lack of extrahepatic biliary obstruction in the RRV-IgG group.

Analysis of the liver immune profile revealed that high dose IgG inhibited Th1 cytokine production from T cells and increased the percentage of regulatory T cells (Figure 3).

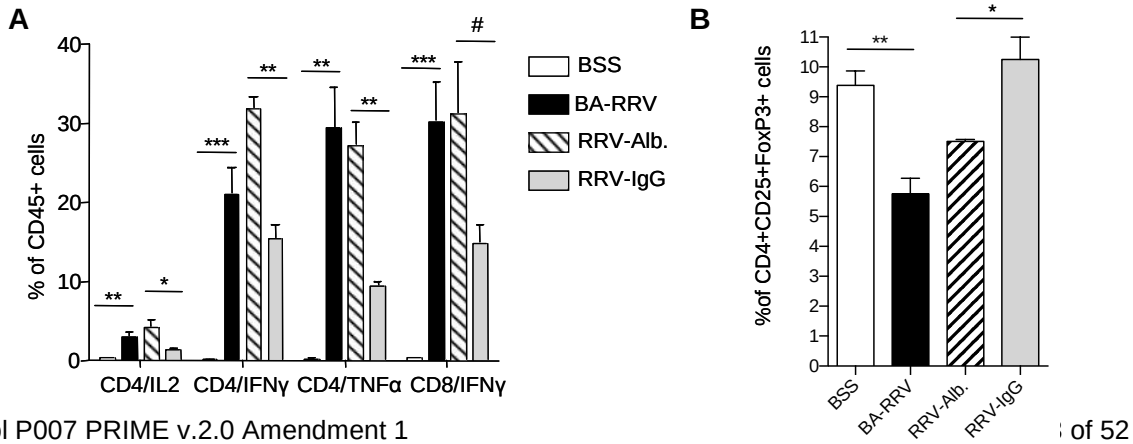


Figure 3. High dose IgG inhibits inflammatory cytokine production and expands regulatory T cells (Tregs). A. Intracellular cytokine staining. Liver immune cells were stained with cell surface markers, stimulated with PMA/ionomycin and IL-2, IFN-γ, TNF-α were quantified by intracellular staining and FACS analysis. High dose IgG treatment was associated with significantly reduced inflammatory cytokine production by $CD4^+$ T cells (**P<0.005, ***P<0.0005). **B. Treg analysis.** Percoll gradient-purified liver immune cells were stained with CD4, CD25 and FoxP3 to identify Tregs. (*P<0.005, **P<0.0005). BA mice had depressed levels of Tregs that return to normal with high dose IgG therapy.

In summary, high dose IgG therapy in the murine model of BA dramatically decreased Th1 cell-mediated inflammation and biliary obstruction. To that end, testing high dose IVIG therapy to diminish intrahepatic bile duct injury in infants with BA, thus prolonging survival with their native liver, appears to be justified.

4. RESEARCH DESIGN AND METHODS

4.1. Overview

The overall hypothesis to be tested is that therapy with IVIG following HPE will be feasible, well tolerated and safe and will improve bile drainage and short-term outcome in infants with biliary atresia. This hypothesis will be tested by a multicenter prospective phase 1/2A open trial. In this trial the feasibility, tolerability and safety of IVIG therapy will be assessed in this patient population, efficacy will be estimated and exploratory mechanistic research studies will be performed. After IRB-approved written consent is obtained from the parent or guardian, the subject will be enrolled and will receive three monthly intravenous doses of IVIG at designated intervals over the first 60 days following HPE and will be followed for 360 days after enrollment. Blood will also be obtained during this study to assess potential mechanisms by which the IVIG may alter or reduce bile duct inflammation and injury and improve bile flow. The study will be conducted in a manner to allow for assessment of feasibility, acceptability, tolerability and safety in 29 enrolled infants. All infants in this trial will also be treated with standardized doses of other routine treatments for BA during this trial (ursodeoxycholic acid, trimethoprim-sulfamethoxasole, fat-soluble vitamin supplements). Subjects in this study will not receive corticosteroid therapy for treatment of biliary atresia, as this is of unproven benefit at the present time. All subjects will receive standard clinical care that is routinely used for infants with biliary atresia, which will include nutritional support. This routine clinical care will not be modified by participation in this study.

4.2. Study Population

All infants with an established diagnosis of BA, excluding those with Biliary Atresia Splenic Malformation syndrome, who are seen at one of the ChiLDREN study sites and who undergo a standard surgical HPE before age 120 days will be eligible for the trial. Parents/guardians will be approached about participation in this clinical trial after a decision is made by the attending physician at the ChiLDREN site for the infant to undergo an exploratory laparotomy with possible HPE, or within 5 days after HPE.

Definition of HPE. *Open abdominal surgery in which a Roux-en-Y loop of jejunum is anastomosed to the porta of the liver after a careful surgical dissection to locate patent bile duct remnants in the porta. For purposes of this protocol the term HPE excludes the gall bladder Kasai procedure and excludes laparoscopic HPE.*
Definition of BA. *For the purposes of this protocol, BA is defined as a fibroinflammatory obliteration of the lumen of one or more segments of the extrahepatic biliary tree within the first 120 days of life*. Diagnosis is established at time of surgical exploration by either a) an intraoperative cholangiogram showing blockage of part of or the entire extrahepatic biliary tree or b) failure to be able to perform an intraoperative cholangiogram because of the absence of a gall bladder lumen. Diagnosis is confirmed by surgical exploration of the extrahepatic biliary tree and by subsequent histological examination of the bile duct remnant demonstrating an obstructed or absent lumen. Rarely, post-operative histological examination of the excised biliary remnant will demonstrate that a disease process other

than BA caused the biliary obstruction. If this is found in a subject already enrolled in this trial, the subject will be removed from the study and replaced by another enrollee. In the event that the diagnosis of BA is established during exploratory laparotomy, but the surgeon does not perform a HPE (as may occur in cases of advanced cirrhosis), the patient will not be eligible for this study.

The maximum number of patients to be entered in the study at all clinical sites will be 29 (excluding those replaced because they were found to be ineligible owing to post-op histological information).

4.2.1. Inclusion criteria

- Infant under 120 days old with established diagnosis of BA and enrolled in the ChiLDREN prospective database study (Prospective Study of Biliary Atresia Epidemiology [PROBE])
- Standard HPE operation has been performed for BA within the previous 3 days
- Post-conception age ≥ 36 weeks at time of enrollment
- Weight at enrollment ≥ 2000 gm
- Written informed consent to participate in the study obtained within 3 days of completion of HPE. *(Note: Families of potential study subjects may be approached prior to the HPE; however consent can only be signed after the diagnosis of BA is established at surgical exploration and after HPE is performed.)*

4.2.2. Exclusion criteria

- Laparoscopic HPE or “gall bladder Kasai” (cholecysto-portostomy) surgery was performed
- Biliary atresia splenic malformation syndrome (presence of asplenia, polysplenia or double spleen)
- History of a hypercoagulable disorder
- Renal Disease defined as serum creatinine > 1.0 mg/dl prior to enrollment or presence of complex renal anomalies found on imaging
- Evidence of congestive heart failure or fluid overload
- Presence of significant systemic hypertension for age (defined as persistent systolic blood pressure ≥112 mmHg measured on at least 3 occasions following HPE)
- Infants whose mother is known to have human immunodeficiency virus infection
- Infants whose mother is known to be serum HBsAg or hepatitis C virus antibody positive
- Previous treatment with intravenous immunoglobulin therapy for any reason
- Previous treatment with corticosteroid therapy for post-operative treatment of biliary atresia
- Previous treatment with any other investigational agent
- History of allergic reaction to any human blood product infusion
- 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 IVIG

4.2.3. Inclusion of Females and Minorities

Male and female infants of all races and ethnic groups are eligible for this trial.

4.3. Outcome Measures

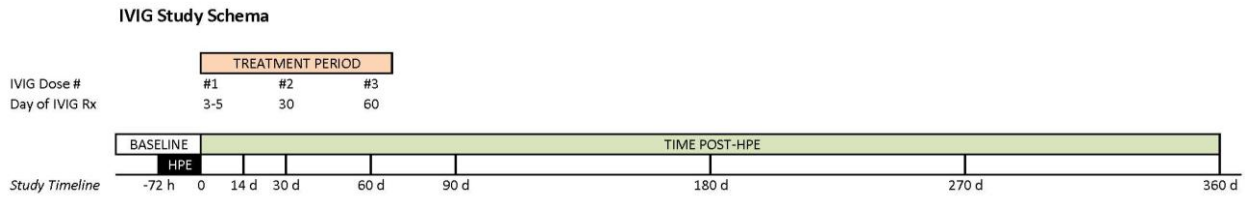
4.3.1. Primary Outcome Measures:

1. Feasibility: Percentage of subjects for whom administration of IVIG is feasible, defined as the successful administration (at least 80% of each dose) of the 3 doses of IVIG at the prescribed times through peripheral IV lines or central lines if already in place for clinical indications.
2. Acceptability: Percentage of subjects for whom the study is acceptable, defined as the ability of the subject's family or guardian to allow intravenous line placements, blood draws, and other study procedures for the study subjects.
3. Safety and tolerability measures:
    a. Percentage of subjects with any serious adverse events (SAEs),
    b. Percentage of subjects with any level 3, 4, or 5 toxicity (per NCI CTEP grading system [http://www.eortc.be/services/doc/ctc/ctcaev3.pdf], and
    c. Percentage of subjects with other expected adverse events (such as allergic reactions, irritability, fluid volume problems, IV infiltration and aseptic meningitis).

4.3.2. Secondary Outcome Measures:

1. Percentage of subjects who survive 90 days after HPE with both their native liver and serum total bilirubin <1.5 mg/dL at 90 days after HPE. *For this study, serum total bilirubin must be measured as a total and not calculated by summing bilirubin components (such as serum indirect plus direct bilirubin or conjugated plus unconjugated bilirubin).*
2. Percentage of subjects who survive 180 days after HPE with both their native liver and serum total bilirubin <1.5 mg/dL at 180 days after HPE. *For this study, serum total bilirubin must be measured as a total and not calculated by summing bilirubin components (such as serum indirect plus direct bilirubin or conjugated plus unconjugated bilirubin).*
3. Percentage of subjects who survive 360 days after HPE with both their native liver and serum total bilirubin concentration <1.5 mg/dL at 360 days after HPE.
4. Percentage of subjects who survive with their native liver at 360 days after HPE.
5. Percentage and absolute number of Tregs (CD4+CD25+FoxP3+), CD3/4 T cells, CD3/8 T cells, NK cells (CD56), NK T cells (CD3/56), CD19/20 B cells, macrophages (CD14/11b), and neutrophils; plasma levels of anti-enolase antibody; and plasma cytokine levels (Th1/Th2 multiplex and IL17) prior to IVIG dose #1 and at 60, 90, 180, and 360 days after HPE.

4.4. Study Design and Intervention



4.4.1. General Study Design

The primary objective of this study is to determine the feasibility, tolerability and safety of administering three doses of IVIG intravenously to 29 subjects with BA who have undergone the HPE surgery within 5 days prior to enrollment. Consent will be obtained, baseline clinical and research blood specimens will be obtained, historical and clinical data obtained, and IVIG will be administered at days 3-5, 30 and 60 days following HPE. When possible, peripheral venous catheters, central venous lines or peripherally inserted central catheters (PICC) lines that were placed for clinical indications will be used for the IVIG administration. Blood draws will be drawn through IV lines whenever possible. If no IV access is available, a peripheral IV will be placed for administration of IVIG and blood draws at the time of each IVIG infusion and removed after the infusion. Analysis of feasibility, tolerability and safety will be ongoing during this trial by the Safety Monitor, DSMB and NIH Project staff (see Section 7.6)

4.4.2. Recruitment for study

All infants with an established diagnosis of BA who are seen at one of the ChiLDREN study sites and who undergoes a standard surgical HPE before age 120 days will be eligible for the trial. Families of infants with a high suspicion of the diagnosis of BA will be approached and provided information about this trial either before the patient undergoes surgical exploration and the HPE surgery or within 3 days of the performance of HPE for the diagnosis of BA. In general, these infants were referred to the ChiLDREN site for clinical evaluation of cholestasis. Therefore, there will not be any specific advertising to increase referrals to the ChiLDREN sites for the purposes of this study.

4.4.3. Consenting for study

Written informed consent will be obtained within 3 days after HPE surgery performed for established biliary atresia. As part of the informed consent process, parents/legal guardians will be informed of the study procedures, the design of the study, the potential risks and benefits of participation and potential side effects of IVIG. This information will be shared with the parent/guardians at the time of enrollment.

4.4.4. Administration of study drug (IVIG)

IVIG will be administered on both an inpatient and outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate adjustment to the IVIG infusion rate and early termination are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's biliary atresia. If a subject has an acute illness at the time that an IVIG dose is due, the subject will be stabilized medically as per local standard of care before the dose of IVIG is administered. If the 30 day post-HPE visit is missed the 60 day post-HPE dose may still be administered.

Dose and timing: All participants will receive the same dose of IVIG at the same intervals in an open-label fashion as outlined in Table 2 as long as the subject does not have any increased risk for toxicity for any IVIG infusion (see Section 7.4). The day of the HPE will be considered the day that the surgery is completed (the day that the patient leaves the operating room) and this will be called Day 0. The first dose of IVIG will be given on Day 3-5 following the surgery while the subject is still in the hospital. Consent will need to be obtained before the first IVIG infusion.
IVIG will be initiated on day 3-5 after HPE surgery (HPE is day 0) at a dose of 1 gm/kg body weight by slow intravenous infusion over at least 4-8 hours, or according to local clinical guidelines for rate and time of infusion. The same dose (1 gm/kg) and duration of infusion will be repeated on day 30 and on day 60 after HPE. Ideally the first dose will be administered on day 3 after HPE, however the first dose may be administered on day 4 or 5 after HPE if there is a clinical situation that precludes start of the study medication on day 3. Dose #1 must be given while the subject is still hospitalized. Dose #2 and #3 may be given as outpatient infusions at the study site if the subject has been discharged from the hospital, as expected. The dose will be based on the weight of the subject on the day of the infusion. If the subject has an intercurrent illness or infection that would preclude giving the 2nd or 3rd IVIG dose at the specified times, the subject will be stabilized or treated for this illness and then the IVIG will be given if the investigator believes it will be tolerated. Since there is a 10 day window for administering doses #2 and #3 of IVIG, we anticipate that all doses will be able to be given during this window. If dose #2 is missed for reasons other than an IVIG related adverse event, dose #3 may still be given. Standard precautions will be taken for administering IVIG, including administering IVIG in a controlled setting by experienced nursing staff, and with the immediate availability of intravenous diphenhydramine, intravenous or subcutaneous epinephrine, and oral or rectal acetaminophen. Local site standard protocol for administration of IVIG will be followed, which may include premedication with acetaminophen or diphenhydramine.

A single dosage level of 1 gm/kg of IVIG was chosen for this study (rather than a range of escalating doses) because 1) this dose is effective and commonly used to treat other autoimmune or immune-mediated diseases in children and adults, 2) this dose can be administered intravenously over 6-8 hours during an outpatient research visit, and 3) this dose is usually not associated with significant side effects in children. To attempt to study several escalating doses in this study would imply that there was a therapeutic target for serum IgG that we would be trying to achieve and that the proper dose could be determined based on PK/PD studies. However, there is no target serum level of IgG that is

known to be effective for BA or for other diseases and, thus, PK/PD studies would be difficult to interpret. Therefore, we chose to use a high dose of IVIG that is effective in other immune-mediated diseases in children and adults and that should be well tolerated by the infants and their families.

Route of Administration: Dose #1 of IVIG will be administered through peripheral IV, central venous catheter or PICC line, whichever is in place for clinical indications on days 3-5 following HPE. It is mandatory that dose #1 be administered during the inpatient post-operative period following HPE. It is anticipated that doses #2 and #3 of IVIG will be administered in the outpatient setting, although these doses could be administered in the inpatient setting if the subject had been admitted to hospital for a clinically indicated reason. If no IV is in place at the prescribed time for doses #1, #2, or #3 of IVIG, then a peripheral IV will be started for the study drug administration and removed after administration of the IVIG. If the family does not allow placement of a peripheral IV for a dose of IVIG, then that dose of IVIG will not be given but the subject will remain in the study and follow the rest of the study protocol, including attempts at administering subsequent IVIG doses.

Table 2. Schedule and dosing of IVIG following HPE in infants with biliary atresia.

IVIG Infusion	Day of dosing following HPE*	IVIG dose**
#1	Day 3 (up to day 5)	1 gm/kg body weight
#2	Day 30	1 gm/kg body weight
#3	Day 60	1 gm/kg body weight

*See 4.4.4 Dose and Timing
**Initial dose will be based on subject's weight at time of HPE. Subsequent doses will be adjusted based on subject's weight measured at 30 and 60 days after HPE. HPE = hepatic portoenterostomy; IVIG = intravenous immunoglobulin

4.4.5. Study Medication Early Termination or IVIG Infusion Rate Adjustment

In the event that a subject has a potential side effect of the study drug (such as moderate irritability, erythematous rash, urticaria, respiratory difficulties, hypotension) during IV infusion, the clinical site PI will have the option to slow the IVIG infusion rate or stop the infusion of the study drug. If the study drug is stopped prematurely, the subject will not receive any subsequent doses of IVIG, but will be followed for the 360 day duration of this study. Adjustments can be made in response to an adverse event to slow the IVIG infusion rate in order to administer the entire dose of IVIG. Diphenhydramine, acetaminophen or epinephrine can be given to treat hypersensitivity reactions during IVIG infusions. If a subject suffers a level 3, 4, or 5 toxicity or an SAE with evidence of attribution to the IVIG (see NCI CTEP grading system) either during or after the infusion of IVIG, he/she will not receive any further IVIG infusions but will be followed for the 360 day duration of this study.

4.6. Other perioperative and medical management

All subjects with BA will undergo standard surgical HPE following the technical guidelines agreed upon by the ChiLDREN Surgical Committee. All subjects will receive antibiotics intravenously for at least 2 days postoperatively or until they are able to tolerate oral/enteric feedings. Intravenous antibiotics are routinely used postoperatively at ChiLDREN centers as prophylaxis against ascending cholangitis and to prevent perioperative wound infections. 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 180 days after HPE for cholangitis prophylaxis. In the event of an episode of cholangitis despite antimicrobial prophylaxis or following the termination of the 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 an additional 180 days from completion of the IV antibiotics, or until 360 days (end of the study). In the unlikely event that the subject develops a hypersensitivity reaction to TMP/SMZ manifested as a skin rash or fevers (see section 7.4.3), the medication will be discontinued promptly. The subject may receive all remaining IVIG infusions and continue with follow-up visits as per protocol.

Other medications and treatments that are part of the routine BA clinical care following HPE will be continued as outlined in Section 4.7.

4.7. Routine clinical care guidelines for ChiLDREN

Routine clinical care guidelines were established for infants enrolled in the ChiLDREN prospective database study (PROBE; P003) 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.

4.7.1. Physical Exam

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

- Height or recumbent length, weight, occipital-frontal head circumference
- Vital signs.
- Skin examination for rash, urticaria, jaundice, xanthomas, palmar erythema, and vascular pattern.
- Abdomen examination to determine liver size and texture, spleen size, presence of ascites.
- Examination of extremities

4.7.2. Diet

When the total serum 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 <12 months of age) and whole milk (if ≥ 12 months of age) or breast milk can be continued if the preference of the family. MCT-containing formula or breast milk will

be continued if the total bilirubin is ≥1.5 mg/dL and the child is 12 months of age or older. When growth is inadequate, measures will be taken for nutritional rehabilitation according to medical management used at each ChiLDREN clinical center. Solid foods will be initiated at the discretion of the subject's primary care provider. MCT-containing formula will not be supplied or paid for by this study, since it is standard of clinical care.

Growth	Age (mo)	Total Bilirubin (mg/dL)	Diet
Adequate	<12	≥1.5	MCT-containing formula or breast milk
Adequate	<12	<1.5	Standard formula or breast milk
Adequate	≥12	≥1.5	MCT-containing formula [or breast milk]
Adequate	≥12	<1.5	Whole milk or breast milk
Inadequate	N/A	N/A	Nutritional rehabilitation

4.7.3. Vitamin supplementation

Supplementation with fat-soluble vitamins is part of the standard clinical care of infants with BA. Therefore, it is recommended that all subjects in this study with total bilirubin ≥1.5 mg/dL should receive the following:

a. AquaDEKs® vitamin drops or similar vitamin preparation: 2 ml orally per day
b. Vitamin K : 2.5 mg orally co-administered with AquaDEKs® vitamin drops on Mondays, Wednesdays and Fridays
c. Other individual vitamin supplements as needed to maintain normal serum levels of fat-soluble vitamins

AquaDEKs® and Vitamin K may be stopped when the total bilirubin is <1.5 mg/dL (normal total serum bilirubin). Serum vitamin levels and prothrombin time/INR will be measured during follow-up visits after HPE as per normal clinical care at the study sites. When an abnormal vitamin value is obtained, the dosage of the specific vitamin will be augmented or reduced, as appropriate, per the local study site standard of clinical care. Repeat levels will be obtained as per local standard of clinical care. This study will not pay for the vitamin supplements used in this study nor for the serum measurements of fat soluble vitamins.

4.7.4. Ursodeoxycholic acid

- Ursodeoxycholic acid suspension: 20 mg/kg/day divided BID orally until the end of the 360 day post-HPE visit.

This medicine may improve bile flow and is the standard of care for biliary atresia. *This study medication will be provided by the study to the families at no charge*.

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

4.7.5. Antibiotics for prophylaxis against ascending cholangitis (see also Section 4.6)

- TMP/SMZ: 4-5 mg TMP/kg/day orally for 180 days following HPE.

This is considered a study medication and will be provided by the study to the families at no charge.

In the unlikely event that the subject develops a hypersensitivity reaction to TMP/SMZ it will be discontinued. The subject will receive all remaining IVIG infusions and continue with follow-up visits as per protocol. .

4.7.6. 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). IVIG has been shown to interfere only with live virus vaccines but not killed virus vaccines. Only killed virus vaccines will be administered to infants under one year of age in this trial. Participants may receive live attenuated viral vaccines at 12-15 months of age (MMR and Varicella vaccinations) because it is very unlikely that substantial amounts of IVIG would still be circulating in the participant at that time. This is based on the last IVIG dose being given 60 days after the HPE procedure, the latter which is expected to be performed at 40 to 100 days of age in the majority of participants with the latest it can be performed in this trial being 180 days of age. The Rotavirus vaccines (live attenuated virus vaccines) are <u>not</u> recommended in children with significant abdominal disorders or following abdominal surgery, and therefore are not recommended for study participants..

<u>Development of infectious diseases and vaccine-preventable diseases</u>

Subjects will be prospectively monitored for the development of infectious diseases and vaccine-preventable diseases. 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.

4.7.7. <u>Prohibited medication</u>: Corticosteroid therapy

The use of corticosteroid therapy (IV or oral, chronic or pulse) for post-operative treatment of BA will <u>not be permitted</u> during this trial and will be treated as a protocol violation. Because the published studies to date do not demonstrate a benefit or the safety of corticosteroid therapy following HPE for BA, and because this therapy may have effects that could potentially confound the analysis of the results of this clinical trial, the investigators have agreed not to use this medication for treatment of BA in participants enrolled in this trial. Corticosteroids may be used at the discretion of the treating caregiver for treatment of aseptic meningitis related to the use of IVIG in this protocol, or for other non-BA related indications, such as reactive airways disease, and their use will be recorded on case report forms. Since these are generally short courses of corticosteroids, we do not believe these courses would significantly interfere with the assessment of the study drug so the subject will remain in the study.

4.8. Visit Schedule for this clinical trial

Subjects will be evaluated in the outpatient clinic (or in hospital if still in hospital from original HPE surgery) 14 days after HPE, at 30 and 60 days after HPE (at time of IVIG infusions), and then at 90, 180, 270 and 360 days after HPE in the outpatient clinic or during inpatient admissions, or at the time of liver transplantation if before 360 days after HPE. The inclusion of the 14 day time point will allow for careful monitoring for any side effects of the first IVIG infusion. During each visit, information will be captured in the ChiLDREN database. This database will capture data particularly related to the course of BA and to potential adverse effects of IVIG, on the interval medical history, physical findings, diet and medication records, laboratory findings, interval sentinel events, illnesses or hospital admissions, according to the schedule in Table 3 (below).

Table 3. Schedule of evaluations for the trial of IVIG therapy in infants with BA

Evaluation	Recruitment or Baseline	Initial Admission and initial IVIG infusions	14 days post HPE	30 and 60 days post HPE	90 and 180 days post HPE	270 days post HPE	360 days post HPE	At Liver Trans-plant
Windows for visits		Initial dose to be given on days 3-5 post HPE	± 3 days	± 5 days	± 20 days	± 20 days	± 30 days	
Informed consent & Eligibility	X							
Intake/Medical History	X							
Intravenous Infusion of IVIG*		X (dose #1)		X (doses #2 and #3)				
Ursodeoxycholic acid therapy		X	X	X	X	X	X	
Trimethoprim-sulfamethoxasole therapy			X	X	X			
Diagnosis and surgery	X							
Medication Record	X	X	X	X	X	X	X	X
Physical Exam, Growth Measures	X	X	X	X	X	X	X	X
-Complete blood count		X (before IVIG dose #1)	X	X	X	X	X	X
-LFTs, GGT, PT/INR -Electrolytes,	X**	X	X	X	X	X	X	X
BUN creatinine		X	X	X	X	X	X	
Research Blood tests - mechanistic studies		X (before IVIG dose #1)		X (before dose #3)	X		X	
Serum IgG level				X (before dose #3)	X	X	X	X prior to TX
Interval Medical History			X	X	X	X	X	X
Interval Adverse Events		X	X	X	X	X	X	X

*dose #1, #2, and #3 administered on days 3-5, 30 and 60 post-HPE, respectively, see Section 4.4.4.
**May use clinical labs from pre-HPE in not performed post.

4.9. Surgical procedures and laboratory tests

Results of surgical procedure(s) and laboratory tests performed for the initial diagnosis of BA will be obtained from the medical records and captured as part of the PROBE database. Tests collected at baseline will include hematologic and biochemical analysis (such as complete blood count, liver function tests [including a measurement of total serum bilirubin], prothrombin time/INR, etc). The following laboratory tests will be obtained in all patients, at the time points shown in Table 3, in order to assess study outcomes and for routine clinical care: liver function tests, electrolytes, BUN, serum creatinine, complete blood count, and prothrombin time/INR. These laboratory tests will be performed using standard automated assays in certified clinical laboratories located at each ChiLDREN clinical center.

4.10. Research Blood Tests

The following research tests (see Summary Table below) will be performed on 4.0 ml of blood obtained at time points in Table 3, prior to infusion #1 and #3 of IVIG and at days 90, 180 and 360 post Kasai (except for liver transplant visit). The purpose of these tests is to determine possible effects of the IVIG on the immune system of the treated infant that may provide clues as to its mechanism of action.

1. Shipment of 3.0 ml whole blood at room temperature to the laboratory of Cara Mack, MD University of Colorado School of Medicine, Aurora, CO, for isolation of peripheral blood mononuclear cells (PBMCs).
2. Flow cytometry studies on PBMCs (see 4.10.1) will include analysis for T-cells, and B-cells, monocyte/macrophages, and neutrophils. These tests will be performed in the laboratory of Cara Mack, MD University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO.
3. 1.0 ml of blood will be obtained and plasma isolated at local site and stored at -70^{o}C.
4. The frozen plasma will be batched and shipped to the CTRC Core Laboratory at Children's Hospital in Aurora, CO for measurement of circulating cytokine levels in plasma by Luminex platform for panel of 13 cytokines (analyzed in the Children's Hospital CTRC Core Laboratory).
5. In addition, an aliquot of the frozen plasma(obtained from the CTRC) will be subjected to analysis for anti-enolase antibodies by ELISA in laboratory of Cara Mack, MD at University of Colorado School of Medicine, Aurora, CO.
6. Total serum IgG (measured at local site and paid for by this study) obtained before dose 3 and then at follow-up visits.

Test	Volume & type	Processing	Laboratory to perform Assay
Isolation of PBMCs	3.0 ml. whole blood	Aurora, Colorado	Cara Mack
Flow cytometry for CD4, CD8, Tregs, and NK	Isolated PBMCs	None	Cara Mack
Isolation of plasma	1.0 ml. whole blood	Local Site	N/A
Circulating cytokines	150 µL plasma	None	CTRC
Anti-enolase antibodies	100 µL plasma	None	Cara Mack
Total serum IgG	0.4 ml serum (1.0 ml	Local Site	Local Site

	blood)		

4.10.1. FACS Analysis of PBMCs

The PBMC flow cytometry analysis will include detailed surface marker expression for the following: T cells, regulatory T cells (Tregs), B cells, moncytes/macrophages, and neutrophils. T cells are defined based on expression of CD3 (present on all T cells), CD4 (helper T cells), CD8 (cytotoxic T cells) and CD56 (natural killer T cells). T cell subset analysis will include the *state of the T cells*: naïve (CD45RA) versus memory (CD27), and the degree of T cell activation based on CD69 and HLA-DR expression. Tregs are defined based on surface expression of CD4 and CD25 and intracellular expression of FoxP3. B cells are identified based on B cell maturation markers CD19, CD20 and CD27. Monocytes/macrophages are identified based on CD14 expression. One mechanism of action of IVIG includes down regulation of IFN-γ Receptor 2 on monocytes, resulting in diminished macrophage activation.[26,27] Therefore, macrophage expression of IFN-γ R2 (CD119) will be determined. Neutrophils are defined based on CD66a and CD16b expression. B cells, macrophages and neutrophils all express the activating Fc-γ receptor (Fc□RIII; CD16) and the inhibitory Fc-γ receptor (FcγRIIB; CD32B). IVIG has been shown to down regulate FcγRIII and increase expression of FcγRIIB resulting in inhibition of activation of these cells.[26,27] Thus, FcγRIII and FcγRIIB expression levels on B cells, macrophages and neutrophils will be determined.

4.11. Preparation and administration of study medication

4.11.1. Preparation, packaging, and labeling

IVIG that will be used in this study will be Gamunex-C® (immune globulin [human],10%) , from a single lot. The IVIG will be shipped periodically to the research pharmacy at each clinical site and stored under standard conditions until used for this study. The IVIG will be labeled "For Investigational Use Only"".

Other study medications (ursodeoxycholic acid and TMP-SMZ will be dispensed by the clinical site research pharmacist during this trial. At each follow-up visit, dosing will be adjusted according to the infant's weight. Changes in infant's weight and dosage will be captured in the database and will be communicated to the family by the research coordinator.

4.11.2. Intravenous formulation

A standard intravenous formulation of IVIG (Gamunex-C®) from a single manufacturer single lot (Grifols Therapeutics, Inc.) as a liquid purified form of human immune globulin will be used for this study.

4.12. Adherence to protocol and withdrawal from the study

4.12.1. 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 attempt at enrollment of all eligible patients, retention of study subjects, 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. Compliance with oral medications will be assessed by the site research coordinator by determining if the subject took at least 80% of their doses.

4.12.2. 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 at any time during the study. Subjects will be removed from the study for any of the following reasons:

- Withdrawal of consent.
- Patient is lost to follow-up

4.12.3. Discontinuation of study medication

IVIG will be discontinued, but patients 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 patient to remain on study medication
- There is a serious adverse event or severe adverse event (level 3, 4 or 5 AE) with evidence of attribution to the IVIG, as outlined in the Data and Safety Monitoring Plan.

Any subject who is withdrawn from the study will no longer receive IVIG and will receive standard medical care. The subject will continue to be followed per protocol even if the IVIG is discontinued, unless the family or investigator withdraws the subject completely from the study.

4.12.4 Subjects receiving a Liver Transplant

If a subject receives a liver transplant prior to 360 days post-HPE, any remaining IVIG infusions will be discontinued and the subject will continue to be followed only for serious adverse events. Serious adverse events will be reported as per section 7.2 and 7.3 of the protocol.

4.13. 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 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 will make an effort to minimize attrition due to logistical considerations.

4.14. Research versus standard clinical care

An 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 4, participation in this trial will specifically require the following which may be outside of standard clinical care: 1) treatment with IVIG, 2) outpatient visit at 14 days after HPE, 3) serum concentration of electrolytes, BUN, creatinine, IgG level, and 4) research blood draws. All other outpatient visits and laboratory studies may be routinely performed as part of standard clinical care of infants with biliary atresia, as summarized in Table 4 below. All study procedures described in the protocol, regardless of whether conducted as part of standard clinical care or not, are mandatory.

Table 4. Outline of care related to this clinical trial versus standard clinical care

Health care item	Conducted by*
Medical encounters/visits	
• 14 days after HPE	T
• 30, 60, 90, 180, 270 and 360 days after HPE	CC
Standard diet and nutritional support	CC
Vitamin supplementation	CC
Ursodeoxycholic acid	T
Antibiotic prophylaxis for ascending cholangitis	T
Administration of IVIG	T
Childhood immunizations	CC
Laboratory monitoring	
• Complete blood count	CC
• LFTs	CC
• Prothombin Time/INR	CC
• Serum electrolytes, BUN, creatinine level	T
• Blood for mechanistic research studies	T
• Serum IgG level	T

* T=Collected or administered by this clinical Trial;
CC=Collected or administered as part of standard Clinical Care.
HPE=hepatic portoenterostomy surgery

5. STATISTICAL ANALYSIS AND DATA MANAGEMENT

5.1. Summary of Study Design

This study is a multicenter prospective phase 1/2A open-label trial in which the feasibility, acceptability, tolerability and safety of IVIG therapy will be assessed post-HPE in infants with biliary atresia, preliminary efficacy (activity) will be estimated, and exploratory

mechanistic research studies will be performed. Twenty-nine subjects will be enrolled into the trial, which involves three intravenous doses of IVIG at designated intervals over the first 60 days following HPE with follow-up for 360 days after enrollment.

5.2. Sample size

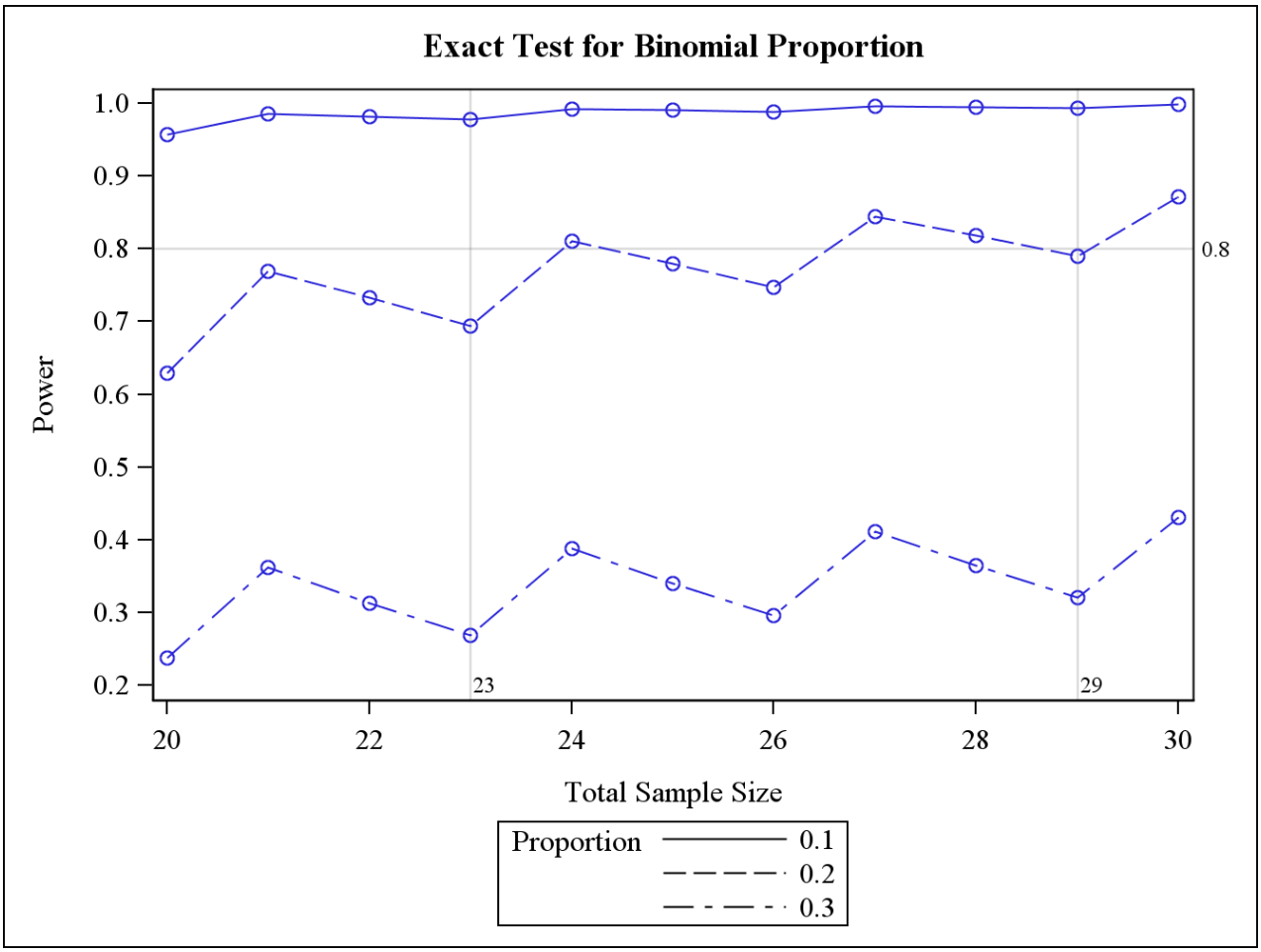
This Phase 1/2A study is primarily sized based on the precision to estimate the feasibility, tolerability and safety of IVIG in the population of infants with biliary atresia; the sample size of 29 subjects has been calculated, with 20% loss-to-follow-up expected (resulting in 23 subjects). With this sample size, we also calculated the power to compare preliminary efficacy (activity) outcomes between the IVIG subjects in this study and a comparable population of historical controls from another ChiLDREN study.

5.2.1. Feasibility, Acceptability, Tolerability and Safety

To determine the feasibility, acceptability, tolerability and safety profile of IVIG treatment after HPE for BA (Aim 1), we will determine the proportion of participants who have feasibility, acceptability, tolerability and safety problems with IVIG treatment. For convenience below, we discuss power and precision in terms of adverse events (reflecting the focus on safety), although the same considerations apply to each of feasibility, acceptability, and tolerability. We anticipate that 23-29 subjects will receive IVIG. With this number of treated subjects, we have greater than 98% power to observe at least one subject with a specific adverse event when the true event rate is 20%, 91 to 95% power to observe at least one subject with a specific adverse event when the true event rate is 10%, and 69 to 77% power to observe at least one subject with a specific adverse event when the true event rate is 5%, based on calculations from the binomial distribution.

The power to reject the null hypothesis that the proportion of subjects with either a specific adverse event or any adverse event is 40% or higher, if the true proportion is 10%, 20% or 30% and using a one-sided 10% Type I error rate is provided in Figure 4. With 23 treated subjects, there is 70% power to detect a 40% or higher proportion if the true proportion is 20%, and 79% power to detect this difference with 29 treated patients (exact one-sample binomial test, SAS 9.3). These considerations also apply to the other outcomes described in Aim 1 – feasibility, acceptability and tolerability.

Figure 4. Power to Detect Proportion of Outcomes ≥ 40%, if the True Proportion is 10%, 20%, or 30% for Various Sample Sizes and one-sided Type I error of 10%



It is important to note that the level used for the null hypothesis in these calculations (40% or higher) is arbitrary and used for purposes of illustration only. Depending on the nature of the adverse event, a much higher rate might well be acceptable if the event is relatively minor and there is clear benefit in terms of lower serum bilirubin or higher survival without liver transplantation, while a much lower rate of a very serious and unusual adverse event might be unacceptable even in the presence of higher survival without liver transplantation.

5.2.2. Preliminary Efficacy (Activity).

The use of concurrent placebo or active control subjects was considered for this study, however it is not feasible to treat infants in a research study with an IV placebo (normal saline) and the availability of robust patient-level data for historical controls from another ChiLDREN study were felt to justify the bias issues inherent in the use of historical controls.

Placebo subjects from the ChiLDREN START (Steroids in Biliary Atresia Randomized Trial) study will be used as a historical comparative group to compare with subjects in this study who receive IVIG. Approximately 70 subjects in START received placebo from 2006-2011, but were otherwise treated similarly to the subjects in the current trial, except they received no IVIG. It is anticipated that the START randomized double-blind study will be unblinded in early 2013. With the anticipated length of this IVIG study (24-30 months enrollment and 12 months of follow-up), we will be able to use the START placebo subjects as the comparative group.

A one-sided testing strategy was chosen given that we are only interested if IVIG improves the preliminary measures of efficacy.

Hypothesis 2a states that treatment with IVIG will improve the probability of an infant having good bile flow 90 and 180 days after HPE for BA as evidenced by total serum bilirubin concentration. The primary endpoint for this hypothesis is the proportion of subjects who survive 180 days after HPE with both their native liver and serum total bilirubin <1.5 mg/dL at 180 days after HPE. Based on the ChiLDREN PROBE prospective study, approximately 51% of BA subjects who had an HPE before 4 months of age had good bile drainage at 6 months following the HPE procedure, as determined by a serum total bilirubin level <1.5 mg/dL and survival with their native liver. We anticipate the same improvement in the placebo subjects in the ChiLDREN START study.

Assuming 20% loss-to-follow-up in the ChiLDREN START study and in this study (i.e., 23 subjects in the IVIG group and 56 subjects in the START placebo group), there is 74% power with a one-sided Type I error rate of 10% to detect a difference of 25.5% -- a 50% improvement with treatment (i.e., 76.5% in the IVIG group and 51% in the placebo group). This is based on Fisher's exact test for two proportions (SAS 9.3). The corresponding power for sample sizes of 29 and 70 in the IVIG and placebo groups (if there were no drop-outs) is 82%. Alternatively, we have 80% power to detect a difference of 28% in this outcome (79% in the IVIG group and 51% in the placebo group) with sample sizes of 23 and 56 in the IVIG and placebo groups, reflecting a possible 20% drop-out rate. The corresponding power for sample sizes of 29 and 70 in the IVIG and placebo groups, if there were no drop-outs, is 88%.

Hypothesis 2b states that treatment with IVIG will improve the probability of an infant having good bile flow 360 days after HPE for BA as evidenced by the proportion of subjects who survive 360 days after HPE with both their native liver and serum total bilirubin <1.5 mg/dL at 360 days after HPE. In the prospective PROBE study, approximately 68% of BA patients who had an HPE by 4 months of age survived to 12 months of age with their native liver and had serum bilirubin <1.5 mg/dL. We assume that a comparable proportion of placebo subjects in START will have this outcome. There is 76% to 85% power to detect a 33% relative improvement in this outcome with the anticipated sample sizes described above (ranging from 23 and 56 in the IVIG and placebo groups, respectively, when adjusting for 20% drop-out, to 29 and 70 in the IVIG and placebo groups, respectively, if there were no drop-out), based on Fisher's exact test for two proportions (SAS 9.3) and a one-sided Type I error rate of 10%. This relative

improvement corresponds to approximately 90% of the IVIG subjects having improved bile flow at 360 days after HPE, compared to an anticipated 68% in START placebo patients.

Hypothesis 3 states that survival with native liver at 360 days following HPE is improved in infants treated with IVIG compared to historical controls. Since 68% of PROBE BA patients who had their HPE by 4 months of age survived to 12 months of age with their native liver and with serum total bilirubin < 1.5 mg/dL, the power calculations for Hypothesis 2b apply identically to Hypothesis 3.

5.2.3. Biomarkers. There are no data to inform the precision to estimate the exploratory biomarkers studied for the first time in IVIG-treated human subjects with BA.

5.3. Data Analysis

General
The data for analytic variables will be examined to identify unusual values, patterns of missing values and whether their distributions are non-Gaussian (i.e., have significant skewness and kurtosis). 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. Descriptive statistics (means, SD, and percentages with appropriate one-sided confidence intervals [CI]) and graphical displays (histograms, scatterplots, Kaplan-Meier curves) will be used to summarize study variables.

Aim 1: To determine the feasibility, acceptability, tolerability and safety profile of IVIG treatment after HPE for biliary atresia.

Hypothesis 1: Administering IVIG will be feasible, acceptable and well tolerated in infants with BA without significant toxicity.

The percentage of subjects (and one-sided 90% Clopper-Pearson lower confidence bounds) in which the study is feasible and acceptable will be calculated. Point estimates and one-sided 90% Clopper-Pearson lower confidence bounds will be calculated for safety and tolerability parameters.

Aim 2: To obtain preliminary evidence whether IVIG therapy is associated with lower serum bilirubin concentration after HPE.

***Hypothesis 2a*:** The probability of an infant having good bile drainage at 90 and 180 days after HPE (as defined by survival 90 or 180 days after HPE with both their native liver and serum total bilirubin level <1.5 mg/dL at 90 or 180 days after

HPE) will be greater in infants who are treated with IVIG compared to historical controls.

The proportions of subjects who have survived with their native liver and have bilirubin <1.5 mg/dL will be calculated at both time points. Each proportion will be compared to that of historical controls from the placebo group in the START study using Fisher's exact test. Given that previous studies have found age at HPE to be predictive of later clinical outcomes, we will perform an exploratory logistic regression analysis, including IVIG treatment (vs control) and age at HPE as covariates. Odds ratios and one-sided 90% Wald upper confidence bounds will be presented.

***Hypothesis 2b*:** The probability of an infant having good bile drainage at 360 days after HPE (as defined by survival 360 days after HPE with both their native liver and serum total bilirubin level <1.5 mg/dL at 360 days after HPE) will be greater in infants who are treated with IVIG compared to historical controls.

The same methods will employed as in Hypothesis 2a.

Aim 3: To obtain preliminary evidence whether IVIG treatment after HPE will improve survival without liver transplantation at 360 days after HPE.

Hypothesis 3: Survival without transplantation will be greater at 360 days after HPE in infants treated with IVIG compared to historical controls.

The proportion of subjects who have survived with their native liver at 360 days after HPE will be estimated among the IVIG treated subjects and the historical placebo controls by Kaplan-Meier methods. Time to liver transplantation or death will be calculated as the number of days from the day of HPE to the first of liver transplantation, death, or last day of follow-up without these events. Subjects will be censored if these events don't occur by the last day of study follow-up. The difference between the two Kaplan-Meier estimates will be computed and a 90% upper confidence bound will be calculated for the difference, using the pooled variance (based on each group's standard error calculated with Greenwood's formula).

Aim 4: **To explore mechanisms of action of IVIG treatment in biliary atresia.**

Hypothesis 4: IVIG treatment in BA is associated with an increase in circulating regulatory T-Cells and reduction of inflammatory cytokines and specific autoantibodies.

The percentage and absolute numbers (with corresponding two-sided 80% CIs) of circulating regulatory T-cells, circulating cytokine levels and IgM anti-enolase autoantibody levels (and two-sided 80% CIs) (detailed in section 4.3.2) will be calculated at each time point measured and each will be compared to the baseline values before

IVIG administration using the paired *t* or nonparametric Wilcoxon signed rank tests. In addition, trends over time (prior to IVIG and 60, 90, 180 and 360 days after HPE) will be explored using linear or non-linear methods. Changes from baseline in these parameters will be compared between those subjects who achieve normal serum bilirubin at 180 days after HPE and those that do not, and between those that survive with native liver at 360 days after HPE and those that do not by the 2-sample *t* or nonparametric Wilcoxon rank sum test. Although the hypothesis is stated in terms of increases or decreases, thus suggesting one-sided CIs, two-sided 80% CIs will be presented that allow testing of both increases or decreases for each parameter. No adjustments for multiple comparisons will be made, owing to the exploratory and hypothesis-generating nature of this aim.

5.4. Data Management

The Statistical Analysis of Biomedical and Educational Research (SABER) unit 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 provides and maintains computerized data management systems with capabilities for central data entry, remote site data entry, editing, modifying, and reporting of data.

5.4.1. Data Collection

The DCC maintains a password-protected website for all studies in ChiLDREN. All transmissions to and from the website are encrypted using SSL. Each subject is identified by a study specific subject identification number. Data collection does not contain any personal subject identifiers, except dates, such as date of birth, which are necessary for research purposes. An investigator and/or study coordinator can only view data in the study database from his/her clinical site.

5.4.2. Quality assurance

The DCC staff monitors and reports on data quality, protocol adherence, and recruitment status. The DCC staff conducts site visits for data review on a periodic basis for quality assurance. During these visits the clinical monitor reviews the regulatory file, checks all informed consent documents, and reviews procedures with the site coordinators. Interim site visits may be made to centers with low compliance or high error rates. The DCC is responsible for reporting on safety and data quality and timeliness to the NIDDK and the NIDDK appointed Data and Safety Monitoring Board. The DCC staff assists with the preparation of study materials, including the final protocol and the manuals of operation for each study to optimize clinical site performance in study conduct.

5.4.3. Training

The DCC staff will verify that clinical site staff are trained and/or certified in study conduct and procedures. The DCC has developed general and project-specific manuals of operations to assist investigators and study coordinators in the conduct of this trial, entering and transferring data, and collecting, processing and shipping samples. Clinical coordinators will participate in a protocol initiation and electronic data capture system training session provided by the DCC. The Project Manager/Clinical Monitor will review the study protocol and data entry system, and check all regulatory documents prior to site initiation.

6. HUMAN SUBJECTS RESEARCH CONSIDERATIONS

6.1. Risks to study subjects

6.1.1. Involvement of subjects
Subjects will be recruited into this study at the time that BA is suspected and exploratory surgery is scheduled or within 3 days following HPE. Subjects will be treated with three infusions of IVIG and followed as outpatients for 360 days following HPE.

6.1.2. Planned duration of the entire study
Based on the anticipated enrollment of 29 subjects per year by the CHILDREN centers involved in this trial, we estimate completing enrollment within 18-24 months, with an additional 6 months to gather primary outcome follow-up data on subjects enrolled in the final year. However, the testing of secondary outcome measures (such as survival with native liver at 360 days after HPE) will extend the overall duration of the study to 2.5 to 3 years from the initiation of enrollment.

6.1.3. Duration of participation for each subject
Each subject will remain in the clinical trial for 360 days. The use of IVIG will be limited to the first 60 days. This will be followed by very close follow-up in outpatient clinics for an additional 300 days (total 360 days post-HPE follow-up). 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.

6.1.4. 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 for this study.

6.1.5. Potential risks to subjects
There are three potential sets of risks associated with this clinical trial. The first set derives from blood draws, and includes amount of blood, as well as pain, bruising, or superficial phlebitis. Most of the scheduled venipunctures are part of routine tests necessary for the proper care of infants following HPE; serum levels of electrolytes, BUN and creatinine and IgG will be included to monitor for possible adverse effects of IVIG treatment. The second set of risks derives from expected adverse events associated with IVIG administration or from the other standard of care medications. The third set of risks derives from peripheral IV insertion and use. Several of these are discussed in more detail:

6.1.5.a. Amount of blood

Blood is drawn as part of the routine clinical care of the BA patient following HPE, to monitor for liver function and adverse events. Blood will also be drawn for mechanistic research studies for this clinical trial. The cumulative amount of blood to be drawn for research purposes is outlined in Table 5 below.

Table 5. Total amount of blood drawn from infants in the clinical trial

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	5.0	5.0	5.0
2 weeks post op	1.0	6.0	6.0
30 days post op	1.0	7.0	7.0
60 days post op	6.0	13.0	13.0
90 days post op	6.0	12.0	19.0
180 days post op	6.0	6.0	6.0
270 days post op	2.0	2.0	2.0
360 days post op	6.0	6.0	6.0
At liver transplant	1.0		

NOTE: Blood volume for clinically indicated tests: Approximately 2.2-3.3 ml of blood may be removed from the child at each visit to evaluate hepatic function, , PT/INR and complete blood count and differential. More may be withdrawn to perform additional clinically indicated lab tests.

At each follow up visit, 0.6 ml of blood will be drawn for a complete blood count and differential, 1.0 ml for hepatic function tests, and 0.6-1.7 ml for a PT/INR. 1.0 ml will be drawn for safety related serum electrolytes, BUN and creatinine. At the initial blood draw prior to IVIG and follow-up visits at 60, 90, 180, and 360 days post-HPE, 4.0 ml of blood will be drawn for mechanistic research studies. An additional 1.0 ml of blood will be drawn at 60, 90, 180, 270 and 360 days post-HPE for serum IgG level.

6.1.5.b. Side effects of IVIG

The second set of potential risks derive from reported side effects of IVIG which include headache, irritability, flu-like symptoms, fever, nausea, fluid overload, allergic reactions, local infusion site reactions, viral contamination of the product, interference with live vaccines, aseptic meningitis, renal dysfunction, thrombotic events, hypotension and hypoglycemia. All of these side effects are uncommon or not serious in infants and young children (45-46). Despite these potential adverse events, this clinical trial is justified by the devastating nature of BA on the well-being of affected children and on the progression to end-stage liver disease in most patients with the prospects for improved care of future patients with BA if IVIG is found to be beneficial. In this context, IVIG treatment may result in reduced hepatic inflammation, improved bile flow, allow for better

growth, and increase long-term survival with the native liver. To minimize potential adverse events to subjects, it is recommended that the IVIG be diluted to a 5% solution when administered into a peripheral vein if this is acceptable by the local institutional policy. In addition, the clinical site staff will monitor all subjects closely during each IVIG infusion for side effects, and promptly adhere to their institutional action plan and that outlined in the Data Safety Monitoring Plan (DSMP) if side effects are identified (see Section 7.4).

6.1.6. Alternative treatments considered

IVIG treatment is being considered in this study as an adjuvant treatment for children with biliary atresia. There is currently no other available treatment for BA of proven efficacy and safety that is not being used in this trial. At present, other adjuvant therapies may be under development, such as anti-inflammatory agents that target specific components of the immune system, anti-fibrogenic medications and others. We are aware of only one ongoing trial of one such medication (corticosteroids trial of ChiLDREN – the START study, NIDDK IND #71411) that has fully enrolled its participants and from which results may become available prior to completion of the PRIME trial. These results may impact the PRIME study and adjustments may need to be made in the PRIME protocol after the START results are finalized. Preventive approaches to biliary atresia may also become available in the future as new discoveries are made on pathogenic agents causing biliary atresia.

6.2. Adequacy of protection against risks

6.2.1. 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 inclusion and exclusion criteria will also protect subjects against potential adverse effects of IVIG 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.

6.2.2. Description of recruitment plan

See Section 4.4.2

6.2.3. 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; the original signed consent will be retained in the subject's medical record and a copy 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.

6.2.4. Assent

Assent will not be sought from study subjects because they will be infants at entry and during the duration of this study.

6.3. Potential Benefits

6.3.1. Description of possible health benefits to subjects

The use of IVIG as an adjuvant therapy following HPE has the potential to reduce hepatic and bile duct inflammation and increase bile drainage in infants with biliary atresia following the HPE surgery. This is based on the beneficial anti-inflammatory effects of IVIG in a variety of other immune-mediated or autoimmune diseases in children and in adults. We anticipate that improved bile drainage 90, 180 days or 360 days after HPE will lead to better long-term outcome, however this study is not designed to test that hypothesis. The design of this clinical trial within CHILDREN will allow us to generate data on short term clinical outcomes that may inform the need for a larger randomized more prolonged clinical trial to define efficacy and safety of IVIG. Given the relatively low toxicity profile of IVIG in infants and children, we believe that the potential therapeutic benefit for future patients with biliary atresia justifies the use of this treatment in this phase 1/2A research study.

6.3.2. Description of any incentives or rewards offered for participation

Parents or guardians of infants enrolled in the study will receive a small stipend in compliance with local policies per outpatient visit to reimburse them for meals and/or parking fees according to costs at each clinical site. The supplies of IVIG will be provided to each study site. In addition, the clinical site research pharmacy will provide a supply of prescribed doses of ursodeoxycholic acid, and trimethoprin-sulfamethoxasole. The provision of these items at no cost to the family will help minimize variations in routine clinical care among CHILDREN clinical sites, and may serve as an incentive to study participation.

6.3.3. Tests performed as part of research

Laboratory tests to evaluate side effects of the IVIG (e.g., electrolytes, BUN and creatinine, serum total IgG), will be paid for by the research grant. Mechanistic research blood tests will also be paid for by the research grant.

6.4. Summary of importance of knowledge to be gained from this research

Knowledge gained from this phase I/IIa clinical trial may increase the generalizable knowledge about the mechanisms involved in the pathogenesis of biliary atresia and its potential response to IVIG as a therapeutic agent. The risks of this study represent a minor increase above minimum risk. Currently there is no direct evidence of a benefit of IVIG in biliary atresia (it has not been reported to have been used in this disease), however many other immune and autoimmune-mediated inflammatory diseases have

been shown to respond to IVIG, justifying the plausibility of IVIG as a therapy for biliary atresia. Mechanistic studies in this protocol may provide insight into immunologic processes involved in the pathogenesis of BA that may be altered by IVIG therapy. It should be emphasized that biliary atresia currently has very poor treatment options but has very serious clinical consequences. The results of this study may provide the justification for further testing of a readily available, safe therapy for treatment of biliary atresia, i.e., IVIG. The experimental design of this trial will provide important information about short term feasibility, tolerability and safety of IVIG administration in this population of young patients. The results of this study could also provide some initial evidence of potential benefit of IVIG in biliary atresia. This combined new knowledge gained from this study should be of value in determining if a larger phase 2 or 3 clinical trial of IVIG should be entertained in infants with BA.

7. DATA AND SAFETY MONITORING PLAN (DSMP)

7.1. Summary of DSMP

The DSMP aims to monitor this research protocol with emphasis on patient safety and data integrity. To this end, implementation of 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 the following:

- Verification that patient 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 patient safety issues. To this end, a Study Medical Safety Officer, independent of all investigators in the study, 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).

7.2. Adverse events (AE) and serious adverse event (SAE)

7.2.1. Adverse events (AE) – Overview

IVIG has not been used in infants with BA following HPE to our knowledge. However, IVIG has been used to treat immune deficiencies, Kawasaki disease, neonatal hemochromatosis and idiopathic thrombocytopenic purpura (ITP) in infants and young children previously, and has been tested in small preterm infants as a therapy to reduce the risk for infection and sepsis. In this study, we will monitor for potential adverse effects appropriately at each scheduled visit. Patients 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 the package insert of IVIG and reviews of the published clinical trials of IVIG in the diseases mentioned above (45-46). Notably, some of the potential adverse effects of IVIG may also be present in infants with BA due to the natural progression of disease to biliary cirrhosis, such as fever, rashes, and impaired response to routine immunizations. Therefore, it is important in this study that the frequency, severity and seriousness of adverse effects, as well as their likely causality, be assessed in subjects receiving study medication.

7.2.2. 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

7.2.3. Serious Adverse Events (SAE) or Serious Suspected Adverse Reaction

Serious Adverse Event or Serious Suspected Adverse Reaction

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

- Death,
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

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

Suspected Adverse Reaction
A Suspected Adverse Reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. “Unexpected”, as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Expected Adverse Event or Expected Adverse Reaction
Expected events are any adverse experiences that have been identified in nature or severity in the current investigator brochure and/or protocol.

7.3. AE and SAE reporting plan

An independent physician not involved in the study will serve as the Medical Safety Officer, reviewing all SAEs promptly after being reported in the database by the clinical sites. The Medical Safety Officer will prepare a final SAE narrative report for each SAE which will be distributed to the NIDDK Program Director and DSMB Chair. In addition, the DSMB will review all AEs during their regularly scheduled meetings, or on an expedited basis as determined by the NIDDK Program Director, who will solicit the input of the Chair of the DSMB as needed

All adverse events are reported to the DCC by completion of the Adverse Events Form. All SAEs as defined previously require expedited event notification within 24 hours of occurrence or identification to the DCC.

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 report. 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 to the DSMB for review during their regularly scheduled meetings. The FDA definitions and requirements for expedited reporting will be used to determine if any individual SAE warrants notification to the FDA and to the IRBs 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.

On behalf of the NIDDK , the Data Coordinating Center will submit an expedited safety report to the FDA for all serious unexpected suspected adverse reactions (SUSARs). That is, when the SAE is unexpected and may be related to the study drug based on evidence of causality. This report will include information on frequency of similar events along with a narrative of similar events to provide context for the individual report. Copies of the expedited safety report will be provided to NIDDK, DSMB, and site investigators.

7.4. Monitoring and management for specific AEs

Several side effects of IVIG may occur in the subjects during this trial. The following section outlines the plan for monitoring and management of these AEs. This monitoring will be conducted after HPE but 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.

7.4.1. Elevation in Blood Pressure related to Increased Fluid Volume

Although unlikely, hypertension could develop due to the fluid volume administered with each IVIG infusion in this study (20 ml per kg body weight over 6-8 hours for each infusion). Vital signs will be continually monitored during the IVIG infusions per institutional protocol. If hypertension develops (systolic BP ≥112 mmHg) during an IVIG infusion, the infusion rate will be reduced or stopped and BP will be measured again in 30 minutes. If BP remains elevated, intravenous furosemide (1 mg/kg/dose) will be an option based on the clinical judgment of the treating physician if fluid volume is considered the likely cause of the hypertension. Alternatively, if the patient is asymptomatic, BP can be re-measured for 30 minutes without treatment. Blood pressure will continue to be monitored each 15 minutes until resolution of the hypertension. If the IVIG infusion must be stopped completely, the subject will not receive further doses of IVIG.

Additional treatment options for the hypertension include:

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

7.4.2. 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 patient is being followed as an outpatient, or evaluation by the clinical inpatient or outpatient team if the patient is in hospital receiving IVIG infusion at the time of the event. If the severe irritability develops during an IVIG infusion, the infusion will be slowed down or stopped. Following evaluation for irritability and if no obvious cause is identified, the subject will be treated symptomatically and will not receive additional doses of IVIG. Consideration for a lumbar puncture and evaluation for the rare aseptic meningitis syndrome (see 7.4.4) that has been reported with IVIG therapy will be based on the clinical discretion of the treating physician. The irritability may also be a manifestation of headaches that occur in 20-30% of adults who receive comparable doses of IVIG.

7.4.3. Hypersensitivity reaction to trimethoprim-sulfamethoxazole

If the patient develops a hypersensitivity reaction to TMP/SMZ manifested by a suspicious skin rash or other acute allergic symptoms, the medication will be discontinued promptly. The subject will continue to receive remaining IVIG infusions and followed as per protocol.

7.4.4. Aseptic meningitis syndrome (AMS)

AMS may occur infrequently with IVIG treatment. Discontinuation of IVIG treatment has resulted in remission of AMS within several days without sequelae. Administration of corticosteroids may also be helpful, the decision made upon consultation with neurology or infectious diseases specialists. The syndrome usually begins within several hours to two days following IVIG treatment and is characterized by the following symptoms and signs: severe headache, nuchal rigidity, drowsiness, fever, photophobia, painful eye movements, nausea and vomiting. Cerebrospinal fluid (CSF) studies are frequently positive with pleocytosis up to several thousand cells per cu mm, predominantly from the granulocytic series, and with elevated protein levels up to several hundred mg/dL, but negative culture results. Subjects enrolled in this study who exhibit any of these symptoms or signs will have a thorough neurological examination performed including

CSF studies, to rule out other causes of meningitis. Because AMS may occur more frequently in association with high doses (2 g/kg) and/or rapid infusion of IVIG, the risk of AMS will be lowered in this study by using 1 g/kg doses of IVIG and using slow infusion rates. The occurrence of AMS will be an indication to discontinue any further IVIG infusions in the subject.

7.4.5. Renal Dysfunction

Development of acute renal failure may occur during IVIG administration in patients who are volume depleted at the time of IVIG administration or in patients with underlying renal dysfunction. For this reason an exclusion criteria for this trial is the presence of underlying renal dysfunction or significant congenital renal malformations. Renal dysfunction and acute renal failure occur more commonly in patients receiving IVIG products containing sucrose; the preparation used in this clinical trial does not contain sucrose. To prevent development of acute renal failure, we will assure that patients are not volume depleted prior to the initiation of the infusion of IVIG. Periodic monitoring of renal function and urine output will be performed during outpatient follow up. Renal function (measurement of blood urea nitrogen (BUN)/serum creatinine) will be measured prior to the initial infusion of IVIG and before each subsequent dose. If renal function worsens (doubling of baseline serum creatinine that exceeds the upper limit of normal) in a subject during this trial, IVIG infusions will be discontinued.

7.4.6. Fluid volume overload

Because of the fluid volume administered with each IVIG infusion in this study (20 ml per kg body weight of a 5% Gammunex-C solution over 4-8 hours for each infusion), individuals with expanded fluid volume, or where fluid volume may be a concern, will not be enrolled in this study. On the day of doses #2 and #3, the physical exam must be completed by a physician prior to the infusion to rule out signs of fluid overload or congestive heart failure. IV fluids will be reduced by 20 ml per kg per day for the day that dose #1 is administered, and if IV fluids are being administered for the days that doses #2 and #3 are given. If fluid volume problems develop during an IVIG infusion, the infusion will be stopped and appropriate steps will be taken to reduce fluid intake and consideration of use of intravenous diuretic medications (e.g. furosemide I mg/kg IV). If fluid volume status has improved at the time of the subsequent IVIG dose, the dose may be given.

7.4.7. Thrombotic events

Patients at risk may include those with a history of atherosclerosis, multiple cardiovascular risk factors, advanced age, impaired cardiac output, coagulation disorders, prolonged periods of immobilization and/or known or suspected hyperviscosity. Since none of these are likely to be present in the subjects enrolled in this clinical trial, we believe the risk of thrombosis is minimal. Subjects will be evaluated for thrombosis if coolness of an extremity or poor perfusion of another body part is noted clinically or if an acute pulmonary event (e.g., pulmonary embolism) or central nervous system event occur. If a subject develops a thrombosis that is thought to be related to the IVIG, no further IVIG infusions will be given.

7.4.8. Hypersensitivity Reactions (Fever, rash, urticaria)

Severe hypersensitivity reactions may rarely occur with IVIG products, manifested by fever, erythematous rash, urticaria, breathing difficulties and hypotension. In case of hypersensitivity, IVIG infusion will be discontinued immediately and appropriate treatment will be initiated. Medications such as epinephrine will be available at time of IVIG infusion for immediate treatment of acute hypersensitivity reactions. Some centers give prophylactic acetaminophen and diphenhydramine prior to IVIG given for clinical indications, to prevent these reactions. This will be allowed as per local protocol if this is the standard of care for IVIG infusions at any study site. Patients with known antibodies to IgA (those with congenital IgA deficiency) may have a greater risk of developing potentially severe hypersensitivity and anaphylactic reactions, however this has not been observed in infants treated for Kawasaki Disease who do not routinely get tested for IgA deficiency. In addition, infants under 4 months of age may normally have unmeasurable IgA, thus determining IgA deficiency at this age may not be possible. IVIG will not be used in subjects who have a history of hypersensitivity reaction to IVIG. If a subject develops a hypersensitivity reaction to the IVIG, no further infusions of IVIG will be given.

7.4.9. Transmissible infectious agents

Because IVIG is made from human blood, it may carry a risk of transmitting infectious agents, e.g., viruses, and theoretically, the Creutzfeldt-Jakob disease (CJD) agent. IVIG products have almost no risk of HIV or HCV transmission. All infections that are suspected by an investigator to possibly have been transmitted by the IVIG product will be reported as AEs and will also be reported to the manufacturer of the IVIG preparation used in this study. If a subject develops an infection possibly transmitted by the IVIG, the subject will not receive any subsequent IVIG infusions.

7.4.10. Hemolysis

IVIG products may contain blood group antibodies which may act as hemolysins and induce in vivo coating of red blood cells (RBCs) with immunoglobulin, causing a positive direct antiglobulin reaction and, rarely, hemolysis. Delayed hemolytic anemia can develop subsequent to IVIG therapy due to enhanced RBC sequestration, and acute hemolysis consistent with intravascular hemolysis, has been reported. Thus, subjects in this study will be monitored for clinical signs and symptoms of hemolysis and for anemia by complete blood count and total serum bilirubin. If signs and/or symptoms of hemolysis are present after IVIG infusion, a hematology consultation will be obtained to assist with management and the subject will not receive any subsequent IVIG infusions.

7.4.11. Transfusion-related Acute Lung Injury (TRALI)

Noncardiogenic pulmonary edema may occur in patients following treatment with IVIG products. TRALI is characterized by severe respiratory distress, pulmonary edema, hypoxemia, normal left ventricular function, and fever. Symptoms typically occur within 1 to 6 hours after IVIG infusion. Patients will be monitored for pulmonary adverse reactions following IVIG infusions in this study. If TRALI is suspected, appropriate tests will be performed for the presence of anti-neutrophil and anti-HLA antibodies in both the IVIG product and patient serum. TRALI will be managed with oxygen therapy and

adequate ventilatory support. If a subject develops an episode of TRALI, he/she will not receive any subsequent IVIG infusions.

7.4.12 Adverse Events Associated with Peripheral IV Catheters

Peripheral intravenous lines that are placed for clinical purposes will be used for IVIG infusions and blood draws whenever possible. However, IV lines may be needed for purposes of this study, especially for IVIG infusions #2 and #3. The risks of placing an IV line in an infant include some redness, swelling, pain or bruising at the IV site. IV fluid infiltration of the IV sites may also occur. Rarely, infection or thrombosis of the vessel entered will occur. For these reasons, IV insertion sites will be monitored each 15-30 minutes during the IVIG infusions of this study and the IV lines will be removed promptly at completion of the infusion visit. Infiltrations will require removal of the IV line and the site will be treated with a cold compress. In the very unlikely event of a thrombosis of the vessel entered, the IV will either be removed or a vascular ultrasound examination will be performed to determine if a thrombosis is present. If thrombosis is found, hematology will be consulted and local recommendations will be followed for treatment of the thrombosis.

7.5 Stopping Criteria

Feasibility, tolerability and safety of this study will be monitored continuously during the course of this study by the Medical Safety Officer, the DSMB and the NIH Project Staff. If at any time, the frequency or nature of SAEs or AEs becomes concerning, enrollment in the study will be put on hold and a more thorough analysis of SAE and AE frequency, causation and risk will be made and a decision made regarding continuation or stoppage of the study.

8. DATA AND SAFETY MONITORING BOARD (DSMB)

Data and safety will be monitored by the NIDDK in conjunction with an NIDDK-appointed Data and Safety Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the study. The description of DSMB activities is included in the DSMB charter.

Food and Drug Administration

The investigators, sponsor, and DCC, will be conducting this study with the oversight of the Food and Drug Administration (FDA) under an Investigational New Drug (IND) application. The trial will not begin until the IND application is in effect.

The investigators will complete a Statement of Investigator (FDA Form 1572) and must obtain IRB approval per the Code of Federal Regulations.

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 and one in Canada 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 TMP/SMZ 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 (Table 6).

Table 6. Total Planned Enrollment:

TARGETED/PLANNED ENROLLMENT: Number of Subjects The Entire Study (29)			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	2	2	4
Not Hispanic or Latino	14	11	25
Ethnic Category Total of All Subjects*	16	13	29
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	3	2	5
White	12	10	22
Racial Categories: Total of All Subjects*	16	13	29

9.C. Inclusion of Children

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

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