Supplementary Online Content

Bezerra JA, Spino C, Magee JC, for the Childhood Liver Disease Research and Education Network (ChiLDREN). Use of corticosteroids after hepatoportoenterostomy for bile drainage in infants with biliary atresia: the START randomized clinical trial. *JAMA*. doi:10.1001/jama.2014.2623

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1: Inclusion and exclusion criteria for START

Inclusion Criteria

1. Portoenterostomy or gall bladder Kasai operation for biliary atresia within the previous 72 hours
2. Post-conception age ≥ 36 weeks
3. Weight at enrollment ≥ 2000 gm
4. Written informed consent to participate in the study obtained prior to or within 72 hours of completion of portoenterostomy

Exclusion Criteria

1. Known immunodeficiency
2. Diabetes mellitus
3. Presence of significant systemic hypertension for age (persistent systolic blood pressure ≥112 mmHg)
4. A serum indirect (unconjugated) bilirubin ≥ 5 mg/dL for infants under 4 weeks of age or ≥ 7 mg/dL for infants between 4 and 8 weeks of age
5. Known sensitivity to corticosteroids
6. Documented bacteremia or other tissue infection which is felt to be clinically relevant
7. Known congenital infection or disease with herpes simplex virus, toxoplasmosis, or cytomegalovirus inclusion disease of the liver
8. Infants whose mother is known to have human immunodeficiency virus infection
9. Infants whose mother is known to be HBsAg or hepatitis C virus positive

10. 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
11. Any other clinical condition that is a contraindication to the use of corticosteroid (e.g., bowel perforation)
12. Infants who have received the live attenuated rotavirus vaccine (e.g. Rotateq) within 5 days prior to proposed administration of study drug

eTable 2: Clinical care guidelines for postoperative care for the duration of the study

Diet

1. Maternal breast milk or MCT-containing formula: Participants with total bilirubin ≥1.5 mg/dL and the child is <12 months of age, as long as the child's growth is adequate
2. Maternal breast milk or standard infant formula: Participants with total bilirubin <1.5 mg/dL and the child is <12 months of age, as long as the child's growth is adequate
3. Pregestimil® formula (provided to the study by Mead Johnson Nutrition, Evansville, IN, as a part of a cooperative agreement with the NIDDK): To be continued until 24 months of age if the total bilirubin is ≥1.5 mg/dL and the child is ≥12 months of age
4. When growth is inadequate, measures will be taken for nutritional rehabilitation according to medical management used at each ChiLDREN clinical center.

Vitamin Supplementation

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

1. AquADEK® (provided to the study by Axcan Pharma U.S, Inc., Mont-Saint-Hilaire, Qc, Canada, as a part of a cooperative agreement with the NIDDK) vitamin drops: 2 ml orally per day until 2 years of age
2. Vitamin K : 2.5 mg orally coadministered with AquADEK® vitamin drops on Mondays, Wednesdays and Fridays
3. AquADEK® and Vitamin K may be stopped when the total bilirubin is <1.5 mg/dL. Serum vitamin levels and prothrombin time/INR will be measured during the follow-up visit at one month after portoenterostomy. When an abnormal value is obtained, the

dosage of the specific vitamin will be augmented or reduced, as appropriate, and the level will be rechecked in 4 weeks

Ursodeoxycholic Acid

1. Ursodeoxycholic acid (provided to the study by Axcan Pharma U.S, Inc., Mont-Saint-Hilaire, Qc, Canada, as a part of a cooperative agreement with the NIDDK): 20 mg/kg/day divided BID orally until 2 years of age.
2. Ursodeoxycholic acid: discontinued if serum total bilirubin >15 mg/dL

Antibiotics for Prophylaxis Against Ascending Cholangitis (Definition at end of eTable 3)

1. Trimethoprin/Sulfamethoxazole (TMP/SMZ): 4-5 mg TMP/kg/day orally for 6 months
2. Dose to be adjusted based on renal function
3. If allergic to TMP/SMZ: participant will receive oral neomycin 25/mg/kg twice a day orally. Because neomycin provides no prophylaxis against PCP, the subject will also undergo a blinded taper and discontinuation of the study drug/placebo. Our choice to discontinue the study drug/placebo is based on our concerns that the subjects would be exposed to additional risks that would derive from adding another antimicrobial for PCP prophylaxis.

Ranitidine

Ranitidine (Zantac®, provided to the study by GlaxoSmithKline, Philadelphia, PA, as a part of a cooperative agreement with the NIDDK): 12.5 mg orally twice daily (2-6 mg/kg/day) during administration of study drug/placebo

Routine Childhood Immunizations

1. Routine primary immunizations will be given to all children by their primary care provider as recommended by the Committee on Infectious Diseases of the American

Academy of Pediatrics (AAP), except that immunizations will not be given for the first 4 weeks after portoenterostomy (a period of time when the corticosteroid dose is 2-4 mg/kg/day). The normal immunization schedule will then be resumed, with immunizations to be given prior to one year of age being delayed by up to 4 weeks.

2. If there is a need to catch-up with routine immunization schedule, it is anticipated that the catch-up schedule recommended by the Committee on Infectious Diseases of the AAP will be used by the primary care provider
3. Rotavirus vaccine: Not to be given due to the history of abdominal surgery, as recommended in the package insert
4. Additional vaccines that are recommended for children with chronic liver disease are Hepatitis A and annual influenza vaccine, and will be administered as recommended by AAP guidelines

Steroid Pulses

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

Definition of Ascending Cholangitis

A. Definite cholangitis: Must meet all three (with or without positive blood or liver culture)

1. Fever > 38°C in a child with no other obvious source of infection
2. Decreased stool pigmentation in a child who previously had stool pigmentation
3. Elevation of direct bilirubin by 25% or at least >1.0 mg/dL above previous baseline levels

B. Possible cholangitis with fever: Child with fever > 38°C with no other obvious source of

infection with at least one of the following:

1. Decreased stool pigmentation in a child who previously had stool pigmentation
2. Elevation of direct bilirubin by 25% or at least >1.0 mg/dL above previous level baseline
3. Rise in 2 or more of AST, ALT, alkaline phosphatase or GGTP to 1.5X the upper limit of normal or >25% above baseline values if previously elevated

C. Possible cholangitis without fever (temperature less than 38°C): Decreased stool pigmentation in a child who previously had stool pigmentation with at least one of the following:

1. Elevation of direct bilirubin by 25% or at least >1.0 mg/dL above previous level baseline
2. Rise in 2 or more of AST, ALT, alkaline phosphatase or GGTP to 1.5X the upper limit of normal or >25% above baseline values if previously elevated

eTable 3: List of a priori expected adverse events for START

Expected adverse events

1. Cataracts
2. Hypertension
3. Hyperglycemia
4. Hypokalemia
5. Impaired wound healing
6. Gastrointestinal bleeding
7. Pancreatitis
8. Increased crying and decreased sleeping
9. Increased risk of infection is defined by vaccine preventable infections
10. Decreased response to immunizations
11. Weight gain as defined as weight Z-score
12. Poor height growth as defined as height Z-score
13. Bone fractures
14. Bacteremia
15. Fungemia

eTable 4: Comparison of demographic characteristics of START participants and those assessed for eligibility who did not consent to participate in START

Characteristic	Did Not Consent to Participate in START (N=116)	Consented to Participate in START** (N=141)	P value*
Age – Mean ± SD, Days	63.4 ± 30.18	68.7 ± 29.93	0.14
Male – n (%)	47 (41%)	69 (49%)	0.18
Race – n (%)			0.58
White	68 (59%)	91 (65%)	
Black	20 (17%)	19 (13%)	
Other	28 (24%)	31 (22%)	
Ethnicity – n (%)			0.88
Hispanic	32 (28%)	36 (26%)	
Non-Hispanic	84 (72%)	104 (74%)	
Refused	0 (0%)	1 (<1%)	

*Chi-square or Fisher's exact test for categorical variables and two-sample t-test for continuous variables.

**Note that one participant consented but was not randomized.

eTable 5: Impact of age at time of HPE on primary outcome

End Point: Good Bile Drainage at 6 Months Post-HPE[a]	**Steroids**	**Placebo**	**Adjusted Relative Risk (95% CI)**	**P Value**
Entire Cohort[b]	**N=70**	**N=70**		
[Original Main Effects Model], N (%)	41	34	RR = 1.14	0.43
	(58.6%)	(48.6%)	(0.83, 1.57)	
Entire Cohort[c]	**N=70**	**N=70**		
[Interaction Model] – n (%)				
Treatment				0.50
Age at HPE (<70, ≥70 days)				0.07
BASM				0.63
Treatment * Age at Time of HPE				0.67
Age at HPE < 70 days[d]	**N=39**	**N=37**		
[Age-Specific Separate Model], N (%)	28	21	RR = 1.23	0.36
	(71.8%)	(56.8%)	(0.79, 1.89)	
Age at HPE ≥ 70 days[d]	**N=31**	**N=33**		
[Age-Specific Separate Model], N (%)	13	13	RR = 1.09	0.79
	(41.9%)	(39.4%)	(0.59, 2.00)	

[a]Good bile drainage defined as serum total bilirubin < 1.5 mg/dL at 6-months post-HPE in a subject alive with native liver

[b][Originally Planned Main Effects Model] Relative risk and P value for treatment success (good bile drainage) from a log-binomial model with the following covariates: treatment group, age of

the infant at HPE (continuous variable) and BASM as fixed effects, and site as a random effect [originally planned analysis]

[c][Interaction Model] Relative risk and P value for treatment success (good bile drainage) from a log-binomial model with the following covariates: treatment group, age of the infant at HPE (dichotomous variable: <70 days; ≥ 70 days), BASM as fixed effects, and interaction of treatment group and age of the infant at HPE (dichotomous), and site as a random effect

[d][Age-Specific Separate Models] Relative risk and P value for treatment success (good bile drainage) from a log-binomial model with the following covariates: treatment group, and BASM as fixed effects, and site as a random effect

eTable 6: Serious adverse events (SAEs) by type and treatment group

Variable	Steroids N=70	Placebo N=70	TOTAL
Anemia	0	0	0
Cardiovascular	0	0	0
Congenital	1	1	2
Gastrointestinal	6	3	9
Hepatic	41	30	71
Immunological	1	0	1
Infectious	129	109	238
Metabolic	1	2	3
Miscellaneous	5	1	6
Neoplastic	1	0	1
Neurological	1	0	1
Nutritional	8	11	19
Orthopedic	2	2	4
Pulmonary	0	1	1
Surgical	8	2	10
Total SAEs	204	162	366
Total subjects with SAEs	57	56	113
Total randomized subjects	70	70	140
% with SAEs	81.43%	80.0 %	80.71%
P value*			>0.99

* P value from Fisher's Exact test comparing percentages of subjects with SAEs by treatment group.

eTable 7: Unexpected adverse events (AEs) by type and treatment group

Variable	Steroids N=70	Placebo N=70	TOTAL
Anemia	0	0	0
Cardiovascular	0	0	0
Congenital	0	0	0
Death	0	0	0
Dermatological	12	11	23
Febrile events	21	27	48
Gastrointestinal	19	19	38
Gynecological	0	0	0
Hematological	0	4	4
Hepatic	1	1	2
Injury	1	3	4
Immunological	2	1	3
Infectious viral	25	9	34
Infectious cholangitis	0	0	0
Infectious surgical	0	0	0
Infectious	38	24	62
Metabolic	1	2	3
Miscellaneous	2	4	6
Musculoskeletal	0	0	0
Neoplastic	0	0	0
Neurological	0	0	0

Variable	Steroids N=70	Placebo N=70	TOTAL
Nutritional	0	6	6
Orthopedic	1	0	1
Pulmonary	32	16	48
Surgical	0	0	0
Urinary	4	2	6
Wound non-infectious	0	0	0
Total unexpected AEs	159	129	288
Total subjects with unexpected AEs	36	36	72
Total subjects randomized	70	70	140
% with unexpected AEs	51.43%	51.43%	51.43%
P value*			>0.99

* P value from Fisher's Exact test comparing percentages of subjects with unexpected AEs by treatment group.

eTable 8: List of first SAEs during study treatment period by treatment group

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Placebo	Congenital	Right inguinal hernia	Unlikely	Expected	Resolved	None	8
Placebo	Gastrointestinal	Vomiting, dehydration	Unlikely	Expected	Resolved	None	11
Placebo	Gastrointestinal	Difficulty swallowing and reflux	Unlikely	Expected	Resolved	None	31
Placebo	Hepatic	Coagulopathy	Unlikely	Expected	Resolved	None	5
Placebo	Hepatic	Ascites (hypoalbuminemia and poor nutrition)	Unlikely	Expected	Resolved	None	11
Placebo	Hepatic	Elevated bilirubin	Unlikely	Expected	Resolved	None	59
Placebo	Infectious	Fungemia and streptococcal bacteremia/TPN and NG tube feeding	Possibly	Expected	Resolved	Blinded Taper	11
Placebo	Infectious	Possible cholangitis without fever	Possibly	Expected	Resolved	None	14
Placebo	Infectious	Possible cholangitis with fever	Possibly	Expected	Resolved	None	17
Placebo	Infectious	Rule out cholangitis	Unlikely	Expected	Resolved	None	18

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Placebo	Infectious	Rule out cholangitis	Unlikely	Expected	Resolved	None	25
Placebo	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	27
Placebo	Infectious	Fever with URI symptoms	Unlikely	Expected	Resolved	None	28
Placebo	Infectious	Definite cholangitis with positive culture	Unlikely	Expected	Resolved	None	29
Placebo	Infectious	Possible cholangitis without fever	Unlikely	Expected	Resolved	None	31
Placebo	Infectious	Viral gastroenteritis	Unlikely	Expected	Resolved	None	32
Placebo	Infectious	Fever, rule out cholangitis	Unlikely	Expected	Resolved	None	33
Placebo	Infectious	Possible cholangitis with fever	Possibly	Expected	Resolved	None	35
Placebo	Infectious	Fever	Possibly	Expected	Resolved	None	38
Placebo	Infectious	Fever and lethargy after recently starting diuretics	Unlikely	Expected	Resolved	None	55
Placebo	Infectious	Definite cholangitis with Bacteremia (E. Coli); C. Difficile colitis	Unlikely	Expected	Resolved	None	55
Placebo	Infectious	Definite cholangitis with	Possibly	Expected	Resolved	Unblinded	60

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
		bacteremia				Taper	
Placebo	Infectious	Fever, suspected viral syndrome	Unlikely	Expected	Resolved	None	60
Placebo	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	66
Placebo	Infectious	Possible cholangitis with fever - polymicrobial bacterial infection	Unlikely	Expected	Resolved	None	67
Placebo	Infectious	Fever	Unlikely	Expected	Resolved	None	71
Placebo	Infectious	Fever and positive blood culture	Unlikely	Expected	Resolved	None	78
Placebo	Infectious	Fever	Possibly	Expected	Resolved	None	78
Placebo	Infectious	Possible cholangitis without fever, worsening ascites	Unlikely	Expected	Resolved	None	81
Placebo	Infectious	Definite cholangitis with positive culture (E. Coli sepsis)	Possibly	Expected	Resolved	Blinded Taper	89
Placebo	Orthopedic	Fever and right humerus	Possibly	Expected	Resolved	None	42

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
		fracture					
Placebo	Surgical	Postoperative small bowel obstruction	Unlikely	Expected	Resolved	None	12
Steroids	Gastrointestinal	Ileus, possible obstruction secondary to edema	Unlikely	Expected	Resolved	None	7
Steroids	Gastrointestinal	Intussusception	Unlikely	Unexpected	Resolved	None	7
Steroids	Gastrointestinal	Vomiting	Unlikely	Expected	Resolved	None	53
Steroids	Hepatic	Ascites	Unlikely	Expected	Resolved	None	8
Steroids	Hepatic	Ascites	Unlikely	Expected	Resolved	None	19
Steroids	Hepatic	Ascites causing tachypnea	Unlikely	Expected	Resolved	None	32
Steroids	Hepatic	Liver failure	Unlikely	Expected	Resolved	None	82
Steroids	Immunological	Febrile illness (likely side effect of immunization)	Unlikely	Expected	Resolved	No	79
Steroids	Infectious	Varicella	Unlikely	Unexpected	Resolved	Blinded taper	3
Steroids	Infectious	Fever, candidemia	Possibly	Expected	Resolved	Unblinded taper	10
Steroids	Infectious	Rule out cholangitis	Unlikely	Expected	Resolved	None	10

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Steroids	Infectious	Possible cholangitis without fever - Bacteremia (coag negative Staph)	Unlikely	Expected	Resolved	None	11
Steroids	Infectious	Infected hematoma	Unlikely	Expected	Resolved	None	13
Steroids	Infectious	Antibiotic therapy for rise in bilirubin	Unlikely	Expected	Resolved	None	17
Steroids	Infectious	Possible cholangitis with fever	Possibly	Expected	Resolved	None	17
Steroids	Infectious	Viral gastroenteritis	Unlikely	Expected	Resolved	None	18
Steroids	Infectious	Fever, upper respiratory tract viral infection; failure to thrive	Unlikely	Expected	Resolved	None	20
Steroids	Infectious	Vomiting, hypothermia, r/o cholangitis	Possibly	Expected	Resolved	None	23
Steroids	Infectious	Fever	Unlikely	Expected	Resolved	None	24
Steroids	Infectious	Definite cholangitis with bacteremia	Possibly	Expected	Resolved	None	27
Steroids	Infectious	Fever	Unlikely	Expected	Resolved	None	28
Steroids	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	28

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Steroids	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	31
Steroids	Infectious	Meningitis	Possibly	Expected	Resolved	Blinded taper	36
Steroids	Infectious	Fever, rule out cholangitis	Unlikely	Expected	Resolved	None	36
Steroids	Infectious	C. Difficile infection (secondary ileus)	Unlikely	Expected	Resolved	None	37
Steroids	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	52
Steroids	Infectious	Urinary tract infection with septic shock, Klebsiella bacteremia	Possibly	Expected	Resolved	None	56
Steroids	Infectious	Possible cholangitis with fever	Possibly	Expected	Resolved	None	58
Steroids	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	61
Steroids	Infectious	Fever	Unlikely	Expected	Resolved	None	63
Steroids	Infectious	Viral Infection	Unlikely	Expected	Resolved	None	65
Steroids	Infectious	Fever, rule out cholangitis	Possibly	Expected	Resolved	None	75
Steroids	Infectious	Fever of unknown etiology	Unlikely	Expected	Resolved	None	76
Steroids	Infectious	Fever	Unlikely	Expected	Resolved	None	78
Steroids	Infectious	Bacteremia	Unlikely	Expected	Resolved	None	86

Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Steroids	Infectious	Possible cholangitis with fever	Unlikely	Expected	Resolved	None	90
Steroids	Neurological	Cerebrovascular accident	Unlikely	Unexpected	Resolved	None	19
Steroids	Nutritional	Failure to thrive, hypoglycemia	Unlikely	Expected	Resolved	None	22
Steroids	Surgical	Evisceration of appendix	Unlikely	Expected	Resolved	None	3
Steroids	Surgical	Leak from jejuno-jejunostomy	Possibly	Expected	Resolved	Blinded taper	5
Steroids	Surgical	Perihepatic fluid collection	Unlikely	Expected	Resolved	None	8
Steroids	Surgical	Leak at jejuno-jejunal anastomosis and peritonitis/cholangitis, bile leak, bowel perforation, ostomy creation, central line infection	Unlikely	Expected	Resolved	Blinded taper	9
Steroids	Surgical	Cholecystostomy tube dislodgement or blockage; positive RSV during hospitalization	Unlikely	Expected	Resolved	None	10

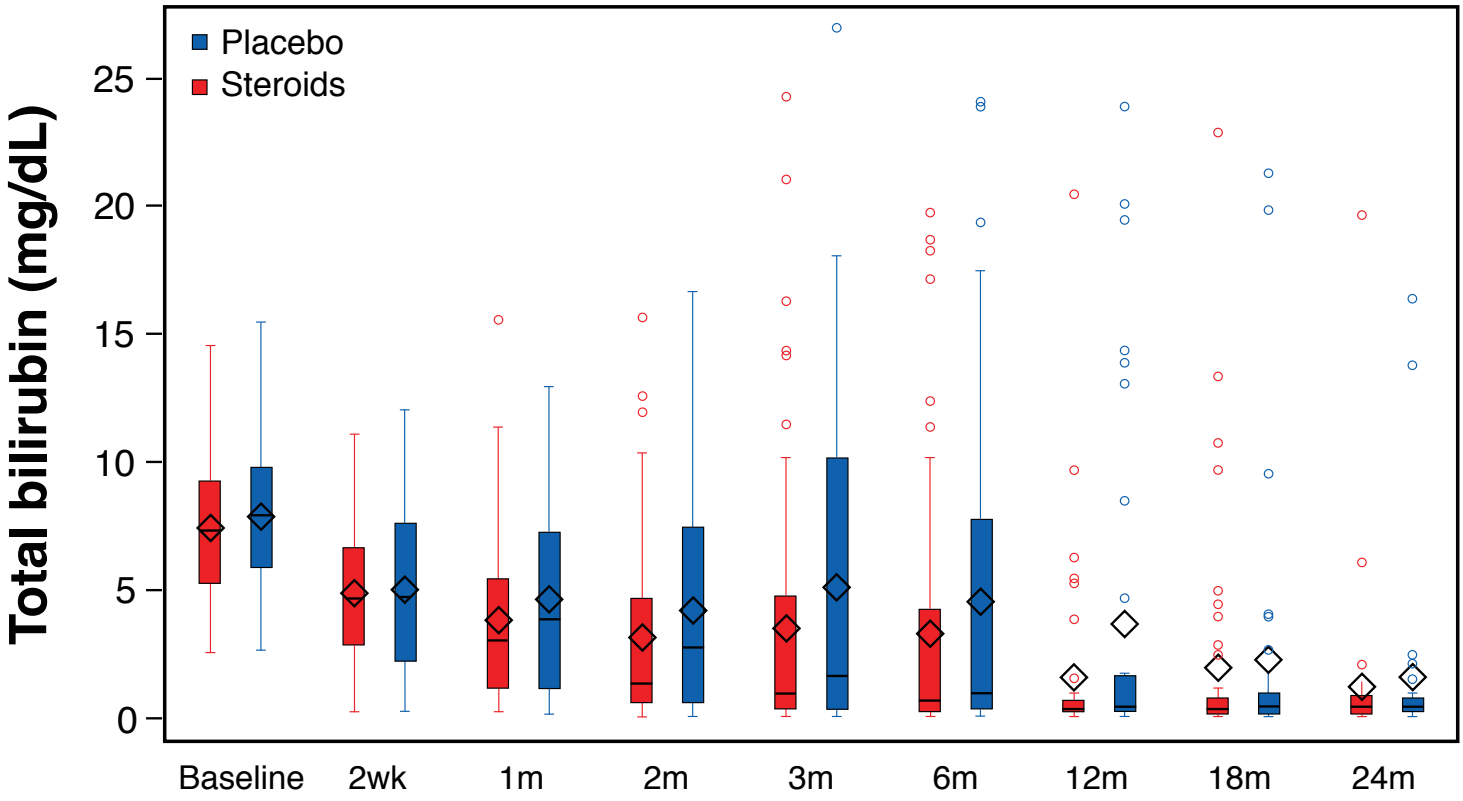
Treatment Group	SAE Primary Category	SAE Description	Related to Study Drug	Expected or unexpected	Resolved	Change in Study Drug	Time from Kasai to First SAE on Study Drug (days)
Steroids	Surgical	Kasai revision	Unlikely	Expected	Resolved	None	37

eTable 9: Inadequate response to immunizations by treatment group at 18 months of age.

Variable	Steroids Number (%)	Placebo Number (%)
Hepatitis B	1/11 (9.1%)	1/9 (11.1%)
Diphtheria	0	0
Tetanus	0	0
H Influenza	3/25 (12.0%)	3/18 (16.7%)
Polio Virus 1	15/30 (50.0%)	6/21 (28.6%)
Polio Virus 2	10/30 (33.3%)	7/21 (33.3%)
Polio Virus 3	12/30 (40.0%)	7/21 (33.3%)
Subjects With ≥1 Decreased Response	17	10
Total Subjects With Decreased Response	33	26
% Relative to the Number Assessed	51.5%	38.5%
P value*	0.43	

*P value from Fisher's exact test comparing percentage of subjects with inadequate response to immunizations by treatment group. Note: Inadequate response to immunizations is defined as any nonprotective titer to a specific antigen, with the denominator determined by having a result provided for that antigen.

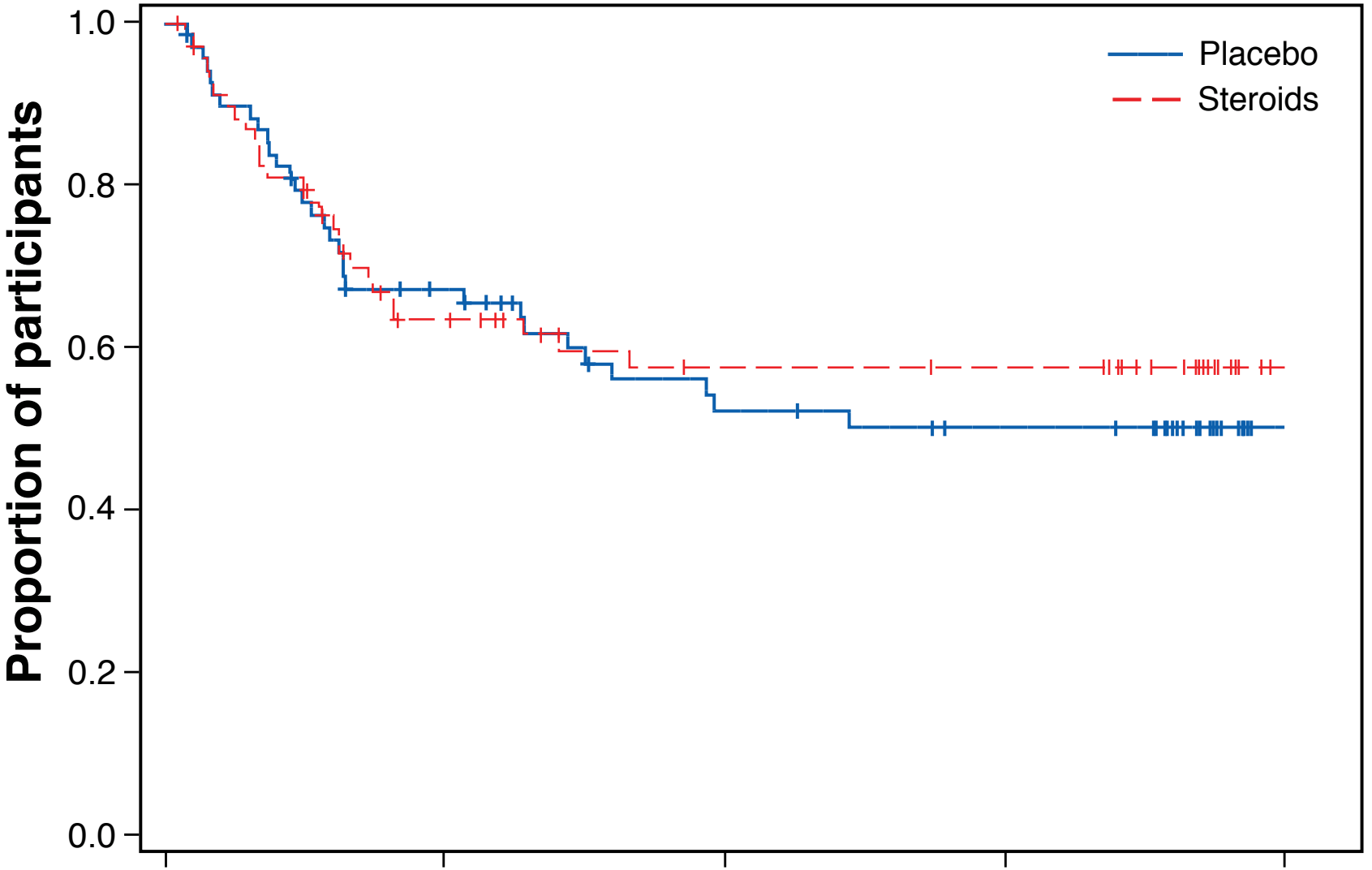
eFigure 1: Levels of serum total bilirubin throughout the duration of the study by treatment group. There are no statistically significant treatment differences for any of the time points.



Number of participants									
Placebo	70	60	66	60	65	58	36	33	30
Steroids	70	59	67	64	63	59	41	46	35

Diamond = mean; dash = median; box = interquartile range (IQR); whisker = 1.5 x IQR; circles = outliers beyond 1.5 x IQR

eFigure 2: Time to first cholangitis SAE by treatment group



Number of participants					
Placebo	70	41	27	23	1
Steroids	70	38	28	27	0

Logrank P=0.63