PEDIATRIC HYDROXYUREA CLINICAL TRIAL
(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

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PEDIATRIC HYDROXYUREA PHASE III CLINICAL TRIAL
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CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 OVERVIEW

The Pediatric Hydroxyurea Phase III Clinical Trial (BABY HUG) was designed as a Phase III, two-year study treatment, double-blind, randomized placebo-controlled trial including 200 children at 14 clinical centers. The final child was randomized into the BABY HUG Treatment Study in September 2007. At the end of their child's two years of masked study treatment, all parents/guardians decided whether their child was to be treated with open-label hydroxyurea (HU) therapy, without regard to and without knowledge of their child's randomized treatment assignment. When originally consenting to participation in the BABY HUG treatment study (BH) and the BABY HUG Follow-up Study I (BHFU I), parents/guardians were told explicitly of the investigators' intention to request permission to continue to follow their child for many years, in order to evaluate possible long-term effects of treatment.

The purpose of BHFU I was to provide structured follow-up of the children enrolled in BH, in order to characterize the long-term toxicities and unexpected risks (if any) associated with treatment with hydroxyurea at an early age. Ideally this unique group of children will be intensively followed for growth, development, and clinical status at least through puberty or early adulthood to document any alterations in the natural history of sickle cell disease (SCD) associated with early HU therapy. BHFU I was the initial installment and BABY HUG Follow-up Study II (BHFU II) is the second installment in that effort.

All parents/guardians of BHFU I participants who completed at least 24 months of follow-up, will be offered enrollment of their child into BHFU II. All children enrolled will be followed to a common termination date of December 31, 2016. This plan will provide five years of follow up after the cessation of BHFU I for each child. Although all parents/guardians have been offered treatment with open-label hydroxyurea for their child, participation in BHFU II will not be contingent upon their subsequent treatment choice.

In BHFU II, parents/guardians will be asked to consent to periodic reporting of clinically obtained information on their child including growth parameters, blood test results, transcranial Doppler (TCD) or other clinically obtained routine studies, and details of sickle cell disease related hospitalizations and health events ("passive" follow-up). Collection and ongoing evaluation of growth and clinical data are key to the determination of long-term effects of hydroxyurea. Blood cells, serum, and urine will be collected at entry to BHFU II and on a second occasion at study exit. This will provide samples for surrogate markers of toxicity and clinical efficacy, such as measures of renal and spleen function and markers of DNA damage (see Exhibit 1-1). The remaining blood sample will be separated, aliquots made and stored for future studies. Surplus blood and serum samples will be de-identified and stored in an NHLBI-sponsored repository (BioLINCC) for future studies with links to the de-identified clinical database of the BABY HUG Treatment Study. In addition, parents/guardians will be invited to have their child participate in an optional "active" reassessment at approximately 10 years of age (+/- 3 months from the subject's 10^{th} birthday). At that time, age-appropriate neuropsychological testing (WISC-IV, Vineland, Peds QOL and Conner CPT II), abdominal ultrasonography, radionuclide liver-spleen scan, TCD, pulmonary function testing, echocardiography/brain natriuretic peptide and MRI/MRA will be performed (see Exhibit 1-1) These studies will allow simultaneous assessment of unexpected nephrotoxicity or splenic enlargement and possible prolonged protection from organ dysfunction. In the active group, there will also be annual blood draws for markers of toxicity and clinical efficacy, such as measures of renal and spleen function and markers of DNA damage

(see Exhibit 1-1). Data collected in this follow-up study will be descriptively analyzed according to the original treatment assignment (HU versus placebo), as well as the subsequent independent decision by families concerning use of open-label HU in the follow-up period.

Data collected in the passive follow-up portion will determine whether early hydroxyurea treatment is associated with long-term toxicities and provide limited data regarding long-term efficacy. Data collected in the active follow-up portion will identify long-term effects on organ dysfunction, and determine if duration of treatment and age of initiation (early vs. late) affect hydroxyurea's efficacy and toxicity. Information obtained from BHFU II is vitally important to understanding the risks and benefits of early treatment, and ultimately for creation of an optimal paradigm for hydroxyurea therapy in young children with sickle cell anemia.

Exhibit 1-1
Schedule of Blood and Urine Collection and Special Studies

APPENDIX A																							
Schedule of Blood and Urine Collection and Schedule of Special Studies																							
BABY HUG Follow-up Observational Study II																							
Time Point	**Clinical Data Reports**	**Blood Collections**									**Urine Collection**			**Other Tests**									**Physical Exam**
	6 Month Data Abstraction Form	HbF **core**	CBC, Retics, Diff **local**	LDH, Billi, ALT **local**	HJB/ Reticulocyte micronuclei **core**	Pitted Cells **core**	Stored Blood Sample **core**	VDJ, DNA Extraction **core**	Cystatin C **core**	Creatinine and BUN **core**	Urine Osmo **local**	Stored Urine **core**	Microalb: Creat Ratio **core**	Liver/ Spleen **central**	Abdom Sono **central**	TCD **central**	Pulm Func Tests **central/CBC local**	Cardiac Echo **central/BNP local**	MRI/ MRA Brain **central**	Neuro-psych: Vineland, WISC-IV, Connor CPT II, Peds QOL **local**	Questionnaire CRF **local**	
PASSIVE FOLLOW-UP CARE																							
Study Entry	7/1/2012	X	X	X	X	X	X^	X	X	X		X	X										
Every 6 Months for 60 Months	x																						
Every 12 Months for 60 Months																					X		
When Performed as Part of Clinical Care (not paid as part of the study)														X*	X*	X*	X*	X+*	X*	X*			X*
End of Study	X	X	X	X	X	X	X^	X	X	X		X	X										
ACTIVE FOLLOW-UP CARE																							
Study Entry	X	X	X	X	X	X	X^	X	X	X	X	X	X										X
Every 6 Months for 60 Months	X																						
Every 12 Months for 60 Months		X	X	X	X	X			X	X											X		X
Once during the study at age 10														X	X	X	X	X+	X	X			
End of Study	X	X	X	X	X	X	X^	X	X	X	X	X	X										X

^ The Stored Blood and Urine samples will be collected, separated and aliquots made and stored for future study.
+ Brain Natiuretic Peptide (BNP) will be performed locally once during the study at the time of an Echocardiogram.
* Results for 'Other Tests' performed in the passive group should be reported to the DCC on the appropriate Case Report Form and images obtained as a result of 'Other Tests' that are performed on subjects enrolled in the passive group should be sent to the appropriate Core/central reviewer for central reading.

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CHAPTER 2

SUBJECT RECRUITMENT, ELIGIBILITY, AND INFORMED CONSENT

2.1 RECRUITMENT

Only children who were enrolled into BHFU I may be recruited for enrollment in BHFU II. When a child was consented for screening in BH and when they enrolled in BHFU I, parents/guardians were made aware that the Investigators wished to maintain contact with enrolled children after the treatment and follow-up studies ended for inclusion in a second and subsequent follow-up study.

2.2 ELIGIBILITY

All children who completed at least 24 months of follow-up visits in BHFU I are eligible for BHFU II. Exclusion and inclusion criteria have already been evaluated for these subjects in BH, so explicit eligibility criteria evaluation is not required again. Please note that a child on a chronic transfusion program is eligible for this study, however, one who is a recipient of a stem cell transplant is ineligible for BHFU II. The child will be enrolled in BHFU II when s/he is declared eligible by the Data Coordinating Center (DCC). The DCC will declare a child eligible upon verification that s/he participated in BHFU I for at least 24 months.

2.3 INFORMED CONSENT

A new informed consent from parents/guardians will be required for participation. Consent will be requested upon local IRB approval of the Protocol and Consent Form and upon Clinical Center certification by the DCC.

Individual Clinical Center consent forms will be prepared based on the model informed consent form in the protocol. The model consent form will be approved by the Project Officer of the National Heart, Lung, and Blood Institute (NHLBI) and the Observational Study Monitoring Board (OSMB). Each final consent form will be reviewed by DCC staff and the Project Officer to ensure all required elements of the consent form have been addressed.

The Clinical Center Principal Investigator (PI) will offer participation to each eligible family that was in BHFU I. The family will be given adequate time and privacy to review the consent form. They will have the opportunity to have all of their questions and concerns addressed by the PI.

An ombudsman, required for the BABY HUG Treatment Study consent process, may be present but is not required. A copy of the signed consent form will be given to the parent/guardians and placed in the child's medical record. The original will be maintained in study files by the PI or designee.

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CHAPTER 3

CLINICAL CENTER PROCEDURES

3.1 SCHEDULING

3.1.1 General Principles for Scheduling "Active Group" Tests

Any active blood draws should be spaced as necessary; a general guideline for research blood draws is no more than 3cc/kg/8 weeks. When possible, blood draws should be grouped around clinical blood draws and IV starts in order to minimize the number of times the child is subjected to needle insertion. Blood will be drawn at entry and at the end of the study for all subjects, and additionally every 12 months for the active subject group only.

For maximum attention and cooperation, neuropsychological testing should be performed when the child is alert and not hungry or sleepy.

Some of the special study tests for the active subject group may require scheduling 4-6 weeks in advance in some institutions. Therefore, it will be important to plan ahead for subjects who elect active follow-up.

3.1.2 Visit Schedule

3.1.2.1 All Subjects

All subjects will have a baseline visit at study entry and one at exit. Any standard clinical visits in between this period will be captured in medical chart reviews at 6 month intervals. The Visit Schedule (See Exhibit 3-1) indicates the dates when the visits and abstractions are to be conducted for each subject.

3.1.2.1.1 Baseline

At study entry, all subjects will have six blood specimens collected and tested centrally: Hemoglobin F (HbF)(0.5 mL), Howell-Jolly Bodies (HJB)/Reticulocyte Micronuclei, VDJ/DNA Extraction (4.0 mL), Pitted Cell (0.1 mL), Cystatin C (1.0 mL), Creatinine/BUN (4.0 mL) and stored blood (5.0 mL) (for long-term storage in the repository). A urine specimen (1-2mL) will also be collected for the Microalbumin:Creatinine ratio and for long-term storage in the repository. Locally performed blood tests will include CBC, reticulocytes, differential, LDH, bilirubin, and ALT. Subjects will be asked about their medical history, and any adverse (clinical) events they have been experiencing. Height and weight will be measured if it is the standard of care for the Clinical Center. Refer to Exhibit 1-1 for a complete listing of required blood draws and special studies.

Baseline tests should be performed within three (3) months of the consent date. Record the tests on Form 03 (Central Lab Collection) and Form 04 (Local Lab Data). In Active subjects only, an additional urine sample will be collected for the urine osmolality testing to be performed by the local laboratory.

3.1.2.1.2 Medical Record Abstraction

Every 6 months, Clinical Centers will abstract information from subjects' medical records and enter it through the Data Management System (eCOS) via Form 10 (Data Abstraction). The initial abstraction period will be retrospective to January 1, 2012 (as is permitted by the study consent form) and all follow-up and abstraction will end on December 31, 2016.

Following are the tests, events and reports to be abstracted every 6 months if available:

- Lab results*
- TCD
- MRI
- MRA
- CT

- EEG
- PFT
- Liver/Spleen Scan
- Echocardiogram
- Abdominal Sonogram
- Neuropsychological Testing
- Hospitalizations, clinic, and ER visits
- Surgical Procedures
- Transfusions
- Physical Exam
- Hydroxyurea usage and associated toxicities

*For subjects who have more than one chemistry or fetal hemoglobin test done in a six month period, record the result which was obtained closest to the end of the visit window on Form 10.

3.1.2.1.3 Annual Visits (Active and Passive Subjects)

All subjects (active and passive) will be administered a questionnaire (Form 15) regarding their health status including questions pertaining to enuresis, obstructive sleep apnea/snoring and priapism (if applicable). This questionnaire is administered by BHFUII personnel beginning at the first annual visit.

3.1.2.1.4 Study Exit

Because the BABY HUG Follow-up Study II will end on December 31, 2016, the exit procedures should occur within the last three months of the study (October 1, 2016 – December 31, 2016). The following lab tests will be performed for this visit:

- Hemoglobin F, 0.5 mL blood (central lab)

- Howell Jolly Bodies (HJB) by flow cytometry/Reticulocyte Micronuclei, 1.0mL blood (central lab)
- Pitted cell count, 0.1mL blood (central lab)
- Blood specimen for repository storage, 5.0mL blood (central lab)
- VDJ/DNA Extraction, 3.0mL blood (central lab)
- Cystatin C, 1.0mL blood (central lab)
- HPLC creatinine/BUN 1.0mL blood (central lab)
- Microalbumin: creatinine urine ratio , 5.0mL urine (central lab)
- Urine specimen for repository storage (central lab)
- CBC, reticulocytes, differential (local lab)
- LDH, bilirubin, ALT (local lab)

Please see Chapter 4 for the specifics of collecting and shipping the blood and urine specimens and Chapter 5 for procedural instructions for Active Group tests.

3.1.2.2 Active Subjects

3.1.2.2.1 10 Years of Age Visit

Active subjects are those who agree to one extra set of tests at approximately 10 years of age (±3 months). These subjects can decline any specific test(s) they choose and still be considered active. However, the goal is that all tests will be performed. The active group investigation at the 10 year of age visit entails the following extra set of lab and clinical tests:

- Liver-spleen scan
- Abdominal ultrasound
- Transcranial Doppler

- Pulmonary Function Testing with hemoglobin done locally on the same day and reported on Form 31 for PFT result
- Cardiac Echocardiogram with Brain Natriuretic Peptide (BNP) done locally on the same day and reported on Form 32
- MRI/MRA
- Neuropsychological tests: WISC-IV, Vineland, Conner CPT II and Peds QOL

Please see Chapter 5 for procedural instructions.

3.1.2.2.2 Annual Visits (Active Subjects Only)

Active subjects also agree to have the following tests/procedures performed every 12 months:

- Blood specimens (HbF, CBC, Reticulocytes, Differential, LDH, Bilirubin, ALT, HJB/reticulocyte micronuclei, Pitted cell count, Cystatin C, HPLC creatinine and BUN) – Complete Form 04 to record locally obtained results and Form 03 for centrally obtained results (refer to Appendix A for a description of the blood test requirements).
- Complete Physical Examination (Form 14) – Refer to section 5.9 “Physical Examination” for specific instructions.

3.1.2.3 Passive Subjects

If the procedures listed in section 3.1.2.2 “Active Subjects” are performed as a part of standard clinical care in a Passive subject, the procedure performed closest to 10 years of age should be reported to the DCC on the relevant case report forms and the images/CD should be sent to the appropriate core lab for central review. If only one of each of the listed procedures is performed clinically during the study, then it should be sent to the specified core lab for central review.

3.2 FORMS

The following list delineates each form number, its name and when it is expected.

Exhibit 3-1
Visit Schedule Forms Grid

	FORM REQUIREMENTS		FREQUENCY											
FORM NUMBER	FORM NAME	CONTENTS	Entry		q 6 months		q 12 months		Age 10		As part of Clin Care		End of Study	
			A	P	A	P	A	P	A	P	A	P	A	P
01	ENROLLMENT	Consent Info	■	■										
03	CENTRAL LAB COLLECTION (Labels/Dates only)	HbF	■	■			■						■	■
		Stored Blood	■	■									■	■
		Creatinine/BUN	■	■			■						■	■
		Microalb:Creat (Urine)	■	■									■	■
		Pitted Cells	■	■			■						■	■
		HJB/Retic Micro	■	■			■						■	■
		VDJ/Genetic Mods	■	■									■	■
		Cystatin C	■	■			■						■	■
		Stored Urine	■	■									■	■
04	LOCAL LAB DATA (Results)	CBC	■	■			■						■	■
		Retics	■	■			■						■	■
		Diff	■	■			■						■	■
		LDH	■	■			■						■	■
		Bilirubin	■	■			■						■	■
		ALT	■	■			■						■	■
		Urine Osmolality	■										■	
10	DATA ABSTRACTION	6 Month Data	■	■	■	■							■	■
12	STUDY EXIT	Early or Scheduled Exit											■	■
13	TCD PERFORMANCE	TCD Performance							■			■		
14	PHYSICAL EXAMINATION	Physical Exam	■				■					■	■	

15	QUESTIONNAIRE	Sleep Apnea, Priapsim, Adherence, Enuresis													
20	SPECIAL STUDIES PERFORMANCE	Liver/Spleen													
		Abdominal Sono													
		TCD													
		PFT													
		Cardiac Echocardiogram													
		MRI													
		MRA													
		VINELAND													
		WISC IV													
		CONNOR CPT II													
		PEDS QOL													
21	LIVER/SPLEEN SCAN PERFORMANCE	L/S Local Performance													
23	ABD SONO CENTRAL PERFORMANCE	Abd Sono Performance													
25	SAE	Serious Adverse Event													
26	STEM CELL TRANSPLANT	Transplant Info													
27	VINELAND	VINELAND													
28	WISC IV	WISC IV													
29	PEDS QOL	PEDS QOL													
30	CONNOR CPT II	CONNOR CPT II													
31	PFT PERFORMANCE	PFT (local)													
32	ECHO PERFORMANCE	ECHO (local)													
		BNP (same time as Echo)													
33	MRI/MRA PERFORMANCE	MRI Performance													

3.3 REPORTS

Refer to the Data Management Manual of Operations for types of reports found in the electronic Data Management System (eCOS).

3.3.1 Visit Schedule

Please see Exhibit 3-1 for the Visit Schedule Forms Grid. The Visit Schedule indicates the time periods for data abstraction, when specimens should be collected and when special studies should be performed. The time clock for the Visit Schedule begins with the date the subject signs consent.

3.3.2 Delinquent Forms

If a subject is not seen within his/her time window, the Clinical Center Coordinator will need to record a protocol deviation and report it to the DCC. Additionally, the Clinical Center Coordinator will need to report this to the BABY HUG Follow-up Study II DCC for a discussion regarding what tests and procedures to perform in the new timeframe; this decision will be made by the Steering Committee.

3.4 ADVERSE EVENT MONITORING

3.4.1 Adverse Events

Adverse (clinical) events will be recorded via abstraction on Form 10 (Clinical Data Report) and then on Form 25 (AE/SAE report) or Form 50 (Major Event) as appropriate.

3.4.2 Serious Adverse Events

3.4.2.1 Definition

A **Serious Adverse Event** (SAE) is any one of the following:

1. Death
2. Life-threatening event

3. Prolonged hospitalization (greater than 7 days)

4. Splenic sequestration crisis

5. Stroke, TIA

6. Acute chest syndrome

7. ICU admissions

8. Unexpected adverse event and that is related to HU

SAEs that are SCD-related have been added to the FDA-defined list. Item # 3 has been modified from the FDA definition because frequent hospitalizations occur as a consequence of having sickle cell anemia (even without being enrolled in a clinical trial).

3.4.2.2 Monitoring

Standard clinical trial prospective SAE reporting for the events listed in section 3.4.2.1 will occur only during the five days following the 10 year assessments of active follow-up subjects. These are the only SAEs that are to be reported during the BHFU II. The OSMB will perform a semi-annual systematic review to determine if one treatment group (or HU exposure type) has more reports of SAEs than the other treatment group (or HU exposure type). See Chapter 9, section 9.2 for more information.

3.4.2.3 Reporting SAEs

Record all SAEs (see section 3.4.2.1) on Form 25 (AE/SAE Form). The duration of an event includes both the beginning and ending day of the event.

An unexpected adverse event is an event not previously identified in nature, severity, or frequency in the risk information described in the Protocol, informed consent or listed in the current hydroxyurea product labeling. Each unexpected BABY HUG Follow-up Study II SAE, suspected as related to hydroxyurea usage, must also be reported on a U.S. FDA MedWatch Form 3500A.

Detailed information on completion of MedWatch Forms and an electronic template of the form are available at:

- http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf. Submit the MedWatch form by uploading to eCOS as a source document to the SAE Form 25 (or e-mail to the BABY HUG Follow-up Study DCC at: BabyHug@neriscience.com)
- The MedWatch form must be submitted to the BABY HUG Follow-up Study DCC within 24 hours of the Clinical Center becoming aware of the event.
- All corresponding medical records (i.e. discharge summary, death notice, labs) must be de-identified and uploaded in to the system with the data entry of the form. No subject PHI must be on those forms when they are entered in eCOS.

Serious Adverse Events will be reported to the FDA under the BABY HUG IND as well as to the OSMB. All safety reporting to the IND will comply with 21 CFR 312.32 and as updated by the Final Rule: Investigational New Drug Safety Reporting Requirements for Human Drug and Biological Products and Safety Reporting Requirements for Bioavailability and Bioequivalence Studies in Humans (2010).

3.4.3 "Major" Event Reporting Requirements

In addition to reporting serious adverse events, the NHLBI requires that all "major" events be reported to the DCC as soon as Clinical Center Staff become aware of the event. All events should be reported by data entering the appropriate forms in eCOS (the DCC's data management system). All corresponding medical records (i.e. discharge summary, death notice, labs, etc.) must be de-identified and uploaded in to the system with the data entry of the form. No subject PHI must be on those forms when they are entered in eCOS. The NHLBI has defined a "major" event as an event which would meet the definition of a reportable serious adverse event as described in section 3.4.2.1, but which does not occur following the performance of a BHFU II

active assessment study (e.g. liver/spleen scan, abdominal sonogram, MRI/MRA, cardiac echocardiogram, neuropsychology test, for both active and passive participants). The reported information will be communicated to the Observational Study Monitoring Board (OSMB) Chairman for informational purposes and comment, whereas Serious Adverse Events will continue to be reported to the FDA under the BABY HUG IND as well as to the OSMB.

3.5 MAINTENANCE OF STUDY RECORDS

3.5.1 Subject Records

Study-related subject records must be kept in lockable cabinets or file drawers, preferably in the Study Coordinator's office. The study does not mandate a specific system of subject record filing. Thus, each Coordinator should develop a well-organized filing system that optimizes the ability to easily retrieve specific files.

The source documentation for the BABY HUG Follow-up Study II is the subject's medical record, special test results with study forms (e.g. liver/spleen scans – Form 21, and abdominal sonograms – Form 23, etc.), lab result reports (electronic or on paper), paper Questionnaire (Form 15), Neurological Exam Worksheet, and the neuropsychological test booklets.

3.5.2 Coordinator Files

Material contained in the subject files that are maintained by the Coordinator should include, but are not necessarily limited to the following:

- Signed original consent forms
- Signed original HIPAA forms (if not incorporated in to the consent form)
- Record of family honoraria
- Printouts of the entered study forms (not required)
- Copies of neuropsychological evaluations

- Current medical records or a system for reviewing computerized records including laboratory records must be in place
- Copies of specimen and film transmittal forms

3.5.3 Clinical Center Records

The Study Coordinator should also designate a bookshelf or desktop space to keep a collection of binders for study materials including:

- Protocol, with all amendments, and signed/dated Investigator Signature pages
- Manual of Operations
- Forms
- Address Directory
- Numbered Memos (may be stored electronically, but should be referenced for accessibility) and signed acknowledgements
- Steering Committee Minutes (may be stored electronically, but should be referenced for accessibility)
- Correspondence (may be stored electronically, but should be referenced for accessibility)
    - With other Coordinators
    - Coordinator Conference call minutes
    - With the Coordinating Center
- Specific Clinical Center requirements
    - IRB and other administrative materials and approved documents

- Local consent form
- FDA Form 1572 and respective CVs and Medical Licenses
- Financial Disclosures
- Certifications
- Site Signature Log with Delegation of Authority
- Local special study performance directions and notes (e.g., TCD) (may be stored electronically, but should be referenced for accessibility)

3.6 TRANSFER OF SUBJECTS

3.6.1 Notification of Transfer

If a subject moves from one BABY HUG Follow-up Study II Clinical Center to another BABY HUG Follow-up Study II Clinical Center, the Study Coordinator of the original Clinical Center will discuss the pending transfer with the subject. The Study Coordinator will contact the new Clinical Center and provide the subject with the contact information for the Study Coordinator at the new Clinical Center.

A memo and PART 1 of Form A: Notification of Transfer Subject (Exhibit 3-4) must be completed by the original Clinical Center's PI and sent to the PI of the new Clinical Center informing him/her of the potential transfer. This will serve as a formal notice to the new Clinical Center that a subject is transferring. The memo should include:

1. Approximate date the subject is scheduled to move
2. Date of the last clinic visit
3. The Visit Schedule
4. A medical summary and other pertinent source documents

5. Subject's new address, telephone number and other relevant contact information. The subject's family should also provide contact information for a relative not residing in their household.

6. Copies of relevant study records

The new Clinical Center's Study Coordinator should also receive a copy of this memo. The DCC should receive a copy of Form A: Notification of Transfer Subject.

3.6.2 Release of Medical and Study Records

Once a Clinical Center becomes aware of a subject's intent to transfer to a new Clinical Center, they may request that the subject's family sign a medical records release form. The form must contain the name of the subject, the signature of the parent and the party to which the subject's medical information is to be released. The medical records, study documentation and labels must remain at the original Clinical Center until the subject's family completes a medical release form.

The PI or designee at the original Clinical Center should forward the subject's medical records to the PI or designee at the new Clinical Center after the medical release form is signed. A copy of the subject's consent form, medical records and medical record abstractions should be sent to the new Clinical Center as soon as possible, prior to the subject's first clinic visit at the new Clinical Center.

The originating Clinical Center will retain the subject's original BABY HUG Follow-up Study II records and send the new Clinical Center copies of all documents on file. The Study Coordinator at the original Clinical Center should clear all edits prior to the transfer of the subject. The subject must retain the original BABY HUG Follow-up Study II subject identification number throughout the course of the study, even though it was assigned at the original Clinical Center.

3.6.3 Consenting the Subject at the New Clinical Center

The new Clinical Center must re-consent the subject to the follow-up study using their locally approved IRB consent. The subject must be informed that being at the new Clinical Center may come with new regulations, possibly including different IRB regulations and different state regulations. The subject becomes the responsibility of the new Clinical Center only after the informed consent for the new Clinical Center is signed. Once transfer is complete, transferred subjects will be counted in the subject totals of the new center at which they are now receiving care.

3.7 CAPITATION REPORT

The Capitation Report is the billing statement for each Clinical Center, which tallies, for example, the number of medical abstraction forms completed, such that payment can be released to each Clinical Center. The Capitation Report with calculated billing amounts signed off by DCC staff will be sent from the DCC to the Clinical Centers quarterly (March, June, September, and December). The PI will need to verify the information, and send the DCC signed Capitation Report to NHLBI (the direct payor), or prime contractor in the case of the three subcontracted sites, with an invoice from their business office.

Form A Rev. 5/1/12

Exhibit 3-4
BABY HUG Follow-Up Study II
Notification of Transfer Subject

PART I: FOR ORIGINAL CLINICAL CENTER COMPLETION

1. Subject ID: ___ ___ ___ ___

2. Date Form Initiated: ___ ___ ___ - ___ ___ - ___ ___ ___ ___
   Month Day Year

3. Original Clinical Center: ___ ___

   New Clinical Center: ___ ___

4. Anticipated Date of Last Clinic Visit at Original Center: __ __ __ - __ __ - __ __ __ __
   Month Day Year

5. Anticipated Date of Transfer: ___ ___ ___ - ___ ___ - ___ ___ ___ ___
   Month Day Year

6. Signature of Principal Investigator: _____

7. Is this transfer
   Permanent ……(1)
   Temporary ……(2)

PART II: FOR NEW CLINICAL CENTER COMPLETION

1. Date Consent Form Signed: ___ ___ ___ - ___ ___ - ___ ___ ___ ___
   Month Day Year

2. Date First Clinic Visit Completed: ___ ___ ___ - ___ ___ - ___ ___ ___ ___
   Month Day Year

3. Signature of Principal Investigator: _____

PART III: FOR DCC COMPLETION

1. Date subject transferred: ___ ___ ___ - ___ ___ - ___ ___ ___ ___
   Month Day Year

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 4

COLLECTION AND SHIPMENT OF SPECIMENS FOR CENTRAL AND LOCAL LABORATORIES

4.1 OVERVIEW

The purpose of this chapter is to provide guidelines for the collection, preparation and shipment of blood and urine specimens to the BABY HUG Follow-up Study II Core Laboratories. Exhibit 4-1 summarizes the required Core Laboratory specimens. It should be noted that all specimens that will be shipped to the Core Laboratories must be shipped Monday through Thursday only. Core Laboratories cannot process specimens over the weekend.

Exhibit 4-1
Summary of Core Laboratory Specimens

Specimen	Volume	Collection tube type	Frequency		Laboratory	Shipping Condition/Timing
			Active	Passive		
Urine for Microalbumin: Creatinine Ratio and Stored Urine	10 mL	Cryovial	Entry, Exit	Entry, Exit	AU/Niren Patel	Ship Upon Collection with Frozen Gel Pak
Stored Blood Sample	5.0 mL	EDTA lavender top	Entry, Exit	Entry, Exit	AU/Niren Patel	Ship Upon Collection with Frozen Gel Pak
Cystatin C	1.0 mL	Red top	Entry, q12 months, Exit	Entry, Exit	CCHMC/Thad Howard	Ship Upon Collection with Frozen Gel Pak-**DO NOT PLACE FROZEN GEL PAK IMMEDIATELY UP AGAINST CYSTATIN C TUBES**
HbF	0.5 mL	EDTA lavender top	Entry, q12 months, Exit	Entry, Exit	AU/Niren Patel	Ship Upon Collection with Frozen Gel Pak
Howell Jolly Bodies/ Reticulocyte Micronuceli	1.0 mL	EDTA lavender top	Entry, q12 months, Exit	Entry, Exit	CCHMC/Thad Howard	Ship Upon Collection with Frozen Gel Pak
Pitted Cells	0.1 mL	EDTA lavender top, transfer to vial w/gluteraldehyde	Entry, q12 months, Exit	Entry, Exit	CMC Dallas/John Burns	Frozen Gel Pak Ship Batched within 2 weeks of collection–**DO NOT FREEZE SPECIMENS**
Creatinine and BUN	1.0 mL 4.0 mL	Red top Red top	Entry, Exit q12 months	Entry, Exit	AU/Niren Patel	Ship Upon Collection with Frozen Gel Pak
VDJ/ DNA Extraction	3.0 mL	EDTA lavender top	Entry, Exit	Entry, Exit	CCHMC/Thad Howard	Ship Upon Collection with Frozen Gel Pak

4.2 LABELS

The BABY HUG Follow-up Study II Data Coordinating Center (DCC) will provide each Clinical Center with pre-printed labels for all specimens: blood, urine and images/CDs. The BHFU II labels contain randomized 13-digit alpha-numeric characters to ensure the Core Laboratories remain "masked" to subject identification.

The Clinical Center staff will receive sets of label sheets grouped by Subject ID Number. When a child enrolls in the BHFU II, he/she maintains the Subject ID Number that was assigned to him/her in the BABY HUG Treatment Study and subsequently, BABY HUG Follow-up Study I. The set of label sheets with that Subject ID Number shall be used exclusively for that child for all of his/her specimens and images/CDs throughout the entire study. These child-specific label sheets enable the DCC to link particular specimens and images/CDs to the child when the label number is entered into the database. A single label *number* is to be used per specimen.

The labels in the first column have the Subject ID Number (e.g., 9998). These labels will not be used. Each row on these label sheets consists of duplicate labels with the same label number and label code, with the addition of aliquot numbers. The first label, which excludes an aliquot number, should be affixed to the specimen. **The remaining labels should be cut from the sheet and shipped with the specimen to the central laboratory.**

4.3 GENERATING SHIPPING MANIFESTS

Study forms, shipping manifests and data files are used to track processing, shipment and receipt of biological specimens and imaging CDs used in the BHFU II from Clinical Centers to Core Laboratories, and the Central Repository. All blood specimens collected and shipped must be handled according to universal precautions.

All shipments to Core Laboratories must be accompanied by a shipping manifest. Shipping manifests are generated using the DCC's electronic Data Management System (eCOS)

after specimen or imaging study information (e.g. label number, date collected, time collected, etc.) has been recorded on the appropriate electronic case report form (e.g. Form 03 - Central Lab Collection, Form 21 – Liver/Spleen Scan Performance, etc.), indicating that a sample was shipped. Once the form is completed/saved, then a shipment manifest will be generated by eCOS and sent via email to the Study Coordinator. The coordinator must print each emailed manifest, **black-out the Subject ID**, and then place a copy of in the manifest(s) into the shipment box. The coordinator should also print a copy of the manifest to be retained with the subject's study records at the Clinical Center. The specimens/images must be prepared for shipment according to the specific Core Laboratories requirements. Once the shipment is ready, then the Study Coordinator will email the intended Core Lab recipient, Cc'ing the DCC (BabyHug@neriscience.com), notifying them of the shipping date and including the FedEx tracking number in the *Subject Line* of the email. The Core Lab will reply to the originating Study Coordinator's notification email, and the DCC, confirming receipt and condition of specimen(s)/file(s).

4.4 HEMATOLOGY, BIOCHEMISTRY AND URINE SPECIMENS

4.4.1 Specimen Collection

All specimens are to be collected using **Standard Precautions**. The Core Laboratory can analyze specimens received up to 48 hours after collection. After this time, the specimens must be discarded. Clinical Centers will need to send specimens in a timely manner.

4.4.1.1 HbF

HbF is to be collected for at entry and exit for all subjects, and an additional specimen should be collected every 12 months in the active group.

4.4.1.2 HPLC Creatinine and BUN

HPLC Creatinine and BUN are to be collected at entry and exit for all subjects, and additionally for the active subjects every 12 months.

4.4.1.3 Stored Blood Sample

For those who have consented, stored blood specimens will be collected at entry and at exit for both active and passive subjects.

4.4.1.4 Urine for Microalbumin: Creatinine Ratio

Urine specimens are to be collected at entry and at exit, for the urine Microalbumin: Creatinine ratio (MCR) for all subjects. For those who have consented, a volume of each MCR urine specimen will be stored at the Hematology Core Laboratory. Urine is to be transported in a cryovial and shipped to the Augusta University (AU) Core Laboratory with the blood specimens. Please label the vial appropriately with a label provided by the DCC. The subject does not need to have water withheld prior to collection of the MCR/stored urine sample. However, a center may divide the MCR/stored urine sample and submit a portion locally for urine osmolality (water deprivation preferred, see section 4.9.1 for instructions).

4.4.2 Shipping Procedure

1. Gel packs must be frozen prior to shipping specimens to the AU Core Laboratory.
2. Place one frozen gel pack in the bottom of the styrofoam-lined shipping box.
3. Put the tubes (stored blood, creatinine HPLC, HbF and/or urine) in the foam insert located in the white plastic container, seal the plastic bag and tighten the orange lid.
4. Place the container inside the styrofoam-lined shipping box on top of the frozen gel pack.
5. Place another frozen gel pack on top of the plastic container.
6. Fill the void inside the shipping box with crumpled or shredded paper.
7. Cover the Styrofoam-lined box with the styrofoam lid.

8. Fill out Form 03. Follow instructions in Section 4.3 Generating Shipping Manifests. All specimens can be shipped together in the same box.

9. Insert a copy of the manifest(s), **after blacking-out the Subject ID**, in the shipping box between the Styrofoam and the outer corrugated box, **as well as the remaining corresponding labels containing the aliquot numbers.**

10. Close the outer box and tape shut with appropriate shipping tape.

11. Attach a FedEx Airbill with the following address:

    Niren Patel (706) 721-9640 telephone
    Augusta University
    989 St. Sebastian Way
    Room EF-201
    Augusta, GA 30912

12. Send an email to Niren Patel at AU (NPATEL@augusta.edu) and the DCC (BabyHug@neriscience.com) with the tracking number in the Subject Line of the email. The lab will send confirmation, and condition of sample(s), once received. If the shipment is not received in the AU Core Laboratory at the expected time, the Core Laboratory Coordinator or a designee will pursue tracking the shipment and notify the Clinical Center and the DCC.

13. Blood specimens not received within 48 hours of collection will not be processed and must be redrawn.

4.4.3 Stored Blood Aliquot Procedures

Stored blood specimens will require 5 mL collected in an EDTA lavender-top tube. Tube(s) must be labeled with the same 13-digit alpha-numeric labels provided by the DCC. The specimens will be divided into plasma and cell pellet aliquots and stored frozen -70° F at the

Hematology Core Laboratory until shipped to the NHLBI Specimen Repository (BioLINCC) and labeled using those labels sent with the specimens from the Clinical Centers. Additionally, this laboratory will conserve residual plasma and cell pellets, which will also be shipped to the NHLBI Specimen Repository (BioLINCC).

4.5 PIT COUNT

4.5.1 Specimen Collection

Pitted Cell specimens are to be collected at entry and exit for all subjects, and additionally every 12 months for active subjects only. Requests for 2% glutaraldehyde tubes should be made to Lindsay Therrian at (214) 456- 1495 or Lindsay.Therrian@childrens.com. Please note the expiration date on the tubes when planning a subject's visit and allow one week's notice for shipping. Coordinator should provide the number of tubes needed, as well as a current email address. Ms. Therrian will notify the Coordinator as to when they should expect the shipment as it must be refrigerated upon arrival.

4.5.2 Preparation of Whole Blood Sample for Pitted Red Blood Cell Count

IMPORTANT: The pitted cell count sample should be prepared within one hour of collection of the blood sample into the EDTA lavender-top tube.

1. After the subject's whole blood sample is drawn and placed in an EDTA lavender-top tube, the sample is immediately used for preparation of the sample for the pitted cell count.
2. Put on gloves.
3. **Gently invert the lavender-top (EDTA) tube of subject blood 10 times to mix the sample.**
4. Remove stopper from the tube of blood.

5. Hold the provided plastic pipette vertically, squeeze the bulb and insert into the specimen. Carefully remove a small amount of the subject sample. Use a clean pipette for each subject sample. Wipe excess blood off of the outside of the pipette.

6. Place two small drops (approximately 100 µL) of fresh blood into 0.5 milliliters (mL) of 2% glutaraldehyde.

7. Recap the plastic glutaraldehyde tube firmly.

8. Gently invert the tube of blood/gluteraldehyde mixture ten times to ensure mixing of specimen. Place into specimen bag.

9. Recap the EDTA lavender-top tube. Discard the pipette into a biohazard container.

10. Label the glutaraldehyde tube and bag with one of the subject's 13-digit label numbers. On a blank label provided by the DCC, write the date and time of preparation, and the initials of the person who prepared the sample. Record the same information on Form 03 (Central Lab Collection). Follow instructions in Section 4.3 Generating Shipping Manifests.

11. Place the prepared sample in a refrigerator at 2^{o} **-** 8^{o} centigrade for storage until shipping, which must be within two weeks of collection. **DO NOT FREEZE SPECIMENS.**

4.5.3 Shipping Procedure

*** Please ship specimens on Monday through Thursday ONLY ***

1. Please contact John Burns via email (joburn@childrens.com), and Cc the DCC (BabyHug@neriscience.com) to notify him of your intent to ship the (batched)

specimen(s). Include the ship date and tracking number in the *Subject Line* of the email. Alternative contact is via telephone at 214-456-6779.

2. Use frozen gel packs for shipping the specimen.

3. Ship using the IATA-provided cold pack container with frozen gel packs to arrive the next morning (Tuesday - Friday only) to:

   John Burns
   Special Testing Laboratory
   Children's Medical Center of Dallas
   1935 Medical District Drive, D200
   Dallas, Texas 75235

4. Enclose one copy of the shipping manifest(s) generated from eCOS with sample(s). **Remember to <u>black-out</u> the Subject ID**. Retain a copy of each manifest in the local study records.

5. The Pitted Cell Core Laboratory will notify the Clinical Center, and DCC, upon receipt. If specimens are not acceptable then instruction will be provided with actions to take regarding recollection of the specimen.

4.6 VDJ/DNA Extraction

4.6.1 Specimen Collection

VDJ/DNA Extraction specimens are to be collected at entry and exit for all subjects.

4.6.2 Shipping Procedure

Follow the procedure below for specimen preparation for VDJ/DNA Extraction:

1. Collect 3 mL of peripheral blood in an EDTA lavender-top vacutainer. This specimen can only be collected Monday - Thursday.

2. Affix one of the child's designated BABY HUG Follow-up Study II labels to the tube.

3. Fill out Form 03 (Central Lab Collection). Follow instructions in Section 4.3 Generating Shipping Manifests.

4. The Core Laboratory should be notified by e-mail (Thad.Howard@cchmc.org), Cc'ing the DCC (BabyHug@neriscience.com), the night of shipment with the ship date and a FedEx tracking number in the *Subject Line* of the email. The Core Lab will email upon receipt and if there is any issue with the package delivery and/or specimen.

5. Place a frozen gel pack above and below the container in the styrofoam package.

6. Place the shipping manifest(s) in the shipping box between the styrofoam and the outer corrugated box, **as well as the remaining corresponding labels containing the aliquot numbers**. Remember to black-out the Subject ID. Retain a copy of each manifest in the local study records.

7. Ship at room temperature the day of collection using overnight delivery to:

Thad Howard
Cincinnati Children's Hospital Medical Center
Ware Lab, CBDI - Hematology
3333 Burnet Ave, Room R1553 MLC 7015
Cincinnati, OH 45229
Phone: (513) 803-0965 or Cell (901) 652-4533
Fax: (513) 803-5095

4.7 HOWELL-JOLLY BODIES (HJB)/Reticulocyte Micronuclei

4.7.1 Specimen Collection

HJB/Reticulocyte Micronuclei specimens are to be collected at entry and exit for all subjects and additionally every 12 months in the active group only.

4.7.2 Shipping Procedure

Follow the procedure below for specimen preparation for HJB (same process as VDJ, different amount):

1. Collect 1.0 mL of peripheral blood in an EDTA lavender-top vacutainer. This specimen can only be collected Monday - Thursday.

2. Affix one of the child's designated BABY HUG Follow-up Study II labels to the tube.

3. Fill out Form 03 (Central Lab Collection). Follow instructions in Section 4.3 Generating Shipping Manifests.

4. The Core Laboratory should be notified by e-mail (Thad.Howard@cchmc.org), Cc'ing the DCC (BabyHug@neriscience.com), the night of shipment with the ship date and a FedEx tracking number in the *Subject Line* of the email. The Core Lab will email upon receipt and if there is any issue with the package delivery and/or specimen.

5. Place a frozen gel pack above and below the container in the styrofoam package, **as well as the remaining corresponding labels containing the aliquot numbers**.

6. Place a copy of the manifest(s) in the shipping box between the styrofoam and the outer corrugated box. **Remember to black-out the Subject ID**. Retain a copy of each manifest in the local study records.

7. Ship at room temperature the day of collection using overnight delivery to:

Thad Howard
Cincinnati Children's Hospital Medical Center
Ware Lab, CBDI – Hematology
3333 Burnet Ave, Room R1553 MLC 7015
Cincinnati, OH 45229
Phone: (513) 803-0965 or Cell (901) 652-4533
Fax: (513) 803-5095

4.8 CYSTATIN-C

4.8.1 Specimen Collection

Cystatin C specimens are to be collected at entry and exit for all subjects and additionally every 12 months in the active group only.

4.8.2 Shipping Procedure

Follow the procedure below for sample preparation for Cystatin-C:

1. Draw 1.0mL of blood into a small red-top (no anticoagulant) tube.

2. Hold the red-top tube at room temperature for 15-30 min.

3. Label the tube using a freezer-safe label provided by the DCC.

4. Fill out Form 03 (Central Lab Collection). Follow instructions in Section 4.3 Generating Shipping Manifests.

5. The day of collection, add specimen to plastic transport container. Place a frozen gel pak above and below the container in the styrofoam package supplied. Include a copy of the shipping manifest(s), remembering to black-out the Subject ID, **as**

well as the remaining corresponding labels containing the aliquot numbers. Retain a copy of each manifest in the local study records.

6. You may ship Cystatin C specimens to CCHMC, along with HJB/VDJ/DNA specimens. However, do not place the Cystatin C specimens immediately up against frozen gel paks. If the packages are prepared as described above in sections 4.6.2 and 4.7.2, then the Cystatin C specimens may be safely shipped with other specimens.

7. The Core Laboratory should be notified by e-mail (Thad.Howard@cchmc.org), Cc'ing the DCC (BabyHug@neriscience.com), the night of shipment with the ship date and a FedEx tracking number in the *Subject Line* of the email. The Core Lab will email upon receipt and if there is any issue with the package delivery and/or specimen. Shipment should be made only Monday through Thursday.

The shipping address for Cystatin-C is:

Thad Howard (Cystatin C)
Cincinnati Children's Hospital Medical Center
Ware Lab, CBDI – Hematology
3333 Burnet Ave, Room R1553, MLC 7015
Cincinnati, OH 45229
Phone: (513) 803-0965 or Cell (901) 652-4533
Fax: (513) 803-5095

4.9 LOCAL LABORATORY SAMPLE PREPARATION AND COLLECTION

4.9.1 Urine Osmolality Specimen Collection

As noted in section 3.1.2.1.1, at entry and exit all subjects will have CBC, Reticulocytes, Differential, LDH, Bilirubin, and ALT samples drawn and tested locally. Results will be recorded

on Form 04. The active group will, in addition, have a urine sample collected for urine osmolality, which will be performed locally. A urine collection cup, a plastic bag to place the urine cup in once the sample is collected, and a copy of the instructions in Exhibit 4-2 should be provided to the family at the visit prior to that on which urine specimens will be collected. The parent/guardian should be instructed to follow the directions as outlined on Exhibit 4-2, complete the questions asked and return the form with the urine. NPO (nothing by mouth) is reported on Form 04 as whole hours, truncated (6.6 hours is reported as 6 hours). The urine sample may be divided and used for both the locally performed urine osmolality and the centrally performed microalbumin:creatinine ratio. The microalbumin:creatinine ratio sample for central analysis (see section 4.4.1.4) may also be performed on a random urine sample.

Exhibit 4-2
Urine Concentrating Ability Instructions and Worksheet

We need to collect urine at least 6 to 8 hours after the last time a child had anything to eat or drink

Use the worksheet below to record the information requested in the following instructions.

1. Have your child void or pass urine at about bedtime.
2. Write down the date and time of the fluid (even water) before this void.
3. After at least 6 hours from the last fluid, when your child (you) awakens have your child urinate in cup. We need at least an inch of urine in the cup.
4. Close the lid tightly on the cup and place in the plastic bag provided.
5. Bring the urine sample with you to your child's clinic visit no more than 4 hours after they gave the urine sample. Leave the urine at room temperature until giving it to your child's nurse or research staff. If more than 4 hours, then refrigerate until the sample can be delivered.

Special things to know:

1. If your child passes urine during the night you may collect the morning urine, however, make note of it in the "Problems", below.
2. If your child drinks anything during the night you may collect the morning urine, however, make note when/what was consumed under "Problems", below.
3. If your child has something to eat before the morning urine is collected, but it does not contain a lot of liquid, then DO collect the urine sample. Make note of it under "Problems", below.

Time of last fluid: Date _____ and hour _____ am pm

Time of urine collection: Date _____ and hour _____ am pm

Please tell us about any problems you had collecting this sample.

Problems:_____

_____

Thank you!

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 5

PROCEDURES FOR SPECIAL STUDIES IN THE ACTIVE GROUP

5.1 INTRODUCTION

In addition to the collections specified in the BABY HUG Follow-up Study II Protocol Section 9.2.1 for the passive group, active subjects will also have the collection of many of the same primary and secondary endpoint measurements as in the BABY HUG Treatment Study. These will include: HbF, pit counts, HJB, VDJ, liver-spleen scan information (quantitative and qualitative), abdominal sonogram, multi-digit serum creatinine (for the calculation of the Schwartz estimate of GFR), Cystatin C, and neuropsychological testing. Note that the tests and procedures described in this chapter **apply only to the active group** of subjects in the follow-up study and should be collected according to Exhibit 1-1 of the MOO (Appendix A of Protocol).

As permitted by the family, primary and secondary endpoints will be measured when the subject is approximately 10 years old (±3 months). Additional blood draws will be made and a complete physical examination with structured neurological assessment (Form 14) will be performed annually. The schedule of child visits during which specimens will be collected for laboratory testing is shown in Exhibit 1-1 of the MOO. A questionnaire (Form 15) will be administered which addresses health-related issues including enuresis, obstructive sleep apnea/snoring and priapism.

5.1.1 Special Studies Performance

It is extremely important that the instructions included in this section be followed precisely each time a special study is performed. Please provide a copy of these instructions to the

technician prior to performance of any special study. **It is also very important that all subject identifying information be excluded from all images stored and shipped outside of the Clinical Center for review.**

5.2 LIVER-SPLEEN SCAN

5.2.1 Procedure

Radiopharmaceutical: 99m Tc Sulfur Colloid.

Dose: 0.05 mCi/kg (preferred minimum dose 0.5 mCi; a dose of 1.0 mCi or more acceptable if that is the local practice).

Injection Site: Direct venous administration. Scan should begin within 15 minutes of injection.

Equipment: Large field of view gamma camera.

Collimator: Low energy or general (have consistent use of collimator type for a subject).

Computer Set up: **400K Image:** Static 400 K counts, 256 X 256 byte mode.

Timed Image: Static fixed time views for the same amount of time as the static 400K counts true posterior image.

Scanning Technique:

1. Subject in supine position on table.

   Collimator should be in contact with subject (anterior head touching or as close as possible).

2. 400K Image: **true anterior/posterior** views.

   Timed Image: **left anterior oblique/right posterior oblique** views (obliquity should create maximum separation

of liver and spleen with minimal to no overlap of organs; record camera angle). Quantitative Analysis:

400K Image: Draw region of interest around spleen, then liver on the **anterior** view. Record counts, number of pixels in ROI, and counts/pixel for each organ. Take a picture.

3. 400K Image: Repeat the same for the **posterior** view. Take a picture.

4. 400K Image: Calculate the geometric mean counts of spleen and liver from both views. (See Section 5.2.2.) Do the same for counts/pixel. Take a picture or record on film.

5. 400K Image: Generate the total spleen to liver ratio (See Section 5.2.2.) and the counts/pixel spleen to liver ratio. Take a picture(s) or record on film.

6. Timed Image: Repeat step 1 using **left anterior oblique** view.

7. Timed Image: Repeat step 1 using **right posterior oblique** view.

8. Timed Image: Calculate the geometric mean counts of spleen and liver from both views. Do the same for counts/pixel. Take a picture or record on film.

9. Timed Image: Generate the total spleen to liver ratio and the counts/pixel spleen to liver ratio. Take a picture(s) or record on film.

Processing Scans:

1. 400K Image:

- Include proper identification.
- Label the anterior view #1.
- Label the posterior view #2.
- Label the anterior view with region of interest around spleen and liver with counts and counts/pixel recorded on the film as #3.
- Label the posterior view with region of interest around spleen and liver with counts and counts/pixel recorded on the film as #4.
- Label as #5 the geometric mean counts and the geometric mean counts/pixel of spleen and liver from both views.
- Label as #6 the total counts and counts/pixel spleen to liver ratios.

Please do not draw ROIs if the spleen cannot definitively be seen.

2. Timed Image:

- Include proper identification.
- Label the left anterior oblique (LAO) view #7.
- Label the right posterior oblique (RPO) view #8.
- Label the LAO view with region of interest around spleen and liver with counts and counts/pixel recorded on the film as #9.
- Label the RPO view with region of interest around spleen and liver with counts and counts/pixel recorded on the film as #10.
- Label as #11 the geometric mean counts and geometric mean counts/pixel of spleen and liver from both views.
- Label as #12 the total counts and counts/pixel spleen to liver ratios.

5.2.2 Calculations for Quantitative Assessment

The geometric mean counts of the spleen and the liver, and the spleen-liver ratio, will be recorded on the films and calculated according to the following formula:

Spleen geometric mean = $\sqrt{AxC}$

Liver geometric mean = $\sqrt{BxD}$

Spleen-liver ratio = $\frac{\sqrt{AxC}}{\sqrt{BxD}}$

For the total counts of spleen-liver ratios (400K and Timed Images), the following applies:

A = anterior (400K Image) or LAO (Timed Image) spleen count

B = anterior (400K Image) or LAO (Timed Image) liver count

C = posterior (400K Image) or RPO (Timed Image) spleen count

D = posterior (400K Image) or RPO (Timed Image) liver count

The counts/pixel geometric means and the spleen-liver ratios (400K Image and Timed Image) will also be calculated according to the above formula.

5.2.3 Labeling and Shipping of CDs

A hard copy of the liver/spleen scan CD will be sent to two central reviewers to assess splenic uptake at a later date. In the meantime, use the following procedure to label the CDs and store a copy of the CD in a secure location until further notice:

1. Remove all subject Personal Identifying Information (PII) on all CDs, to the best extent possible.

2. Affix to each CD a label with the 13-digit alpha-numeric characters as affixed to the BABY HUG Follow-up Study II form requesting the scan (Form 21). All CDs must have the same label number.

3. Data enter Form 21 into eCOS.

4. When ready to ship, follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the manifest(s), a copy of which will be placed in the shipping package with the CD. **Be sure to <u>black-out</u> the Subject ID** and retain a copy of the manifest(s) in the local research file.

5.3 ABDOMINAL SONOGRAM

5.3.1 Procedure

<u>Scheduling and NPO Guidelines</u>

Because it is preferable that subjects be NPO (nothing by mouth) (except for clear liquids) for six hours before the sonogram, schedule them as the first exam of the day. It is acceptable to give clear liquids (Pedialyte, water, clear juices) as needed, but no milk, prior to the sonogram, as indicated by each Clinical Center's standard of care.

<u>Probe Selection</u>

Use the highest frequency, non-linear probe that is available and that gives the best image quality.

<u>Image Annotation</u>

When the subject is not supine, note on the image the position that the subject is in (i.e., left decubitus, right decubitis, prone or upright).

Scanning Protocol

A. Gall Bladder

i. With the subject in the supine position, take multiple longitudinal and transverse images representative of the entire gallbladder.

ii. With the subject in either the left or right lateral decubitus position, take multiple transverse and longitudinal images representative of the entire gallbladder.

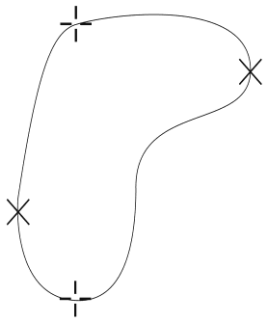
iii. If there appears to be gallbladder wall thickening (above 3mm) measure the gallbladder wall on a transverse image and obtain a color Doppler image of the gallbladder wall.

iv. Measure the diameter of the common bile duct (CBD) in the region of the porta hepatitis. Confirm that you are measuring the CBD with color Doppler.

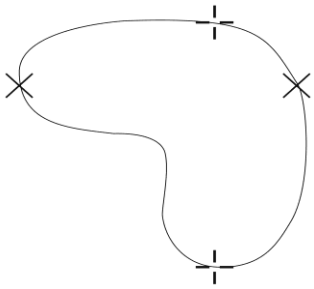
B. Liver

i. Include one longitudinal image of the liver obtained with the right kidney in the field of view. The image should be obtained through the middle portion of the kidney and/or where the greatest kidney length can be obtained. Please include a longitudinal measurement of the liver at this location.

C. Spleen (See Exhibit 5-1 for the complete checklist.)



Example of a longitudinal spleen image: Note the correct cursor placement for anterior-posterior measurement (+) and longitudinal measurement (x)



Example of a transverse image of the spleen: Note the correct cursor placement for transverse measurement (x).

i. Obtain measurements during relaxed respiration.

ii. In the transverse coronal plane, measure the greatest length and AP diameter at the hilum of the spleen.

iii. In the transverse plane, measure the greatest transverse diameter of the spleen at the level of the hilum.

iv. Multiply the above three measurements together then multiply the result by a factor of 0.53 to obtain an estimate of spleen volume.

D. Kidneys

i. In the supine position, obtain a longitudinal image of each kidney with the liver and spleen in the field of view in order to compare renal echogenicity to liver/spleen echogenicity.

ii. For the remainder of renal scanning, place the subject prone whenever possible. Alternatively, if necessary, scan in the upright position, with the probe on the subject's back. If unable to scan with a posterior approach, scan the kidneys in the supine position. Obtain the following:

1. Longitudinal images representative of the entire kidney
2. Transverse images representative of the entire kidney
3. Maximum renal length measurements

iii. Maximum transverse measurement at the level of the renal hilum

iv. Maximum anterior-posterior measurement in BOTH the longitudinal and transverse planes

5.3.2 Labeling and Shipping of CDs

A hard copy of the abdominal sonogram CD will be sent to a central reviewer at a later date. In the meantime, use the following procedure to label all the CDs and store a copy of the CD in a secure location until further notice from the DCC:

1. Remove all subject Personal Identifying Information (PII) on all CDs, to the best extent possible.
2. Affix to each CD a label with the same 13-digit alpha-numeric characters as affixed to the BABY HUG Follow-up Study II form requesting the sonogram (Form 23).
3. Data enter Form 23 into eCOS.
4. When ready to ship, follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the shipping manifest(s), a copy of which should be placed in

the shipping package with the CD. **Be sure to <u>black-out</u> the Subject ID** and place another copy of the manifest(s) in the local research record.

5.4 NEUROPSYCHOLOGY MEASURES

The neuropsychological measures consist of three measures administered by a licensed psychologist or a graduate student, intern, fellow, or master's level psychometrician under the supervision of a licensed psychologist; these include:

1. A test of intellectual function (Wechsler Intelligence Scale for Children, 4th Edition; WISC-IV);
2. A computerized test of attention and concentration (Conners Continuous Peformance Test, 2nd Edition; CPT-II); and
3. The Vineland Adaptive Behavior Scales, Interview Edition, Survey Form (VABS-I).

IN ADDITION, a designated BABY HUG staff member (coordinator) will administer four measures of quality of life:

1. PedsQL™ Generic Core Scales;
2. PedsQL™ Multidimensional Fatigue Scale;
3. PedsQ™L Sickle Cell Disease Module; and
4. PedsQL™ Family Information Form).

5.4.1 Wechsler Intelligence Scale for Children, 4th Edition (WISC-IV)

Instructions for administering and scoring the WISC-IV are found in the test manual: Wechsler, D. (2003). WISC-IV (Wechsler Intelligence Scale for Children, Fourth Edition) Administration and Scoring Manual. San Antonio: Harcourt Assessment (PsychCorp).

The WISC-IV must be individually administered by a licensed psychologist trained in its use. The test must be administered in a quiet location on a table with chairs of appropriate height for the size of the child. Interruptions during the test are not permitted, unless directed by the test administrator, since some of the subtests are timed. The test equipment will be provided by the study. For each child, two test booklets are required for test administration and scoring: The Record Form and the Coding and Symbol Search Booklet. A stopwatch or alternative electronic timer will be needed.

The test takes approximately 1.5 hours to complete, and consists of 10 subtests (Block Design, Similarities, Digit Span, Picture Concepts, Coding, Vocabulary, Letter-Number Sequences, Matrix Reasoning, Comprehension, and Symbol Search). The supplemental subtests (Picture Completion, Cancellation, Information, Arithmetic, and Word Reasoning) are NOT administered. The 10 subtests result in a scaled score that ranges from 1 to 19.

The 10 subtests determine a Full Scale IQ score, Verbal Comprehension Index, Perceptual Reasoning Index, Working Memory Index, and Processing Speed Index. These indices are reported as standard scores, with scores ranging from 40 to 160.

The WISC-IV can be hand scored, which will require using the Norms and Conversion Tables found in Appendix A of the WISC-IV Administration and Scoring Manual. Alternatively, a computerized scoring program for the WISC-IV can be used. If the WISC-IV is computer scored, the raw scores entered into the program should be checked twice for accuracy before data are entered into the Case Report Form.

Scores are to be transferred on to BABY HUG Follow-up Study II Form 28. Data for all scores on listed Form 28 should be entered.

Within two weeks of completion of the WISC-IV, the completed BABY HUG Follow-up Study II Form 28 is to be data entered in eCOS. All original examination forms should be kept in

the research files of the study following institutional IRB and HIPAA policies related to the storage and maintenance of psychological testing raw data.

5.4.2 Conners Continuous Performance Test, 2nd Edition (CPT-II)

The CPT-II is a computerized test of attention and concentration. The test must be installed onto a single computer that will be available for use for all study participants. The computer should have access to a printer so that a hard copy of the test results can be printed. The CPT-II test must be administered by a licensed psychologist or graduate student, intern, fellow, or master's level psychometrician under the supervision of a licensed psychologist. Instructions for installation and administration are provided by the study along with the test manual and software.

The child's BABY HUG acrostic should be entered into the program instead of the child's name, and the school/facility should be BABY HUG Ext 2. The administration instructions are provided in the test manual: Conners, C.K., & MHS Staff. (2000), Conners' CPT-II Continuous Performance Test II, Computer Program for Windows Technical Guide and Software Manual. North Tonawanda, NY: MHS.

The CPT-II takes approximately 20 minutes to set up and administer. The test is very simple. Letters of the alphabet are presented at 1, 2, or 4 second intervals. The child is told to press the space bar every time any letter except a designated letter (say X) is shown on the screen. When the X appears, the child has to inhibit pressing the space bar. Because this is a test that requires sustained, focused attention and measures speed of responding, it is critical that the test be administered in a quiet place with no interruptions. Interruption invalidates the test.

When completed, the child's data should be saved electronically and a hard copy printed out.

Scores are to be transferred on to BABY HUG Follow-up Study II Form 30. Data for all scores on listed Form 30 should be entered.

Within two weeks of completion of the CPT-II, the completed BABY HUG Follow-up Study II Form 30 is to be data entered in eCOS. All original examination forms should be kept in the research files of the study following institutional IRB and HIPPA policies related to the storage and maintenance of psychological testing raw data.

5.4.3 Vineland Adaptive Behavior Scales (VABS)

The psychologist at each Clinical Center will administer the *Vineland Adaptive Behavior Scales: Interview Edition*, *Survey Form* to the child's parent or guardian at approximately 10 years of age for Active subjects only. (NOTE: The VABS is being used for this study instead of the newer version (VABS-II) to permit measurement with the same instrument across assessment points, beginning with the original BABY HUG study.)

The Data Coordinating Center (DCC) will purchase sufficient Record Booklets for each Clinical Center.

The BABY HUG Follow-up Study II psychologist will administer the Communications, Daily Living Skills, and Socialization Domains to the child's parent or guardian. The Motor Skills Domain items are not administered to children over age 5. The psychologist should strictly adhere to the standardized directions given in the Survey Form Manual for administration of the questionnaire. Administration instructions and scoring directions are included in the test manual: Sparrow, S.S., Balla, D.A. & Cicchetti, D.V. (1984). *Vineland Adaptive Behavior Scales, Interview Edition, Survey Form Manual,* Circle Pines, MN: AGS-American Guidance Service. The responses are to be recorded and scored on the Record Booklet by the psychologist administering the test, and then transferred to BABY HUG Follow-up Study II Form 27 (Vineland). The Standard Score, the 95% Band of Error and the National Percentile Rank must also be recorded on the booklet and the

BABY HUG Follow-up Study II form. If the child is older than 5 years old, then the Motor Skills section of the examination should be skipped and documented as "Not Done" on the Form 27.

Within two weeks of completion of the Vineland test, the completed BABY HUG Follow-up Study II Form 27 is to be data entered in eCOS. All original examination forms should be kept in the research files of the study following institutional IRB and HIPPA policies related to the storage and maintenance of psychological testing raw data.

If a child's parent/guardian would like the results of the examination, they may be given the result and interpretation by the local examiner.

5.4.4 Quality of Life Measures

The quality of life measures consist of four paper and pencil questionnaires that may be administered by a trained staff member (e.g., coordinator) for the study. It is important to assess whether the parent and/or child is able to read. If not, the coordinator should take the parent/child to a quiet, private room and offer to read the items aloud. It is critical that this is done in a manner that is not embarrassing to either the parent or the child.

The quality of life measures include:

1. PedsQL™ Family Information Form. (To be completed by the child's parent). This is a single page questionnaire that captures demographic information about the child and parents, as well as 5 questions about the impact of the child's health condition on the child and 4 questions about the impact of the child's health condition on the parents.
2. PedsQL™ Generic Core Scales (To be completed by the child and the parent). This is a single page questionnaire that includes questions related to problems with Health and Activities (8 questions), Feelings (5 questions), Getting Along with Others (5 questions), and School (5 questions). One questionnaire is completed by the

parent (Parent Report for Children ages 8-12) and one by the child (Child Report ages 8-12). Each item is rated on a 5-point scale (0=Never to 4=Almost Always).

3. PedsQL™ Multidimensional Fatigue Scale. (To be completed by the child and the parent). This is a single page questionnaire that includes questions related to General Fatigue (6 questions), Sleep/Rest Fatigue (6 questions), and Cognitive Fatigue (6 questions). One questionnaire is completed by the parent (Parent Report for Children ages 8-12) and one by the child (Child Report ages 8-12). Each item is rated on a 5-point scale (0=Never to 4=Almost Always).
4. PedsQ™L Sickle Cell Disease Module. (To be completed by the child and the parent). This is a two page questionnaire that includes questions related to the subject's pain, pain impact and pain management; his/her worrying and emotions; effects of treatment; and communication issues related to sickle cell disease. One questionnaire is completed by the parent (Parent Report for Children ages 8-12) and one by the child (Child Report ages 8-12). Each item is rated on a 5-point scale (0=Never to 4=Almost Always).

Scoring of the PedsQL™ is straightforward. Instructions are provided in the PedsQL™ Scoring Algorithm. Each of the items is "reverse-scored" and then translated into a 100 point scale such that a 0 = 100, 1=75, 2=50, 3=25, 4=0. The scores are then summed and divided by the number of items answered. At least 50% or more of the items have to be completed. Thus, if a child responded to Item 1=0, Item 2=0, Item 3=3, Item 4=4, Item 5=2, and Item 6 was not answered, then the scoring would be as described in the example below.

EXAMPLE: *In the past **ONE month**, how much of a **problem** has this been for you…*

ABOUT MY HEALTH AND ACTIVITIES (problems with...)	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than one block	(0)	1	2	3	4
2. It is hard for me to run	(0)	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	(3)	4
4. It is hard for me to lift something heavy	(0)	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	(1)	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4

Item 1: 0= 100

Item 2: 0= 100

Item 3: 3= 25

Item 4: 4= 0

Item 5: 1= 75

Item 6: Missing

Sum: 100+100+25+75=300/5= 60

This is done for each subscale, and the total score is calculated using all the items. Higher scores are associated with better quality of life.

Scores are to be transferred on to BABY HUG Follow-up Study II Form 29. Data for all responses should be entered on Form 29 and final scores will be calculated by the DCC.

Within two weeks of completion of the quality of life instruments, the completed BABY HUG Follow-up Study II Form 29 is to be data entered in eCOS. All original examination forms should be kept in the research files of the study following institutional IRB and HIPAA policies related to the storage and maintenance of psychological testing raw data.

5.5 TRANSCRANIAL DOPPLER ULTRASONOGRAPHY (TCD)

5.5.1 TCD Performance

1. The BABY HUG Follow-up Study II coordinator should provide the TCD examiner with a copy of the TCD form (Form 13). A 13-digit alpha-numeric label should be affixed to the form.

2. The TCD should be performed according to the Clinical Center standard procedures and the data captured on CD and prepared for shipment to the TCD Core Laboratory at the Medical University of South Carolina.

3. All subject Personal Identifying Information (e.g. name, date of birth, social security number, hospital ID number, etc.) should be removed from the CD to the best extent possible.

5.5.2 TCD Shipment

CD(s) with the TCD images will be sent to the TCD Core Laboratory at a later date. In the meantime, use the following procedure to label all the CDs and store a copy of the CD in a secure location until further notice from the DCC:

1. Remove all subject Personal Identifying Information (PII) on all CDs, to the best extent possible.

2. Affix to each CD a label with the same 13-digit alpha-numeric characters as affixed to the BABY HUG Follow-up Study II form requesting the TCD (Form 13).

3. Data enter Form 13 into eCOS.

4. When ready to ship, follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the shipping manifest(s), a copy of which should be placed in

the shipping package with the CD. **Be sure to black-out the Subject ID** and place another copy of the manifest(s) in the local research record.

5. Once shipment has been approved, send all CDs and shipping manifests via FedEx to the TCD Core Laboratory and email (adamsrj@musc.edu), Cc'ing the DCC (BabyHug@neriscience.com), with the ship date and tracking number. The TCD Core Lab will confirm, with the originating center coordinator and DCC, upon receipt.

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Charleston SC 29425

5.6 CARDIAC ECHOCARDIOGRAPHY AND BRAIN NATURIETIC PEPTIDE

5.6.1 Echocardiogram Performance

Echocardiograms performed as part of the BABY HUG follow up study **are for research purposes only, and** will not include a clinical interpretation. The CD-ROM with the echocardiographic data will not be returned to the site. The ECHO measurement data generated at the central reading facility will be sent to the site, however, there may be a delay in doing the measurements and reporting them to the site. Therefore, if there is a clinical indication for an echocardiogram, the site may wish to perform a complete clinical ECHO locally. If a clinically-indicated echocardiogram occurs within a study window, the echocardiogram can be used for the study as long as the original data are available for central reading and all of the components of the echocardiogram that are required are included.

The guidelines below are intended to assist the Clinical Center staff with capturing all of the required study information. Please provide Form 32 (ECHO Performance) and this section of the Manual of Operations to the Echocardiographer prior to the performance of the echocardiogram. The following information will be required to obtain a complete assessment of the echocardiogram.

Height and Weight. Measurement of height and weight are critical to the interpretation of the echocardiographic measurements. Height and weight should be measured on the day of the echocardiogram.

Equipment. The echocardiogram requires a high-resolution ultrasound machine using a transducer appropriate for body size and equipped with pulsed Doppler, 2-D directed M-mode, tissue Doppler, and ECG. Recording of systolic, diastolic, and mean blood pressure requires an automated blood pressure recorder such as the Dinamap Vital Signs Monitor, Critikon, Inc.

Image De-Identification. To assure full compliance with HIPAA requirements, patient identifiers (such as name, date of birth, medical record number, etc.) will not to be included in the submitted echocardiograms, to the best extent possible. The patient's "Label ID" (see 4.2 LABELS) and the date of the echocardiogram will uniquely identify each of the exams. These data should be embedded in the image data in addition to inclusion on the echocardiogram submission form to assure participant identification. The method of inclusion of the patient's Label ID depends on the specific ultrasound machine but entering the Label ID as the patient last name will generally assure that the information will be embedded on the image.

Methods. The 2-D echo and Doppler exams are preferentially recorded in DICOM format and transferred to a CD or DVD for submission to the core lab.

All measurements will be made at the core lab and no measurements are required from the clinical sites. A good quality electrocardiographic recording is required to be present on all

echocardiographic images. Each of the individual recordings should include 6-8 cardiac cycles in order to include at least 2 respiratory cycles. M-mode and pulsed Doppler recordings are used to measure time intervals and therefore should be recorded at a sweep speed of 100mm/s. The following recordings should be obtained:

1. *Apical long-axis 4-chamber view of the left ventricle.* The transducer should be positioned at the true apex with the plane of interrogation bisecting the mitral valve. The image depth setting should be reduced to maximize the size of the image of the left ventricle but care must be taken to include the entire left ventricle throughout the cardiac cycle. Dual focus should be used to optimize the apical endocardium. At least 6-8 cardiac cycles should be recorded.

2. *Two-D parasternal long-axis images of the aortic root.* The transducer is positioned at the level of the aortic root, oriented in the axial plane of the aortic root and ascending aorta using the parasternal long-axis view. Zoom mode is activated over the aortic root to include the aortic valve annulus and aortic valve and root in the image At least 6-8 cardiac cycles should be recorded.

3. *Two-D parasternal long-axis images of the right ventricle inflow tract (RVIT).* Place the continuous wave Doppler through the Tricuspid valve within the Tricuspid Regurgitation jet (if there is any). The sweep speed should be adjusted to 100mm/s. At least 6-8 cardiac cycles should be recorded.

4. *Two-D images of the left ventricular short axis*. The transducer is positioned at the level of the maximum true short-axis diameter, which is usually at the level of the mitral valve leaflets in young children and at the level of tips of the leaflets in older children. The optimum position for the transducer can generally be determined in the parasternal long-axis view and then the transducer turned into a short axis orientation. The image depth

setting should be set to include the largest possible image of the left ventricular short axis but must include the posterior epicardium throughout the cardiac cycle. At least 6-8 cardiac cycles should be recorded.

5. *Two-D directed m-mode.* From a parasternal short axis image of the left ventricle, the m-mode sample cursor is centered as the diameter of the circular short axis of the left ventricle simultaneously with automated blood pressure. The blood pressure protocol is presented in detail below. The highest supported sweep speed should be selected. The size of the m-mode image of the left ventricle should be maximized by reducing the size of the 2-D image or if possible by switching to a display mode that includes only the m-mode image. At least 6-8 cardiac cycles should be recorded with at least four blood pressure recordings.

6. *Pulsed spectral Doppler samples of the mitral valve inflow.* Mitral valve inflow is recorded from the 4-chamber apical image of the left ventricle with the sample site positioned at the tips of the mitral valve. The screen size of the positive deflection should be maximized and the Doppler should be recorded at high sweep speed. The 2-D update trigger should be reduced to every 4-5 beats to allow at least 2 sequential beats to be recorded without blank space in the Doppler signal. At least 6-8 uninterrupted cardiac cycles should be recorded.

7. *Pulsed spectral Doppler samples of the tricuspid valve inflow.* Tricupid valve inflow is recorded from the 4-chamber apical image of the right ventricle with the sample site positioned at the tips of the tricuspid valve. The screen size of the positive deflection should be maximized and the Doppler should be recorded at high sweep speed. The 2-D update trigger should be reduced to every 4-5 beats to allow at least 2 sequential beats to be recorded without blank space in the Doppler signal. At least 6-8 uninterrupted cardiac cycles should be recorded. Tricuspid regurgitation velocity should be obtained

from apical 4 chamber. It should be documented as velocity (m/sec) as well as pressure gradient (mmHg).

8. *Apical 4 chamber view of the right ventricle*. Place the continuous wave Doppler through the Tricuspid vale within the Tricuspid Regurgitation jet (if there is any). The sweep speed should be adjusted to 100mm/s. At least 6-8 cardiac cycles should be recorded.

9*. Tissue Doppler.* Tissue Doppler samples from the left and right ventricular free walls and the ventricular septum in proximity to the atrioventricular valve annulus are obtained in color 2D and spectral modes. A 2-D apical 4-chamber view is obtained and tissue color Doppler mode is activated. For each of the 3 sample sites, the spectral Doppler sample site is positioned as close to the atrioventricular valve annulus as possible as long as the sample remains within the ventricular myocardium throughout the cardiac cycle. The length of the pulsed Doppler sample volume can be increased if necessary to obtain a myocardial tissue Doppler sample throughout the entire cardiac cycle. At least 6-8 cardiac cycles should be recorded a sweep speed of 100mm/s with a continuous signal free of Doppler dropout.

10. *Aortic Doppler.* From an apical 5-chamber view, color Doppler is used to orient the spectral Doppler sampling site within the center of the aortic outflow at the level of the aortic valve annulus. At least 6-8 consecutive beats should be recorded at a sweep speed of 100mm/s.

11*. Pulsed spectral Doppler samples of pulmonary vein inflow.* The Doppler sample site is positioned within the pulmonary vein just at the junction with the left atrium and the recording of 6-8 consecutive beats is made at a sweep speed of 100mm/s. The particular pulmonary vein that is sampled is not important although typically the right or left inferior vein is most easily sampled.

12. *M-mode color-Doppler recording of the left ventricular diastolic flow propagation velocity.* First, the maximum velocity of the early mitral inflow wave is obtained from the preceding pulsed Doppler samples (see # 4 above). This number is important because it is used to set the aliasing velocity on the color m-mode for the flow propagation tracing. The maximum mitral valve inflow velocity is typically between 50 and 100 cm/sec. The color m-mode velocity should be set for about ½ the maximum mitral valve inflow velocity [gathered in (6)], which is typically 25-50 cm/sec. The aliasing velocity settings on most ultrasound machines are available in steps (not a continuous range), so the value that is closest to ½ the maximum inflow velocity needs to be selected. The steps to obtaining this measurement are as follows. From an apical 4 chamber view of the mitral valve inflow, two-dimensional color Doppler mode is used to determine the position and direction of flow of the mitral inflow jet. The color Doppler sector must be large enough to include the entire length from the level of the mitral valve annulus to the left ventricular apex. The transducer is moved to a position of maximum alignment with the direction of left ventricular inflow and the m-mode color Doppler mode is activated with the sampling position aligned with the center of the inflow signal. The scale setting for the color Doppler m-mode is reduced to the target aliasing velocity. If the correct scale setting has been achieved, visual inspection of the signal will confirm an aliasing boundary on the color Doppler m-mode at about the level of the anterior mitral leaflet. It is difficult to obtain a stable recording of this signal so at least ten to fifteen cardiac cycles of the color Doppler m-mode sample should be recorded at the highest sweep speed setting.

Blood Pressure Measurement. Blood pressure (BP) should be obtained simultaneously with the m-mode recording since it will be used for wall stress calculation. When an automated blood pressure device is used, the device can be switched on and allowed to run during the m-mode sample, with a total of 4 blood pressure recordings. The first recording is the least accurate as

the machine uses this value to set the target range for future samples, and is therefore discarded. The subsequent 3 samples of systolic, diastolic, and mean blood pressures are to be submitted.

Interpretation. Videotape or digital recordings and blood pressure data will be sent to a central reading center for analysis of LV size, function, loading conditions and contractility.

No local interpretation is required**, nor will a clinical interpretation be returned to the site.**

To assist the Echocardiographer in collecting the required data, the following images are recommended by the central reading center:

> *Baby HUG FUS II: Estimate and breakdown of the number and type of echocardiogram images that should be included (minimum of about 29 total images) for a BABY HUG echocardiogram.*

1. ***2-D Parasternal long axis view of the Aortic Root*****:** At least 1 image
   - Parasternal long axis without zoom
   - Parasternal long axis with zoom
2. ***2-d Parasternal Long-axis of the RVIT (tricuspid valve)*****:** At least 3 images (if regurgitation present)
   - Parasternal long-axis view of the jet without color
   - Parasternal long-axis view of the jet with color
   - Parasternal long-axis view with continuous wave Doppler
3. ***2-D Parasternal Short axis view of the LV*****:** At least 1 image
   - Parasternal short axis view at the level of mitral valve leaflet (for young children)
   - Parasternal short axis view at the level of the tips of the leaflets (for older children)
4. ***2-D directed M-mode*****:** At least 1 image
   - M-mode of the LV in short axis view with correct sweep speed and blood pressure stated in the image

5. ***Apical 4 chamber view of the LV*** **:** At least 2 images
    - Apical 4 chamber view without Dual focus
    - Apical 4 chamber view with Dual focus
6. ***Pulsed spectral Doppler samples of the mitral valve inflow:*** At least 2 images
    - Apical 4 chamber view with pulse wave Doppler inactivated and positioned at the tips of the mitral valve
    - Apical 4 chamber view with activated pulse wave Doppler
7. ***Pulsed spectral Doppler samples of the tricuspid valve inflow:*** At least 2 images
    - Apical 4 chamber view with pulse wave Doppler inactivated and positioned at the tips of the tricuspid valve
    - Apical 4 chamber view with activated pulse wave Doppler
8. ***Continuous wave Doppler sample of the tricuspid valve regurgitation (if any) :*** At least 2 images
    - Apical 4 chamber view of the TR jet with color only
    - Apical 4 chamber view of the TR jet with color and continuous wave Doppler
9. ***Tissue Doppler:*** At least 6 images
    - Apical 4 chamber view of the left, right and septal ventricular free walls with color only
    - Apical 4 chamber view of the left, right and septal ventricular free walls with color flow and spectral mode
10. ***Aortic Doppler:*** At least 2 images
    - Apical 5 chamber view with color flow only
    - Apical 5 chamber view with color flow and spectral Doppler
11. ***Pulsed spectral Doppler samples of pulmonary vein inflow:*** At least 3 images
    - Apical 4 chamber view with color flow
    - Apical 4 chamber view with color flow showing the Pulse wave Doppler position.

- Apical 4 chamber view Pulse Doppler wave forms

12. ***M-mode color-Doppler recording of the LV diastolic flow propagation velocity:*** At least 2 images

- Apical 4 chamber view with 2-D color mode and a adjusted color Doppler sector
- Apical 4 chamber view with 2-D color mode, adjusted color Doppler sector and activated m-mode

5.6.2 Echocardiogram Shipment

CD(s) with the ECHO images will be sent to the ECHO Core Laboratory at a later date. In the meantime, use the following procedure to label all the CDs and store a copy of the CD in a secure location until further notice from the DCC:

1. Remove all subject Personal Identifying Information (PII) on all CDs, to the best extent possible.

2. Affix to each CD a label with the same 13-digit alpha-numeric characters as affixed to the BABY HUG Follow-up Study II form requesting the ECHO (Form 32).

3. Data enter Form 32 into eCOS.

4. When ready to ship follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the shipping manifest, a copy of which should be placed in the shipping package with the CD. **Remember to <u>black-out the patient ID</u>** and place another copy of the manifest(s) in the local research record.

5. Send all CDs and shipping manifests, in batches, via FedEx to the Echo Core Lab:

   Jason Czachor
   Clinical Research Center of Michigan
   Children's Hospital of Michigan

3901 Beaubien Blvd, Room # 5177 Carls Building

Detroit, MI 48201

5.7 PULMONARY FUNCTION TESTING

Pulmonary Function Testing (PFT) will be performed on active subjects according to the protocol outlined in this section. Clinical Center staff should provide the PFT examiner with BABY HUG Follow-up Study Form 31 and this section of the MOO prior to performance of the test. Hemoglobin from a standard local CBC needs to be performed on the same day as the PFTs and is paid for by the study.

NO CENTRAL READING of PFT data will be performed, so close attention to the details of interpretation of the raw data acquired during the test is required. Be sure that the PFT examiner reads and understands the tables to calculate the values required for reporting in eCOS for BHFU II. Recommendations for procedure modifications can be considered by the BHFU II Steering Committee.

The Form 31 is to be started by the BABY HUG Follow-up Study II Coordinator and signed by the Coordinator, completed in consultation with the local PFT examiner (and local supervising pulmonologist if required by the site) once all values are provided. Please work with the PFT examiner to be sure that there is agreement about the racial norms that should be used in evaluating the results according to the tables below.

Coordinators should remind families in advance about bronchodilator use as noted in Section 5.7.1.2 e: if the subject takes asthma medications, they should use the preventer as usual, but not use the bronchodilator (rescue) medication within 4 hours of the pulmonary function testing.

5.7.1 Spirometry

5.7.1.1 Standardization

a. Equipment and procedures are based on the ATS recommendations for accuracy and precision (1, 2).

b. All technicians should be certified pulmonary function technologists (Nat'l Board of Resp. Care) or meet the recommendations for personnel qualifications issued by the ATS (4). At a minimum, all technicians performing pulmonary function testing must be "certified" as properly trained by the center PI or by the medical director of the pulmonary function laboratory.

c. Standing height should be determined by the formula (preferred method) height = arm span / 1.06

   1. Height should be measured with stadiometer using standard technique.

   2. Height estimate by arm span can be used alternatively for those where standing height cannot be obtained (e.g. wheelchair bound).

Reference equations

The recently published predicted values of Hankinson et al (3) will be used. The general form of the prediction equation is:

$$\text{Predicted Value} = Z + A\,(\text{Age}) + B\,(\text{Age}^2) + C\,(\text{Height}^2)$$

Table 5-1
Values for Reference Equations

	Ethnicity	Gender	Z	A	B	C
FVC	Caucasian	Male	-.1933	.00064	-.000269	.00018642
		Female	-.3560	.01870	-.000382	.00014815
	African-American	Male	-.1517	-.01821	0	.00016643
		Female	-.3039	.00536	-.000265	.00013606
	Mexican American	Male	.2376	-.00891	-.000182	.00017823
		Female	.1210	.000307	-.000237	.00014246
FEV_1	Caucasian	Male	.5536	-.01303	-.000172	.00014098
		Female	.4333	-.00361	-.000194	.00011496
	African-American	Male	.3411	-.02309	0	.00013194
		Female	.3433	-.01283	-.000097	.00010846
	Mexican-American	Male	.6306	-.02928	0	.00015104
		Female	.4529	-.01178	-.000113	.00012154
FEV_6	Caucasian	Male	0.1102	-0.00842	-0.000223	0.00018188
		Female	-0.1373	0.01317	-0.000352	0.00014395
	African-American	Male	-0.0547	-0.02114		0.00016429
		Female	-0.1981	0.00047	-0.000230	0.00013497
	Mexican-American	Male	0.5757	-0.02860		0.00017840
		Female	0.2033	0.00020	-0.000232	0.00014106
PEF	Caucasian	Male	1.0523	0.08272	-0.001301	0.00024962
		Female	0.9267	0.0629	-0.001031	0.00018623

	Ethnicity	Gender	Z	A	B	C
	African-American	Male	2.2257	-0.04082		0.00027333
		Female	1.3597	0.03458	-0.000847	0.00019746
	Mexican-American	Male	0.0870	0.06580	-0.001195	0.00030243
		Female	0.2401	0.06174	-0.001023	0.00022203

Age is in years at last birthday.

Height is standing height in cm. (Arm span should be done if indicated by laboratory protocol).

PFT predicted values are in liters.

Predicted values for Asian-Americans will use a factor of 0.94 applied to the Caucasian predicted value (1, 3). Other ethnic groups will be as for Caucasians.

A subject's ethnic identity is self-defined.

5.7.1.2 Subject Preparation

a. Subject seated during testing.

b. Nose clips worn.

c. Subject to loosen restrictive clothing.

d. Subjects should avoid a heavy meal for at least two hours prior to testing.

e. Pre-BD testing: At least four hours since last short-acting BD and at least 12 hours since last long-acting BD.

f. Bronchodilator dose to evaluation airway responsiveness:

-Albuterol 2.5 mg via nebulizer.

- Albuterol MDI 4 puffs (90 mcg/puff) via spacer.

g. Post-BD testing: At least fifteen minutes and no longer than one hour following administration of albuterol.

General Instructions for Forced Vital Capacity (FVC)

a**.** Instruct subject, including demonstration. Emphasize the necessity for deep, full inspiration, a hard and forceful "blast" and a complete expiration for at least six seconds.

b. Perform test until ATS acceptability and reproducibility criteria are met. Reproducibility criteria will reflect updated ATS Spirometry standard of 150 ml for FVC and FEV1. For subjects with a FVC of 1.0 liter or less, reproducibility criteria is ±100 ml as referenced by Miller et al., Standardization of spirometry, ERJ, 2005; 26, 3199-338.

c. Acceptability criteria and problems with suggestions on how to fix them.

Test start: peak flow rate should be reached within 80 msec and the peak flow should be "sharp" on FV curve. Back extrapolation no more than 150 cc or 5% of the VC (whichever is greater). Trials with excessive back extrapolated volume should be rejected. Patients may need coaching to get this right (e.g. "BLAST" it out).

Cough: This can cause flow irregularities. Reject test when cough is within the first one second (FEV_1 will not be accurate). Cough in the later part of the VC is not a reason per se to reject the effort. Often cough can be reduced by asking the patient to exhale slightly less forcefully.

Test end: When the expiratory effort lasts at least six seconds and the volume time curve plateaus for >1 second with a volume of >0.025L. Pediatric patients

may have shorter exhalation times and effort of 3-6 seconds may be acceptable. Subjects with severe obstructive lung disease may continue to exhale for ten or more seconds. If flow reaches less than 40 ml/sec, however, it is acceptable to cease the effort during prolonged expiratory efforts. Subjects may need coaching to continue the expiratory effort. Occasionally, premature glottis closure causes abrupt test end. Subjects may need to relax and try again with slightly less than maximum effort.

5.7.1.3 Reproducibility

Two criteria are used to determine how well each acceptable effort compares with the largest acceptable effort.

FEV_1: The second largest FEV_1 should be within .15 L of the largest acceptable FEV_1.

FVC: The second largest FVC should be within .15 L of the largest acceptable FVC. At least three acceptable and two "reproducible" efforts should be obtained. If this cannot be obtained after approximately eight attempts, then the testing should be halted and the subject reported to the coordinator or investigator.

5.7.1.4 Reporting

The largest acceptable FEV_1 and FVC are reported. The FEF 25-75 should be taken from the best flow volume effort (largest FEV1 + FVC). These do not have to be taken from the same maneuver. The ratio of FEV_1/ FVC is calculated as the ratio of the largest FEV_1 and the largest FVC. At least two quality assurance values will be reported as well, the total expiratory time (FET100%) and back extrapolation volume (Vext). FET100% will be reported from the largest FVC and Vext will be reported from the trial with largest FEV_1. They will not be reported as data, but will serve the PFT center as quality assurance indicators.

5.7.2 Lung Volume Airway Resistance and Diffusing Capacity Measurements

5.7.2.1 Lung Volume General Methodology

Equipment and procedures should follow ATS/ERS task force recommendations. Lung volumes and airway resistance will be measured using a constant volume plethysmograph (DuBois box). ATS standards for plethysmographic assessments can be found in Wanger et al. (ERJ, 2005; 26: 511-512). Although there are no standard reference values (most studies are in small patient populations and Caucasians) suggested reference values in children and adolescents are from Zapletal et al. (Z Erkrank Atm-ORG 1977; 149: 343-371). Reproducibility criteria: FRC pleth values should be repeated at least 3 times and should be within 5% agreeance. Mean value for FRC pleth values should be reported.

5.7.2.2 Lung Volume Reference Equations

The equations of Crapo et al. (6) will be used for predicted values. A scaling factor of .88 will be used for African-Americans. Asian-Americans will use a scaling factor of 0.94. Other ethnic groups will use the predicted of Caucasians.

Lung volumes and airway resistance will be measured within one hour post-BD. It is often easier for subjects with severe airflow obstruction to perform the plethysmographic studies prior to the post BD spirometry.

5.7.2.3 Diffusing Capacity (Single Breath) Methodology

DLCO is measured by standard techniques (10) in which a tracer gas, either helium, methane or neon is introduced for the measurement of alveolar volume (V_A).

Equipment specifications are outlined in the ATS statement on diffusion capacity (Macintyre et al., ERJ, 2005;26: 720-735). Patients will be seated.

Maneuver should be performed more than 30 minutes following any exercise, more than two hours following a meal and more than 15 minutes following bronchodilator. Subject should breathe at ambient FiO2 (21%) for at least ten minutes prior to testing. Deep inspiration prior to test should be avoided as it can result in increased CO uptake. Patients who cannot be safely taken off or tolerate removal of supplemental oxygen should not be tested and this data noted as missing with the reason documented. Breath-holding time should be 9-11 seconds, washout volume at least .5 L for subjects with vital capacity less than two liters or low VC and .75-1 liter for a vital capacity greater than two liters.

At least two maneuvers should be performed, and should agree within 10% or 3 ml/min/mmHg. A four minute pause should occur between each test. Larger periods may be needed in patients with obstructive airway disease (to clear test gas). No more than four trials should be attempted.

Breath-hold time is calculated using the method of Jones and Meade (12), where the beginning and end of inspiration is determined from the extrapolation of the best fit linear regression of volume vs. time during inspiration. Breath-hold includes 70% of inspiratory time and 50% of the sample collection time and should be 9-11 seconds.

V_A is calculated from simultaneous measurement of single breath tracer gas dilution. Anatomic dead space will be estimated as 2.2 ml/kg (1 ml/lb). The dead space volume of the circuit is supplied by the manufacturer of the equipment.

The mean of two acceptable maneuvers is reported as the data point, both Hgb corrected and uncorrected. Since hemoglobin (Hgb) may vary considerably among individuals in this study, a correction will be made for Hgb in the LTRC

database. Both the corrected and uncorrected values will be reported. The Hgb obtained from the CBC will be used.

Hemoglobin correction will be according to the Cotes equation per ATS/ERS standards equation for <15 yrs of age should be

DLadj = DLCO * (1.7Hb/(9.38 + Hb))

Adjustment for carboxyhemoglobin need not be generally done.

CO transfer coefficient will be calculated as DCO/V_A.

a. Diffusing Capacity Reference Equations

The equations used will be of Crapo and Morris (12):

Males: DLCO (ml/min/mmHg) = .416 (Height) - .219 (Age) -26.34

Females: DLCO (ml/min/mmHg) = .256 (Height) -.144 (Age) -8.36

No race/ethnic adjustment is used for DLCO.

5.7.2.4 Hemoglobin (local)

Hemoglobin from a standard CBC should be performed at the time of the PFT and the results of which should be recorded on Form 04 by the study Coordinator.

5.8 MRI/MRA (without contrast)

Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) studies will be collected for several reasons. It is important to determine each patient's brain and cranial artery anatomy because abnormalities of the brain and cranial arteries have been found in sickle cell anemia patients even at the young age of those enrolled in the BABY HUG Follow-up Study II. It is important to know if each patient's cranial vessel anatomy remains stable and the brain grows normally over the period of the patient's participation in the study because large doses of

hydroxyurea have been demonstrated to retard organ growth (including brain growth) in murine models and many of the children enrolled in BHFU II were exposed to hydroxyurea at a very early age. Because stroke and silent infarction of the brain are documented severe outcomes of sickle cell anemia, it is important to know if any cerebral infarctions or hemorrhages occur.

The BABY HUG Follow-up Study II MRI and MRA studies are proposed to be performed as follows. Recommendations for procedure modifications can be considered by the BABY HUG Follow-up II Steering Committee.

5.8.1 MRI

1. Axial T1 weighted image spin echo (SE)

   TR between 400-800 msec

   TE between 10-30 msec

2. Axial FLAIR - T2 weighted images

   either conventional SE or fast (turbo) spin echo images

   approximately 5 mm thick with minimal slice gap

3. Coronal FLAIR

   The same protocol as utilized in the axial plane should also be performed in the coronal plane.

4. Diffusion weighted imaging (required only at time of suspected neurological event)

   B=1000 or greater

   axial images in X, Y, and Z planes

5.8.2 Cranial MRA

Anatomic Coverage

- An axial (transverse) plane of acquisition should be used.
- The study must include the juxtasellar and supraclinoid internal carotid arteries and the horizontal portion of the middle cerebral arteries in the Sylvian cistern. This can usually be achieved by centering the slab on the supraclinoid ICA.

Echo Time (TE)

The TE should be minimized ($\leq$ 5 msec if possible). This reduces intravoxel phase dispersion and resultant loss of flow related signal. Loss of flow related signal can mimic or exaggerate vascular stenosis, and is particularly problematic in patients with SCD due to their increased flow rates.

Spatial Resolution

Use of the smallest feasible voxel size (small FOV 15-20 cm, 256-512 matrix) and shortest obtainable echo time (TE) minimize flow related loss of intravascular signal. Unfortunately, when one attempts to use a small FOV or increase the read matrix, the minimum obtainable TE usually increases. While both are important, minimizing TE should take priority over voxel size considerations.

- The highest reasonable resolution should be used. A matrix of 256 x 256 is acceptable. Higher resolution matrices (256 x 512) are preferable as long as TE is not prolonged > 5 msec.
- The minimum feasible field of view should be used, optimally in the 15-20 cm range. A rectangular field of view can be used, if available, to reduce scan time or increase resolution.

- At least 60 partitions, ≤ 1 mm thick should be acquired.

Other Parameters

- The flip angle is system and parameter specific and should be optimized on each imaging system.
- Variable flip angle techniques (also known as TONE, ramped-RF) may be used if available, provided there is no significant TE penalty on the system.
- A presaturation pulse may be used cephalad to the slab to eliminate visualization of venous structures.
- Where available, magnetization transfer preparation may be used, understanding that MTC usually increases the minimum TR.

5.8.3 Labeling and Shipping of CD

A CD containing all MRI and MRA studies will be sent to a central reviewer for interpretation at a later date. The Clinical Center may keep a digital copy of the MRI/MRA studies in the subject's file. In the meantime, use the following procedure to label the CDs and store a copy of the CD in a secure location until further notice from the DCC:

1. Remove all patient Personal Identifying Information (PII) on all CDs, to the best extent possible.
2. Affix to *each* MRI CD a label with the same 13-digit number as already affixed to the MRI section of Form 33.
3. Data enter Form 33 into eCOS.

4. Follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the shipping manifest, a copy of which should be placed in the shipping package with the CD. **Be sure to <u>black-out</u> the Subject ID.**

5. Store a copy of the CD in a secure location until further notice.

5.9 PHYSICAL EXAMINATION

All active subjects are expected to have a complete physical examination performed at study entry, exit and at annual visits. Please refer to your subject's visit schedule to ascertain the appropriate visit window. The physical examination results are to be recorded on study Form 14 (Physical Examination). Clinical Center staff should make every attempt to obtain all of the information requested on the form, at least for active group subjects. Some, but not all, of this data may be abstracted from a physical examination performed for clinical care for subjects in the passive group and should be reported on the same form, if available at entry, exit and annually.

Items included in the physical examination are general anthropometric measurements, vital signs including oxygen saturation and appearance. There are specific elements of a general physical exam which should be evaluated by the person performing the physical examination (site PI or trained designee) and coded as per Form 14. The examiner may wish to use the Form 14 as their source document, or create an electronic template in their institution's electronic medical record that records and interprets the data in this manner. The intent is to capture important physical examination facts, not minutiae that are described in BABY HUG prescribed verbiage by the person performing the exam. The data submitted is thus ready for analysis and does not require later interpretation.

a. Neurological Examination

The Neurological Examination in BABY HUG FU II will be performed, by site PI or trained designee MD/PNP, according to the Worksheet included in this chapter (Exhibit 5–2). The exam is designed to be performed by a pediatric hematologist or pediatric nurse practitioner rather than a neurologist and should be able to be completed in 10 minutes or less. Please maintain the completed worksheet as the source documentation of the examination. After performance of the suggested evaluations the examiner will be asked for their interpretation of patient status categorized as:

1. Normal, OR
2. Deficit with little or no impact on function, OR
3. Abnormal with functional limits or missing function with reference to 7 specific domains of neurologic function:

- Behavior/Mental Status,
- Language,
- Cranial Nerves,
- Deep Tendon Reflexes,
- Motor, Power, and Tone,
- Fine Motor Coordination, and
- Gait

Please note scoring guidelines are provided to give the examiner a general idea about what can usually be accomplished by a child in the age group of subjects in BHFU II. The final interpretation should be based on the examiner's overall sense of the child's performance in the area rather than on passing a specific test or displaying a certain skill. Only the interpretation of 1, 2, or 3 above is reported for each of the domains listed on the Physical Examination Form (Form 14).

5.9.1 Question by Question Commentary:

Level of Consciousness – complete on all children. A decreased level of consciousness may make interpretation of subsequent skills difficult. If decreased level of consciousness is temporary, an attempt should be made to repeat the exam when child is more alert.

M1. Behavior/Mental Status

Seven specific issues to be assessed. Here the examiner subjectively evaluates activity level, interpersonal interaction, attention and affect over the entire exam. First objective question is serial numbers: at least 3-5 correct answers should be given by age. Right left orientation should be correct. Memory items should be given at this time and recall checked while examining child for reflexes and cranial nerves in about 5 minutes.

M2. Language

Speech development is informally assessed in speaking with the child during the exam. For naming use the pictures at the end of the worksheet and ask the child to point to the object named. Comprehension is assessed by starting with simple commands listed, and escalating to multistep commands; most subjects in the BHFU II age group should be able to follow at least 2 step commands. Letter recognition, reading and writing commands are as on the second page of the worksheet and can be executed on front and back of the last page.

M3. Cranial Nerves

This is relatively standard pediatric neurologic assessment, and the worksheet is to guide your evaluation and scoring of this domain.

M4. Deep Tendon Reflexes

This is a standard neuromuscular assessment, and the worksheet is to guide your evaluation and scoring of this domain.

M5. Motor, Power, and Tone

This is relatively standard neuromuscular assessment, and the worksheet is to guide your evaluation and scoring of this domain. Be sure to note if there are any involuntary movements (tremor, posturing or spasticity) that would be important in evaluation of an abnormal examination for this domain.

M6. Fine Motor Coordination

Here there are specific skills that are part of the usual neurologic examination. The examiner is instructed to demonstrate the skill and then ask the patient to perform. Small balls of scrap paper, 2-3 mm in size, will be needed for this part (not provided).

M7. Gait

Start with the child walking down a hall or across room. This should be normal gait by the age of BHFU II subjects. Then ask the child to perform each of the listed maneuvers. Examiner may need to demonstrate if subject does not appear to understand the verbal direction.

5.9.2 Tanner Staging

Tanner Staging is the only assessment of pubertal development to be performed in BHFU II, and will be an important overview of how participants are maturing. The usual lower limit of onset of physiologic puberty in hematologically usual children is 8 years for females, and 9 years of age for males. So many BABY HUG subjects may enter and progress through puberty during FU II.

Please refer to the images in Exhibit 5–3 from (New Engl J Med 2008; 358:2366-2377) and the written description of each of the stages below. Please record in the primary source documentation of the physical examination your final assignment of the actual Tanner stage (I-V)

for pubic hair (both genders), breasts (female) and genitals (male). Only the Roman numeral stage will be reported to the BHFU II data set on Form 14.

Tanner stage is assessed independently for pubic hair and breasts (female) or genitals (male) as development does not always track together. Stage II is the onset of puberty, Stage I is pre-pubertal or early childhood anatomy.

5.9.2.1 Pubic Hair (males and females):

I. Child or pre-adolescent with no different hair pattern than on abdomen

II. Sparse growth of long, fine hairs at base of penis (males) or labia majora (females)

III. Darker, curly, coarser hair extending to junction of pubis

IV. Smaller amount of adult pattern hair spreading laterally across pubis

V. Adult distribution with inverse triangle pattern, possible spread to medial thighs and onto abdomen

5.9.2.2 Breasts (females)

I. Preadolescent form with papilla only

II. Breast bud with papilla as mound, aerola widening

III. Papilla and aerola larger and darker, no separation of aerola from breast contour

IV. Aerola and papilla project separately from the breast contour

V. Recession of aerola in line with breast contour, papilla only projection

5.9.2.3 Genitals (males)

I. Preadolescent, penis usually less than 3 cm

II. Scrotum reddens and enlarges, testes start to enlarge, penis does not change

III. Scrotum enlarges further, testes enlarge, penis lengthens up to 6 cm

IV. Scrotum darkens, penis increases in circumference and length up to 10 cm, glans develops

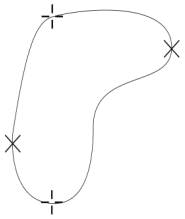
V. Darkening of scrotum, further testicular and penile enlargement

Exhibit 5-1
Checklist for BABY HUG Follow-up Abdominal Sonography

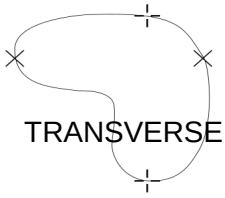
Spleen (see diagrams below)

- ☐ In the coronal plane, measure at the level of the hilum:
  - ☐ Length
  - ☐ AP diameter
- ☐ In the transverse plane, measure at the level of the hilum:
  - ☐ Transverse

Proper placement of cursors for spleen measurements



LONGITUDINAL



TRANSVERSE

Kidneys

- ☐ In the supine position obtain a longitudinal image of each kidney with liver/spleen in field of view for comparison of echogenicity.
- ☐ In the prone position obtain:
  - ☐ Longitudinal images of the entire kidney
  - ☐ Transverse images of the entire kidney
  - ☐ Maximum renal length measurement
  - ☐ AP measurement in BOTH longitudinal and transverse planes.
- ☐ At level of renal hilum:
  - ☐ Transverse measurement

Gall bladder

- ☐ Obtain longitudinal and transverse images of entire GB in supine position.
- ☐ Obtain longitudinal and transverse images of entire GB in decub position.
- ☐ If GB wall thickening >3 mm:
    - ☐ Measure GB wall on transverse image
    - ☐ Obtain color Doppler image of GB wall
- ☐ Measure common bile duct in region of the porta hepatis.
    - ☐ Confirm measurement with color Doppler.

Liver

- ☐ With right kidney in field of view, measure the liver length where the greatest length can be obtained.

Exhibit 5–2
Worksheet - BABY HUG Follow-Up Study II
PRCA Neurological Outcome Measure

WORKSHEET – does not require data entry

(CHILDREN AGED 3 YEARS AND OLDER)

SAVE THIS DOCUMENT AS YOUR PRIMARY SOURCE DOCUMENTATION

OF THE NEUROLOGICAL EXAMINATION

PART I: IDENTIFYING INFORMATION

1. Patient's ID Number: ___ ___ ___ ___ 2. Current Clinic: ___ ___

3. Patient's Letter Code: ___ ___ ___

4. Date of assessment: ___ ___ - ___ - ___ ___ ___
   Month Day Year

5. Exam Performed and Scored by: _____
   Name

6. Age of child: _____ Yrs _____ mo

INSTRUCTIONS: Check appropriate column for each item: Normal, Abnormal, or Not Done. Guidelines for Scoring are given as suggestions, and need not be viewed as absolute criteria for scoring. Each section should be assessed and an overall score assigned, this is all that will be entered into the database. Record Overall Scores on Form 14 (Physical Examination). *******Maintain this worksheet as part of your primary source documentation.*******

LEVEL OF CONSCIOUSNESS

TEST ITEM	Normal	Abnormal	Notes
Level of Consciousness			

M1. BEHAVIOR/MENTAL STATUS

TEST ITEMS	**Normal**	**Abnormal**	**Not Done**	**Guidelines for Scoring**
Activity Level				**Abnormal =** Excessively quiet, shy, removed, hyperactive, fidgety, gets up, uncontrollable, spills, into everything
Interpersonal interaction				**Abnormal =** Clings to parent, aloof, withdrawn, gaze avoidance, punches
Attention				**Abnormal** = Short, distractible, flits, ignores, preoccupied, disorganized, inattentive
Affect				**Abnormal** = Extremely shy, pouts or clings excessively or cries a lot for no reason, angry, totally flat, gaze avoidance, hyperactive, no sustained attention
Serial Numbers				Age **4-8** years: **Ask**: "Start at 20 count backwards" Age **8+ - 13** yrs: **Ask**: "Start at 50 count backwards by 3's" Age **13** yrs & up: **Ask**: "Start at 100 count backwards by 7's". **Normal =** at least 3-5 correct answers.

Right/Left Orientation				Test in children older than 6 years age: "Show me your left hand" and "Show me your right hand". **Normal** is to know each hand.
Memory, Delayed Recall				**Instructions**: "I need you to memorize 3 words and will ask you to repeat them in 5 minutes. The words are **"Chair", "Candle", "Dog".** Ask the child to repeat.

				Normal is 2-3. **Normal** is to remember 2-3.

M1. Behavior/Mental Status: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M2. LANGUAGE

TEST ITEMS	Normal	Abnormal	Not Done	Guidelines for Scoring
Speech Development				**Age-appropriate** sentences, etc.
Naming				Show patient attached sheet with pictures. Ask child to identify: bicycle, ball, pencil
Comprehension				**Simple Tasks**: Ask child to: *a. Close your eyes b. Touch your nose c. Point to the floor and then ceiling* **Complex 3 Step Command**: ask child to listen to the complete instruction, remember it, then do all 3 activities together when prompted: *"Blink twice, stick out your tongue, then touch your finger to your nose"*
Letter Recognition / Reading				See page 5. Ask patient to identify letters A, B, H
Writing				Ask the patient to write 'The cat is black' on the worksheet on page 6.

M2. Language: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M3. CRANIAL NERVES

TEST ITEMS		Normal	Abnormal	Not Done	Guidelines for Performance
Pupillary Light Reflex	Right				Direct and Consensual
	Left				
Ocular Motility	Right				Move pen or red object or light smoothly from right to left and back testing full range. Watch for nystagmus or dysconjugate eye movements
	Left				
Facial Sensation	Right				Touch each side with light touch and cold object asking if child can feel or for older, 'is it the same on both sides' comparing forehead, cheek and chin R/L
	Left				
Facial Movements	Right				Ask patient to smile, count to 10 watching mouth symmetry Maximal eye closure strength *"Squeeze eyes shut as tightly as you can"*
	Left				
Swallow, Palate and Gag	Right				Observe during open mouth crying or demonstrate with tongue protruded 'Say 'ahhhhh."
	Left				

M3. Cranial Nerves: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M4. DEEP TENDON REFLEXES

TEST ITEMS		Normal	Abnormal	Not Done	Comments
Biceps	Right				
	Left				
Bronchioradials	Right				
	Left				
Patellar	Right				

	Left				
Ankle Jerk	Right				
	Left				
Ankle clonus	Right				
	Left				

M4. Deep Tendon Reflexes: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M5. MOTOR, POWER AND TONE EXAM

	Normal	Abnormal	Not Tested	Comments
Neck/Trunk Muscles				Shoulder shrug
Right Arm				
Left Arm				
Right Leg				
Left Leg				

	Yes	No
Are there any involuntary movements present (tremor, posturing, spacticity)	(1)	(2)

M5. Motor, Power and Tone Exam: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M6. FINE MOTOR COORDINATION

TEST ITEMS		Normal	Abnormal	Not Done	Guidelines for Scoring
Pincer Grasp	Left				Encourage to pick up small 2-3 mm. ball of rolled up paper
	Right				
Rapid Sequential Finger Movements	Left				**Demonstrate**: thumb touches tip of individual fingers back and forth 5 times *"As fast as you can"*
	Right				
Finger To Nose Testing	Left				**Demonstrate**: Ask child to touch their right index finger to their nose as fast as you can, then repeat using left index finger.
	Right				
Rapid Foot Tap	Left				

	Right				**Demonstrate**: feet flat on floor, foot taps floor X 20 *"As fast as you can"*
Drawing	Left				Ask the child to copy the three figures on page 6. Most children should be able to draw complete figures that are closed objects that resemble the model figure. Normal is being able to draw all 3; abnormal is an incomplete figure in 1 or more of the model figures.
	Right				

M6. Fine Motor Coordination: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)
Abnormal with functional limits or missing function	(3)

M7. GAIT

TEST ITEMS		**Normal**	**Abnormal**	**Not Done**	**Guidelines for Scoring** **Normal after 16 months of age.**
Gait Walking					By $\geq$ 16 mos.
Gait on Heels					Normal is 10 steps.
Gait on Toes					Normal is 10 steps.
Hop on Foot repetitively	Left				25 x (age 7 yrs to 8 yrs.) 50 x (age 9 yrs or older)
	Right				
Station on one leg sustained	Left				Test age 7 and up. Count seconds out loud and compare stability.
	Right				

M7. Gait: Overall Score

Normal	(1)
Deficit with little or no impact on function	(2)

Abnormal with functional limits or missing function	(3)

PICTURES FOR NAMING TEST OF LANGUAGE (M2)

Ask patient to identify:

Identify the letters (M2, Page 2)

A, B, C, D, E, F, G, H, I, J, K, L

Please write the sentence “the cat is black” (M2, Page 2)

MODEL FIGURES TO COPY (M6, Page 4)

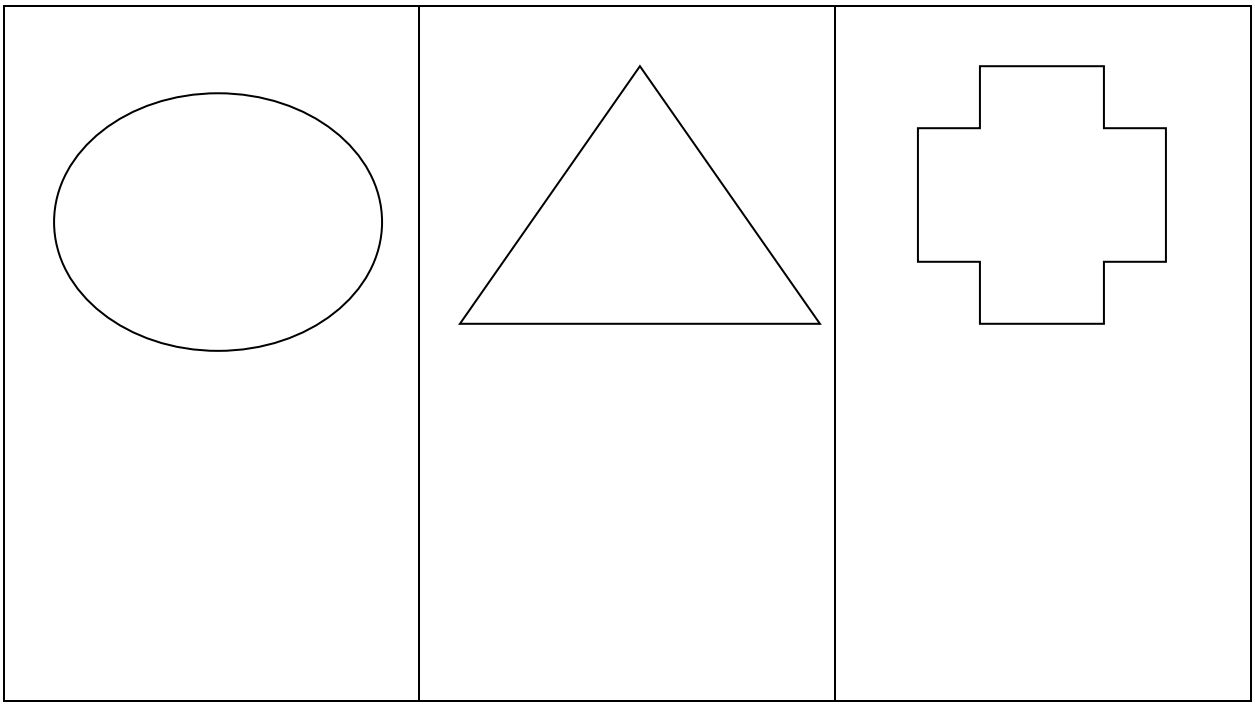
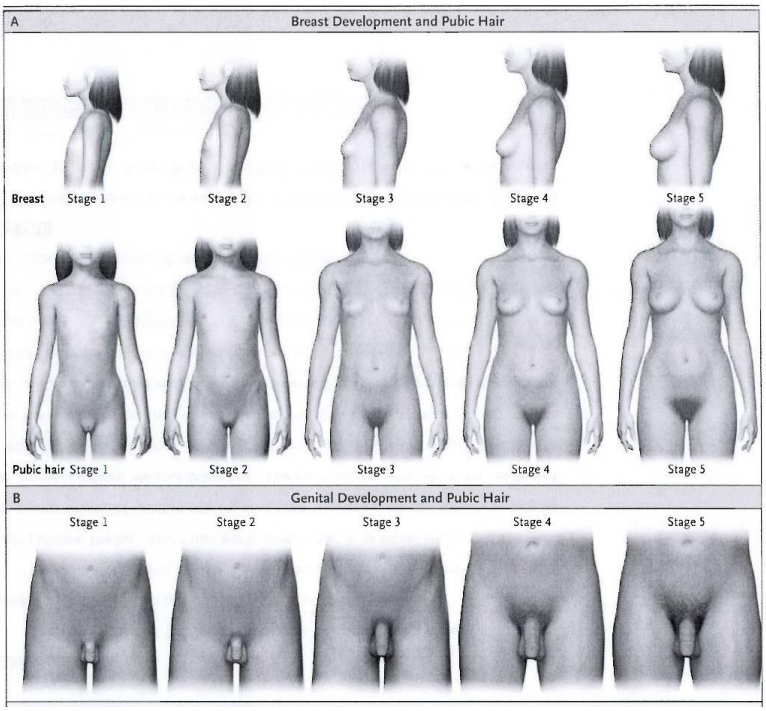


Exhibit 5–3
BABY HUG Follow-Up Study II
Tanner Staging Illustration



*Reference: Carel JC, Leger J. Clinical Practice: Precocious Puberty. N Engl J Med 2008; 358:2366-77.

Exhibit 5-4
Diagnosis Code Listing for Completion of BH FUS II Form 32
Echocardiogram Performance

Diagnoses Codes, Alphabetical

DIAGNOSIS	SYSTEM	CODE
ADD/ADHD	Neurological	D-2278 M
Aortic Arch Anomaly	Cardiovascular	D-1640
Aortic Atresia	Cardiovascular	D-1641
Aortic Valve Regurgitation	Cardiovascular	D-1642
Arrhythmia, cardiac (including atrial fibrillation, atrial flutter, PACs, PAT, PVCs, unspecified)	Cardiovascular	D-1609 M
ASD (Atrial Septal Defect)	Cardiovascular	D-1632
Asthma	Respiratory	D-1700
Atrial Septal Defect (ASD)	Cardiovascular	D-1632
Atrial Septal Defect, Ostium Primum	Cardiovascular	D-1643
Atrial Septal Defect, Secundum	Cardiovascular	D-1665
Autism	Neurological	D-2276 M
Bacteremia, Sepsis, septicemia bacterial	Other Infect. Disease	D-2702
Bacterial Bone or Joint Infection	Bone/Joint	D-2316
Bicommissural Aortic Valve (Bicuspid Aortic Valve)	Cardiovascular	D-1644
Bicuspid Aortic Valve (Bicommissural Aortic Valve)	Cardiovascular	D-1644
Bronchiolitis	Respiratory	D-1701
Bronchitis	Respiratory	D-1702
Bronchopulmonary Dysplasia	Respiratory	D-1703
Cardiac Arrhythmia, (including atrial fibrillation, atrial flutter, PACs, PAT, PVCs)	Cardiovascular	D-1609 M
Cardiomyopathy, Dilated	Cardiovascular	D-1634 M
Cardiomyopathy, HIV related	Cardiovascular	D-1600
Cardiomyopathy, Hypertropic	Cardiovascular	D-1635 M
Cardiomyopathy, postpartum	Cardiovascular	D-1617
Cardiomyopathy, unspecified (not HIV related)	Cardiovascular	D-1601 M
Cardiorespiratory Arrest	Cardiovascular	D-1677

DIAGNOSIS	SYSTEM	CODE
Cardiovascular accident (CVA, stroke [ischemic, hemorrhagic, thrombotic])	Neurological	D-2220
CARDIOVASCULAR, OTHER (Specify in narrative.)	Cardiovascular	D-1699
Central Hypoventilation	Neurological Additional	D-3203 M
Cerebral Palsy	Neurological	D-2283
Cholecystitis	Gastrointestinal	D-1900
Cholelithiasis	Gastrointestinal	D-1932
Cleft lip/palate	Mouth, Tongue, Throat	D-1512
Coarctation of Aorta	Cardiovascular	D-1645
Cognitive Delay/Learning Disability	Neurological	D-2280
Congenital anomaly, not specified	Unclassified	D-2915
Congenital anomaly, other, specify	Unclassified	D-2925
Congenital Heart Disease, patent ductus arteriosus (PAD)	Cardiovascular	D-1628
Congenital Heart Disease/Defect, unspecified	Cardiovascular	D-1629
Congenital Heart Disease/Defect (include tetralogy of Fallot, Ebstein's anomaly, right ventricular hypoplasia/atresia, left ventricular hypoplasia, pulmonary atresia, aortic atresia)	Cardiovascular	D-1627
Congenital hip defect	Bone/Joint	D-2306
Congestive Heart Failure, acute	Cardiovascular	D-1619
Congestive Heart Failure, chronic	Cardiovascular	D-1618
Congestive Heart Failure, unspecified	Cardiovascular	D-1602
Coronary Arteriovenous Fistula	Cardiovascular	D-1662
Coronary Artery Anomaly	Cardiovascular	D-1646
Croup	Respiratory	D-1704
Deep Venous Thrombosis (DVT)	Cardiovascular	D-1614
Dehydration	Unclassified	D-2912
Delayed motor examination without focal findings	Neurological	D-2253
Depression, Unspecified	Neurological Additional	D-3200 M
Developmental Delay	Neurological Additional	D-3219 M
Diabetes, Insulin-dependent (not related to pregnancy)	Endocrine	D-2400 M
Diabetes, non-Insulin-dependent (include oral hypoglycemics) (not related to pregnancy)	Endocrine	D-2401 M
Diabetes, Unspecified	Endocrine	D-2416 M
Diarrhea, acute	Gastrointestinal	D-1904

DIAGNOSIS	SYSTEM	CODE
Diarrhea, chronic or recurrent	Gastrointestinal	D-1905
Diarrhea/Vomiting, chronic and episodic	Gastrointestinal	D-1964 M
Double Outlet Right Ventricle	Cardiovascular	D-1647
DVT (Deep Venous Thrombosis)	Cardiovascular	D-1614
Great Vein Anomaly	Cardiovascular	D-1649
Growth Failure	Endocrine	D-2419 M
Heart Block	Cardiovascular	D-1637 M
Heart murmur	Cardiovascular	D-1630
Hypertension, chronic, no medications	Cardiovascular	D-1621
Hypertension, chronic, on medications	Cardiovascular	D-1622
Hypertension, unspecified (not related to pregnancy)	Cardiovascular	D-1605 M
Hyperthyroidism	Endocrine	D-2402
Hypoparathyroidism	Endocrine	D-2420 M
Hypothyroidism	Endocrine	D-2403
IBD (Inflammatory Bowel Disease)	Gastrointestinal	D-1950
IBS (Irritable Bowel Syndrom)	Gastrointestinal	D-1951
Mitral Regurgitation	Cardiovascular	D-1652
Mitral Stenosis	Cardiovascular	D-1653
Mitral Valve Prolapse	Cardiovascular	D-1654
Myocardial Abscess	Cardiovascular	D-1606
Myocarditis	Cardiovascular	D-1607
Osteomyelitis	Bone/Joint	D-2301
Partial Anomalous Pulmonary Venous Connection	Cardiovascular	D-1655
Patent Foramen Ovale (PFO)	Cardiovascular	D-1670
PDA (patent ductus arteriosus, Congenital Heart Disease)	Cardiovascular	D-1628
PDA ligation	Procedures/Surgery	D-3005
Persistent Left Superior Vena Cava	Cardiovascular	D-1663
PFO (Patent Foramen Ovale)	Cardiovascular	D-1670
Pleural Effusion	Respiratory	D-1711
Pleuritis/Pleurisy	Respiratory	D-1712
Primary Pulmonary Hypertension	Cardiovascular	D-1616
Pulmonary Embolus	Respiratory	D-1718
Pulmonary hypertension	Cardiovascular	D-1623

DIAGNOSIS	SYSTEM	CODE
Pulmonary Tuberculosis, active (on medication)	Respiratory	D-1722
Pulmonary Tuberculosis, inactive by chest x-ray	Respiratory	D-1737
Pulmonary Tuberculosis, inactive, previously treated	Respiratory	D-1721
Pulmonary Valve Stenosis	Cardiovascular	D-1664
SBE (Endocarditis, bacterial subacute)	Cardiovascular	D-1620
Seizure Disorder, unspecified	Neurological	D-2215 M
Thyroid, enlarged	Endocrine	D-2413
Tricuspid Atresia	Cardiovascular	D-1661
Tricuspid Regurgitation	Cardiovascular	D-1659
Tricuspid Valve Prolapse	Cardiovascular	D-1669
Truncus Arteriosus	Cardiovascular	D-1660
Unexplained exercise intolerance	Neurological	D-2263 M
Valvular Abnormality, specified (include mitral valve prolapse (MVP) mitral stenosis, mitral regurgitation, aortic stenosis, aortic insufficiency/regurgitation, tricuspid insufficiency/ regurgitation)	Cardiovascular	D-1626
Ventricular Septal Defect (VSD)	Cardiovascular	D-1613
VSD (Ventricular Septal Defect)	Cardiovascular	D-1613
WPW (Wolff-Parkinson-White) Syndrome	Cardiovascular	D-1638 M

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

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CHAPTER 6

CENTRAL READING GROUPS

6.1 INTRODUCTION

Liver-spleen scans, abdominal ultrasounds, cardiac echocardiogram, MRIs and MRAs will be centrally evaluated by individuals independent of the BABY HUG Follow-up Study II Clinical Centers for future review. The procedures for central review are described in this chapter.

6.2 LIVER-SPLEEN SCAN

6.2.1 Overview

The liver spleen scans will be read by two nuclear medicine specialists who will independently assess each liver/spleen scan that is performed at approximately 10 years of age as having normal, decreased (<50% decreased or >50% decreased) or absent spleen function. In case of disagreement, a scan will be sent to a third reviewer. The two readings out of the three that are in agreement will be the single final reading. This qualitative assessment of spleen function will be used for determining the primary endpoint outcome: worsened or not worsened (includes improved) spleen.

If a subject has had a splenectomy, the liver-spleen scan is not required.

6.2.2 Scans Required for Central Reading

Scans will meet the following specifications.

<u>400K Image</u>

- Proper identification (Subject 13-digit label number and date)

- An anterior view labeled #1
- A posterior view labeled #2
- An anterior view with region of interest around spleen and liver with counts and counts/pixel recorded on CD labeled #3
- A posterior view with region of interest around spleen and liver with counts and counts/pixel recorded on CD labeled #4
- The geometric mean counts and the geometric mean counts/pixel of spleen and liver from both views calculated and recorded on CD labeled #5
- The total and counts/pixel spleen to liver ratios recorded on CD labeled #6

Timed Image

- Proper identification (Subject 13-digit label number and date)
- A left anterior oblique (LAO) view labeled #7
- A right posterior oblique (RPO) view labeled #8
- A LAO view with region of interest around spleen and liver with counts and counts/pixel recorded on CD labeled #9
- A RPO view with region of interest around spleen and liver with counts and counts/pixel recorded on CD labeled #10
- The geometric mean counts and geometric mean counts/pixel of spleen and liver from both views calculated and recorded on CD labeled #11
- The total and counts/pixel spleen to liver ratios recorded on CD labeled #12

6.2.3 DCC Scan Processing Procedure

Procedures for the central review processing procedures are currently under review and will be disseminated once approved by the Steering Committee.

6.2.4 Guidelines for Qualitative Grading of Liver-Spleen Scans

The central readings will be based on qualitative, visual assessments comparing uptake in the spleen to that in the liver. The reader will rate the spleen uptake on the posterior and LAO views, as compared to uptake in the left lobe of the liver and provide a qualitative assessment of spleen function. The measurement will be recorded on Form 40 (Liver-Spleen Scan Central Reading) as follows:

- *Normal*: normal spleen function (uptake proportionate to liver);
- *Decreased:* spleen function but decreased (uptake disproportionately lower than liver) – separated into two categories:
    - *Moderately Decreased is* less than 50% decreased
    - *Markedly Decreased is more than 50% decreased*;
- *Absent:* spleen function absent (no appreciable uptake above background level.

6.2.5 Liver-Spleen Primary Outcome

The readings as applied in Table 6-1 determine for each child whether spleen function has improved, worsened or not worsened after exit from the BABY HUG Treatment study or since the BABY HUG Follow-up I Study. These three categories (Improved, Not Worse, and Worse) contribute to the possible responses for the spleen primary outcome.

TABLE 6-1
Liver-Spleen Primary Outcome Determination

Spleen Function at Baseline		Spleen Function After Exit from the treatment study		
		Normal	Decreased	Absent
Normal		Not Worse	Worse	Worse
Decreased	Moderately	Improved	Not Worse	Worse
	Markedly	Improved	Not Worse	Worse
Absent		Improved	Improved	Not Worse

6.2.6 Guidelines for Quantitative Grading of Liver-Spleen Scans

A quantitative assessment of liver-spleen uptake will provide additional information about spleen function that may be used as a secondary endpoint in the data analysis. The total count spleen-liver geometric means and ratio will be recorded on the films for both the 400 K Image and Timed Image. The counts/pixel spleen-liver geometric means and ratios (400K Image and Timed Image) will also be calculated. A spleen-liver ratio greater than 0.2 using total counts is often considered normal, while below 0.2 is often considered reduced splenic function. Using counts/pixel, a spleen-liver ratio in the 0.7-0.9 range is considered normal.

6.3 ABDOMINAL ULTRASOUND

6.3.1 Overview

Abdominal ultrasound imaging will be performed at approximately 10 years of age. The evaluation is tailored specifically to determine splenic volume and echogenicity, renal volumes and echogenicity and to assess the gallbladder and biliary system. The imaging will be centrally reviewed by one pediatric radiologist.

6.3.2 Assessment of the Spleen

6.3.2.1 Splenic Parenchyma

Representative images of the entire spleen will be obtained in the longitudinal and transverse planes and the parenchyma will be assessed for normal vs abnormal echogenicity.

6.3.2.2 Splenic Volume

Table 6-2 shows normal values to use to evaluate splenic volume [1].

TABLE 6-2
Splenic Volume

Body length	Splenic Volume Mean values	Standard deviation
56 – 70 cm	18.02 cc	7.54
71 – 85 cm	29.63 cc	14.48
86 -100 cm	32.53 cc	16.09
101-110 cm	30.29 cc	7.24
111-120 cm	50.27 cc	15.2
121-130 cm	43.52 cc	12.73
131-140 cm	60.07 cc	18.86
141-150 cm	56.74 cc	24.07
151-160 cm	75.85 cc	10.65
161-170 cm	74.80 cc	18.20

6.3.3 Assessment of the Kidneys

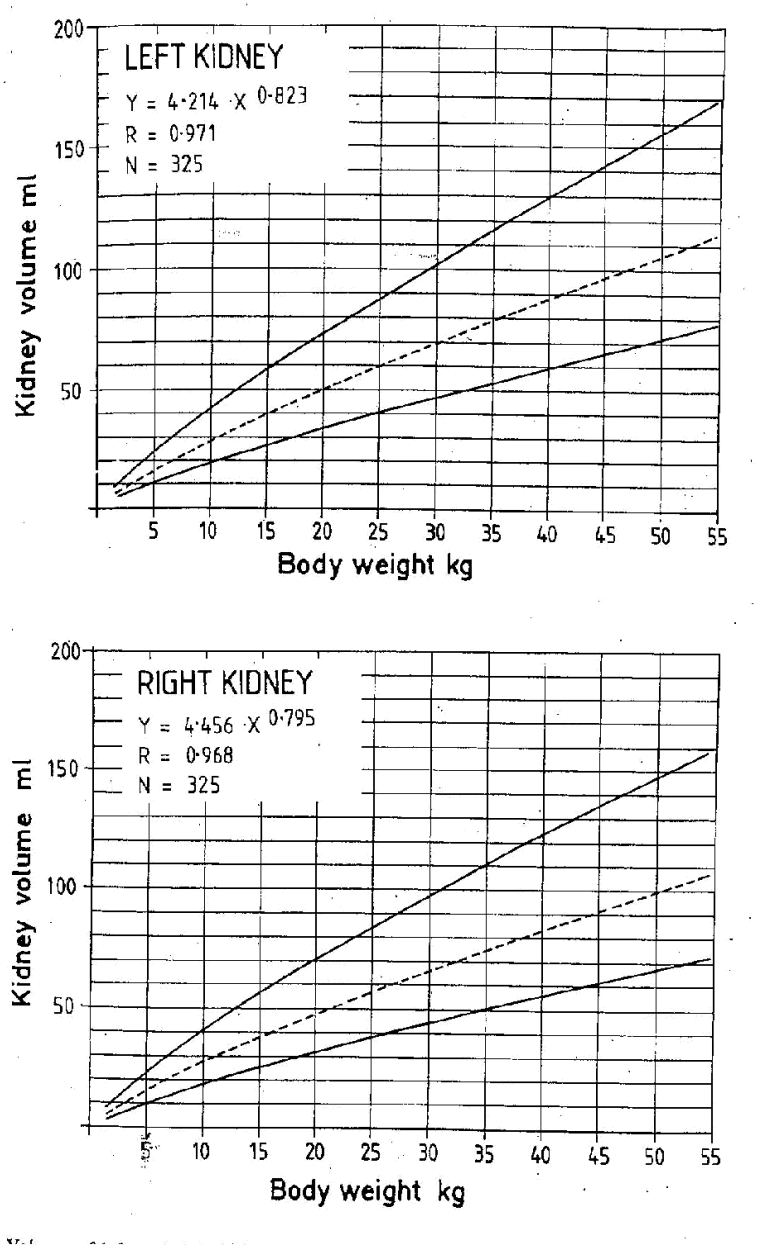
6.3.3.1 Renal Parenchyma

Representative images of both kidneys will be obtained in the longitudinal and transverse planes and the cortical echogenicity will be determined to be either normal or abnormal.

6.3.3.2 Renal Volume

Figure 6-3 will be used as standards to evaluate the renal volumes [2].

Figure 6-3
Kidney Volume



6.3.4 Gall Bladder and Biliary System

The gall bladder wall will be considered abnormally thickened when it measures > 3 mm. The common bile duct will be considered to be dilated when it measures > 4 mm.

6.3.5 Liver

There are no published normal liver lengths or volumes for children. For purposes of determining whether the liver is enlarged, a comparison of the liver length to the length of the right kidney will be used. If the inferior tip of the liver extends below the inferior tip of the right kidney on a longitudinal image obtained in the region of the right kidney with the longest dimension, the subject will be considered to have hepatomegaly.

6.3.6 Processing Scans for Central Reading

The ultrasound images will contain the following information

- Proper identification (Subject 13-digit label number and date)
- The probe frequency
- Annotate the image with the position of the subject if other than supine/recumbent
- Annotate the image as transverse or longitudinal
- Measurements of the spleen in the transverse, anterior-posterior and longitudinal dimensions
- Measurements of the kidneys in the longitudinal, transverse and anterior-posterior dimensions
- Measurement of the gall bladder wall
- Measurement of the common bile duct in the region of the porta hepatis

6.3.7 Handling the Ultrasound Images

Clinical Center staff retain original ultrasound images on site until further notice of shipment for central review. Procedures for the central review processing are currently under review and will be disseminated once approved by the Steering Committee.

The ultrasound images will be accompanied by the following information:

- Proper Identification (Subject 13-digit label number and date)
- Subject's Age
- Subject's Body Length
- Subject's Body Weight
- NPO Status (how long they were held NPO)

6.4 TRANSCRANIAL DOPPLER (TCD) EXAM

6.4.1 TCD Exam Form

Transcranial Doppler will be performed at approximately 10 years of age. The TCD should be performed according to the Clinical Center standard of care and recorded on CD.

Once the TCD has been performed, BABY HUG Follow-up Study II Form 13 (TCD Exam) will be completed by the coordinator. Upon completion of Form 13, follow the instructions in Section 4.3 Generating Shipping Manifests to prepare the shipping manifest. The coordinator will affix one of the subject's label numbers to the form. Form 13 will remain with the subject's binder at the BABY HUG Follow-up Clinical Center.

6.4.2 Processing the TCD Exam

Have the TCD examiner copy the TCD exam to CD. A copy of the TCD diskette will be left at the BABY HUG Follow-up Study II Clinical Center and a copy will be prepared for shipment to the TCD Core Laboratory for review. The coordinator will affix a duplicate label (with the same label affixed to Form 13) to the TCD diskette.

6.4.3 Sending the TCD Exam

Each Clinical Center will be given FedEx billable stamps for sending the TCD diskette to the Medical University of South Carolina (MUSC). The coordinator will include a copy of the shipping manifest in the package with the CD.

6.4.4 TCD Reading and Archiving

The TCD exam will be read and interpreted using standardized procedures by the TCD Center at the MUSC.

After MUSC staff interprets the exam, the results will be retained until the procedures for sharing the data with the DCC, for statistical analysis, have been approved.

MUSC will retain the diskette for archiving with the BABY HUG Follow-up Study II records.

6.5 MRI/MRA

6.5.1 MRI/MRA Performance and Forms

BABY HUG Follow-up Study II MRI/MRAs should be performed according to the guidelines in Section 5.8 of this Manual of Operations.

6.5.2 MRI/MRA Processing

The MRI/MRAs will be read by two specialists who will independently assess each MRI/MRA scan that is performed at approximately 10 years of age and will record their assessments using BABY HUG Follow-up Study II Forms 43 (MRI) and 44 (MRA). In case of disagreement, a scan will be sent to a third reviewer. The two readings out of the three that are in agreement will be the single final reading.

6.5.3 MRI/MRA Shipment

Procedures for the central review processing procedures are currently under review and will be disseminated once approved by the Steering Committee. MRI/MRA CDs will be retained at the originating Clinical Center until further notification.

6.6 ECHOCARDIOGRAM

6.6.1 Echocardiogram Performance

BABY HUG Follow-up Study II echocardiograms should be performed according to the guidelines in section 5.6 of this Manual of Operations.

6.6.2 Echocardiogram Processing

Clinical Center staff will forward one copy of each echocardiogram on CD to the ECHO core laboratory with a shipping manifest. The ECHO lab will log receipt of the CD.

The echocardiograms will be read by a specialist who will independently assess each Echocardiogram that is performed at approximately 10 years of age. The results will be retained by the Core Lab until the procedures for sharing the data with the DCC, for statistical analysis, have been approved.

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CHAPTER 7

DATA COLLECTION, ENTRY, EDITING, STORAGE AND ARCHIVAL

SEE EXTERNAL DATA MANAGEMENT MANUAL OF OPERATIONS

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CHAPTER 8

TRAINING AND CERTIFICATION

8.1 INTRODUCTION

In multi-center studies, procedures must be standardized within each Clinical Center and among the participating Clinical Centers to assure that findings from all centers are comparable and, therefore, can be pooled. Certification of Clinical Centers and their staff indicates that they have been instructed in the collection of study data. Specifically, each Clinical Center participating in the BABY HUG Follow-up Study II must be certified to enroll and collect data from subjects. Staff who will be responsible for enrolling subjects, completing data collection forms, performing data entry, collecting specimens and/or sending study specimens to Core Laboratories must be certified. This chapter specifies the requirements for certification of Clinical Centers and their personnel and the responsibilities of the Data Coordinating Center (DCC) for coordinating the certification program.

8.2 TRAINING

Training for the BABY HUG Follow-up Study II is organized by the DCC. Training sessions for form data entry will be conducted via the internet using WebEx. Additional training and discussions will be held during Coordinator conference calls and Steering Committee Meetings.

8.3 CLINICAL CENTER CERTIFICATION

In order for a BABY HUG Follow-up Study II Clinical Center to be certified to enroll subjects and collect specimens and study data, the following requirements must be met:

1. Approval by DCC and the NHLBI Project Officer of the Clinical Center consent form (prior to submission to IRB and have any revisions).

2. Documentation of approval by local Institutional Review Board (IRB) of the BABY HUG Follow-up Study II Protocol and Consent Form, by submission of copies of approvals to the DCC. A copy of the IRB-approved Consent Form must be sent to the DCC each time it is revised. Notification of annual IRB approval is also required.

3. Approval by local institution of a Health Insurance Portability and Accountability Act of 1996 (HIPAA) Privacy Rule Authorization form.

4. Certification of at least one Principal Investigator and one Clinic Coordinator or Data Manager.

Personnel who were certified in the BABY HUG Follow-up Study I will be able to transfer their certification with some modification. The certification requirements for the BABY HUG Follow-up Study I can be reviewed in Chapter 8 of the BABY HUG Follow-up Study I Manual of Operations.

8.4 BABY HUG FOLLOW-UP STUDY II CERTIFICATION OF CLINICAL CENTER PERSONNEL

The BABY HUG Follow-up Study II requires that at least the following individuals be certified:

- Principal Investigator
- Co-Investigator (if part of the study at that site)
- Clinic Coordinator
- Data Manager (if part of the study at that site)

8.4.1 Principal Investigator

8.4.1.1 BABY HUG Follow-up Study I Certified

If the Principal Investigator was previously certified for the BABY HUG Follow-up Study I, his/her certification can be transferred to the BABY HUG Follow-up Study II with the additional requirement that he/she needs to complete and submit:

1. An updated Conflict of Interest Statement, Form 110 (Exhibit 8–1). Please note that the Conflict of Interest Statement must updated and submitted any times changes have occurred. The DCC should be notified annually if there are no changes to report.

2. NERI's Data Access Authorization Form (Exhibit 8-4).

8.4.1.2 Non-BABY HUG Follow-up Study I Certified

If a new, non-BABY HUG Follow-up Study I certified Principal Investigator joins the BABY HUG Follow-up Study II, he/she must meet the following requirements:

1. Approval requested and granted from NHLBI.

2. Training by a certified Principal Investigator or by the DCC.

3. Successful completion of the BABY HUG Follow-up Study II general knowledge test (see

Exhibit 8-2).

4. Submission of a Request for Principal Investigator Certification, Form 111 (see Exhibit 8-3).

5. Submission of NERI's Data Access Authorization Form (Exhibit 8-4).

6. Submission of Conflict of Interest statement, Form 110 (Exhibit 8–1). Please note that the Conflict of Interest Statement must updated and submitted any times

changes have occurred. The DCC should be notified annually if there are no changes to report.

7. Complete and sign a new Statement of Investigator (FDA 1572). Submit to the DCC along with:

   - Signed/dated Curriculum Vitae.
   - Valid Medical License.
   - Valid Human Subjects Training Certificate (e.g. CITI).
   - Signed Signature Log with current study staff.
   - In cases where the PI has changed during study, also submit the IRB letter of change acknowledgement.

8.4.2 Co-Investigator (if applicable)

8.4.2.1 BABY HUG Follow-up Study I Certified

If the Co-Investigator was previously certified for the BABY HUG Follow-up Study I, his/her certification can be transferred to the BABY HUG Follow-up Study II except he/she needs to submit:

1. An updated Conflict of Interest Statement, Form 110 (Exhibit 8–1). Please note that the Conflict of Interest Statement must updated and submitted any times changes have occurred. The DCC should be notified annually if there are no changes to report.
2. NERI's Data Access Authorization Form (Exhibit 8-4).

8.4.2.2 Non-BABY HUG Follow-up Study I Certified

If a new, non-BABY HUG Follow-up Study I certified Co-Investigator joins the BABY HUG Follow-up Study II, he/she must meet the following requirements:

1. Approval requested from NHLBI and granted.

2. Training by a certified Principal Investigator or by the DCC.

3. Successful completion of the BABY HUG Follow-up Study II general knowledge test (see

4. Exhibit 8-2).

5. Submission of a Request for Co-Investigator Certification, Form 111 (Exhibit 8-3).

5. Submission of NERI's Data Access Authorization Form (Exhibit 8-4).

6. Submission of Conflict of Interest statement, Form 110 (Exhibit 8–1).

7. PI to add as Sub-Investigator to the Statement of Investigator (FDA 1572). Submit to the DCC along with:

    - Signed/dated Curriculum Vitae.
    - Valid Medical License.
    - Valid Human Subjects Training Certificate (e.g. CITI).
    - Signed Signature Log with Delegation of Authority.

8.4.3 Clinic Coordinator

8.4.3.1 BABY HUG Follow-up Study I Certified

If the Clinic Coordinator was previously certified for the BABY HUG Follow-up Study I, his/her certification can be transferred to the BABY HUG Follow-up Study II except he/she needs to submit:

1. An updated Conflict of Interest Statement, Form 110 (Exhibit 8–1). Please note that the Conflict of Interest Statement must updated and submitted any times

changes have occurred. The DCC should be notified annually if there are no changes to report.

2. NERI's Data Access Authorization Form (Exhibit 8-4).

8.4.3.2 Non-BABY HUG Follow-up Study I Certified

If a new, non-BABY HUG Follow-up Study I certified Clinic Coordinator joins the BABY HUG Follow-up Study II, he/she must meet the following requirements:

1. Training by a certified Clinic Coordinator or the DCC.

2. Successful completion of the BABY HUG Follow-up Study II general knowledge test (see

Exhibit 8-2).

3. Submission of a completed BABY HUG Follow-up Study II Data Access Authorization Form (see Exhibit 8-4).

4. Successful completion of BABY HUG Follow-up Study II Forms 03 (Central Lab Collection) for a standard set of patient information (see

Exhibit 8-5).

5. Submission of a Request for Clinic Coordinator/Data Manager Certification, Form 112 (see Exhibit 8-6).

6. Submission of Conflict of Interest statement, Form 110 (Exhibit 8–1).

7. PI to add as Sub-Investigator to the Statement of Investigator (FDA 1572). Submit to the DCC along with:

   - Signed/dated Curriculum Vitae.

- Valid Medical/Nursing License (if applicable).
- Valid Human Subjects Training Certificate (e.g. CITI).
- Signed Site Signature Log with Delegation of Authority.

8.4.4 Data Manager (if applicable)

8.4.4.1 BABY HUG Follow-up Study I Certified

If the Data Manager was previously certified for the BABY HUG Follow-up Study I, his/her certification can be transferred to the BABY HUG Follow-up Study II except he/she needs to submit:

1. An updated Conflict of Interest Statement, Form 110 (Exhibit 8–1). Please note that the Conflict of Interest Statement must updated and submitted any times changes have occurred. The DCC should be notified annually if there are no changes to report.
2. NERI's Data Access Authorization Form (Exhibit 8-4).

8.4.4.2 Non-BABY HUG Follow-up Study I Certified

If a new, non-BABY HUG Follow-up Study I certified Data Manager joins the BABY HUG Follow-up Study II, he/she must meet the following requirements:

1. Training by a certified Clinic Coordinator or the DCC.
2. Successful completion of the BABY HUG Follow-up Study II general knowledge test (see Exhibit 8-2).
3. Submission of a completed BABY HUG Follow-up Study II Data Access Authorization Form (see Exhibit 8-2).

4. Successful completion and data entry of BABY HUG Follow-up Study II Forms 01 (Enrollment Form) and 03 (Central Lab Collection) for a standard set of patient information (see

Exhibit 8-5).

5. Submission of a Request for Clinic Coordinator/Data Manager Certification, Form 112 (see Exhibit 8-6).

6. Submission of Conflict of Interest statement, Form 110 (Exhibit 8–1).

7. PI to add as Sub-Investigator to the Statement of Investigator (FDA 1572). Submit to the DCC along with:

    - Signed/dated Curriculum Vitae.
    - Valid Medical License (if applicable).
    - Valid Human Subjects Training Certificate (e.g. CITI).
    - Signed Signature Log.

8.4.5 Database Access (Content Only Privileges)

Clinical Centers may request “Review Only” privileges for Clinical Center staff who will not be required to perform data entry, but need access to study documents. Submission of a completed BABY HUG Follow-up Study II Data Access Authorization Form (Exhibit 8-4) is required. The DCC will provide this form to the Clinical Center when notified that a new staff member requires access to the database.

8.5 ROLE OF THE DATA COORDINATING CENTER IN CERTIFICATION

The tasks related to the certification program for which the DCC staff has responsibility are:

a. Documentation of certification procedures;

b. Coordination of, participation in, and instruction in training sessions;

c. Distribution, receipt and review of certification materials;

d. Documentation of the completion status of certification requirements for Clinical Centers and Clinical Center staff;

e. Certification of Clinical Centers and Clinical Center staff; and

f. Issue certification numbers to Clinical Center staff.

8.5.1 Processing Requests for Certification of Clinical Center Staff

An individual at the DCC processes all requests for certification. Upon receipt of a request for certification, the DCC reviews the materials in the certification file maintained for each Clinical Center to assure that all required materials have been received. Requests for certification are then reviewed by designated DCC staff.

8.5.2 Certification of Clinical Center Staff

DCC staff is responsible for review of certification materials submitted for Clinical Center staff.

8.5.3 Notification of Certification

After review of submitted materials, if certification is recommended, the DCC will assign a unique BABY HUG Follow-up Study II staff number to the individual. Individuals transferring their BABY HUG Follow-up Study I certification to the BABY HUG Follow-up Study II will maintain their existing certification number. An updated listing of Clinical Center Certification Numbers, Form

113 (see Exhibit 8-7) will be sent to the Clinical Center with the new (or transferred) certification number listed next to the individual's name as personnel are certified during the BABY HUG Follow-up Study II.

8.5.4 Processing Requests for Certification of Clinical Centers

Requests for Clinical Center certification are also logged at the DCC and each is reviewed to assure that the required staff has been certified and that all requirements have been met. The DCC notifies each Clinical Center of certification so that they may begin subject enrollment by forwarding a completed copy of BABY HUG Follow-up Study Form 114, Notification of Clinical Center Certification (Exhibit 8-8).

8.5.5 Liaison Activities

The Certification Coordinator maintains regular telephone communications with staff in each Clinical Center to detect and help to resolve any problems encountered in the certification process. Problems which the DCC is unable to resolve are referred to the Steering Committee.

8.6 REVIEWING CLINIC COORDINATOR CERTIFICATION

If a Clinic Coordinator fails to meet the standards necessary for conduct of the BABY HUG Follow-up Study II, the DCC staff will review the problem(s) with the Steering Committee with a request to the Chairman that the Principal Investigator be contacted to review the problem(s) and solicit any explanation(s). If the DCC staff document no improvement within two months of the date the problem is reviewed by the Steering Committee Chairman with the site Principal Investigator, the DCC staff will notify the appropriate individual in writing that his/her certification has been suspended, and other Clinical Center staff will be responsible for the integrity of the performance of the tasks of that coordinator. Copies of this letter will be sent to the Principal Investigator and the Steering Committee. A staff member who has had certification suspended will be re-certified when all of the following conditions have been met: (1) at least 5 forms or one year's work have been reviewed and co-signed by the Principal Investigator and all are

satisfactory, (2) any outstanding edit messages and memoranda responses have been received and are satisfactory, and (3) current work is satisfactory and is submitted in a timely fashion.

In the extenuating circumstance when no certified Clinic Coordinator is available at the Clinical Center due to illness or other unexpected events or while new staff are being recruited and certified, BABY HUG Follow-up Study II forms will be accepted by the DCC if each form is reviewed and co-signed by the Principal Investigator.

Exhibit 8–1
BABY HUG Follow-up Study II
Financial Disclosure

I, the undersigned, certify that:

1. As of _____, neither I, nor my spouse or dependent children own or will buy or trade stock or stock options in any of the companies* providing medication, equipment or financial support in the trial. In addition, I do not have a retainer-type consultant position with any of the companies.*

2. I agree to disclose financial interests as outlined in the BABY HUG Follow-up Study II Policy on Conflict-of-Interest during my participation in the BABY HUG Follow-up Study II.

If response is no to questions 1 or 2, an explanatory letter is required.

_____ _____

Typed or Printed Name Signature

_____

Date

*Companies include: Bristol-Myers Squibb

Exhibit 8-2
BABY HUG Follow-up Study II Certification Test

1. Which subjects were eligible for the BABY HUG Follow-up Study II?
   a. BABY HUG patients who were on hydroxyurea
   b. All BABY HUG patients
   c. All BABY HUG Follow-up Study I patients with at least 24 months of follow-up
   d. Any 5 year old child with sickle cell disease at your Clinical Center who is on hydroxyurea

2. The BABY HUG Follow-up Study II will follow subjects until:
   a. Completion of the Y10 Visit
   b. January 2015
   c. June 2015
   d. December 2016

3. The BABY HUG Follow-up Study II is divided into two arms. What are they? Select all that apply:
   a. Hydroxyurea
   b. Placebo
   c. Active
   d. Passive

4. The purpose of the BABY HUG Follow-up Study II is:
   a. To ensure that all subjects are given the same formulation of hydroxyurea.
   b. To ensure that hydroxyurea is being administered appropriately.
   c. To follow-up subjects who were enrolled in BH and BHFU I.

5. How frequently is subject data abstracted in the BABY HUG Follow-up Study II?
   a. Every 2 weeks
   b. Once a month
   c. Every 6 months

d. Once a year

e. Every other year

6. Are subjects who have a stem cell transplant during BABY HUG Follow-up Study II allowed to continue in the study?

a. Yes, but subject will only be assessed annually for vital status and graft-vs-host-disease (captured on Form 26 from the date of reconditioning).

b. No, the subject must be withdrawn.

7. Circle all of the subjects/families that will NOT require re-consent on the amended Protocol Version 9 Consent:

a. Subjects with data to be entered into the eCOS Database

b. Subjects entered into the C-TASC database but subsequently withdrawn from the study prior to September 30th, 2015

c. Deceased subjects

d. Subjects undergoing Bone/STEM Cell transplantation

_____

NAME

_____ _____

SIGNATURE DATE

Exhibit 8-3
BABY HUG Follow-Up Study II
Request for Investigator/Co-Investigator Certification

Clinical Center: _____ Clinical Center No.: ☐☐

Certification as BABY HUG Follow-up Study II Co-Investigator is requested for:

Name _____

The individual named above has (all MUST be checked):

* ☐ Successfully completed the BABY HUG Follow-up Study II general knowledge test.

☐ (*Co-Principal Investigators only*) attended a BABY HUG Follow-up Study training session on

_____ or received training from _____

Date(s) Name

who is a fully certified BABY HUG Follow-up Study II Principal Investigator

*To be submitted with this form if not previously submitted.

Principal Investigator: _____

Signature

Date: _____

Exhibit 8-4
BABY HUG Follow-up Study II Data Access and Electronic Signature Authorization Form

neri

Data Access and Electronic Signature Authorization

Study Name			
Staff First Name		Staff Last Name	
Site #			
Site Name			
Address			
City		State/Province	
Country		Email	
Telephone		Fax	
Study Role		Data Access Level	Data Entry ☐ Read Only ☐
Authorized to perform Electronic Signature?	Yes ☐ No ☐	Signatory Level	Principal Investigator ☐ Designee ☐

If Yes, complete the Certification of Electronic Signature section. If Designee, PI must complete the attestation section.

CERTIFICATION OF ELECTRONIC SIGNATURE

Pursuant to Section 11.100 of Title 21 of the Code of Federal Regulations, this is to certify that the **Site** named above intends that all electronic signatures executed by **Staff** named above on electronic case report forms in the Electronic Data Capture (EDC) system for the **Study** named above are the legally binding equivalent of traditional handwritten signatures.

Staff named above acknowledges that his/her unique username and password will be kept in a secure location and will not be disclosed to another party. By providing an electronic signature to completed Case Report Forms of subjects entered into the EDC system, the **Staff** named above verifies that all data provided are true and accurate to the best of his/her knowledge.

Signature: _____

Printed Name: _____ **Date:** _____

PRINCIPAL INVESTIGATOR ATTESTATION FOR DESIGNEE

I, the Principal Investigator signed below, authorize the Designee named above to execute electronic signatures for the **Study** named above. I acknowledge that this authorization does not waive my responsibility as PI to ensure subject safety and accuracy of all data collected for this study at this site.

Signature: _____

Printed Name: _____ **Date:** _____

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Exhibit 8-5
Subject Narrative Practice Data for Form 03

For the practice forms, a "dummy" subject ID will need to be used so that the system will not confuse the practice test with real data. Please request a "dummy" subject ID from the DCC.

Please replace the "XXXX" wherever subject ID is mentioned below. At the end of the forms, use the certification number provided by the DCC. You will also be given a list of labels to use for this certification. Each label can only be used once, and there should be a label given to each sample. The collection times can be made up.

Medical Record Date: December 12, 2016

Patient XXXX from Clinic 99 with Patient Letter Code XYZ returns to clinic today to have BABY HUG Follow-up Study II exit studies performed. A urine specimen was collected, labeled with label number **(label)** and shipped to the Augusta University AU for processing. 5 ml of blood was drawn, labeled with label number **(label)** and shipped to the AU for processing. There was insufficient blood available to send specimens to the remaining BABY HUG Follow-up Study core laboratories. The family agreed to return on December 19, 2016 to have the remaining specimens collected.

Medical Record Date: December 19, 2016

Patient XXXX from Clinic 99 with Patient Letter Code XYZ returns to clinic today to have the remaining required blood specimens collected. 1 ml of blood was collected in a red top tube, labeled with label number **(label)** and shipped to Cincinnati Children's Hospital Medical Center (CCHMC) for Cystatin C processing. 0.1 ml of blood was collected, labeled with label number **(label)** and shipped to UTSW for processing. 0.5 ml of blood was collected in a lavender top tube, labeled with label number **(label)** and shipped to AU for HbF processing. 1.0 ml of blood was collected in a red top tube, labeled with label number **(label)** and shipped to the AU for processing. 1.0 ml of blood was collected in a lavender top tube, labeled with label number **(label)** for HJB processing. 3.0 ml of blood was collected in a lavender top tube, labeled with label number **(label)** and shipped to CCHMC for VDJ Testing, along with the Cystatin C and HJB specimens.

Exhibit 8-6
BABY HUG Follow-Up Study II
Request for Clinic Coordinator/Data Manager Certification

Clinical Center: _____ Clinical Center No.: ☐☐

Certification as BABY HUG Follow-up Study II Clinic Coordinator is requested for:

Name _____

The individual named above has (all MUST be checked):

* ☐ Attended a BABY HUG Follow-up Study II Training Session on (Date):_____

OR

☐ Received equivalent training at a Clinical Center by (Name) _____

who is a fully certified BABY HUG Follow-up Study II Clinic Coordinator.

* ☐ Successfully completed the BABY HUG Follow-up Study II general knowledge test.
* ☐ Completed a BABY HUG Follow-up Study II Data Authorization Access Form.
* ☐ Successfully completed and data entered BABY HUG Follow-up Study Form 03 for the standard patient narrative, and submitted the form printouts to the DCC.
* ☐ Attended BABY HUG Follow-up Study II eCOS training via:

Webinar with DCC on: _____ OR Training video & slides on: _____

*To be submitted with this form if not previously submitted.

Principal Investigator: _____

Signature Date

Exhibit 8-7
BABY HUG Follow-Up Study II
Clinical Center Certification Numbers

Clinical Center: _____ Clinical Center No.: | | |

Name	Certification No.	Position
		- 0 1	Principal Investigator
		-	
		-	
		-	
		-	
		-	
		-	
		-	
		-	
		-	
		-	

_____	☐☐ - ☐☐	_____
_____	☐☐ - ☐☐	_____
_____	☐☐ - ☐☐	_____
_____	☐☐ - ☐☐	_____
_____	☐☐ - ☐☐	_____

Issued by: _____

DCC Staff Member

Date: _____

Exhibit 8-8
BABY HUG Follow-Up Study II
Notification of Clinical Center Certification

Clinical Center: _____ Clinical Center No.: | | |

Principal Investigator: _____

YOUR CLINICAL CENTER HAS BEEN CERTIFIED TO BEGIN CONSENTING SUBJECTS FOR THE BABY HUG FOLLOW-UP STUDY II.

Approved by: _____

Date: _____

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 9

STUDY MONITORING AND REPORTING RESULTS

9.1 INTRODUCTION

Monitoring will occur on an as needed basis and will be conducted by the OSMB and Steering Committee.

9.2 MONITORING FOR ADVERSE EVENTS

Clinical events (hospitalizations and specific other sickle cell related events) will be retrospectively abstracted from the medical records of study participants at specified intervals (two times per year). Clinical Centers will be asked to classify the event based upon BABY HUG criteria using simple yes/no data forms. Definitions of clinical events can be found in Appendix F of the BABY HUG Treatment Study Protocol except for the post-hoc Steering Committee definition of splenic sequestration which is defined as follows:

Splenic Sequestration: 2 cm or more increase from last visit in palpable spleen size AND a decrease in Hb of 2 g/dL or more below the 3-month rolling average.

For children who agree to active Follow-up, standard clinical trial prospective, serious adverse event (SAE) reporting for the items listed in Chapter 3, Section 3.4.2 of this Manual of Operations must be captured for the five days following completion of each of the active studies using BABY HUG Follow-up Study II Form 25 (Serious Adverse Event). If the SAE is related to the study drug (HU) and unexpected then it must be submitted to the DCC using a MedWatch

3500A. The NHLBI has also required that “Major” Events be reported to NHLBI and the OSMB Chairman (as indicated in Chapter 3, Section 3.4.3 of this Manual of Operations). See Chapter 3.

9.3 MONITORING DATA QUALITY

Integrated into the data entry system are real time validations, including both inter- and intra-instrument data checks. Inconsistent or questionable values are flagged during entry, and a query is automatically generated to the data entry staff. These queries provide the information necessary to investigate any data entry errors or resolve questions regarding out-of-range or questionable values. Second-level query tracking allows monitors and data manager’s real time access to unresolved queries as well as the date and time of query generation and resolution.

9.4 MONITORING SPECIMEN AND CD QUALITY

On a continual basis, all specimens received in the Core Laboratories are inspected on arrival including labeling with comparison to shipping manifests in the shipments. Inconsistencies in labeling and problems with specimen condition (e.g., cracked tubes, clotted CBC specimens, insufficient quantities) are communicated to the Clinical Center staff within 24 hours and reported to the DCC. Laboratory results are checked for validity prior to entry in the Follow-up study II database. The core laboratory is queried regarding out-of-range results.

Liver-spleen scan, abdominal sonogram, MRI/MRA, TCD and echocardiogram CDs are examined for adequacy by the central readers. Images judged to be inadequate for reading are returned to the Clinical Center for reprocessing. Within 10 days of receipt, the films should be reprocessed.

9.5 INSTITUTIONAL DATA AND SAFETY MONITORING AND REPORTING

Accrual and safety data will be under continual review by the DCC, NHLBI and NICHD.

Accrual and safety data will also be reviewed annually by each Clinical Center's IRB. Prior to implementation of this study, the Protocol and the proposed subject consent forms will be reviewed and approved by the local IRB. This committee will also approve all amendments to the Protocol or informed consent, and conduct continuing annual review so long as the BABY HUG Follow-up Study II is open.

Every six months, data is retroactively abstracted for review by treatment group by the OSMB. A report will be forwarded to the OSMB at these times and their recommendations will be expeditiously implemented. The OSMB reserves the right to recommend early termination of the study for considerations of safety or efficacy.

9.6 CLINICAL CENTER MONITORING

9.6.1 Protocol Deviations

DCC staff are responsible for monitoring for protocol deviations and for notifying all appropriate BABY HUG Follow-up Study II personnel or appropriate committees of any deviations when the DCC becomes aware of them (e.g., performing special studies outside of the BABY HUG Follow-up Study II window). If a clinical center discovers a protocol deviation, email one of the BABY HUG Follow-Up Study II staff at the DCC with the information (BabyHug@neriscience.com).

9.6.2 Performance Reports

Performance reports are prepared by the DCC staff and distributed to the Clinical Centers semi-annually. These reports include information on forms entered, edited and printed, special study procedures performed, laboratory specimens collected, and protocol deviations.

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 10

DATA COORDINATING CENTER RESPONSIBILITIES

10.1 INTRODUCTION

The Data Coordinating Center (DCC) is organized and staffed as part of the New England Research Institutes, Inc. (NERI) to serve the needs of the BABY HUG Follow-up Study II. The DCC staff fulfills key roles in developing and implementing the study's statistical design, data collection and management, and analysis of study results. The major responsibilities of the DCC, which serves as the central data repository for the information collected under the common study protocol, are:

(1) serve as communication center for the study;

(2) provide the Internet data entry system for all study data;

(3) maintain a central database of data integrated from all Clinical Centers, Core Laboratories and central reviewers; and

(4) generate analyses to monitor for evidence of efficacy, adverse effects, safety monitoring and adherence to the study protocol. The objectives and procedures designed to achieve these obligations are presented in this chapter.

10.2 OBJECTIVES OF THE DATA COORDINATING CENTER

The general aims of the BABY HUG Follow-up Study II DCC are to:

- Serve as a collaborating partner with the other Investigators in the organization, design, conduct, and analysis of the study.

- Provide biostatistical expertise to the study in the area of design and operation of this multicenter study.
- Work with the other Investigators and consultants to draft and revise as necessary the Protocol, Manual of Operations, and study forms.
- Assist the NHLBI in maintaining an Investigational New Drug (IND) application with the FDA for hydroxyurea usage in young children.
- Provide expertise in the area of Internet data entry and statistical analysis.
- Develop and implement the required data processing procedures for handling all study forms, core laboratory results and other materials.
- Develop, implement and maintain quality control procedures to detect and correct deficiencies in data collection, processing or analyses.
- Provide facilities and staff to carry out appropriate analyses to monitor the study for evidence of adverse effects.
- Establish and maintain subcontracts with Core Laboratories and medical consultants as needed by the study.
- Serve as the communication center for the study.
- Prepare progress reports and assist in preparation of publications.

10.3 PLANNING, TRAINING AND MEETING SUPPORT

The members of the BABY HUG Follow-up Study II DCC play a major role in the organization and conduct of Investigator meetings, subcommittee meetings, and conference calls held during all phases of the study. The BABY HUG Follow-up Study II DCC staff provide training to the BABY HUG Follow-up Study II Clinical Center staff in the collection and processing of data,

as necessary. In addition, the BABY HUG Follow-up Study II DCC staff provide logistical support for orientation and training sessions.

10.4 STUDY DOCUMENTS AND SUPPLIES

DCC staff are responsible for the coordination of the preparation of the Protocol and Manual of Operations. DCC staff and the Principal Investigators will collaborate in the development and finalization of these documents. DCC staff will periodically review current procedures and develop additional procedures as needed throughout the course of the study. The DCC will update the Protocol and Manual of Operations as study policies and procedures are added, deleted or changed. Updated revisions will be provided to all Clinical Centers and Core Laboratories.

The DCC staff prepare all the BABY HUG Follow-up Study II forms. All forms changes will also be prepared by the DCC.

DCC staff will assist the National Heart, Lung, and Blood Institute staff in maintaining the Investigational New Drug (IND) application to the Federal Drug Agency (FDA) for hydroxyurea in this age group of children.

The DCC posts the most recent version of the Protocol, Manual of Operations, and Case Report Forms on the BABY HUG Follow-up Study II website. (See Chapter 14 for information on the website.)

10.5 CLINICAL CENTER COMMUNICATIONS

The DCC serves as the communication center for the study.

The DCC staff are a resource for the numerous telephone inquiries and written inquiries concerning the study procedures from study Investigators and Clinical Center personnel.

The DCC issues numbered memoranda to Principal Investigators and/or coordinators to impart study-wide policies, procedures, announcements, operational issues, etc., and to request information.

The DCC staff provide logistical support for meetings, conference calls and training sessions. They are responsible for preparing handouts and other materials for meeting participants as well as for preparing and distributing the minutes of these meetings.

DCC staff maintain and distribute a BABY HUG Follow-up Study II Address Directory. This directory contains a listing of study personnel from each Clinical Center, each Core Laboratory, all study Consultants, as well as personnel from the Program Offices at the NHLBI and NICHD and the Data Coordinating Center. This directory is updated periodically and posted to the study website.

Protocol exceptions for eligible subjects will be considered by the Steering Committee and may be granted as long as study integrity is not compromised. If the committee arrives at a recommendation to grant an exception to the Protocol, the local IRB will be notified and the exception will be conducted in accordance with the local IRB's Standard Operating Procedures.

10.6 DATA COLLECTION AND STORAGE

The BABY HUG Follow-up Study II Manual of Operations provides a description of study design, organization, methods, definitions, and procedures used in data collection. The DCC staff in conjunction with appropriate BABY HUG Follow-up Study II Investigators coordinate the preparation of this document.

10.6.1 Data from the Clinical Centers

The DCC programming staff are responsible for developing an Internet data entry system. (See Chapter 7 for a description of the Web-based electronic data capture system, eClinicalOS [eCOS].) Clinical Center staff submit data from study visits and data abstractions into the BABY

HUG Follow-up Study II database via eCOS. Data must be entered into the database within specified time frames before being declared delinquent or missing. (See Chapter 7 on Data Entry.)

10.6.2 Data from the Core Laboratories

Specimens are sent directly to the Hematology, Biochemistry, Pitted Cell, VDJ and Cystatin-C Core Laboratories from the Clinical Centers. Specimen transmittal information is contained in forms entered into the BABY HUG Follow-up Study II database via eCOS. All Core Laboratory data are transmitted electronically to the DCC. The DCC programming staff are responsible for developing and implementing the procedures to receive and store the data from the Core Laboratories. A regular schedule for transmission of these data is established.

10.6.3 Data from Central Readers

All CDs are sent from the Clinical Centers, and CD shipping information is contained in forms entered into the BABY HUG Follow-up Study II database via eCOS. Clinical Center staff send the CDs to the respective central readers who complete the appropriate reading form. The process for return of the centrally read data is under review and will be disseminated upon approval by the Steering Committee.

10.6.4 Storage System for Study Documents

All important study documents are posted on the BABY HUG Follow-up Study II website which is updated regularly. This includes study reports, minutes of meetings and conference calls, numbered memos, Forms, the Protocol, and the Manual of Operations.

10.7 DATA MANAGEMENT AND MAINTENANCE

The DCC data management staff have designed and implemented the Internet data entry system, eCOS to be used in the Clinical Centers. The Clinical Center staff are responsible for data entry, data editing, and corrections, if necessary, of all study forms. These procedures are

described in Chapter 7. The DCC staff are responsible for storing and analyzing all received study data.

10.7.1 Form Data

Forms are edited for acceptable codes, valid ranges and logical consistency by electronic checks during data entry at the Clinical Center. All ranges and logical consistency checks are executed upon submit.

All corrections made are electronically audited. The audit file includes the old and new values for the field, date of the correction and who made the correction. The form is automatically marked as corrected as soon as responses to edits have been made and accepted.

10.7.2 Core Laboratory Results

Results from core laboratories are checked for acceptable codes and valid ranges. Data outside the ranges are not imported into the database until verified.

10.8 DATA ANALYSES AND REPORTS

Details of the data analysis plan are contained in the BABY HUG Follow-up Study II protocol. Semi-annual reports to the NHLBI and OSMB (or others, as needed) are based on the data.

10.9 DATA PUBLICATION AND REPORTING

DCC staff will assist, if requested, the participating Clinical Center staff in the preparation of publications which have received prior approval according to study procedures (see Chapter 13).

Upon request of the National Institutes of Health (NIH), any and all of the above data are made available to the NIH to access and utilize at any time after the completion of the BABY HUG Follow-up Study II. At that time, any and all data requested by the NIH are transferred to the National Heart, Lung, and Blood Institute (NHLBI).

10.10 SUBJECT PRIVACY, CONFIDENTIALITY OF DATA, AND DATA SECURITY

Because of the importance of protecting study data at the DCC from theft or unauthorized perusal or alteration, access to computer files is restricted through the use of assigned individual usernames and passwords. Protection of the computer files from catastrophic loss is accomplished by a backup system.

To maintain subject privacy, the study records submitted outside of the originating Clinical Center should not contain participants' names, addresses or other identifying information. Each participant record is identified by a unique four-digit Subject ID Number and a three-character Subject Letter Code. Names and addresses corresponding to the identifying codes are kept on file at the Clinical Center.

10.11 QUALITY CONTROL

10.11.1 Certification

The DCC staff has developed, implemented and monitored the BABY HUG Follow-up Study II staff certification program outlined in Chapter 8, including specific DCC responsibilities. The DCC staff maintain a roster of certified staff for each Clinical Center.

10.11.2 Quality Assurance of Clinical Center Data

Clinical Center personnel are trained during training sessions or are instructed by a certified staff member on the BABY HUG Follow-up Study II Data Management System (eCOS). As part of the certification process, they enter the data for selected forms using a standard subject narrative provided by the DCC. The data records from these forms are compared to the master file at the DCC and discrepancies noted.

10.11.3 Performance Reports

Performance of the Clinical Centers is assessed in reports. These reports include consideration of the following:

1. Number of study forms that have been entered and passed edit checks.

2. Number of study visits that have been completed during the ideal or extended window. (For the BABY HUG Follow-up Study II, this will include the visit for the active group at 10 years of age.)

3. Number of Data record abstractions completed within each 6 month or 1 year window.

The DCC staff compare performance and quality of submitted materials for items such as forms past due, studies not performed, or labs not collected, etc. among Clinical Centers.

10.11.4 Site (or Audit) Visits

In addition to preparing the Clinical Center performance monitoring reports, the DCC staff ensure data quality by conducting periodic site monitoring visits to the Clinical Centers. The data on subject medical records are compared against listings of data residing on the BABY HUG Follow-up Study II database for selected forms as of the date of the request for a site visit. Using the data as of the site visit request should prevent any audit-prompted revisions of the data form(s). Recertification of Clinical Center personnel responsible for key areas of data collection may also be performed during site visits.

Each of the BABY HUG Follow-up Study II Clinical Centers will be site visited at least once per year. The Site Visit Team will include NHLBI designated staff and DCC staff.

During the site visits, the team will conduct a review of the accuracy of data reported from the medical record for a random sample of cases. The consent forms will be reviewed. The Clinical Center staff will be notified prior to the visit what information should be available. Differences between the medical record and the database will be brought to the attention of the Clinical Center staff and resolved. The results of the data review (Site Visit Reports) will be

submitted to the Principal Investigator of each Clinical Center, and the NHLBI and NICHD Project Officers.

10.11.5 Quality Control of the Data Coordinating Center

DCC staff activities are governed by the NERI Standard Operation Procedures (SOP) for the conduct of NERI business.

10.12 DATA COORDINATING CENTER CONTACTS

DCC staff serve as a resource for all BABY HUG Follow-up Study II Clinical Center staff and Core Laboratory staff. Questions concerning the Protocol, study procedures, form entry or other study issues may be directed to appropriate DCC staff (Principal Investigator, Study Manager, Coordinator or Data Management staff). Names and telephone numbers of current BABY HUG Follow-up Study II DCC staff are given in the Address Directory of NERI Connect.

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 11

CLOSE-OUT PROCEDURES

11.1 STUDY END DATE

The common termination date for this study is December 31, 2016.

If Investigators become aware of new information regarding the use of hydroxyurea during this study, parents/guardians with children on the BABY HUG Follow-up Study II will be advised to stop HU treatment if their children are currently receiving HU.

11.2 DATA CLEAN-UP, CLOSURE AND STORAGE

The DCC will perform close-out visits at each Clinical Center to assure that all of the following tasks have been completed:

IRB Approvals

- Review all IRB approvals.
- Verify that all IRB approvals were obtained for all subjects.

Consent Forms and Enrollment Records

- Review signed consent forms for all subjects enrolled since the last monitoring visit and compare with the Investigator's enrollment records.
- Review eligibility criteria for all subjects.

Study Forms

- CRFs: Collect all remaining CRFs and update any outstanding data correction tabulations.
- Review the site's study files to ensure that all study documentation is current and complete.

Study Materials

- The monitor(s) will discuss with the Clinical Center the disposition of BABY HUG Follow-up Study II materials that should be kept at the site and the procedures that must be implemented to close out the study.

Visit Log

- Make certain all visits by monitors and other authorized personnel have been documented and that the log is complete.

Monitoring Report

- DCC staff will complete the monitoring report and make the final determination that the Investigator's obligations have been met and that all study and regulatory requirements have been fulfilled.

Laboratory

- Verify that all specimens for laboratory studies have been forwarded to the appropriate location(s).

The Clinical Centers will complete the following tasks in preparation for the DCC close-out visit of their site:

IRB Approvals

- The Clinical Center has informed the IRB of the closeout visit and study completion. The Clinical Center has this document ready to show the monitor.

Consent Forms and Enrollment Records

- Make sure the enrollment records are complete and ready for the monitor.

Study Forms

- The Clinical Center has resolved and is ready to provide to the monitor any outstanding DCC data queries from past visits or/and audits.
- Make all remaining corrections to the CRFs.
- The Clinical Center has all forms ready for the monitor to review.

Documentation

- The PI may keep a copy of the Protocol and Manual of Operations or he/she can destroy it. It is recommended that they be retained at the site for any future reference.
- Clinical Center may retain one copy of each unused form. All other copies of forms must be destroyed.

Supplies

As applicable:

- Clinical Center staff must destroy all gluteraldehyde tubes.
- Clinical Center staff must destroy all microtainers sent to the Clinical Center for study use.

- Clinical Center staff must destroy all study label sheets.
- Arrange to have remaining study incidentals and accessories/supplies destroyed.

Investigator's Final Report

- The PI must prepare a final report to the project officers and contract officers at the NHLBI and NICHD. The report should include an enrollment summary, information on SAEs, and any other relevant information about the Clinical Center.
- The PI must prepare a final report to the Clinical Center IRB. The report should contain all the information that is in the report to the sponsors, as well as any other information specifically requested by the IRB.
- If applicable, the PI must notify the Clinical Center's Hospital Administration that the BABY HUG Follow-up Study II is no longer being conducted at the Clinical Center/hospital.
- Submit the final report to NERI within 90 days of the closeout visit.

Storage

- Clinical Center staff must store completed study forms/tests, signed consent forms and all IRB correspondence at the site. Location of materials should be noted in the Clinical Center closeout report. Records must be kept for seven years after the FDA approves the request to relabel hydroxyurea to include a pediatric indication, or if the request is not approved, for seven years after the study is discontinued and the FDA notified in accordance with ICH GCP.

The DCC will verify in a final site visit report to individual sites that each was properly closed.

11.3 FINAL STUDY DATA AND DISSEMINATION OF RESULTS

The OSMB will review the final data analyses regarding the main findings of the study at a planned final meeting. These data analyses will form the basis of the final consensus recommendations from the OSMB, the Steering Committee and the NHLBI. These consensus recommendations will be shared first with the study children's families and will be made public as soon as possible thereafter. The final data analysis report and any databank studies will be available for submission to the FDA and for archiving.

Public data files will be made available according to NHLBI policy.

PEDIATRIC HYDROXYUREA CLINICAL TRIAL
(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II
MANUAL OF OPERATIONS

CHAPTER 12

ORGANIZATIONAL STRUCTURE AND PARTICIPATING UNITS

12.1 INTRODUCTION

The BABY HUG Follow-up Study II will be conducted in fourteen Clinical Centers, a Data Coordinating Center and Core Laboratories. The Clinical Center staff will be trained in accordance with the procedures set out in this Manual of Operations. The objective is to standardize all study procedures carried out in the Clinical Centers and at the operational central units.

Study monitoring will be carried out by the Observational Study Monitoring Board (OSMB), and Steering Committee. Monitoring will include adherence to protocol, achievement of recruitment goals and subject.

An organizational chart for the BABY HUG Follow-up Study II is presented in Exhibit 12-1.

12.2 PARTICIPATING UNITS

12.2.1 Steering Committee

The Steering Committee will comprise the Study Chairman, the Vice-Chairman, the Principal Investigator of the Data Coordinating Center, the NHLBI and NICHD Project Officers, all Clinical Center Principal Investigators, the Coordinator Chair, two Clinical Center Coordinators (by election) and, ex officio, the directors of the Core Laboratories.

12.2.2 Clinical Centers

The collaborating centers are funded by contracts from the NHLBI. A PI and a Coordinator should be identified at each Clinical Center. Exhibit 12-2 lists the Clinical Centers and their current Principal Investigator.

A final recruitment report specifying the number of subjects enrolled by each certified Clinical Center will be distributed after the end of enrollment.

12.2.3 Core Laboratories

The Core Laboratories have responsibility for receiving blood and urine samples, or imaging files, from the Clinical Centers and performing specimen/imaging analyses.

12.2.4 National Heart, Lung, and Blood Institute

The National Heart, Lung, and Blood Institute (NHLBI) staff -- Blood Diseases Branch (Division of Blood Diseases and Resources) and Office of Biostatistics Research (Division of Prevention and Population Sciences) will participate with study investigators and key study personnel in all phases of the study. A member of the Blood Diseases Branch (Division of Blood Diseases and Resources) will serve as a voting member on the Steering Committee, and other study committees as appropriate.

The NHLBI will provide direction in the management of the contracts which fund the study, and assistance in developing solutions to major problems. An OSMB has been appointed by the NHLBI to provide overall monitoring of the study. The NHLBI OSMB reviews the data at six-month intervals. A progress report showing results according to the different treatment types (see Chapter 4 of the protocol) will be forwarded by the DCC to the OSMB at these times and their recommendations will be expeditiously implemented.

12.2.5 Data Coordinating Center

The Data Coordinating Center staff will include the Principal Investigator(s), Project Manager(s), Statistician(s), Data Manager(s), Clinical Research Associate(s) and Administrative Assistant(s). Data Coordinating Center staff for the BABY HUG Follow-up Study II will provide expertise in the areas of study design, quality control, data processing and data analysis. Data Coordinating Center staff will provide biostatistical and epidemiological advice for the overall conduct of BABY HUG Follow-up Study II and will collaborate with the BABY HUG Follow-up Study II Investigators in all phases of the study including participant enrollment and Follow-up, preparing required statistical analyses, generating Core Laboratory work lists, report forms, blood specimen transmittal lists, and progress reports, and, assist in the preparation of manuscripts for publication. Data Coordinating Center staff will undertake the primary responsibility for the collection, processing, storage and analysis of the study data, as well as cooperating with the Steering Committee to ascertain that the provisions of the protocol are carried out by each Clinical Center.

12.2.6 National Institute of Child Health and Human Development

A Memorandum of Understanding was signed between the NHLBI and NICHD during the BABY HUG Treatment study to allow the NICHD to perform pharmacokinetic (PK) studies under the Best Pharmaceuticals for Children Act (BPCA) to support a submission to the FDA for labeling of hydroxyurea for infants and very young children with sickle cell disease.

12.3 STUDY ADMINISTRATION

12.3.1 Study Chairman

A Study Chairman will be elected by the Steering Committee to represent the BABY HUG Follow-up Study II by the Steering Committee. The Study Chairman is Chairman of the Steering Committee. The Study Chairman is responsible for overall conduct of the study.

12.3.2 Steering Committee

The Study Chairman will preside over the Steering Committee which will be responsible for overseeing the conduct of the study and writing of main papers as directed by the OSMB and as approved by the NHLBI. Each clinical center PI will have one vote on the Steering Committee along with the PI of the DCC. The Project Officers of the NHLBI and NICHD will be non-voting members of the Steering Committee.

Meetings at which decisions are reached, either in person or by telephone conference call, will require the presence of at least 8 voting members (PI or designee from at least 7 clinical centers and the DCC). The Committee will plan to meet monthly by telephone and at least once per year face-to-face.

Study Coordinators are encouraged to attend and contribute to the deliberations during the conference calls and face-to-face meetings of the Steering Committee. Arrangements will be made as needed for special sessions for material of particular importance to Study Coordinators.

12.3.3 Observational Study Monitoring Board

OSMB voting members will include experts in sickle cell anemia, the clinical use of hydroxyurea, biostatistics and bioethics, who are not connected with the study, and ex officio (non-voting) members -- the Study Chairman and the Data Coordinating Center Principal Investigator -- and representatives of the NHLBI and NICHD who will attend meetings to present information and receive recommendations. The OSMB will review Data and Safety Monitoring Reports, and make recommendations. The Steering Committee will report any unexpected or unusual findings to the OSMB which may be convened ad hoc for a special review of BABY HUG Follow-up Study II any time circumstances so warrant. The OSMB will meet at least yearly, to review the annual BABY HUG Follow-up Study II report. It will review safety issues as the trial progresses and will evaluate treatment efficacy at pre-specified interim time points. OSMB

meeting logistics will be arranged by an organization determined by the NHLBI (OSMB Coordinating Center).

The BABY HUG Follow-up Study II Clinical Center investigators are excused from the discussion. The OSMB Coordinating Center staff will take summary notes of the OSMB meeting in its entirety. At the end of the presentation of study outcomes and discussion, the Data Coordinating Center staff are excused for the OSMB to meet in a second Executive Session. The NHLBI representative is responsible for recording summary notes of the second Executive Session and the recommendations of the OSMB. At the end of the second Executive Session, the BABY HUG Follow-up Study II investigators rejoin the OSMB for a preliminary review of OSMB recommendations. The OSMB Coordinating Center provides the summary notes and recommendations of the OSMB, in an expeditious and timely manner, to the Data Coordinating Center. The Data Coordinating Center communicates these recommendations to the BABY HUG Follow-up Study II Steering Committee. At the next OSMB meeting, the OSMB votes to accept (or revise) the summary notes recording transactions of the meeting and recommendations.

12.3.4 Endpoints Evaluation Committees

CDs received from the Clinical Centers will be reviewed on a regular basis by committees consisting of experienced clinicians who are familiar with the area of special study evaluations (e.g., liver-spleen scans, abdominal sonograms) and with the spectrum of illness in sickle cell anemia and who have no other connection with this study. They will receive materials for review from and return grading forms for incorporation into the study database.

Exhibit 12-1
Pediatric Hydroxyurea Clinical Trial (BABY HUG) Follow-up Observational Study II
ORGANIZATIONAL CHART

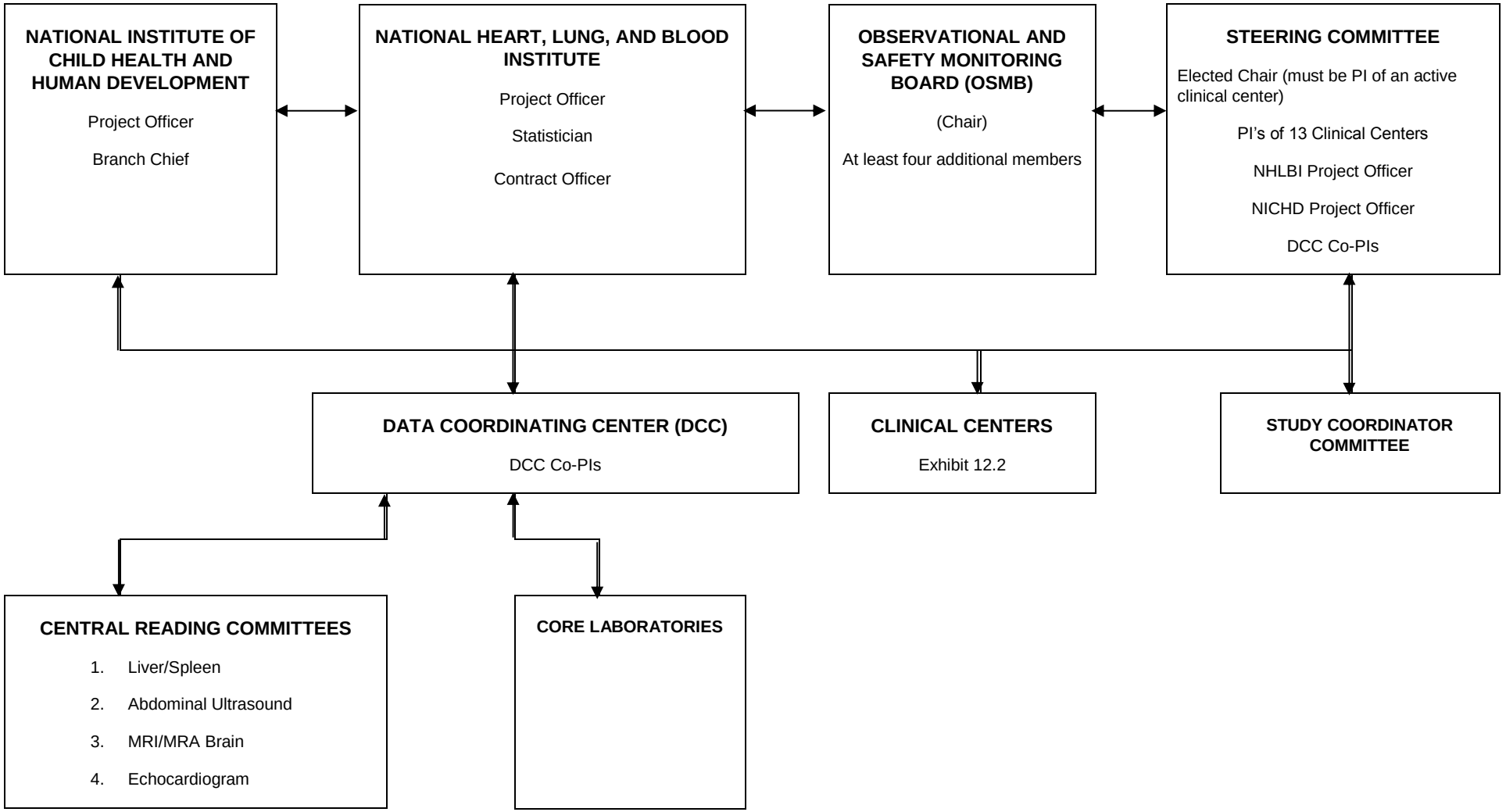

Exhibit 12-2
Participating Clinical Centers

CLINICAL CENTERS

Children's National Medical Center, Naomi Luban, M.D. - 01
(Washington, DC)

Duke University Medical Center, Jennifer Rothman, M.D. - 02
(Durham, NC)

Howard University College of Medicine, Sohail Rana, M.D. - 03
(Washington, DC)

Johns Hopkins University School of Medicine, James F. Casella, M.D. - 04
(Baltimore, MD)

Medical University of South Carolina, Sherron Jackson, M.D. - 05
(Charleston, SC)

St. Jude Children's Research Hospital, Winfred C. Wang, M.D. - 06
(Memphis, TN)

State University of New York - Brooklyn (SUNY), Scott T. Miller, M.D. - 07
(Brooklyn, NY)

University of Miami School of Medicine, Ofelia Alvarez, M.D. - 08
(Miami, FL)

University of Mississippi Medical Center, Suvankar Majumdar, M.D. - 09
(Jackson, Mississippi)

University of Texas Southwestern Medical Center, Zora R. Rogers, M.D. - 10
(Dallas, TX)

University of Alabama, Birmingham, Jeffrey Lebensburger, M.D. - 11
(Birmingham, AL)

Emory University School of Medicine, R. Clark Brown, M.D., Ph.D. - 13
(Atlanta, GA)

Wayne State University, Ingrid Sarnaik, M.D. - 14

(Detroit, MI)

Sinai Hospital of Baltimore, Jason Fixler, M.D. – 43

(Baltimore, MD)

DATA COORDINATING CENTER

New England Research Institutes, Inc.

(Watertown, MA)

Susan Assmann, Ph.D., Principal Investigator

Julie Miller, MPH, PMP, Principal Investigator (contact)

PROJECT OFFICE

Division of Blood Diseases and Resources

National Heart, Lung, and Blood Institute (Bethesda, MD)

Ellen M. Werner, MA. Ph.D, Project Officer

Shimian Zou, PhD, Alternate Project Officer

Myron Waclawiw, Ph.D., Statistician

Catherine Levy, RN, Executive Secretary of the OSMB

CONTRACT OFFICE

Division of Blood Diseases and Resources

National Heart, Lung, and Blood Institute (Bethesda, MD)

Sara Stoops, MBA, Contracts Officer

Arjun Bhalla, Contracts Specialist

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 13

POLICY MATTERS

13.1 INTRODUCTION

Procedural guidelines are established to ensure that all Investigators adhere to the protocol, to facilitate optimum use of data generated by the study, and to ensure optimal use of the resources of the Central Units and Data Coordinating Center.

13.2 PROTOCOL DEVIATIONS

The Data Coordinating Center will create a list of protocol deviations. Protocol deviations are those which impede the progress of the study, such as not filing reports in a timely fashion (form delinquencies) and excessive delays in supplying materials (e.g. scans, other images or event reports) for central review.

The Data Coordinating Center will document protocol deviations in performance reports, as well as notifying the Clinical Centers of them. Repeated protocol deviations which are not corrected will result in reports to the Observational Study Monitoring Board (OSMB), the NICHD and the National Heart, Lung, and Blood Institute (NHLBI).

13.3 CHANGES IN PRINCIPAL INVESTIGATORS

It is expected that changes in Principal Investigators (PIs) will occur in some of the Clinical Centers. These changes may be necessitated by movement of the Principal Investigators to another institution, illness, retirement, or change in responsibility within the same institution. In this situation, retention of the established/experienced nurse coordinator or data manager may

help ensure that the Clinical Center can continue to function effectively. When such a change occurs, it is understood that the contractual arrangement between the NHLBI and the Clinical Center will be reviewed and that a new PI will need to be approved by the Project Officer of the NHLBI. The overall study PI and members of the Steering Committee are available to discuss and provide input as requested by the Clinical Center for decisions that are made in the interim before the new PI will be confirmed.

The Clinical Centers and/or their representatives, the Data Coordinating Center, and the NHLBI and NICHD Project Offices should all participate in any decisions which involve turnover of Principal Investigators and/or Clinical Centers.

13.4 TYPES OF BABY HUG FOLLOW-UP STUDY II RESEARCH

BABY HUG Follow-up Study II research and the resulting presentation and publications may be grouped into the following study categories.

1. Endpoint studies;

2. Data bank studies;

3. Ancillary studies.

The Steering Committee will exercise responsibility for all data bank, and ancillary studies, and for all publications and presentations evolving from BABY HUG research, through the Publications Committee. BABY HUG Follow-up Investigators have agreed that all BABY HUG Follow-up research is collaborative in nature. No investigator will publish BABY HUG Follow-up data from any one Clinical Center or group of Clinical Centers without the written approval of the Publications Committee, the NICHD and the NHLBI.

Investigators at all BABY HUG Follow-up Clinical Centers, including the Data Coordinating Center and the NHLBI and NICHD Program Offices, have equal status with regard to developing

protocols, participating in such studies as are approved and collaborating in the development and publication of research papers based on BABY HUG Follow-up material.

The procedures in this section for endpoint, data bank, and ancillary studies, and for publication of BABY HUG Follow-up research results are similar to those used in other cooperative clinical trials. These procedures are intended to protect the interests of all Investigators and subjects in the trial, namely, to assure that study data conform to the requirements of study design, are accurately presented, authorship is appropriately acknowledged, and the text of all publications is well written with proper attention to the protection of subject privacy. All BABY HUG publications are subject to review and approval by the NHLBI and NICHD.

13.4.1 Data Bank Studies

A data bank study is a study which uses data routinely collected on subjects when they are enrolled in a clinical trial and analyzes these data to answer some scientific question.

13.4.2 Ancillary Studies

An ancillary study is a study which uses supplementary data collected on subjects who are enrolled in a clinical trial, over and above the data collection required by the current Protocol. Such studies are usually restricted to consideration of a specific test technique or involve only supplemental data collected on some or all study subjects.

13.5 CLINICAL CENTER ACCESS TO BABY HUG FOLLOW-UP STUDY II DATA FILES AT THE END OF THE STUDY

At the end of the study, Data Coordinating Center staff will produce a well-documented data CD containing a refined (and reduced) set of the BABY HUG Follow-up Study II data for the purpose of analysis by the BABY HUG Follow-up Study II Investigators and eventual release to the public domain in accordance with NHLBI policy. Clinical Center Investigators may analyze these data in their own centers, but prior to submission of articles for publication must submit the

analyses proposed for publication to the Data Coordinating Center, where they will be reviewed and computations replicated. Clinical Center Investigators who perform their own analyses are responsible for obtaining all support necessary for the data bank or ancillary study outside of regular study resources. The Data Coordinating Center will be the center of study analysis activities as long as the BABY HUG Follow-up Study II Investigators continue in their collaborative efforts.

13.6 PUBLICATIONS

13.6.1 Papers Regarding Overall Study Issues

1. "Overall study issues" are defined as those related directly to assessment/analysis of the study's primary endpoint. The Steering Committee will make writing assignments for initial drafts of such papers; members of the Steering Committee will be invited to assist.

2. The authorship of these papers will include the Investigators, a representative from the Data Coordinating Center, and others as deemed appropriate by the Executive Committee and Publication Committee. Order will be suggested by the lead author and approved by the Publications Committee. Other key personnel with institutional affiliations will be listed as a footnote. These will include the Center Coordinators and others who have a role in the study. Any changes in authorship must be approved by the Executive Committee. NHLBI and NICHD staff may participate as co-authors of the design papers and the primary and secondary analyses papers as appropriate. Criteria for authorship will comply with ICJME policies.

3. Refer to the Manuscript Checklist (Exhibit 13-1) for the steps necessary to propose, develop, and complete a manuscript. All authors must respond with comments or an indication that the manuscript is acceptable within 10 days of distribution of a manuscript draft. Note that active approval or comments are required from all authors.

SC members who are not authors must respond with comments or an indication that the manuscript is acceptable within 10 days of distribution of a manuscript draft, or approval will be assumed.

13.6.2 Other Publications

13.6.2.1 Papers

1. Publications that fall under this policy are those that involve BABY HUG patients and/or include any BABY HUG data, from one or more Centers that participate in BABY HUG or any of its ancillary studies. Local Center manuscripts that involve BABY HUG patients but no study data collected expressly for BABY HUG do not fall under this policy.

2. All study investigators and key personnel, described above, will be encouraged to submit proposals for papers to the Publications Committee. Proposals will be submitted in a defined format, which will include the research questions and hypotheses, the data elements to be used in analysis, and a brief background statement supporting the manuscript's importance (see Exhibit 13-2 Request for Analysis of BABY HUG Research Data). All topics must be reviewed by the Data Coordinating Center (to determine if the study data will support the question) and be approved by the Publications Committee. If more than one investigator submits the same or overlapping proposals, primacy will be determined by the one dated earliest. A listing of projects will be prepared and maintained by the Data Coordinating Center.

3. When approved, one individual will be assigned to serve as Chair of a Writing Committee. Usually, the person proposing the topic will assume this role. However, if the project proposer has already been Chair for another writing committee, he or she may recommend another individual to serve as Chair of the proposed topic.

4. Each Writing Committee will include a representative from the Data Coordinating Center and approximately 5-6 other authors. Approved writing projects will be announced so that Investigators may request membership on the committee. If more Investigators or coordinators wish to participate than can be accommodated, those who are enrolled on fewer writing committees will be given priority. Investigators and coordinators wishing to serve on multiple committees may be asked to prioritize their choices; an attempt will be made to assign topics to those who indicate a high level of interest.

5. For ancillary projects approved by the Steering Committee, the ancillary project P.I. will be the Writing Committee Chair for any manuscripts that arise from his/her research. The Writing Committee membership will be selected by that individual and will consist of some or all of the BABY HUG P.I.s and coordinators who are participating on the project as well as a representative from the Data Coordinating Center. BABY HUG will be acknowledged in any publication that uses BABY HUG data in addition to data obtained as part of the ancillary project. Review of manuscripts by the Publications Committee and NHLBI-NICHD will follow the same process as described above.

6. The Publications Committee will determine the priority with which topics will be analyzed by the Coordinating Center.

7. Once analysis has begun, the manuscript must progress in a timely manner. In general, a draft manuscript should be completed within six months after the data analyses begin. Committee Chairs are encouraged to format their papers and write Introduction, Methods and a preliminary Discussion even prior to Results being available. If the Writing Committee Chair is unable to meet deadlines, s/he or the Publication Committee or the Executive Committee may request that another author

complete the tasks. Under no circumstances should an individual continue as Writing Committee Chair for more than one year after analysis begins, without completion of a manuscript. A replacement Chair must be approved by the Executive Committee.

8. All authors are expected to be full participants in manuscript preparation. If the Writing Committee Chair determines that a member is not participating, s/he may request that person's removal by notifying the Publications Committee.

9. The Publications Committee will maintain a list of Writing Committee Chairs and membership. This will be presented at each meeting of the Steering Committee along with the priority and status of any manuscripts. It is the responsibility of the Publications Committee to recognize potentially overlapping writing projects and consolidate proposals where necessary. If needed, disputes regarding any redistribution of projects or responsibilities can be referred to the Executive Committee for resolution.

10. All publications will include the names of all members of the Writing Committee as masthead authors followed by whichever of the following phrases is appropriate: "for the Investigators of the Pediatric Clinical Trial of Hydroxyurea in Sickle Cell Anemia (BABY HUG)", "for the Investigators of the Pediatric Follow-Up Studies of Hydroxyurea in Sickle Cell Anemia (BABY HUG)", or "for the Investigators of the Pediatric Clinical Trial of Hydroxyurea in Sickle Cell Disease and its Follow-Up Studies (BABY HUG)". The Writing Committee Chair will determine the order of authorship based on effort and contribution. Usually the name of the Writing Committee Chair will be listed first. The Chair may choose to add the names of other individuals to the author list depending on participation in the design/performance of

the project and/or preparation of the manuscript. All publications will acknowledge the support of NHLBI and NICHD.

11. The name, title and affiliation of all key personnel (see section 13.6.1 item 2) will be listed in a footnote to all manuscripts submitted. This listing will be established, maintained, and updated by the DCC.

12. The lead author will usually be designated as corresponding author. The DCC will ensure that all participating centers receive copies of all study publications.

13. All manuscripts will be submitted to the Publications Committee prior to submission for publication. The Publications Committee will choose two to three Investigators as reviewers. The P.I. of the DCC, or a designated DCC statistician, will review the statistical analysis of each manuscript for accuracy even if local statistical resources are used for that data analysis. The review process will be accomplished in a period of no more than two weeks.

14. After suggested changes have been considered, the manuscript will be submitted to the Publication Committee Chair and NHLBI Project Officer or designee prior to submission to a journal. In order to balance the oversight responsibility of the National Heart, Lung, and Blood Institute (NHLBI) with the authorization provided the contractor by the Rights in Data clause of this contract, the NHLBI has established a process to review manuscripts produced under this contract. Please note that the NHLBI does not require contractors to seek the Institute's approval of manuscripts.

    In order to have sufficient time to conduct a meaningful review, the Institute's Project Officer and Contracting Officer must have advance notice of intent to submit a manuscript for publication at least 21 days prior to submission to the publisher. The advance notice should briefly describe the plans for publication of the manuscript.

Concurrently or as soon as possible following this notice, the manuscript should be provided to the Project Officer for OSMB review and for final approval by NHLBI/NICHD. Any comments from the NHLBI/NICHD will be provided in writing within 15 days after receipt of the manuscript by the Project Officer. Comments expressed by the NHLBI about the manuscript shall not be a cause for action under the Disputes clause of the contract by either NHLBI or the contractor, since the NHLBI does not approve manuscripts and draft manuscripts are not contract deliverables.

13.6.2.2 Abstracts

1. Abstracts that fall under this policy are those that involve BABY HUG patients and/or include any BABY HUG data, whether from one or multiple Centers, that were acquired as part of BABY HUG or any of its ancillary studies. Local Center studies that involve BABY HUG patients but no study data do not fall under this policy.

2. Abstracts may be prepared for submission to any appropriate meeting. Usually the topic will be based on or related to one already assigned to an established Writing Committee. Alternatively, topics that differ from those established for Writing Committees may be proposed utilizing the Request for Analysis of BABY HUG Research Data (See Exhibit 13-2).

3. Abstract topics must be approved by the Publications Committee before data will be made available and analysis begun by the Data Coordinating Center. In addition, the Publications Committee Chair, the P.I. of the Study, the NHLBI Project Officer or designee, and the DCC will confer and attempt to balance the desire to get abstracts presented with the need for ongoing statistical analyses for the main study or manuscript preparation. Accordingly, approval in concept may not mean that the DCC can respond to all abstract requests in the time frame desired by the proposer. **Thus, investigators and coordinators are strongly encouraged to plan abstract**

proposals well in advance of deadlines so that there is sufficient time to prepare abstracts.

4. The decision to submit abstracts that arise from Ancillary Projects will be the responsibility of the individual managing the project. Some projects may not require DCC assistance and therefore do not need to go through the above prioritization and approval process. The Writing Committee should be chosen from those Principal Investigators who are project participants.

5. Authorship of abstracts will be determined as for manuscripts (described above), depending on whether the abstract pertains to overall study issues or a sub-issue arising from a writing project or ancillary project.

6. An abstract must be submitted to the Publications Committee Chair at least 14 days prior to the deadline for submission. Abstracts must therefore be sent to potential authors for comment and approval 18-21 days prior to the submission deadline; potential authors who do not respond promptly may be removed. Upon receipt, the Publications Committee Chair will circulate the abstract to the Steering Committee for immediate review, and any comments will be returned to the Writing Committee Chair and Publications Committee Chair within four days. A **final** version will be forwarded for final approval by the Publications Committee Chair and the Project Officer or designee at least seven days prior to submission deadline for OSMB review and approval. If feasible and acceptable to the Executive Committee and NHLBI, accelerated approval of some abstracts may be attempted.

13.7 CONFLICT-OF-INTEREST

BABY HUG Follow-up Study II Investigators and their immediate family will not buy, sell, or hold stock options in any of the companies* providing medication (or making competing products) under study from the time the recruitment of subjects for the trial begins until funding for the study in the Investigator's unit ends and the results are made public; or from the time the recruitment of subjects for the trial begins until the Investigator's active and personal involvement in the study or the involvement of the institution conducting the study (or both) ends.

Each Investigator will agree not to serve as a paid consultant to the companies during these same periods. The guidelines will also apply to the Investigator's spouse and dependents.

Certain other activities are not viewed as constituting prohibited conflicts-of-interest but must be reported annually to the Data Coordinating Center: the participation of Investigators in education activities supported by the companies (permitted only if no honorarium is paid to the Investigator); the participation of Investigators in other research projects supported by the companies; and, occasional scientific consulting to the companies on issues not related to the products in the trial and for which there is no financial payment or other compensation. The BABY HUG Follow-up Study II conflict-of-interest policy will incorporate the NHLBI and U.S. Food and Drug Administration (FDA) policies on conflict-of-interest for Investigators.

The BABY HUG Follow-up Study II Investigators will not accept any restraint on freedom of publication.

*Bristol-Myers Squibb

Exhibit 13-1
BABY HUG MANUSCRIPT CHECKLIST

Writing Project Name: _____

_____

Chair: _____

Step	DATE	ACTION
1		Submit proposal for paper to the DCC using the Analysis Request Form.
2		DCC reviews topic for feasibility. If acceptable, DCC submits request to Publications Committee.
3		Topic approved by the Publications Committee, individual assigned to be the Chair of the Writing Committee.
4		Writing Committee formed: (1 DCC rep, 5-6 other authors) Chair: _____ DCC: _____ Co-authors: _____ _____ _____ _____ _____ _____ Invited authors have 10 days to accept or decline authorship.
5		Analysis begins once request reaches top priority for new analyses. (From this point, a complete first draft manuscript to be completed within 6 months).
6		DCC submits analyses to WC. Writing, additional analyses continue with ongoing discussion between DCC and WC chair.
7		A “final” draft is provided the DCC and reviewed. Within 2 weeks the DCC approves the Manuscript, returns it to the WC Chair for approval for submission to the PC.
8		Publications Committee approves Manuscript and sends notice to WC Chair and DCC.
9		DCC/PI give advance Notice of Intent to submit a Manuscript to NHLBI Project Officer and Contracting Officer at least 21 days prior to journal submission and submits for review by the Project Officer and the Steering Committee.
10		The Project Officer gives final approval or requests revision for NHLBI/NICHD and, if necessary, the OSMB within 15 days.
11		Approval by all authors requested within 10 days.
12		Manuscript approved/revised/denied by the Steering Committee. Approval assumed 10 days after circulation unless comments submitted by a SC member or extenuating circumstances.

Step	DATE	ACTION
13		WC Chair or Lead Author submits Manuscript to the following journal: _____
14		Manuscript accepted/rejected/accepted with revisions. PC, DCC notified by WC Chair/lead author. Only substantive revisions require further review by the DCC, the various committees and Project Officer.
15		WC Chair or Lead Author submits (resubmits) Manuscript to the following journal: _____
16		Manuscript accepted/rejected/accepted with revisions. PC, DCC notified by WC Chair/lead author.
17		Copy of Manuscript received by WFC Chair/lead author who forwards to DCC to distribute to SC and post on website.
18		DCC/lead author assure manuscript is submitted to NIH Manuscript Submission for Public Access, as required.

Exhibit 13-2
Request for Analysis of BABY HUG Research Data

(Please email to BabyHug@neriscience.com and NHLBIBabyHug@mail.nih.gov)

SECTION 1: PROJECT OVERVIEW

Proposed Title:

Date of Request:

Individual Submitting Request:

Summary Description of Project:

Brief Summary of Research Question(s) and Hypothesis/Hypotheses:

Study or Studies to be Used (Randomized Trial, Follow-Up I, and/or Follow-Up II):

Proposed Writing Group Chair (if Different from Individual Submitting Request):

Proposed Writing Group Members (NOTE: NERI will assign statistician(s) to each analysis request):

SECTION 2: DETAILS OF REQUESTED ANALYSES

Attach additional sheets if necessary. NERI will work with the Writing Group to refine this section as needed. However, the initial request should include preliminary answers to as many questions in this section as possible, to provide a starting place for discussion.

A: Research question(s) to address, and specific hypotheses to test.

Please expand on the brief summary from Section 1

B: Population to be analyzed

(e.g. all enrolled subjects? Only those with particular baseline characteristics? Only those with a particular event occurring during the study? Some other subset of subjects?)

C: Time period to be analyzed

(All available data? Specific study visits? Specific calendar period?)

D: Data to be used

Add additional rows if necessary. Please list descriptions of data elements (e.g. Systolic Blood Pressure) rather than question numbers, as the latter may change with form revisions. Indicate any requested recoding, rescaling, or collapsing of variables. For each created or derived variable, indicate the raw data

needed to calculate it and (if not obvious) what formula to use. (NERI and NHLBI Staff are available to help with this.)

Study	Form # and Name	Data Elements	Comments/Formulas

E. Analyses requested

Please provide a brief outline of the analysis to be conducted to address the research questions and hypotheses. This may include specific relations among variables that you would like investigated. Drafts of tables and/or figures are often useful and may be attached as supplements to this document. (N.B. This section is meant primarily to guide the statistician toward an analysis that will be meaningful, understandable, and useful to the investigator. The statistician may supplement or alter the analysis plan to enhance the analysis or to ensure a statistically rigorous approach).

F. Discussion

Provide points that you plan to discuss regarding the limitations, implications, and significance of the findings, expanding on the questions and hypotheses outlined above. The format and content should be similar to that which will be published as Background and Discussion in the manuscript, to be amplified upon analysis completion and conclusions. A comprehensive list of pertinent references should be included below.

G. Key references

<u>SECTION 3</u>: PROPOSED USE OF ANALYSES

FOR MANUSCRIPTS

Proposed journal:

Additional candidate journals:

FOR MEETING PRESENTATIONS

Meeting name:

Location of meeting:

Abstract due date:

Meeting date:

OTHER

Describe:

PEDIATRIC HYDROXYUREA CLINICAL TRIAL

(BABY HUG) FOLLOW-UP OBSERVATIONAL STUDY II

MANUAL OF OPERATIONS

CHAPTER 14

USE OF THE BABY HUG FOLLOW-UP STUDY II WEBSITE

14.1 OVERVIEW

The BABY HUG Follow-up Study II Administrative Website, NERI Connect, is available for use by all BABY HUG Follow-up Study II Clinical Center and Project Office staff. The web address is: connect.neriscience.com (https://connect.neriscience.com/default.aspx). For questions about the website, or to request access, contact the DCC at BabyHug@neriscience.com. Authorized users will be confidentially given a user log-on account and password.

Study Materials can be found under General Documents and include, but are not limited to:

- Protocol
- Manual of Operations
- Data Coordinating Center Documents
- Memos
- Agendas and Minutes
- Publications

14.2 SYSTEM REQUIREMENTS TO ACCESS THE BABY HUG FOLLOW-UP STUDY II WEBSITE

In order to work optimally with the NERI Connect website, the user must have the following basic system requirements:

* Windows XP or higher

* Internet Explorer version 8 or higher

* Office 2007 or higher

* You must add https://connect.neriscience.com to your Internet Explorer's list of trusted sites – there are instructions for doing this available on the logon screen of NERI Connect.

NERI Connect will also work using the Office Web App on Mac systems, iOS devices, and browsers other than Internet Explorer.

Exhibit 14-4
Capitation Report

CAPITATION REPORT
Clinical Site Fixed Price Payment Schedule
BABY HUG Follow-Up Study II

Contractor: _____ Contract No: _____

Date: _____

		Unit Price	No. of Patients	5-Year Maximum Quantity per Subject	Total Amount
	ACTIVE FOLLOW-UP				
1	Patient consent and submission of 6 month data*	$ -		1	$0
2	Patient Incentives*	$ -		13	$0
3	Blood Sample Collection**	$ -		6	$0
4	Physical Exam	$ -		6	$0
5	Liver Spleen Scan	$ -		1	$0
6	Abdominal Ultrasound	$ -		1	$0
7	TCD	$ -		1	$0
8	Vineland	$ -		1	$0
9	WISC-IV	$ -		1	$0
10	Connor CPTII	$ -		1	$0
11	Pulmonary Function Tests (Spirometry)	$ -		1	$0
12	PEDS QOL	$ -		1	$0
13	Microalbumin/Creatinine Ratio Urine	$ -		2	$0
14	Cardiac Echo-cardiogram	$ -		1	$0
15	MRI/MRA Brain	$ -		1	$0
16	CBC, differential, reticulocyte count	$ -		6	$0
17	Bilirubin	$ -		6	$0
18	LDH	$ -		6	$0
19	Spirometry (Post Bronchodilator Response)	$ -		1	$0
20	Pulse Oximetry	$ -		1	$0
21	Diffusion Capacity (DLCO)	$ -		1	$0
22	ALT	$ -		6	$0
23	BNP	$ -		1	$0
24	Urine Osmolality (OSM)	$ -		2	$0
25	Submission of 12 month data	$ -		1	$0
26	Submission of 18 month data	$ -		1	$0
27	Submission of 24 month data	$ -		1	$0
28	Submission of 30 month data	$ -		1	$0
29	Submission of 36 month data	$ -		1	$0
30	Submission of 42 month data	$ -		1	$0
31	Submission of 48 month data	$ -		1	$0
32	Submission of 54 month data	$ -		1	$0
33	Submission of 60 month data or data exit	$ -		1	$0
	Total for Active Follow-Up				**$0**

* Active patients wil be given the incentive for Consent/Entry visit, urine osmolality, each "other" test performed (see Appendix A in protocol) and study exit.

	PASSIVE FOLLOW-UP				
1	Patient consent and submission of 6 month data	$ -		1	$0
2	Patient incentives*	$ -		2	$0
3	Blood and Urine Sample Collection(**)(***)	$ -		2	$0
4	Submission of 12 month data	$ -		1	$0
5	Submission of 18 month data	$ -		1	$0
6	Submission of 24 month data	$ -		1	$0
7	Submission of 30 month data	$ -		1	$0
8	Submission of 36 month data	$ -		1	$0
9	Submission of 42 month data	$ -		1	$0
10	Submission of 48 month data	$ -		1	$0
11	Submission of 54 month data	$ -		1	$0
12	Submission of 60 month data or data exit	$ -		1	$0
	Total for Passive Follow-Up				**$0**

* Passive patients will be given the incentive for Consent/Entry visit and study exit.

** One payment per annual visit window for the following tests: HbF (blood), HJB/Retic Micronuclei, Pitted Cells, Stored Blood Sample, VDJ/DNA Extraction, Cystatin C, and Creatinine and BUN.

*** This price includes the cost of the following tests: CBC, Diff, Retics, LDH, Bilirubin, and ALT

GRAND TOTAL $ -