MFM Neo R01

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

	2. DATE SUBMITTED	Applicant Identifier
	3. DATE RECEIVED BY STATE	State Application Identifier
1. * TYPE OF SUBMISSION ○ Pre-application ○ Application ● Changed/Corrected Application	4. Federal Identifier N/A	

5. APPLICANT INFORMATION *** Organizational DUNS:**

* Legal Name:

Department: Financial Services Division — Division:

* Street1: — Street2:

* City: — County: — * State:

Province: — * Country: USA: UNITED STATES — * ZIP / Postal Code

Person to be contacted on matters involving this application

Prefix: — * First Name: — Middle Name: — * Last Name: — Suffix:

* Phone Number: — Fax Number: — Email.

6. * IDENTIFICATION NUMBER *(EIN) or (TIN)*:

7. * TYPE OF APPLICANT
H: Public/State Controlled Institution of Higher Education

8. * TYPE OF APPLICATION: ● New
○ Resubmission ○ Renewal ○ Continuation ○ Revision

Other (Specify):

Small Business Organization Type
○ Women Owned ○ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)*:

* Is this application being submitted to other agencies? ○ Yes ● No
What other Agencies?

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
BRAIN IMAGING AND DEVELOPMENTAL FOLLOW-UP OF INFANTS TREATED WITH ERYTHROPOIETIN

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

13. PROPOSED PROJECT:
* Start Date: 12/01/2008
* Ending Date: 11/30/2012

14. CONGRESSIONAL DISTRICTS OF:
a. * Applicant — b. * Project

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: — * First Name: — Middle Name: — * Last Name: — Suffix: MD

Position/Title: Professor — * Organization Name:

Department: Pediatrics — Division: Neonatology

* Street1: — Street2: o

* City: — County: — * State: N

Province: — * Country: USA: UNITED STATES — * ZIP / Postal Code:

* Phone Number: — Fax Number: — * Email:

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding $2,500,000.00
b. * Total Federal & Non-Federal Funds $2,500,000.00
c. * Estimated Program Income $0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix: * First Name: Middle Name: * Last Name: Suffix:

* Position/Title: Contract & Grant Administrator
* Organization Name: .r
Department: Financial Services Division
Division:
* Street1:
Street2:
* City:
County:
* State:
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code:
* Phone Number:
Fax Number:
* Email: !

*** Signature of Authorized Representative**

*** Date Signed**

02/05/2008

20. Pre-application File Name: Mime Type:

21. Attach an additional list of Project Congressional Districts if needed.

File Name: 3569-congressional_districts_affected.pdf Mime Type: application/pdf

Congressional Districts Affected:

-all

-all

-all

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Appendix

Number of Attachments in Appendix: 6

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name:

* Street1:		Street2: 1
* City:	County:	* State:
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code:

Project/Performance Site Location 1

Organization Name:

* Street1:		Street2:
* City:	County:	* State:
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code:

Project/Performance Site Location 2

Organization Name:

* Street1:		Street2:
* City:	County:	* State:
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code:

Project/Performance Site Location 3

Organization Name:

* Street1:		Street2:
* City:	County:	* State:
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code:

File Name	Mime Type

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ● Yes ○ No

1.a. If YES to Human Subjects

Is the IRB review Pending? ● Yes ○ No

IRB Approval Date:

Exemption Number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

Human Subject Assurance Number FWA0003255

2. * Are Vertebrate Animals Used? ○ Yes ● No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ○ Yes ● No

4.a.* Does this project have an actual or potential impact on the environment? ○ Yes ● No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5.a.* Does this project involve activities outside the U.S. or partnership with International Collaborators? ○ Yes ● No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract	5172-BRITEstudy_project_summary.pdf	Mime Type: application/pdf
7. * Project Narrative	4997-BRITE_Project_Narrative.pdf	Mime Type: application/pdf
8. Bibliography & References Cited	7508-Final_Reference_08.pdf	Mime Type: application/pdf
9. Facilities & Other Resources	697-Facilities_and_Resources_final_2_.pdf	Mime Type: application/pdf
10. Equipment	4758-Equipment_final_2.pdf	Mime Type: application/pdf

Of the 4 million infants born each year in the United States, approximately 60,000 weigh less than 1500 grams (very low birth weight—VLBW). Twelve percent of these infants sustain brain injury with subsequent developmental delay. Although various neuroprotective strategies have been evaluated, none have been successful. Effective interventions are desperately needed to treat these most vulnerable of infants.

One promising intervention is the use of recombinant erythropoietin (Epo, also known as an erythropoiesis stimulating agent, or ESA). In addition to stimulating red cell production, Epo has been shown to be protective in the developing brain in animal models. We have preliminary data suggesting its efficacy when used in VLBW infants, who are at risk of requiring transfusions, and who are also at risk for brain hemorrhage, hypoxic-ischemic brain injury, and developmental delay. We are currently performing a multicentered study evaluating hematopoietic and short term developmental effects of ESAs in preterm infants randomized to receive Epo, Darbepoetin alfa (a longer acting ESA), or placebo/ control for the first 10 weeks of age. The first enrolled infants will reach 30-36 months in December, 2008. While that study evaluates the safety and general short-term developmental effects of ESAs, there is an unprecedented opportunity to study long term effects of ESA in significant detail, including evaluating the long term developmental effects and the underlying mechanism of neurologic improvement with state of the art multimodal neuroimaging.

This proposal seeks to evaluate long-term developmental effects and underlying neurologic mechanisms of ESAs administered to VLBW infants in the first 10 weeks of life. Our specific hypotheses are: 1) ESAs administered to preterm infants during the neonatal period improve long-term neurodevelopmental outcome, 2) ESAs affect regional brain structure, neurochemistry and neurologic organization as reflected in MR imaging, and 3) neurodevelopmental outcome correlates with neuroimaging parameters. To test these hypotheses, neurodevelopmental outcome will be assessed through a comprehensive neurodevelopmental assessment at 30-36 months (Bayley Scales of Infant Development III, executive function) and 48-60 months (WPSSI III, Early Child Assessment, Executive Categorization Battery). Brain imaging will be performed concurrent with developmental assessments and includes measures of volume (high resolution volumetric analysis), neurochemistry (magnetic resonance spectroscopy) and white matter organization (diffusion tensor imaging). The addition of a testing point at 48-60 months significantly increases the ability to determine if developmental, functional and anatomical differences exist between infants randomized to ESAs, a relatively new interventional strategy used in preterm infants, and those randomized to placebo/control. This proposal addresses our long-term goal of developing effective treatment strategies for neurodevelopmental disorders associated with prematurity through an improved understanding of brain-behavioral relationships.

Approximately 60,000 premature infants are born each year who weigh less than 1,500 grams, many of whom sustain brain damage because of their prematurity. This study is designed to evaluate the long-term developmental effects of one promising neuroprotective treatment, erythropoietin, when given in the neonatal period. Using detailed neurodevelopmental assessments and state-of-the-art neuroimaging, we hope to determine whether this is an effective treatment to prevent brain damage associated with prematurity, and to lay the groundwork for further studies using principles of neuroplasticity to improve the developmental outcome of premature delivery.

Facilities and Resources

Site:

The is a consortium of internationally recognized neuroimaging and neurogenetic researchers based in Its new 50,000 square-foot headquarters is located on the campus of the University of and houses world-class information technology facilities and services, as described below:

LABORATORY: Facilities feature separate laboratory and associated control rooms for a 1.5T mobile Siemens Avanto magnetic resonance (MR) scanner, a 1.5T Siemens Sonata MR scanner, a 3T Siemens Trio MR scanner, a 275-channel VSM magnetoencephalographic (MEG) scanner, a mock MR scanner, and two high resolution electroencephalographic (EEG) systems. A genomics laboratory augments the array of scanning technology with an Illumina BeadStation and Applied Biosystems 7500 real-time thermocycler, as well as standard equipment for DNA extraction, quantification, and storage. These systems are described in more detail under "Equipment," below.

CLINICAL: The is a major resource to the Health Sciences Center, a large full-service hospital with a prominent focus on major psychiatric disorders. The two organizations interface operations to combine patient clinical care with MRI scanning, clinical trials, and clinical research in a seamless unified manner. The is also affiliated with the Center for Alcohol and Substance Abuse Addition (CASAA), a leader in the development and evaluation of cost-effective behavioral treatments for substance use disorders. The has linked data networks with the entire University Hospital system, including CASAA, allowing for easy transfer of patient information including registration and records, clinical visits, neuropsychological testing, and clinical MRI scanning.

ANIMAL: Hall also houses the Biomedical Research and Integrative Neuroimaging (BRaIN) Center, with separate animal facilities and a 4.7T animal MR scanner and a prototype "babySQUID®" neonatal human MEG system.

COMPUTER:

- **Overview:** The 's computing infrastructure consists of approximately 250 computers and 60 Terabytes of network attached storage that consolidates all research data to facilitate easy access and retrieval. Desktop workstations are fed by Gigabit Ethernet connections to a high availability data center that houses core services as well as multiprocessor compute servers.

- **Computing Environment/Apps:** Scientific end users are able to access The s resources using a single sign on environment that enables sharing of data across the Windows, Linux and Macintosh platforms. The data center houses several large shared memory systems (4-8 cores w/24-36GB RAM) as well as two Linux compute clusters (totaling 64 processors). Additionally there are currently over 30 image analysis and neuroinformatics applications installed and administered uniformly across all workstations. Primary data analysis packages are MATLAB, SPM, GIFT/ICA, AFNI, FSL and FreeSurfer. FreeSurfer currently runs on 32 processor cluster.

  The data center is backed by a 30KW Uninterruptible Power Supply (expandable to 80KW). Data center air conditioning is protected by a diesel generator for emergency power and all power and cooling systems are monitored by IT staff 24/7.

- **Network:** All desktop workstations communicate with the 's data center and network attached storage via dual redundant fiber links. The is connected to the Internet (and Internet2) via gigabit

fiber to a 200 megabit per second link on the University of 's campus. A privately managed backup Internet connection provides business continuity in the event of an extended outage. A Virtual Private Network allows researchers and collaborators to access the computing resources remotely through an encrypted link.

- **Acquisition/Storage:** Data acquisition computers across all modalities (1.5T, 1.5T Mobile, 3T, MEG, EEG) have standardized stimulus delivery systems i.e. pupilometry, audio, video and time coding. The mobile data acquisition system includes a group of centrally managed, remotely operated PGP encrypted tablet PCs used for psychological assessment, digital video and document imaging in forensic populations.

  The network attached storage systems are a combination of high availability NetApp filers and Sun ZFS storage pools totaling ~60TB of usable disk space. Research data is stored in a self documenting directory hierarchy (both raw and processed).

- **Backup:** Backup and disaster recovery are major parts of the work done by the IT Group at the . The backup schedule consists of snapshots, monthly and annual archival. A detailed description is included below:

- **Daily Snapshots:** Every two hours a "snapshot" of data is taken. A snapshot is a backup of any changes to files that have occurred in the specified period. There are six snapshots a day and one nightly snapshot taken at midnight. Nightly snapshots are retained for two days. Unless a researcher has specifically requested to opt out of the process, all study volumes and home directories are covered by the snapshot process.

- **Monthly:** Full archival backups are done at the beginning of every month and taken off site. A full backup of all data is also taken mid-month in an on-site rotation. Each off site backup remains out of circulation for one year i.e. June goes next to May, May is next to April etc.

- **Annual:** There is an annual backup every year in May that is held off site for five years.

- **Mail/Web Servers:** The mail server is backed up to tape monthly and retained for six months. The web site is backed up daily and incorporated into the above tape rotation.

OFFICE: research investigators and their staff are housed at Hall in one of the facilities 53 offices. Senior Investigators are provided a private fully equipped office. Junior-level staff are assigned to shared office spaces. staff collaborating on projects are either housed at their current quarters at the University or, when visiting or working at the are hosted in suitable space and/or workstations. Three large fully furnished computer laboratories have been dedicated to spectroscopy, fMR, mMR, and MEG image analysis. In addition, an open gallery with 12 high-performance stations has been installed for use by graduate student, post-doctoral investigators, visiting scientists and other personnel with irregular schedules to work on a first-come, first-served basis. All staff, and are provided administrative support.

OTHER: Investigators have access to extensive library and research resources through including literature search engines and on-line journal access.

:

The **CTSC** (formerly GCRC; see letter of support from the CTSC Medical Director, Dr. is located within the University of Hospital. The CTSC will provide all of the nursing, pharmacy, and outpatient support necessary to complete the portion of the study. The Pediatric Research Nurses will be

available to screen and enroll eligible infants from the Special Baby Clinic, and collect all required data. They will organize and schedule the appropriate follow-up visits for developmental testing and MRI scanning at the Institute, and will be available for nursing support during the MRI scans. The research pharmacy will provide medication if needed for the MRI scans. Outpatient space will be provided for developmental testing.

OFFICE: D has private office space within the Division of Neonatology, Department of Pediatrics. She has access to administrative support, a laptop and desktop computer, and office supplies/resources to complete the project. Drs. , and all have office space and support services to complete the proposed research. All of the investigators have access to library and research resources through ncluding literature search engines and on-line journal access.

Site:

Children's Medical Center utilizes two high field MR scanners located physically within the Medical Imaging department at the hospital. The GE 1.5T MR scanner and GE Signa 3T scanners are equipped with standard imaging and MR spectroscopy (single voxel and 2D CSI multivoxel)pulse sequences and both single channel transmit-receive and 8 channel phase array receive only head coils. The scanners run the HD 12.0 operating system. Both scanners are used routinely as clinical scanners with full sedation and life support equipment. The department has a conscious sedation team who sedates patients and recovers them within the department.

The University of houses the **General Clinical Research Center**, which will provide pediatric nurse coordinator resources for the site investigator. (see letter of support from the G-CRC Medical Director,). The Pediatric Research Nurses has extensive experience in neonatal and pediatric studies, as the Pediatric GCRC is in its 20th year of continuous funding by the NIH. Dr. works closely with the Pediatric Research Nurses in performing all of the developmental follow-up testing for the NIH funded NICHD Neonatal Research Network, of which a participating site. He will have full access to the outpatient clinics and to coordination by the Research Nurses to perform the developmental testing.

Site:

The **Brain Imaging Center** is located at the Campus of the University of Health Sciences Center. The core facility of the Center is a 3 Tesla whole body MRI scanner manufactured by General Electric. The MRI suite has a space of over 2,600 s q-ft. The primary focus of the Center is functional MRI studies of drug addiction. The core team of the Center consists of psychiatrists, psychologists, radiologists, and physicists. A full time MRI technologist, , runs the MRI scanner. The 3T MRI scanner has been fully operational since May, 2003.

and will have access to the computer facility of the Center, which includes three (3) workstations with Linux operating system, seven (7) personal computers, one (1) SGI workstation, one and one (1) Sun Blade 100 workstation. The Center also maintains a data storage system of 1,752 GB for archiving.

There is adequate office space at each of the performance sites for project staff and resources (e.g., telephone, fax, photocopy machines, etc.). The Center has eleven (11) recently-remodeled office rooms, two (2) interview rooms, one exam room, and one (1) conference room, adjacent to the MRI suite. The resources of the office area include telephone, fax, photocopy machines, color printer and black/white printer.

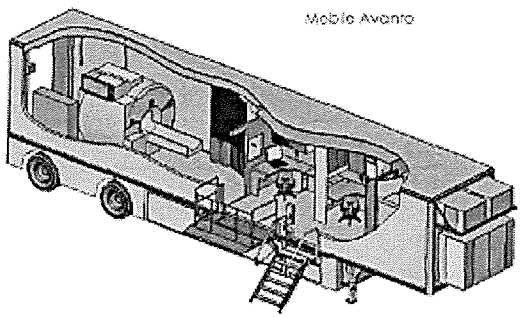
The University of houses the **Pediatric G-CRC**, now known as the CTSC. (see letter of support from Program Director, Pediatric Clinical Translational Research Center). The CTSC has a new 7856 square foot inpatient unit, a 2787 sq ft outpatient area, and a 10,448 sq ft Core Laboratory. There are 6 Pediatric Research nurses who perform inpatient and outpatient research studies. The Pediatric

Research Nurses have extensive experience in neonatal and pediatric studies, as the Pediatric CTSC is in its 45th year of continuous funding by the NIH. works closely with the Pediatric Research Nurses in developmental follow-up studies, and will have full access to the outpatient clinics to perform the developmental testing.

Equipment

Site:

- **Siemens Avanto 1.5 Tesla Mobile MRI Scanner** (see figure): The Avanto is the most advanced system of this strength in the Siemens product line and features an ultra-short 150cm long, whole body superconductive 1.5T magnet, with 5th generation active shielding (AS) technology with counter coils, External Interference Shielding and excellent homogeneity (based on 24 plan plot, 50 cm DSV type, 0.8 ppm. The system comes equipped with a 12-element Matrix head coil capable of ultra-fast parallel acquisition in either 4-channel, 8-channel or 12-channel settings. This system is interfaced to an MR-compatible patient monitoring unit featuring pulsed oximetry, heart rate monitor, O_2, CO_2, NO, and halothane measurement, blood pressure monitor, and EKG (OmniTrak, InVivo Research, Inc. Orlando, FL). This system will allow for unparalleled access to previously difficult to reach populations, including forensic and severely mentally-disordered populations. The Avanto scanner is installed in a trailer that can be transported to suitably equipped sites.



- **Siemens Sonata Maestro 1.5 Tesla MRI Scanner:** The system is equipped with standard transmit and receive RF coils, as well as receive-only surface coils. It is capable of BOLD EPI, diffusion tensor imaging, perfusion and diffusion imaging, and spectroscopy. This scanner is interfaced to an MR-compatible patient monitoring unit featuring pulsed oximetry, heart rate monitor, O2 , CO2, NO and halothane measurement, blood pressure and EKG (OmniTrak, In Vivo Research, Inc. Orlando, FL). TurboFIRE real-time data analysis software is directly interfaced to the scanner operating system.

- **Siemens 3T Trio with Total Imaging Matrix (TIM) Application Suite:** The Trio 32-channel system represents state of the art in MRI hardware. It is capable of BOLD EPI, diffusion tensor imaging, perfusion and diffusion imaging, and spectroscopy. With 32 usable receiver channels as standard, the system allows for the use of current phased array coils (from 4 to 16) to improve sensitivity and speed of acquisition and is ready for future coil designs with more than 16 elements. This system is interfaced to an MR-compatible patient monitoring unit featuring pulsed oximetry, heart rate monitor, O_2, CO_2, NO, and halothane measurement, blood pressure monitor and EKG (OmniTrak, InVivo Research, Inc. Orlando, FL).

- **CTF Omega whole-head MEG and EEG**, with 275 independent squids for magnetic recording in addition to 128 EEG channels.

- **Electrophysiology Equipment**: Two complete electrophysiology systems for recording EEG during the performance of cognitive tasks. These systems are comprised of stimuli presentation computers, electrode caps, amplifiers, digital/analog boards and PCs for data recording. Four head sizes of electrode caps are matintained for each of the two systems. In addition, each system has an SA bioelectric amplifier system capable of amplifying electrical activity from 64 separate channels for recording. Amplifiers are connected to a 16-bit A/D conversion using a custom program implemented on a Pentium II microcomputer running Solaris for Intel. This computer records EEG data and all stimulus and behavioral response codes for later analysis. A custom suite of software for precise presentation of multimodal stimuli, online monitoring of behavioral data, and high resolution EEG acquisition using custom programmed Digitize software is installed. All analyses are completed using the event-related potential software system ERPSS (courtesy of As with the MR scanners, eye-tracking/pupilometry equipment is available during EEG recording.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix | * First Name | Middle Name | * Last Name | Suffix MD

Position/Title: Professor | Department: Pediatrics

Organization Name: | Division: Neonatology

* Street1: | Street2:

* City: | County: | * State: | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code:

*Phone Number | Fax Number | * E-Mail

Credential, e.g., agency login:

*** Project Role:** PD/PI | **Other Project Role Category:**

	File Name	Mime Type
***Attach Biographical Sketch**	67 .pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix | * First Name | Middle Name | * Last Name | Suffix PhD

Position/Title: | Department:

Organization Name: | Division:

* Street1: | Street2:

* City: | County: | * State: | Province:

* Country: USA: UNITED STATES | * Zip / Postal Code:

*Phone Number | Fax Number | * E-Mail

Credential, e.g., agency login:

*** Project Role:** Other Professional | **Other Project Role Category:** Co-Investigator

	File Name	Mime Type
***Attach Biographical Sketch**	.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix | * First Name | Middle Name | * Last Name | Suffix MD

Position/Title: | Department: Neurology

Organization Name: | Division: Pediatric Neurology

* Street1: | Street2:

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
MD	Professor of Pediatrics
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University,	BA		Human Biology
University of		19	Medicine
University of	MD	19	Medicine
University of	Residency	19	Pediatrics
University of	Fellowship	19	Neonatology

A. Positions and Honors

Instruct	19
Assistar	19
Assistar	19
Associa	19
Profess	20
Pediatri	19
Fellow's	19
Fellow's	19
Faculty	19
Faculty	19
Perinata	19
Faculty	19
Glen Pe	20
Champi	20
Swiss N	20
Council	20
U.S. Civ	20
Thrashe	20
NIH Pro	20
Canadia	20
Director,	20
Medical	20
Associat	20
Secretar	20

B. Selected peer reviewed publications

1. C y" in
   th
3. C the
   tr
4. C
   n
5. C
   h
6. B in
   E

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2008 *** End Date:** 11-30-2009 **Budget Period: 1**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.				MD	PD/PI	191,300.00	2.40		2.40	38,260.00	9,948.00	48,208.00
2.				PhD	Investigator	63,000.00	4.80		4.80	25,200.00	6,552.00	31,752.00
3.				MD	Investigator	146,400.00	1.20		1.20	14,640.00	3,806.00	18,446.00
4.				PhD	MRI Physicist	98,612.00	3.00		3.00	24,653.00	6,410.00	31,063.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **129,469.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Coordinator-TBN	6.00		6.00	15,000.00	3,900.00	18,900.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,900.00**
					Total Salary, Wages and Fringe Benefits (A+B)		**148,369.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2008 *** End Date:** 11-30-2009 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2008 *** End Date:** 11-30-2009 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	4,000.00
2. Publication Costs	
3. Consultant Services	18,354.00
4. ADP/Computer Services	3,000.00
5. Subawards/Consortium/Contractual Costs	85,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Outpatient costs (patient travel, patient reimbursement, MRI, testing)	30,000.00
9. Vendor - IHC	22,500.00
Total Other Direct Costs	**163,454.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**316,823.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	50	239,723.00	119,861.50
		Total Indirect Costs	**119,861.50**

Cognizant Federal Agency DHHS,

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**436,684.50**

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: Study_Budget_Justification_final.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2009 *** End Date:** 11-30-2010 **Budget Period: 2**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.				MD	PD/PI	191,300.00	2.40		2.40	39,828.66	10,355.87	50,184.53
2.		R	Lowe	PhD	Investigator	63,000.00	4.80		4.80	26,233.20	6,820.63	33,053.83
3.			Phillips	MD	Investigator	146,400.00	1.20		1.20	15,240.24	3,962.05	19,202.29
4.				PhD	MRI Physicist	98,612.00	3.00		3.00	25,663.77	6,672.81	32,336.58

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **134,777.23**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Coordinator	6.00		6.00	15,615.00	4,060.00	19,675.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**19,675.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**154,452.23**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2009 *** End Date:** 11-30-2010 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2009 *** End Date:** 11-30-2010 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	2,000.00
2. Publication Costs	
3. Consultant Services	18,671.00
4. ADP/Computer Services	3,000.00
5. Subawards/Consortium/Contractual Costs	84,664.55
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Outpatient costs (patient travel, patient reimbursement, MRI, testing)	30,000.00
9. Vendor - IHC	22,500.00
Total Other Direct Costs	**160,835.55**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**320,287.78**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	50	260,123.23	130,061.62
		Total Indirect Costs	**130,061.62**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**450,349.40**

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: Study_Budget_Justification_final.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2010 *** End Date:** 11-30-2011 **Budget Period: 3**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.				MD	PD/PI	191,300.00	2.40		2.40	41,461.64	10,780.46	52,242.10
2.				PhD	Investigator	63,000.00	4.80		4.80	27,308.76	7,100.28	34,409.04
3.				MD	Invenstigator	146,400.00	1.20		1.20	15,865.09	4,124.49	19,989.58
4.				PhD	MRI Physicist	98,612.00	3.00		3.00	26,715.98	6,946.40	33,662.38

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **140,303.10**

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates							
	Graduate Students							
	Undergraduate Students							
	Secretarial/Clerical							
1	Research Coordinator (TBA)		6.00		6.00	16,255.21	4,226.46	20,481.67
1	**Total Number Other Personnel**						**Total Other Personnel**	**20,481.67**
							Total Salary, Wages and Fringe Benefits (A+B)	**160,784.77**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2010 *** End Date:** 11-30-2011 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2010 *** End Date:** 11-30-2011 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	2,000.00
2. Publication Costs	
3. Consultant Services	19,005.00
4. ADP/Computer Services	3,000.00
5. Subawards/Consortium/Contractual Costs	85,600.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Outpatient costs (patient travel, patient reimbursement, MRI, testing)	30,000.00
9. Vendor - IHC	22,500.00
Total Other Direct Costs	**162,105.00**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**329,889.77**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	50	269,289.77	134,644.88
		Total Indirect Costs	**134,644.88**

Cognizant Federal Agency DHHS,

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**464,534.65**

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: _Study_Budget_Justification_final.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2011 *** End Date:** 11-30-2012 **Budget Period: 4**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.				MD	PD/PI	191,300.00	2.40		2.40	43,161.56	11,222.46	54,384.02
2.				PhD	Investigator	63,000.00	4.80		4.80	28,428.41	7,391.39	35,819.80
3.				MD	Investigator	146,400.00	1.20		1.20	16,515.55	4,293.59	20,809.14
4.				PhD	MRI Physicist	98,612.00	3.00		3.00	27,811.33	7,231.20	35,042.53

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **146,055.49**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Coordinator (TBA)	6.00		6.00	16,921.67	4,399.74	21,321.41
1	**Total Number Other Personnel**					**Total Other Personnel**	**21,321.41**
						Total Salary, Wages and Fringe Benefits (A+B)	**167,376.90**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization:

*** Start Date:** 12-01-2011 *** End Date:** 11-30-2012 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	7,000.00
2. Foreign Travel Costs	
Total Travel Cost	**7,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

* ORGANIZATIONAL DUNS:

*** Budget Type:** ● Project ○ Subaward/Consortium

Enter name of Organization: '

*** Start Date:** 12-01-2011 *** End Date:** 11-30-2012 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	2,000.00
2. Publication Costs	
3. Consultant Services	19,355.00
4. ADP/Computer Services	3,000.00
5. Subawards/Consortium/Contractual Costs	87,581.68
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Outpatient costs (patient travel, patient reimbursement, MRI, testing)	30,000.00
9. Vendor - IHC	22,500.00
Total Other Direct Costs	**164,436.68**

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	**338,813.58**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Costs	50	273,231.90	136,615.95
		Total Indirect Costs	**136,615.95**

Cognizant Federal Agency DHHS,

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	**475,429.53**

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: _Study_Budget_Justification_final.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Study Budget Justification

Personnel:

MD, Principal Investigator (2.4 calendar months): Dr is Principal Investigator and Medical Director for this study. Her expertise is in randomized clinical trials of erythropoietin administration to preterm infants, and in research subject safety. As former Medical Director of the research subject Advocate's Office of the and as a long-standing member of the IRRC (the IRB for , she has significant knowledge and understanding of patient safety, consents, DSMPs, and OHRP requirements. She is contact PI for NIH, and as such will be responsible for ensuring completion of annual NIH progress reports. currently the Associate Director-Pediatrics of the and as such will supervise the Research Nurses in subject recruitment through the Special Baby Clinic, and in patient retention through close interaction with the Thrasher Study Coordinator. Dr will lead the group in manuscript preparation and dissemination of findings. She will be responsible for IRB application and approval, coordinating all study materials, and managing the Study Investigators through twice monthly phone conferences and a once yearly Investigators' meeting. She will be responsible for the overall management of the Study.

PhD (6.0 calendar months) has longstanding experience with clinical trials evaluating developmental follow-up of former preterm infants. As a member of the Developmental Care Team for many years at she has significant experience in performing developmental follow-up testing and in participating in RCTs in which developmental testing plays a major role. She will responsible for overseeing the coordination of developmental testing at all three sites, which includes data collection, assisting in supervising the study coordinator in retention and scheduling for patients, and assisting in manuscript preparation. Dr will coordinate efforts of the developmental psychologists at each of the other sites (and), and will be responsible for consistency among testers.

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The monthly co-

investigator phone conferences and is responsibl e for written monthly reports that update the status of the database and any issues related to the Data Management Center.

PhD (0.6 calendar months) is Study Biostatistician, helping with study design and data manag will be responsible annual meetings, ar ty, Dr. is list hours per year.

Research C nanaging the multicen g in overall managemen nd the Manual of O e conferences oving forward in al

Site

te. As such, he will f the study. wi Research Center) to pr nissions and renewals wil Gasparovic at the safely and securely trar project to evaluate anc

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c for the site ough the course of the diatric General Clin he research assistant in r nts. Under s s rate with data in the MRI portion s project to evaluate and

nd be primarily res reaches 30-36 month nd Dr. will be respo ID institute at the Univer

Research A te Research As n, s/he will assist Dr t for the project.

Materials and Supplies:

Video equipment: $2,000 in year one only. T he 3 clinical sites require digital video equipm ent to film the developm ental testing. It is estimated the cost of each video cam era is $600, w ith $200 for digital tapes. $2000 in supplies to cre ate case report forms, including mailing costs to other sites, is requested for each of the four years of the stu dy. Total supply costs over 4 years are $10,000.

Data Storage $3000 per year is requested for terabyte storage of all MRI and patient data at the

Travel:

$5,000 is requested for annual steering committee meetings for the Study Investigators to review data, monitor patient accrual, and facilitate manuscript preparation. In addition, $2000 is requested for the PI to travel to the Pediatric Academic Societies Annual Meeting to present the Study in years 3 and 4. total travel costs for the 4 year period are $24,000.

Patient Care Costs:

Each child involved in this study will undergo a comprehensive developmental assessment and detailed MR imaging study at 30-36 months, and again at 48-60 months. $400 is requested for each developmental assessment. A total of 200 assessments will be performed, for $80,000. will evaluate approximately 40 infants, at a cost of $32,000. The cost is divided evenly over four years ($8000). It is estimated that Dr. will assess 15 children, 2 per child, for approximately 30 assessments, at $400 per assessment. Dr. will assess 45 children, 2 per child, for approximately 90 assessments, at $400 per assessment. The costs of their assessments are listed as consultant costs, and are divided evenly over four years.

$1000 is requested for each detailed MR imaging study. Each child will undergo 2 studies. The total cost of MR Imaging will be $200,000. It is estimated that will perform 90 MR studies, for a total of $90,000. That amount is listed as a vendor charge from divided evenly over 4 years. The remaining $110,000 is divided evenly over 4 years as part of outpatient costs.

Travel reimbursement and incentive is requested for the child and family member to attend each developmental assessment session, and undergo MRI. $100 incentive/compensation and $200 for travel/overnight stay/meals is requested per visit. These costs are divided evenly over four years.

Overall, the cost per patient is $3,400. For 100 patients, total patient care costs will be $340,000, which will be divided evenly over 4 years, as we anticipate enrolling and performing the various studies continuously during most of the 4 year study period.

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**550,604.82**
Section B, Other Personnel		**80,378.08**
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		**630,982.90**
Section C, Equipment		**0.00**
Section D, Travel		**24,000.00**
1. Domestic	24,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**650,831.23**
1. Materials and Supplies	10,000.00	
2. Publication Costs		
3. Consultant Services	75,385.00	
4. ADP/Computer Services	12,000.00	
5. Subawards/Consortium/Contractual Costs	343,446.23	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	120,000.00	
9. Other 2	90,000.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		**1,305,814.13**
Section H, Indirect Costs		**521,183.95**
Section I, Total Direct and Indirect Costs (G + H)		**1,826,998.08**
Section J, Fee		

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:**

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization:

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.				PhD	PD/PI	106,000.00	3.00			27,207.00	9,726.00	36,933.00
2.	Mr.					Co-investigator	127,200.00	0.60			6,530.00	2,334.00	8,864.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **45,797.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
Total Number Other Personnel						**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) **45,797.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:**

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
	Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel **Funds Requested ($)**

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Principal Investigator/Program Director (Last, first, middle):

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:**

*** Budget Type:** ○ Project ● Subaward/Consortium

Enter name of Organization: The ıte

*** Start Date:** 07-01-2008 *** End Date:** 06-30-2009 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	45,797.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Simplified Allocation Method	58.66	45,797.00	26,865.00
		Total Indirect Costs	26,865.00

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	72,662.00

J. Fee	Funds Requested ($)
	0.00

K. * Budget Justification	File Name: _Budget_Justificaiton.pdf (Only attach one file.)	Mime Type: application/pdf

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 0925-0001
Expiration Date: 9/30/2007

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	6604-2._specific_aims_final.pdf
3. Background and Significance	3132-3._background_final.pdf
4. Preliminary Studies / Progress Report	163-4._preliminary_studies_final.pdf
5. Research Design and Methods	1035-5._study_design_and_methods_final_2.pdf
6. Inclusion Enrollment Report	
7. Progress Report Publication List	

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects	7573-8._Protection_of_Human_Subjects_final.pdf
9. Inclusion of Women and Minorities	3829-9._Inclusion_of_Women_and_Minorities_final.pdf
10. Targeted/Planned Enrollment Table	2137-10._Targeted_Planned_Enrollment_Table_final.pdf
11. Inclusion of Children	9124-11._Inclusion_of_Children_final.pdf

Other Research Plan Sections

12. Vertebrate Animals	
13. Select Agent Research	
14. Multiple PI Leadership	
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16. Letters of Support	7168-letters_of_support_2-4-08.pdf
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18. Appendix

2. Specific Aims

Improving the neurodevelopmental outcome for preterm infants continues to be a goal for all neonatal care providers. Advances in neonatal care have led to significant improvements in the survival of infants born less than 1,500 grams, known as very low birth weight (VLBW) infants.[1,2] Of the 4 million infants born each year in the United States, 1.5% (approximately 57,000) are VLBW infants. Twelve percent of these infants sustain a grade 3 or 4 intraventricular hemorrhage (IVH) resulting in a significant increase in the incidence of developmental delay. Moreover, almost one third of preterm infants with normal head ultrasounds also develop cognitive delay.[3,4] Although a variety of neuroprotective treatment strategies have been evaluated, no specific treatment has been identified to reduce or prevent brain injury in these most vulnerable preterm infants.

One potential therapy we are investigating is the use of human recombinant erythropoietin (Epo). In addition to stimulating erythropoiesis, Epo has been shown to be protective in the developing brain in animal models,[5-9] making it possibly beneficial for very premature infants who are at risk for intraventricular hemorrhage, hypoxic-ischemic injury, and developmental delay. The neuroprotective mechanisms of Epo include increased neurogenesis,[10] decreased neuronal susceptibility to glutamate toxicity,[11,12] decreased neuronal apoptosis,[13-17] decreased inflammation,[18,19] decreased nitric oxide-mediated injury,[20-22] increased antioxidant response,[23-25] decreased axonal degeneration,[26] and increased protective effects on glia.[27-29]

We are currently evaluating both hematologic and short term neurodevelopmental outcomes in preterm infants randomized to receive Epo, Darbepoetin alfa (a longer acting Epo; collectively these are known as erythropoiesis stimulating agents, or **ESAs**), or placebo/control for the first 10 weeks of age. This multicenter study is being performed at the University of the University of and at two newborn intensive care units (NICUs) Although evaluation at 18 to 22 months corrected gestational age is an aim of the study funded by the Thrasher Research Fund ("Thrasher Study"), this relatively early follow-up visit is limited to the Bayley Scales of Infant Development 3rd edition (BSID-III) and a neurologic exam. Evaluation involving detailed neurodevelopmental testing combined with MRI analysis at two points in time (30-36 months and 48-60 months) would allow us to determine if developmental, functional and anatomical differences exist between infants randomized to ESAs and those randomized to placebo/control. MRI analysis of ESA treated infants has yet to be performed in any study, and developmental testing has only been performed at 18-22 months.

The specific aims of this proposal, entitled, "**BR**ain imaging and developmental follow-up of **I**nfants **T**reated with **E**rythropoietin", abbreviated the BRITE Study, are designed to evaluate long term neurodevelopmental outcomes of preterm infants receiving ESAs. Our evaluations include 1) a comprehensive battery of neurodevelopmental tests involving executive function and detailed measures of brain-behavior relationships at 30-36 months and at 48-60 months, and 2) detailed MRI imaging at 30-36 months and at 48-60 months, to determine if hypothesized changes in developmental outcomes correlate with differences in brain growth/volume and biochemical function, and to better determine long term outcomes.

We hypothesize that 1) ESAs administered to preterm infants during the neonatal period improve long-term neurodevelopmental outcome, 2) ESAs affect regional brain structure as reflected in MR imaging, and 3) neurodevelopmental outcome correlates with MRI imaging. To this end, our Specific Aims are:

Specific Aim #1: Determine the relationship between ESA treatment and detailed neurodevelopment in children born VLBW.

> **Hypothesis 1a.** Children born VLBW who received ESAs will perform significantly better at both assessment points on measures of executive function and emotional development than placebo/controls
>
> **Hypothesis 1b.** Children born VLBW who received ESAs will demonstrate a positive correlation at both assessment points between peak serum Epo concentrations measured during the original hospitalization and neurodevelopmental outcome

Specific Aim #2: Determine the effect of ESA therapy on structural brain development at 30-36 months and 48-60 months:

> **Hypothesis 2a**. Children born VLBW who received ESAs will demonstrate increased regional brain volumes as determined by morphometric MR analysis, improved white matter organization as determined by DTI, and improved neurometabolic profile on magnetic resonance spectroscopy compared to placebo/controls

Hypothesis 2b. In children born VLBW who received ESAs, a correlation exists between peak serum Epo concentrations measured during the original hospitalization and neuroimaging parameters

Specific Aim #3: Determine the relationship between brain structure and neurodevelopmental outcome of children born VLBW at 30-36 months and 48-60 months.

Hypothesis 3a. regional brain volumes as determined by morphometric MR analysis will correlate with neurodevelopmental outcome at both assessment points

Hypothesis 3b. increased frontal lobe fractional anisotropy as determined by DTI will show a significant correlation with measures of executive function at both assessment points

Hypothesis 3c. increased N-acetylaspartic acid, lactate and choline in the basal ganglia, frontal lobe and anterior cingulate gyrus will correlate with measures of both executive function and emotional development at both assessment points

3. Background

Preterm Morbidity

In the last 15 years there has been significant improvement in the number of extremely low birth weight (ELBW) infants who survive, with now between 60 and 70 percent of ELBW infants living to at least two years of age.[30-36] However this improved survival comes at a cost. Neonatal morbidity is inversely correlated to birth weight and age, and for ELBW infants there is a high risk of neurodevelopmental impairment. Cerebral palsy, deafness, blindness, and developmental delay are frequent sequelae in those born prematurely. Indeed almost half of the ELBW infants go on to develop moderate to severe cognitive intellectual impairment.[31-36] Compounding the risks associated with prematurity are medical problems that often occur in these fragile infants such as intraventricular hemorrhage (IVH), periventricular leukomalacia, retinopathy of prematurity (ROP), chronic lung disease (CLD), necrotizing enterocolitis (NEC), infection, stress, and the use of postnatal steroids.[37] Not surprisingly, survivors of prematurity go on to use a disproportionately high level of special needs services (health and educational) due to their chronic conditions.[38] Given the tremendous financial and personal burden of severe prematurity, there is a desperate need to develop novel neuroprotective strategies for these infants.

Physiologic anemia in neonates and the anemia of prematurity

Erythropoiesis in the fetus is characterized by increased fetal Epo production, resulting in a continuously increasing circulating red cell mass which provides adequate oxygen to growing tissues. In term and preterm infants, improved oxygenation after birth results in decreased erythrocyte production, reflecting a natural adaptation to the extrauterine environment. The hemoglobin concentration decreases over the first 2 to 3 months of life, remains stable over the next several weeks, then slowly rises in the fourth to sixth month of life in response to increased Epo concentrations.[39] The decrease in hemoglobin concentration has thus been termed a "physiologic nadir", rather than true anemia. In preterm infants, adaptive mechanisms to the extrauterine environment are incomplete. VLBW infants have a drop in hemoglobin to significantly lower values than those seen in term infants, and the nadir varies with the degree of prematurity. Epo concentrations are significantly lower in anemic preterm infants than those found in adults, given the degree of anemia.[40-42] The normocytic, normochromic anemia accompanying low Epo concentrations, termed the anemia of prematurity, affects nearly all VLBW infants. Infants with the anemia of prematurity have a decreased ability to increase serum Epo concentrations, despite diminished available oxygen to tissues and the appearance of signs of anemia.[40-44] However, erythroid progenitors are highly sensitive to Epo,[45,46] and concentrations of other erythropoietic growth factors responsible for erythrocyte production (such as interleukin-3 and granulocyte-macrophage colony stimulating factor) were normal.[47]

Erythropoiesis and Epo in preterm infants

The in vitro response of erythroid progenitors from preterm infants lead investigators to begin studies in the early 1990s evaluating Epo administered to preterm infants. Since that time numerous studies evaluating the use of Epo to prevent and treat the anemia of prematurity have been performed. Epo is successful in preterm infants in stimulating erythropoiesis, and transfusion requirements are decreased.[48] Success rates in preventing transfusions in preterm infants are dependent in part on transfusion criteria and the frequency of phlebotomy.[49] Side effects of Epo in published RCTs have not differed from placebo, although retrospective analyses have suggested a relationship between Epo administration and ROP.[50] The development of ROP in preterm infants is likely multifactorial,[51-53] and there is evidence in some adult studies of protective effects of Epo in diabetic retinopathy.[54-56] Prevention of ROP is one of the biggest challenges faced by neonatal caregivers, as more and more babies born ELBW are surviving with morbidities.[57] The relationship between retinal development and elevated vitreal Epo concentrations was first reported by our laboratory,[58] and in vitro investigations continue. All infants enrolled in the Thrasher Study are undergoing complete ophthalmologic exams prior to hospital discharge.

Darbepoetin alfa

A modified version of Epo, known as darbepoetin alfa (Darbepoetin), was developed by Amgen in 1998.[59,60] Darbepoetin has a longer serum half-life than Epo, allowing for decreased dosage intervals to be used. Studies using Darbepoetin once a week to once every three weeks (as compared to 3 doses per week of Epo) in adult oncology and end stage renal failure patients have demonstrated hemoglobin increases equivalent to that of Epo.[61-64] Side effects are similar to Epo, and the production of anti-Epo antibodies has not been reported.[65]

Many hospital formularies were moving from one ESA, Epo, to the newer long-acting ESA, Darbepoetin. Given its potential for similar efficacy with fewer injections, we considered Darbepoetin to be a beneficial alternative to Epo to prevent anemia in preterm infants. Following our in vitro evaluation of fetal and neonatal erythroid progenitor responsiveness to Darbepoetin,[66] we evaluated the pharmacokinetics of darbepoetin administered to preterm infants,[67-70] and provide the results of these studies in the preliminary data section.

ESAs as Neuroprotectants

The initial enthusiasm for Epo came from its effect on erythropoiesis in premature infants. More recently, however, in vitro, animal, and adult clinical studies suggest that Epo plays a potentially more important role in angiogenesis and neurogenesis in the central nervous system.[71,72] It is now known that Epo and Epo receptors are present in non-hematopoietic developing neural tissue in animals as well as humans.[73-75] Similar to the mechanism leading to erythropoiesis, Epo binds to its receptor in non-hematopoietic tissues, activating cellular mechanisms that include cell maturation, division, and inhibition of apoptosis.[76,77]

Studies evaluating Epo in adult and neonatal animal models report the prevention of hypoxic-ischemic brain injury, a decrease in infarction volume, reduced vasoconstriction, decreased neuronal apoptosis, and decreased neurologic deterioration in animals treated with high doses of Epo.[14, 76-81] The mechanisms by which Epo is neuroprotective are under active investigation. Epo has protective effects both in the central nervous system and the peripheral nervous system.[82,83] These broad effects suggest that Epo may have multiple mechanisms of protection, some that affect neurons directly, and others that are likely secondary to Epo effects on other cells or systems. To date, Epo has been shown to increase neurogenesis,[102] decrease neuronal susceptibility to glutamate toxicity,[103,104] decrease neuronal apoptosis,[51,83,105-108] decrease inflammation,[59,109] decrease nitric oxide-mediated injury,[53,110,111] increase antioxidant response, [112-114] prevent axonal degeneration, [115] and increase protective effects on glia.[40,116,117] Epo may also provide neuroprotection by regulating blood flow to the brain following injury, as is suggested by Epo neuroprotection in the model of subarachnoid hemorrhage.[84,85]

Recent studies of ESA neuroprotection have evaluated Darbepoetin.[86,87] Both Epo and Darbepoetin cross the blood-brain barrier at a similar rate as albumin, suggesting that they cross the blood-brain barrier by way of the extracellular pathways.[86] In an adult animal model of ischemia, Darbepoetin conferred behavioral and histological neuroprotection after focal ischemia.[87] In those animal studies, the darbepoetin doses tested **were identical** to the doses being used in the Thrasher Study (10 μg/kg/dose), underscoring the probability that preterm infants enrolled in the Thrasher Study who are randomized to the Darbepoetin arm are indeed receiving neuroprotective doses.

Adult clinical studies also support the neuroprotective potential of Epo: of 1,026 experimental treatments for acute stroke that were recently reviewed, *Epo scored in the top 10* based on the Stroke Therapy Academic Industry Roundtable (STAIR) scale that rates the level of evidence supporting the effectiveness of experimental treatments for acute stroke.[88] These basic and clinical studies offer a tantalizing glimpse into the potential role of Epo in neuroprotection.

Epo and neurodevelopment

Epo receptors are present in the brain of developing human infants from early in gestation,[9] which may explain the particular benefit Epo has for the premature brain. In animal models Epo therapy ameliorates both grey and white matter injury, and has beneficial effects in both the central[5,6] and peripheral nervous system.[82,83] Furthermore, others have shown a direct correlation between the concentration of Epo and the degree of neuroprotection in animal models. **Our group has published the only clinical study showing that the neurodevelopmental outcome of ELBW infants is directly related to their measured serum Epo concentrations.**

Measures of Developmental Outcomes

The use of age-specific measures of development such as the Mental Developmental Index (MDI) began with the introduction of the Bayley Scales of Infant Development.[89] This has served as the standard for developmental evaluations since its inception, and is now in its third revision[90] Many studies use MDI at 18-22 months as a primary outcome variable for RCTs. The correlation between testing at 18-22 months and more long term outcomes at school performance has been reported,[91,92] and better mechanisms to detect children at risk for learning difficulties is felt to be needed. In fact it has been reported that a subnormal MDI on the

Bayley Scales at 20 months is a *poor* predictor of later cognitive skills at school age.[93] The MDI is a good marker for how the infant is performing at 18-22 months, but does not predict long term outcome. The importance of extending beyond traditional tests of cognition was emphasized in a review article by Aylward[94] with recommendations for inclusion of tests of executive function and language. Testing at two points in time allows a trajectory of skills that can not be obtained with testing performed at 18-22 months alone. More complex measures of development can be used at 3 and 4 years[95] as preschoolers have increased skills and attention span compared to 18-22 month olds. These are critical points that must be addressed in RCTs investigating any putative treatment for the neurodevelopmental effects of prematurity; 1) developmental assessments must include assessments of executive function and language and 2) follow up needs to continue to at least 4-5 years of age. This information allows a much improved estimate of developmental prognosis, and is necessary before treatment recommendations can be made.

MRI measures

Evaluating and modifying neuroprotective therapy not only requires comprehensive and timely developmental assessments, but also a probe of the underlying neurologic mechanisms involved. An unprecedented, noninvasive view of the nervous system is now available using new MR techniques, even in difficult to scan populations such as children with cerebral palsy[96,97] or neonates with encephalopathy.[98] It is possible to ask questions regarding the neurologic effects of prematurity and therapeutic interventions on a developing brain, and probe correlations between structure and function through development. For example, regional differences in cerebral *volume* are noted in follow-up studies of survivors of prematurity,[99-101] however these differences take time to manifest. A recent paper by Boardman[102] found that at term-equivalent, overall cerebral tissue volume is similar between premature and term-born infants. Using diffusion tensor imaging to evaluate *white matter structure*, Anjari et al[103] demonstrated regional differences in fractional anisotropy (FA) in premature infants compared to healthy controls at term. Prospective studies of premature children suggest that regional FA done at term-equivalent predicts motor[104] as well as cognitive[105] outcome. Regional *neurometabolite concentrations* are assessed using MR spectroscopy. Miller[106] demonstrated the sensitivity of N-acetylaspartic acid in neonatal hypoxic-ischemic injury as well as in neonates with congenital heart disease.[107] MR spectroscopy also aids prognosis for outcome following pediatric traumatic brain injury[108] and neonatal hypoxic-ischemic encephalopathy.[109] Normative data for neonatal MR spectroscopy and DTI is available.[110]

Not surprisingly, correlation studies suggest a relationship between MR parameters and neurocognitive outcome. Woodward and colleagues evaluated former premature children at two years of age [111], and found that compared to children born full-term, premature children had difficulty encoding new information in working memory which correlated with volume loss in areas specifically related to working memory (i.e., dorsolateral prefrontal cortex, parieto-occipital and pre-motor areas) on MR imaging studies. ***Thus, with a scan time of less than one hour, it is possible to obtain important information regarding regional brain volumes, white matter organization and neurometabolite concentrations in neonates and children. This information will be critical to understand the mechanism of ESAs' effect on neurodevelopment after premature delivery.***

Summary

The neurodevelopmental consequences of extreme prematurity are enormous. Unfortunately, there are no proven neuroprotective treatments available for these children. Developing and evaluating novel neuroprotective strategies is a critical need in neonatal care with tremendous public health implications.

Converging lines of evidence from both animal and human studies demonstrate the potential for Epo as a neuroprotective agent. Mechanisms may include inhibition of apoptosis and increased cell differentiation. However with the exception of our single published study demonstrating the correlation between short-term developmental outcome and Epo concentrations in the neonatal period, there have been no RTCs evaluating long term effect of Epo on various domains of development including language and executive function. Furthermore, there has been no clinical evaluation of the neurologic mechanisms underlying the effect of Epo on development in premature infants.

DTI is ideally suited to evaluate <u>white matter structure</u>, which has been shown to offer prognostic information in premature infants and is a particularly important measure in children at risk of developing cerebral palsy. MR spectroscopy assesses <u>regional neurometabolism</u> which is sensitive to the effects of

neonatal hypoxic-ischemic encephalopathy. Volumetric analysis indicates regional effects of injury and recovery, particularly evident over time in children born prematurely. Integrating these powerful techniques offers an unparalleled opportunity to investigate mechanisms underlying the neurodevelopmental effect of Epo on developing infants.

Our RCT is powered to detect treatment effect at a time when executive function and language can be adequately assessed (specific aim #1). Furthermore, we probe the relationship between underlying neurologic mechanisms and functional outcome (specific aims #2 and #3).

Significance of the BRITE Study

Although the safety and beneficial effect of ESAs on development is supported by preclinical and clinical studies, there is no published trial evaluating long term effects in premature infants. Our randomized, controlled investigation is powered to answer the question: Do ESAs improve clinically relevant developmental functions including long-term language and executive behavior? Our primary outcome measures are well standardized and have functional significance. Thus the outcome of this trial may have an immediate and direct effect on care for premature infants.

Additional significance of this study comes from evaluating the *in vivo* mechanism of action of ESAs on a developing brain. This critical issue is probed for the first time using state-of-the-art neuroimaging techniques, allowing future work to optimize the use of ESAs based on an improved understanding of neuroplasticity and neurodevelopment after premature delivery. Future investigations may include targeted drug delivery systems, additional medication therapy, specific cognitive therapy, stem cell implants or electrical stimulation. These potential future studies will depend on an improved understanding of the neuroplastic mechanisms underlying the behavioral response to ESA therapy.

4. Preliminary Studies

A. Team of Investigators

This study is being conducted by a multidisciplinary team with complementary expertise and experience working together. Neonatology, neurology, early childhood assessment, neuroimaging, statistical analysis and data management are represented. All team members are experienced investigators, most of whom have published together previously. Thus, our group has the necessary experience and commitment to successfully conduct the proposed multi-site clinical trial. The Thrasher Study represents a substantial research investment, and provides a unique population of well-characterized patients, but is not structured to permit a detailed investigation of underlying disease mechanisms or of long term developmental consequences of ESA treatment. This proposal provides a means to capitalize on the current investment in the Thrasher Study, by using the patient cohorts, biological data, and demographic data available to the PI. A letter of support from the Thrasher Research Fund is provided.

Our team is led by Dr . Dr .s neonatologist at , and has studied mechanisms of erythropoiesis in premature infants for the past 20 years. She has conducted clinical translational outcome studies (including multicentered trials) in RCT-format for over 15 years, focusing primarily on Epo and now Darbepoetin administration in preterm infants. s is PI for the ongoing RCT funded by the Thrasher Foundation, which compares the short term effect of two types of erythropoiesis stimulating agents (ESAs) in premature infants. The subject base for the Thrasher study provides a tremendous opportunity to study comprehensive long-term neurodevelopmental effects of ESA treatment, and correlate outcomes with mechanisms of neuroplasticity. Because much of the research team for the BRITE study is already working together under direction, recruiting subjects is greatly facilitated. Other key individuals include Dr . a developmental specialist with longstanding research experience studying developmental follow-up of premature infants. D is the developmental psychologist responsible for the short term follow up analyses of infants enrolled in the Thrasher Study, and as such has evaluated and prepared the developmental assessment teams at the Sites through site visits during the last two years. Drs. and are imaging specialists with expertise in spectroscopy and diffusion tensor imaging respectively. Both have extensive backgrounds designing and analyzing clinical imaging experiments and working in multicenter collaborations. D is a pediatric neurologist and the Medical Director of the Mind Research Network; in this position he will be able to facilitate all aspects of the neuroimaging component of this project. He will also lend his experience conducting clinical trials to the group. The Data Management and Statistical will be directed by Mr. who is on the campus as part of the Mr has been responsible for managing large databases for the , and the . Finally, our statistician is D , who is professor of mathematics and statistics and is the current director of biostatistics for the Clinical and Translational Science Center (CTSC). He has participated in multiple collaborative studies, and has worked previously with all of the BRITE co-investigators.

In addition to our core collaborative group, we are joined by the following individuals. Dr is a Pediatric Neuroradiologist with specific expertise in DTI and Spectroscopy in neonates and former preterm infants. He provides considerable expertise in neuroimaging and will be responsible for all imaging studies performed at the site, and ensuring data transfer to the Network for analysis. In site, Dr is a Pediatric Psychiatrist and the research director of the Brain Imaging Center. He is assisted by Dr. , an MRI Physicist with 20 years experience. D has collaborated previously with Dr and brings experience and collaboration to the proposal.

B. Clinical Studies of ESAs in Preterm Infants

Preliminary Study #1 (al, Pediatrics 2001): RCT of Epo and iron therapy in preterm infants.

National Institute of Child Health and Human Development Neonatal Research Network (NRN) completed a randomized, controlled trial of early Epo and iron therapy in preterm infants <1000 grams[112]. A total of 172 extremely low birth weight (ELBW) infants were enrolled (87 Epo and 85 placebo/control). In that 8 site multicenter study, infants were randomized to Epo (400 units/kg/dose 3x/week) or placebo/control. Transfusions were decreased by the end of the study, and no adverse effects were reported. Moreover, there was no difference between Epo and placebo groups in common neonatal morbidities

such as NEC, BPD, IVH or ROP. This study demonstrated the safety of Epo administered to the smallest and sickest NICU infants, and is the largest study published of Epo-treated ELBW infants. This study demonstrated Dr expertise in performing research on the administration of Epo to preterm infants, and in organizing and orchestrating a complex multicentered trial with the nations' top NIH funded neonatal investigators.

Preliminary Study #2 (Pediatrics 2004): Short term developmental outcome of ELBW infants treated with early Epo and iron.

Working with the same NIH-funded NRN Centers, Dr. performed a follow-up study of the originally enrolled ELBW infants[113]. Infants ≤1000 grams were followed at their initial assessment period of 18-22 months to determine short-term growth and neurodevelopmental outcomes. MDI and PDI were assessed using the BSID II, and overall neurodevelopmental impairment was determined. Of the 72 Epo-treated and 70 placebo/control ELBW infants surviving to discharge, follow-up data (growth, development, re-hospitalization, transfusions) at 18 to 22 months' corrected age were collected on 51 of 72 Epo-treated infants (71%) and 51 of 70 placebo/controls (73%). There were no significant differences between treatment groups in weight or length, or in the percentage of infants weighing <10th percentile either at the time of discharge or at follow-up (see table to right), and no difference was found in the mean head circumference between groups. However, there was an important difference in the incidence of microcephaly between groups. *Over twice as many children developed microcephaly in the placebo group compared to those treated with Epo (32.7% vs 14.3%).* To understand this difference, longer term studies that include probes of neuroplasticity will be required. Given the high correlation of microcephaly with mental retardation, this is potentially a very important finding. Our current proposal therefore includes long term follow up as well as neuroimaging to further explore the relationship between Epo therapy and brain development.

Follow-up Parameter	EPO	Placebo
weight (kg)	9.8±1.3	10.0±1.5
length (cm)	80.2±3.7	80.8±3.3
HC (cm)	47.0±2.0	46.6±1.7
% Wt < 10%tile*	49.0	50.0
% Length < 10%tile*	40.8	36.5
% HC < 10%tile*	14.3	32.7

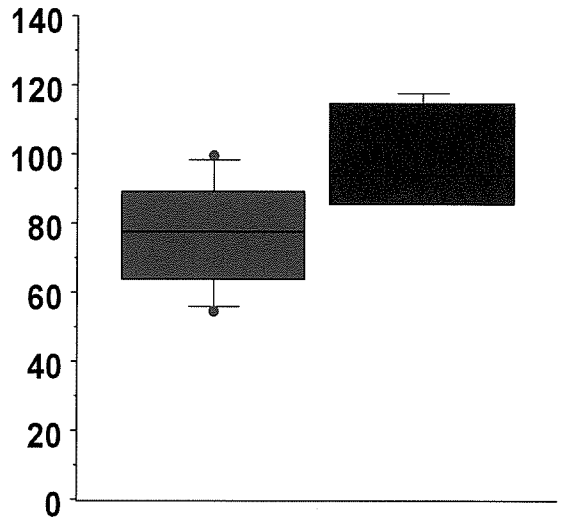
* National Childrens' Health Study guidelines

Preliminary Study #3: (ediatrics 2006). Developmental outcome correlates with neonatal Epo concentration.

To explore the possibility of a neuroprotective effect of high Epo concentrations, Dr group at evaluated the relationship between Epo concentrations and neurodevelopmental outcome in a subgroup of infants enrolled in the NICHD Epo study (summarized above).[114] Of the 20 infants originally enrolled at whom Epo concentrations were measured, 6 infants died and 2 were lost to follow up. Serum Epo concentrations were significantly different between groups during the study:

	Epo	Placebo
Epo concentrations	**2,023±652 mU/mL**	**22±4 mU/mL**
MDI scores	**96±11**	**78±7**
PDI scores	**87±13**	**80±7**
Any NDI	**2/6**	**4/6**

At 18-22 months follow up, Epo recipients had MDI scores of 96±11 and placebo infants had MDI scores of 78±7 (p=0.15). PDI scores were similar between groups (87±13 versus 80±7). There were no differences between groups with respect to anthropometric measurements. Two of six infants in the Epo group and 4 of 6 infants in the placebo group had some form of NDI. Post hoc analysis showed that infants with Epo concentrations ≥500 mU/mL (see figure to the right) had higher MDIs (100±15) than infants with Epo concentrations <500 mU/mL (77±16; p<0.05).



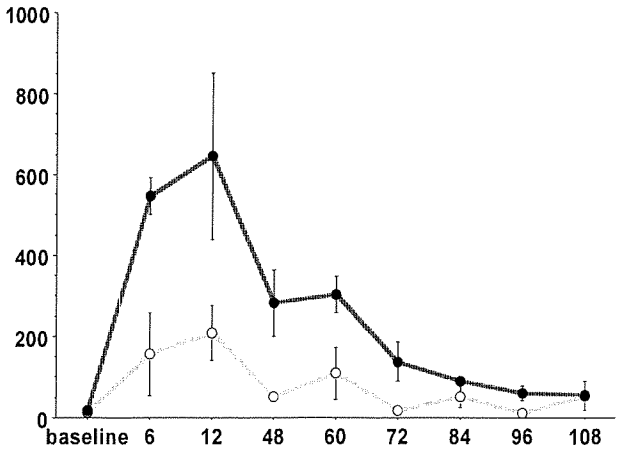
MDI scores at 18-22 months follow up. MDI scores were higher in infants with peak Epo concentrations ≥500 mU/mL (right [red] box) compared to those with Epo concentrations <500 mU/mL (left [blue] box; p<0.05).

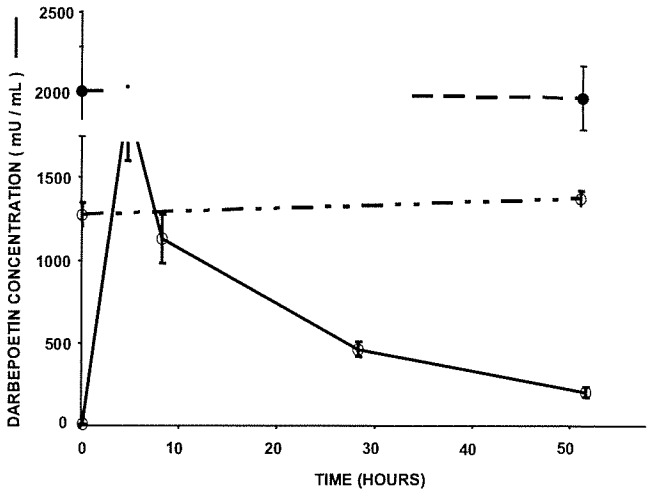
There are two important conclusions to be drawn from this study. First, this study demonstrates that elevated serum Epo concentrations to levels studied in animal models of neuroprotection can be achieved using subcutaneous Epo at doses of 400 units/kg 3x/week, the current Epo dose used in the Thrasher Study. Based on this information, we chose darbepoetin doses that would also achieve Epo concentrations greater than 500 mU/mL.

The second conclusion is that Epo levels correlate with later developmental assessment as measured by the MDI but not the PDI. Prior work has demonstrated that the Psychomotor Development Index as measured by the PDI of the Bayley Scales[89,90] is not a good predictor of development over time, as it is solely a measure of gross and fine motor skills. School achievement is an important marker of later outcome and best measured by tests of cognition and executive function. As reported by Hack[91] the Bayley Scales is not a good predictor of future performance[95] and therefore **testing at older ages as proposed in this study is essential**. The addition of measures of executive function assist in identifying learning disorders associated with prefrontal development, essential for later school success especially in early adolescence. **Therefore our proposal includes long-term follow up and includes a range of developmental assessment tools to ensure that clinically relevant conclusions can be drawn from this work**.

Preliminary *Studies #4 and 5: Single dose pharmacokinetics of Darbepoetin in preterm infants.*

We previously reported significant differences in Epo dosing in preterm infants when compared to adults, due to a much more rapid clearance and a larger volume of distribution,[115,116] and believed that a similar disparity would be evident with Darbepoetin dosing. In order to identify appropriate Darbepoetin dosing in neonates, we evaluated the pharmacokinetics of single dose subcutaneous (SC) and intravenous (IV) Darbepoetin.[67,68] Infants <32 wks gestation and <1500 grams at birth were eligible for participation if they were >21 days old and had a Hgb ≤10.5 g/dL. Twelve infants received a single SC dose of darbepoetin at either 1μg/kg or 4μg/kg. Once before and at pre-set intervals after, blood was obtained for reticulocyte count and serum Epo concentrations. Infants had birth weights of 1,139±245 g (mean±SD), were 29.1±1.2 weeks gestation at delivery, and were 43±12 days old with a Hgb of 9.6±1.0 g/dL when the Darbepoetin was given. Reticulocyte counts increased, and were somewhat greater among the 4μg/kg recipients. The half-life was 30 hours (range, 15 to 56 hours). The highest measured Epo concentrations following SC dosing were 980 mU/mL with 4 μg/kg (left panel, below; x axis represents time in hours, y axis represents Epo concentrations in mU/mL), while the highest doses following IV administration exceeded 1000 mU/mL (right panel; x axis represents time in hours, y axis represents Epo concentrations in mU/mL).

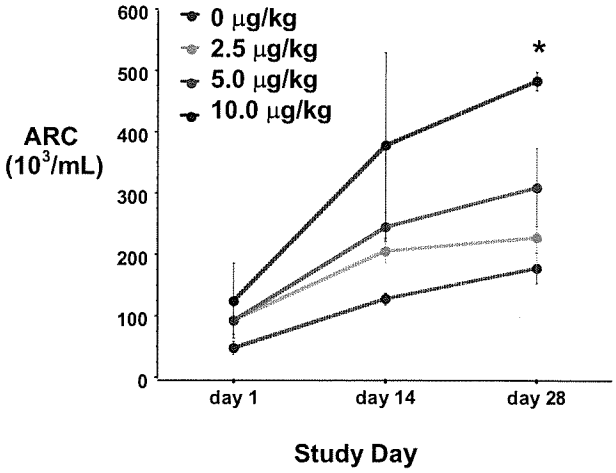




Compared with adults, neonates had more rapid clearance, suggesting that dosing in neonates would require a higher unit dose/kg and a shorter dosing interval than is generally used for adult patients. In addition, we determined that urinary excretion of Darbepoetin was less than urinary excretion of Epo, which might result in higher serum concentrations in those infants randomized to Darbepoetin.[117]

Preliminary Study #6: Randomized, masked, dose response study of Darbepoetin in preterm infants.

We recently completed a randomized, masked, dose response study of darbepoetin in preterm infants.[70] Infants were ≤1,500 grams at birth, were at least 10 days of age, and had consent from parent/guardian. Sixteen Infants were randomized to one of the following doses: 0, 2.5, 5.0, or 10 μg/kg/dose given once a week SC for 4 doses. CBC and retics were obtained at baseline, 2 and 4 weeks. We saw a clear dose response relationship in production of reticulocytes (figure, right), with the greatest response occurring in infants receiving 10 μg/kg dose. No adverse effects were reported in any of the infants. The results demonstrated preliminary safety and efficacy, and confirmed the findings of our pharmacokinetic studies, in that higher doses (than are used in adults for erythropoiesis) resulted in a robust biologic response without adverse effects.

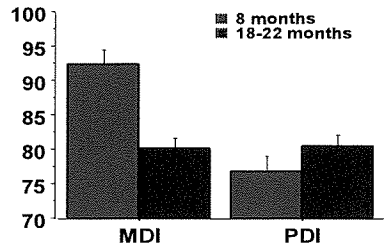


C. Clinical Studies of Developmental Assessment in Preterm Infants

Preliminary Studies #7-9: Studies of Measures of Developmental Outcome Performed by our Group:

In a study evaluating long term developmental outcomes of infants born prematurely, and our developmental investigators found that children born VLBW with mild periventricular, intraventricular hemorrhage (grades I and II) scored significantly lower at 5 to 6 years of age on a combined battery of tests measuring early learning disabilities, in comparison to children born VLBW without hemorrhage.[118] However, these children were all neurodevelopmentally *normal* at 1 to 2 years of age. This indicated that there was an increased risk of learning problems in children born VLBW with mild hemorrhage and also substantiated the importance of follow-up beyond the first two years of life.

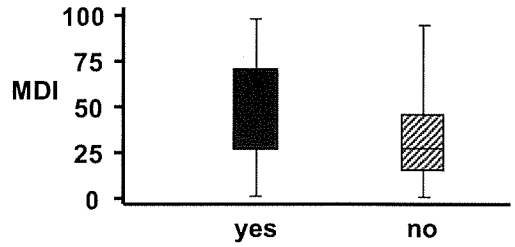
Additional research by s developmental group[119] found a significant drop in MDI scores in a cohort of forty-seven infants born ELBW when tested at 8 months compared to 18 months using the BSID- II[120] (1986 BSID ref) (p=0.006). (figure, right). Low household income and the Bayley Behavior Record emotional regulation score were significantly associated with the changes (p=0.001 and p=0.002 respectively). Results suggested that infant behavior characteristics as well as family socioeconomic status, can adversely affect developmental outcome over time.



Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) at 8 and 18–22 months adjusted age (mean ± SEM). Bayley Scales of Infant Development II; p = .001, MDI versus PDI at 8 months; p=0.001 MDI at 8 versus 18–22 months.

We then investigated the relative strength of maternal responsiveness in predicting infant affect in response to the Still Face paradigm (SF). Fifty infants 6 to 8 months corrected age who were born VLBW were tested with their mothers.[121] When predicting negative infant affect during and after the SF stressor, prior infant negative affect was strongly and uniquely significant. Furthermore, infant positive affect was highly correlated with maternal responsiveness. Our results indicated that self-regulation difficulties might be contributing to increased negative affect in infants born VLBW, and strengthening mother-infant interactions could help increase positive affect resulting in less infant stress.

Preliminary Study #10: Follow-up analysis of infants enrolled in a RCT. In collaboration with investigators involved in a RCT of hydrocortisone treatment in preterm infants (The PROPHET Study), data in the area of early working memory was analyzed in a group of 244 surviving infants born <1000 grams.[122] Those infants that mastered tests of object permanence (a measure of early working memory), had significantly higher scores on the BSID-II in areas of cognition (MDI, shown to the right), emotional regulation and self regulation (Behavior Record scales of the Bayley). Results of this study demonstrated our groups' ability to track and follow-up infants enrolled in a multicentered study. In fact, our developmental group anc rses achieved a 100% follow-up rate for infants enrolled at for the Study.

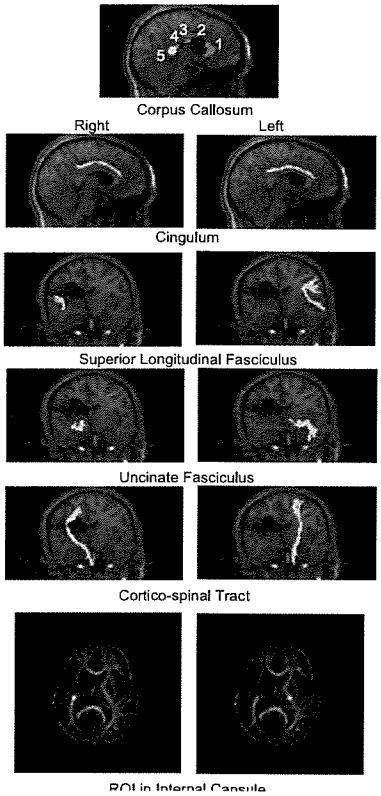


D. Clinical Studies of MR Imaging in Neonates and Children

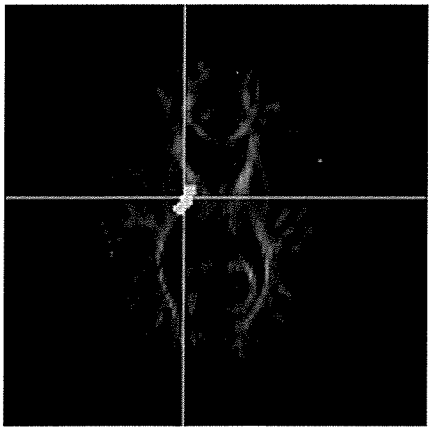
Regional Fractional Anisotropy															
Subject-age 5 yrs	CC1	CC2	**CC3**	**CC4**	CC5	Cing L	Cing R	SLF L	SLF R	UF L	UF R	CST L	CST R	IC L	IC R
Control:10	0.61	0.58	**0.58**	**0.54**	0.6	0.46	0.4	0.38	0.42	0.38	0.39	0.44	0.5	0.55	0.63
L-hemi:14	0.48	0.39	**0.37**	**0.31**	0.54	0.37	0.34	0.34	0.32	0.34	0.34	0.47	0.47	0.52	0.6
Mild R hemi: 8	0.56	0.47	**0.39**	**0.32**	0.5	0.49	0.44	0.4	0.4	0.4	0.39	0.45	0.55	0.6	0.63

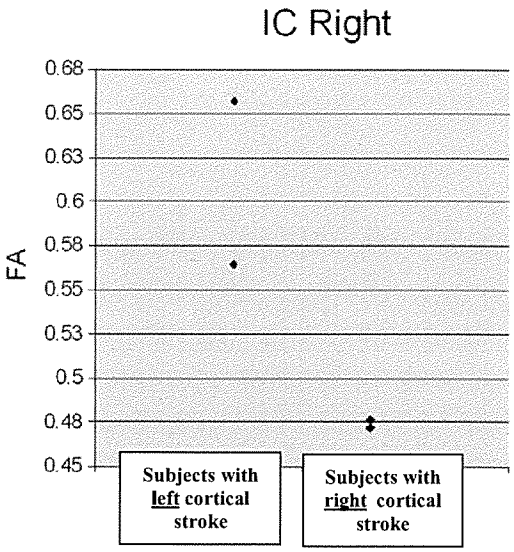
Preliminary Study #11: Diffusion tensor imaging in children with cerebral palsy.

In a technique development study (unpublished data) we performed fMRI and DTI in five children with cerebral palsy as young as five years of age. This study helped develop the DTI sequences to be used for the BRITE study. **Methods:** Fiber tracts were marked following protocols discussed in a paper currently under review, and FA values were determined. The corpus callosum was segmented using methods described by Hofer and Frahm. Tractography of major tracts was done based on the recently published method co-developed by Dr. [23] **Results:** The **figure** (right) demonstrates tractography, showing asymmetric fiber tracks due to the large congenital stroke. The **table** (above) shows FA values from two representative subjects and a control child. There is a striking decrease in FA in both children with cerebral palsy in regions #3 and #4 of the corpus callosum, *even in a child with an almost normal appearing structural MRI scan.* Finally, the **figures** (below) show the difference between the fractional anisotropy (FA) of the right internal capsule in four children with congenital stroke; the two with a left sided cortical stroke demonstrate greater FA of the right internal capsule compared to the two subjects with right sided stroke (where the right internal capsule FA is much less). **Significance:** No firm conclusions can be drawn from such small numbers, but we show that it is feasible to perform DTI in young children with cerebral palsy, including regional assessment of FA and other diffusion measures as well as tractography. DTI is sensitive to abnormalities in children with cerebral palsy, and provides a measure of white matter integrity which is not available through other imaging means. Our proposed study includes DTI measurements of the internal capsule, corpus callosum and major white matter tracts.



DTI in Congenital Stroke

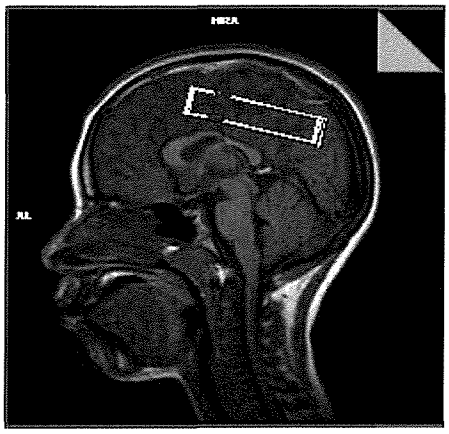


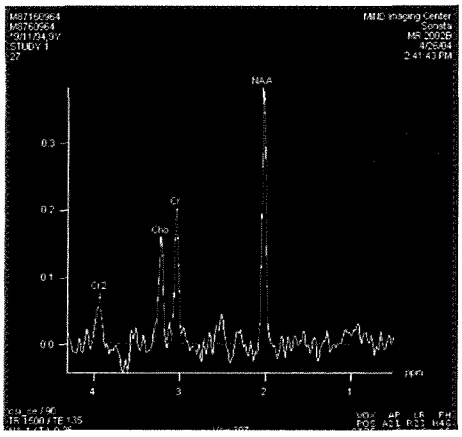


Preliminary Study #12. Magnetic Resonance Spectroscopy in Children with Cerebral Palsy Undergoing a Rehabilitation Intervention.

As part of a pilot study of multimodal neuroimaging in children with cerebral palsy undergoing treadmill training, spectroscopic imaging was performed in four subjects. The spectroscopic data is unpublished, however fMRI and clinical data is included in our previous study.[96] **Methods:** Using ratio data acquired from a slice of white matter directly superior to the lateral ventricles (see Yeo, Phillips, et al for detailed description of method)[124], four subjects age six to 14 with right hemiplegic or asymmetric diplegic (right greater than left)

cerebral palsy underwent spectroscopic imaging before and after a two week intervention. Data were divided into four quadrants. **Results:** The **figures** below demonstrate (left) the location of the acquired SI slab, a typical spectrum (middle), and the regional change in NAA in the four subjects (right). There was a consistent increase in N-acetylaspartic acid (NAA) in the left posterior quadrant of each subject, which is over the primary sensory motor cortex of the involved hemisphere. **Significance:** Interpretation can only be speculative with such few subjects, but this suggests the feasibility of performing spectroscopy studies in young children, and asks the question whether spectroscopy is indeed sensitive to changes in the environment such as occur with an intensive rehabilitation intervention, or as may occur with ESA treatment of premature infants.

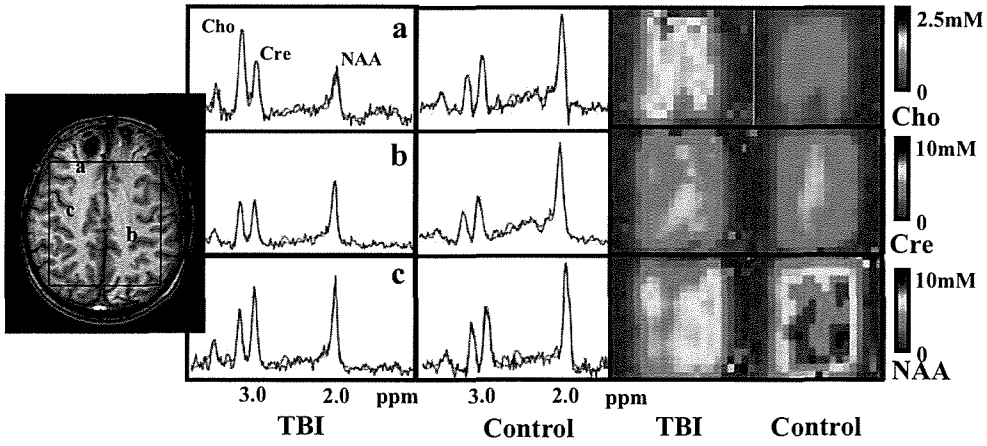




	Pre-therapy mean±SD	Post-therapy mean±SD
Right front	2.023±0.087	2.045±0.149
Left front	2.006±0.127	2.030±0.073
Right back	2.169±0.040	2.061±0.228
Left back	2.009±0.232	2.149±0.153

Preliminary Study #13. Magnetic Resonance Spectroscopy in Children with Traumatic Brain Injury (Yeo, Phillips, 2006).

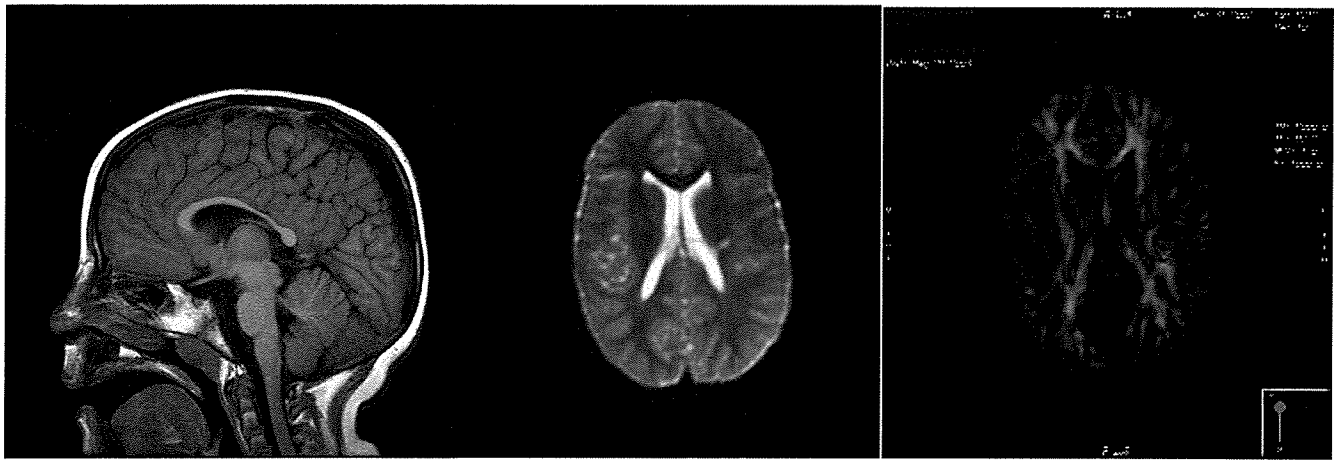
36 children age six to 18 years with mild to severe traumatic brain injury underwent SI and neurocognitive assessment in a longitudinal study.[124] **Methods:** Neurometabolite ratios were obtained from a supraventricular slab of brain located primarily in the white matter. NAA/creatine and choline/creatine ratios were calculated and averaged over the anterior and posterior halves of the brain. **Results:** NAA/creatine was reduced and choline/creatine was increased throughout the brains of children with TBI compared to controls. Over time there was a trend toward normalization of neurometabolite ratios, and initial values correlated with various aspects of the neurocognitive assessments. The **figure** right shows a typical SI dataset, comparing a child with TBI to a control. Note the widespread neurometabolic abnormality after TBI in areas that structurally appear normal on T1 MRI image.



Significance: We show, as others have, that spectroscopy is sensitive to the effects of traumatic brain injury in children. Furthermore it is possible to obtain spectroscopic data from young children, even those requiring sedation due to inability to remain still as can occur after TBI.

Preliminary study #14: MRI Study in a 4 year old child done at

The MRI experiments in this study were performed on a 4 year old female, with seizures, who was born at 24 weeks gestation. This study was performed by Dr. on a GE 3T system. We show below a T1 FLAIR image (Inversion recovery in Siemens terminology), a T2 image, and a color coded fractional anisotropy image. MR data was electronically transferred to where secondary analysis can be performed. By using this already established method of data transfer (the same system is in place for both collaborating sites) we are able to ensure the safety and quality of data available for analysis. The image quality is similar to our Siemens site. We will be transferring images From in DICOM format and doing the analyses at All data transferred to will be managed by the Data Management Center, under the direction of



a) T1 FLAIR **b) T2 weighted** **c) FA Color coded**

E. Summary and Interpretation of Preliminary Studies

Our preliminary studies suggest that Epo improves short term neurodevelopmental outcome as measured by MDI in premature infants. Our prior work also indicates that Epo is safe within established dosing guidelines. Before treatment recommendations can be made however, a long-term RCT is needed to evaluate the effect of both ESAs (Epo and Darbepoetin) on various cognitive domains. Furthermore, understanding the neurologic mechanisms affected by ESAs in former premature infants is necessary to determine how best to modify therapy to achieve optimal neurodevelopmental outcome and to determine how Epo may protect against microcephaly. Our multicenter team has the necessary state-of-the-art neuroimaging tools to investigate potential neurologic effects in these infants.

Accessing the patient population provided by the ongoing Thrasher study provides an unprecedented opportunity to conduct a definitive RCT that determines whether long-term neurodevelopmental outcome is affected by ESA therapy. Our proposal fills a critical gap in the literature by: 1) performing detailed neurocognitive assessments, 2) increasing follow-up to four to five years of age when neurologic outcomes are much clearer, and 3) including assessments of potential mechanisms underlying the long-term effect of ESAs on neurodevelopment. Our neuroimaging experience suggests that DTI and spectroscopic imaging can be acquired in difficult to scan populations (such as children with cerebral palsy, a disorder some of our study population will develop). Furthermore, using sensitive measures of white matter organization (DTI) and neurometabolism (MRS), coupled with standard volumetric analysis, offers a powerful approach to understanding brain—behavioral relationships.

Our proposal uses a population already randomized and treated, thus saving an enormous amount of time and money. We also already have most of the research team in place and working together and therefore many of the coordination logistics have been established. By making use of the considerable neuroimaging resources at the Mind Research Network, our proposal will significantly improve our understanding of the relationship between ESA therapy, developmental outcome and neuroplasticity. Therefore the BRITE study is

both necessary and unique in terms of answering an important clinical question (how do ESAs affect neurodevelopment in former premature infants), while also probing mechanisms of neuroplasticity in former premature infants.

5. Research Design and Methods

A. Subjects

1. Subjects enrolled in Thrasher Study: Infants will be eligible for study if they were originally eligible and enrolled in the multicenter study entitled, "A randomized, masked, placebo controlled study to assess the safety and efficacy of Darbepoetin alpha administered to preterm infants" (the Thrasher Study). Eligibility criteria for the Thrasher study are:

- birth weight 500-1,250 grams, gestational age ≤32 weeks
- hematocrit ≤55%
- ≤48 hours of age
- expected to survive greater than 72 hours
- consent signed by parent or guardian

Exclusion criteria are:

- hemorrhagic or hemolytic disease
- major congenital anomalies (such as trisomy 13 or 18)
- major neurologic abnormality such as hydrocephalus or meningomyelocele
- complex congenital heart disease
- receiving Epo or are enrolled in an Epo study
- evidence of disseminated intravascular coagulation
- clinical seizures are present
- congenital thrombotic disease is suspected
- systolic blood pressures >100 mm Hg (while not on pressor support)

Infants with minor anomalies such as isolated cleft lip, club foot, PDA, or isolated VSD are not excluded from the study.

Infants are randomized to one of three arms: Epo 400 units/kg three times weekly SC, Darbepoetin 10 μg/kg once weekly SC, or sham dosing (the research nurse would pretend to administer a shot behind a privacy screen, then place a bandaid on the infant's thigh). Infants receive study medicine for 10 weeks or until they complete 35 weeks gestation, are transferred or discharged, whichever came first. All infants receive iron, folate and Vitamin E supplements, and serum ferritin is evaluated during the study. CBC and reticulocyte count is performed every 2 weeks. **Epo concentrations are obtained 3 times during the study: baseline, 28 and 42 days into the study.** These data are extremely important as the primary hypotheses of the study hinge on achieving elevated serum Epo concentrations. Serum is collected 6-8 hours after dosing to determine peak concentrations. Epo concentrations are measured by ELISA (R&D Systems), and Darbepoetin is measured as erythropoietin in the ELISA method. Serum is also collected at the end of study for anti-Epo antibody determination. Laboratory data available to the BRITE Study are:

Lab	Day of Study					
	1 (prior to study drug)	14	28	42	56	Final day of study
CBC	X	X	X	X	X	X
Reticulocyte count	X	X	X	X	X	X
Epo	X		X	X		
Ferritin		X		X		
Anti-Epo Ab						X

We anticipate that 80% of infants in the Thrasher study will survive to follow-up, and that approximately 75 infants (25 per group) will be consented (with a new consent for the BRITE Study) and enrolled. Of these 75 infants, approximately 50 received ESAs for 10 weeks, and 25 received sham dosing (three arms in the original study).

Currently, 61 of a planned 102 infants are enrolled in the Study: 11 from 2 from , 38 from The ethnic diversity of infants currently enrolled is as follows:

	Am. Indian or Alaskan Native	Asian/Pacific Islander	Black	Hispanic	White	Total
Female	3	0	0	13	11	27
Male	2	0	2	18	12	34
Total (%)	5 (8%)	0	2 (3%)	31 (51%)	23 (38%)	61
Expected %	**10%**	**1%**	**3%**	**40%**	**46%**	**100%**

The study remains blinded. Screening, enrollment and study coordination are performed through GCRC (CTSC) research nurses in : , and and by IHC neonatal research nurses at the hospitals in The first infant was enrolled in July, 2006, and first follow-up at 18-22 months is planned for March, 2008.

The population of infants enrolled in the Thrasher study is unique for two reasons, and these characteristics may impact long-term outcomes. First, all infants will be exposed to the most rigorous transfusion guidelines published to date (see Thrasher Protocol in the Appendix), so testing short and longer term outcomes is vital in allowing these guidelines to show long term safety and efficacy.[125,126] Second, all infants will have been hospitalized at high altitude centers (>5000 ft.), underscoring the safety and feasibility of implementing a restrictive transfusion strategy at significant elevation, and determining outcomes for our distinct populations in .

2. Recruitment of additional control subjects: In addition to all infants enrolled in the Thrasher Study who survive to their first follow-up visit and consent to the BRITE Study, an ancillary control group of 25 infants will be recruited. Eligibility criteria are:

- infants previously born prematurely with birth weight 500-1,250 grams
- hematocrit ≤55% within first 48 hours after birth
- surviving to discharge

Exclusion criteria are:

- congenital hemorrhagic or hemolytic disease that is unresolved at time of discharge
- major congenital anomalies such as trisomy 13 or 18
- major neurologic abnormality such as hydrocephalus or meningomyelocele
- complex congenital heart disease
- received Epo or were enrolled in an Epo study (other than the Thrasher Study)
- clinical seizures during hospitalization requiring anticonvulsant therapy
- congenital thrombotic disease

Infants with minor anomalies such as isolated cleft lip, club foot, PDA, or isolated VSD are not excluded from the study. Additionally, infants with common morbidities associated with prematurity, such as IVH, ROP, or CLD will not be excluded. A consent signed by the parent or guardian for the BRITE Study will be required. A draft consent form is included in the Appendix.

Infants will be matched as a group to the original control group for gestational age, birth weight and gender. Most importantly, these infants will not have received either Epo or Darbepoetin during their hospitalization, and will have been transfused under the same criteria as all of the infants enrolled in the Thrasher Study. The impact of restrictive transfusion criteria is an area of active research[125,126] (Bell and PINT ref), and effects on neurodevelopmental outcomes are currently being evaluated.

Patients will be recruited at Special Baby clinic, which follows all surviving infants from at 2 to 4 months, 8 to 10 months, and 14 to 18 months. Recruitment will be performed by research nurses through Clinical Translational Science Center (CTSC), in order to keep investigators blinded. In addition to NICU graduates, preterm infants from two other level III NICUs in are seen in follow up clinic by our developmental care team. Approximately 400 infants are seen each year, and over 150 meet entry criteria. These infants are routinely seen at 2 to 4 months of age and again between 14 to 18 months of age. All attempts will be made to recruit ancillary control infants from the outpatient Special Baby Clinic so that images can be obtained and infants examined and tested by the Principal Investigator, . Due to the ethnic differences between the centers after recruiting the first 12 children, we will make sure that the demographic characteristics of the control infants are similar to those across all three sites. As one of the more

significant differences between sites is tha as fewer white subjects than nd , we feel recruiting this ethnic group should not be a problem if randomized enrollment of the control is not effective.

Retention--Travel and Reimbursement for Families: Reimbursement for travel costs (gas, bus, train) will occur for all families for the two assessment days. Overnight stays will be arranged for families greater than 2-3 hours away. Attempts will be made to perform developmental testing prior to MR Imaging, so that the children will be more likely to nap during the procedure. Developmental testing will never be performed if a child receives sedation for their MR Imaging procedure. If the timing is such for the families that testing cannot be performed prior to Imaging, the testing will be postponed until the following morning and an overnight stay will be arranged. For infants enrolled at the site who require an overnight stay, housing is available at the which is 50 yards from the Institute and testing center. For infants enrolled at the site, housing at Ronald McDonald house with free transportation to the testing and neuroimaging center is available. The University of and The Children's Hospital recently moved approximately 20 minutes east of the original campus, and a nev is planned next to the new Children's Hospital. Finally, for infants enrolled at the sites, the house is within ½ mile from the University and Children's Medical Center, with shuttle service available during times that scans would occur at Children's Medical Center.

B. Developmental Testing: Testing will be performed once for each assessment period (30-36 months, and 48-60 months). Testing will be performed in the CTSC outpatient clinic at each site, or, for families living within an hour of each site, testing can be performed at the family's home. Testing will be performed in English or in Spanish, depending on the family's first language. A video tape of the administration of all tests will be submitted and certified by D for each examiner performing developmental testing, to ensure consistency across sites and examiners. The executive function measures will be video-taped and sent to Dr. for scoring. The examiners performing the testing will be masked to the child's original treatment group for the duration of the BRITE Study. Enrollment of ancillary patients through Special baby Clinic will be performed by CTSC Research Nurses so that will remain blinded to each child's original treatment group. For those infants enrolled as ancillary controls at the a, a thorough medical history, demographic questionnaire (information about family composition, ages of family members, number of individuals living in the home, ethnicity, parents' education levels and income) and neurologic exam will be obtained. The data sheets for history and neurologic assessment are included in the attachment section. For those infants enrolled in the Thrasher Study, this information will be available for transfer to the Data Management Center. Test scores will be recorded by the developmental psychologists at each site, and transferred over a secure web site to the Data Management Center under the direction of .

1. First Assessment: 30-36 months

a. **Measures of Overall Cognition, Language and Social-Emotional Development** will occur using the **Bayley Scales of Infant Development III** (BSID-III)[90], which is the most widely used measure of infant development.

   1) **Cognitive**: The **cognitive scale** of the BSID-III will be used for this study, with standard scores (mean of 100 and standard deviation of 15) calculated as an overall index.

   2) **Language**: The **language scale** of the BSID-III will be used, with standard scores (mean of 100 and standard deviation of 15) calculated as an overall index of communicative abilities. In addition the scale can be broken down into separate receptive and expressive language scales that yield z-scores with an average of 10 and standard deviation of 3, for post-hoc analysis if difficulties in this area are noted.

   3) **Social Emotional:** The **social emotional scale**, which is a subscale of the BSID-III, will be used to obtain parent perception of the child's social emotional status[127]. The test yields a standard score with a mean of 100 and standard deviation of 15. It measures self-regulation and interest in the world, communicating needs, and ability to establish relationships, using emotional signals and gestures to communicate.

b. **Measures of Executive Function** will be used to better understand the brain-behavior relationships related to early pre-frontal development. All tests are standardized and have normalized data from children in the age groups to be studied.

1) **Gift Delay**[128]: Children are told they are going to receive a prize but the experimenter forgot to wrap the child's present. The child was asked to turn around in their seat (facing away from the experimenter) so it would be a surprise. Children were reminded not to peek. The experimenter then wrapped a gift box noisily for 60 seconds. Peeking was coded pass/fail as well as by latency (time) to first peek. This test will be video-taped for accuracy in recording of latency. This is considered a measure of orbital-frotal lobe function as it measures inhibition and delayed gratification.

2) **Behavior Rating Inventory of Executive Function- Preschool Version (BRIEF-P)**[129]: The BRIEF-P is 63-item parent report questionnaire that taps into child executive function from two years to five years, 11 months. It contains questions that relate to 5 basic scales: Inhibit, Shift, Emotional Control, Working Memory and Plan/Organize. These scales are also combined to yield the four summary scores of: Inhibitory Self-Control, Flexibility, Emergent Metacognition and a Global Executive Composite. Additionally, the measure contains questions that help to assess Negativity and Inconsistency of the rater. Overall performance is judged according to age-standardized scores that have a mean of 50 and a standard deviation of 10. The BRIEF-P is an ecologically valid and efficient tool for screening, assessing, and monitoring a young child's executive functioning and development.

3) **Categorization and Reverse Categorization**: This will consist of the combination of two subscales increasing in difficulty that tap into the executive domains of rule use or dorsal-frontal brain development.[130] These tasks tap into the same executive function areas and consist of sorting cards into boxes based on varying dimensions (e.g. category, shape, color). These sorting tasks are scored on a pass or fail basis, thus the best score will be 2/2 subscales passed and the worst possible score will be 0/2 passed.

2. Second Assessment: 48-60 months

a. **Measures of Overall Cognition, Language and Social-Emotional Development** will occur using **Wechsler Preschool and Primary Scale of Intelligence-Third Edition** (WPPSI-III).[131] The WPPSI-III is a structured developmental assessment, administered by a trained tester, for use with children aged 2:6 (2 years, 6 months old) to 7:3. The scales involve children pointing at pictures, naming pictures, answering questions about day to day information, building with blocks, and assembling puzzles. The WPPSI generates a Verbal IQ, a Performance IQ, and a Full Scale IQ with a standard score of 100, standard deviation of 15.

   1) **Cognitive**: The **cognitive scale** of the WPPSI-III will be used for this study, with standard scores (mean of 100 and standard deviation of 15) calculated as an overall index.

   2) **Language**: The **language scale** of the WPPSI-III will be used, with standard scores (mean of 100 and standard deviation of 15) calculated as an overall index of communicative abilities. In addition the scale can be broken down into separate receptive and expressive language scales that yield z-scores with an average of 10 and standard deviation of 3, for post-hoc analysis if difficulties in this area are noted.

   3) **Social Emotional:** The **Devereux Early Childhood Assessment (DECA)**[132] is a 37-item parent report measure of child' social skills for use with children ages 2 to 5 years. The scale generates standard scores for subscales that measure: initiative (child's ability to use independent thoughts or action to meet his/her needs), self-control (ability to express emotion in socially appropriate ways), and attachment (mutual, strong, long-lasting relationships with caretakers). The DECA is produced in both English and Spanish.

b. **Measures of Executive Function** will be used to better understand the brain-behavior relationships related to early pre-frontal development. The first three tests described above for measures of executive function will be repeated at the 48-60 month visit. A fourth test, the Bear/Dragon Test, is described below.

   1) **Gift Delay**

2) **Behavior Rating Inventory of Executive Function- Preschool Version (BRIEF-P)**

3) **Progressive Executive Categorization Battery:** This will consist of three four subscales increasing in difficulty that tap into the executive domains of rule use or dorsal-frontal brain development. The first two tasks consist of the Categorization and Reverse Categorization task used in the 30-36 month battery.[130] The third task is the Dimensional Change Card Sort-Separated Dimensions task[133] and 3the fourth is the original Dimensional Change Card Sort.[134] These sorting tasks are scored on a pass or fail basis, thus the best score will be 4/4 subscales passed and the worst possible score will be 0/4 passed.

4) **Bear/Dragon**[135]: This is a simplified Simon Says task in which children are asked to inhibit certain responses to commands. The experimenter introduces children to a "nice" bear puppet (using a soft, high-pitched voice) and a "grumpy" dragon puppet (using a gruff, low-pitched voice). Practice trials are then used and the child passes the trials if they do what the bear directs them to do but not what the dragon directs them. Up to six practice trials are given with verbal rule checks after each trial until the child passes one command by each puppet. After practicing, 10 test trials with the bear and dragon commands in alternating order will occur. A rule reminder is given half way through regardless of performance. Children are seated at a table throughout the task and a score from 0 to 3 is assigned.[136] (Carlson & Moses, 2001). Similar to gift delay this is a measure of inhibition and assesses orbital-frontal skills.

C. Magnetic Resonance Imaging: The following will be performed at 30-36 months and at 48-60 months. The investigators performing the procedure and evaluating the MRIs will be masked to the child's original treatment group for the duration of the BRITE Study.

1. Scanning Procedure:

The imaging protocol is designed so that it can be completed in one 60 minute session. A "child friendly" environment is ensured by having MR technicians experienced in pediatric scanning work with all subjects (imaging facilities in are experienced in pediatric studies). At all sites, conscious sedation is available if necessary for scanning. Imaging will be done at Institute in where is the Medical Director and D and re scientists. In imaging is done at the Brain Imaging Center under the direction of Dr x. (research director) and an MRI Physicist and prior collaborator of Dr 's. At is a pediatric neuroradiologist who is responsible for scanning and data transfer to (see letters of support).

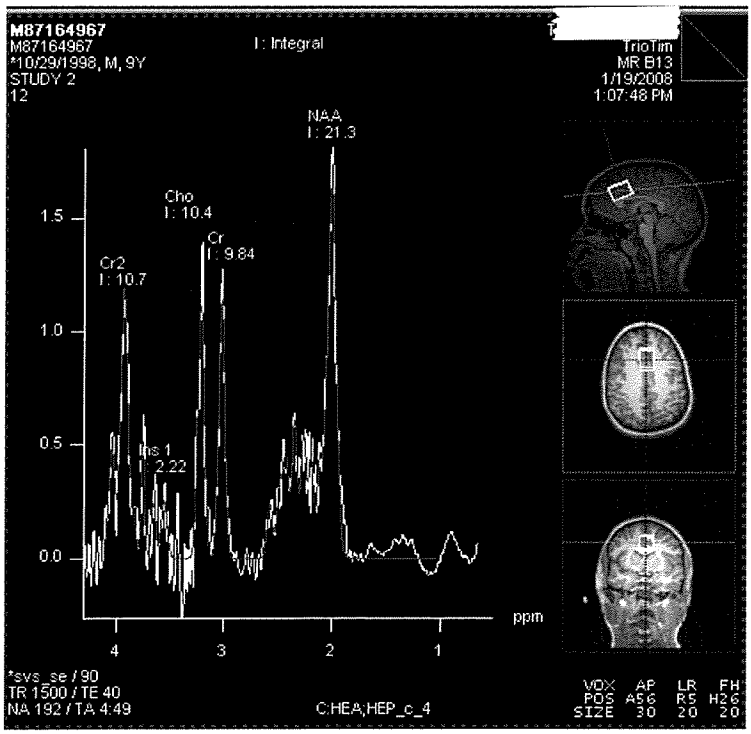
Imaging Protocol: All MRI images are acquired on 3T scanners; a 3T Siemens Trio is used for scanning in Albuquerque, and the Denver and Salt Lake sites use the identical 3T GE system. We will match the imaging protocol across the Siemens and GE sites as closely as possible. Standard sequences available on each scanner will be used. MRI experiments are done in a plane perpendicular to the interhemispheric fissure and angled along the AC-PC line. There will be two structural scans: a high resolution T1-weighted 3 dimensional gradient echo sequence, which serves as the underlay for DTI and MRS results, and a fluid attenuated inversion recovery (FLAIR) sequence for lesion detection.

Post processing data analysis will be done as follows for regional volumetric analysis. Images are aligned to standard MNI template and using the software Freesurfer, an atlas will be used for anatomic segmentation and lesion analysis. Importantly, each image will be reviewed and examined manually by Dr and if the atlas does not segment appropriately (due to the presence of lesions for example), then regions of interest will be manually drawn.

Diffusion tensor imaging (DTI) experiments at the site are conducted with a 12-channel phased array head coil in the axial direction along the AC-PC line. The FOV is 256x256mm with a 2 mm slice thickness, 72 slices, 128x128 matrix size, TE=82ms, TR=9500ms, NEX=1 and with a GRAPPA acceleration factor of 2. The DTI sequence has 30 directions, b=800 s/mm^2, and the scan with b=0 is repeated five times.

The 35 diffusion measurements (30+5) have been organized in five groups of 7, such that each group when included with the previous groups makes an optimal set for calculating the diffusion tensor from those measurements. This strategy ensures that if the subject moves then all the previous measurements can be optimally used for diffusion tensor calculations. The sequence will be repeated twice and the data will be combined to improve signal to noise ratio. The DTI experiment on the GE scanners will be done with an 8-channel radio frequency coil with a 25 direction DTI sequence with a SENSE acceleration factor of 2. The other parameters are identical to the Albuquerque site.

Single voxel proton magnetic resonance spectroscopy (1H-MRS) will be obtained with a point resolved spectroscopy (PRESS) sequence (TR = 2 s, TE = 40 ms, 96 averages, voxel size = 1 cm x 1 cm x 1 cm). Three different anatomical locations will be examined by 1H-MRS. A voxel is placed in the white matter of the right frontal lobe adjacent to the cingulate gyrus, which is an area of white matter often not overtly affected by neonatal injury but with significant implications for cognitive and motor outcome. A second voxel is placed over the gray matter of the anterior cingulate gyrus, a region with potential implications for behavioral/cognitive outcome. A third voxel is placed in the left basal ganglia. The left basal ganglia has been previously reported to show spectroscopic abnormalities correlating with motor and cognitive outcome in children with cerebral palsy with visible lesions of the basal ganglia[136] and also in those without visible basal ganglia lesions.[137] The exact gray-white matter composition of each 1H-MRS voxel is determined by segmentation of the T1 image in post-processing steps. Each 1H-MRS scan takes approximately 3.2 min to acquire. An example of the voxel placement in the anterior cingulated region with the associated spectra is shown in the figure below. The experiment was done on a Siemens 3T TIM system. The total scan time is under one hour.



2. Processing Imaged Data

Processing DTI data: The data processing streamline consists of a) motion and eddy current correction (Flirt in FSL), b) rotation of gradient directions to adjust for image orientation (IDL routine), c) calculation of the diffusion tensor and other DTI parameters (Dtifit in FSL), d) registration of T1 to the DTI images.

Fractional anisotropy (FA), Mean diffusivity (MD), Axial diffusivity (AD), and Radial diffusivity (RD) are the four scalar DTI parameters that will be studied to characterize brain development. In addition major white matter tracts will be individually identified for each subject with the help of DTI-Studio, a public domain software program which does 3D reconstruction of fiber tracts based on FACT (Fiber Assignment by Continuous Tracking) method. We will identify the following major structures: a) corpus callosum divided into 5 segments according to the method of Hofer and Frahm,[138] b) cingulum cingulate gyrus (CGC), c) cortico spinal tract

(CST), d) anterior thalamic radiation (ATR), e) posterior thalamic radiation (PTR), f) superior thalamic radiation (STR), g) inferior longitudinal fasciculus (ILF), g) arcuate fasciculus (AF), h) uncinate fasciculus, i) forceps major (Fmajor), and j) forceps minor (Fminor). The tracks will be identified both on the left and the right side as appropriate.

Processing 1H-MRS data: 1H-MRS data will be processed using the spectral fitting routine LCModel[139] to determine the relative levels of N-acetyl aspartate, glutamate, creatine, choline, and myo-inositol. Since the MR relaxation times of these metabolites have not been reported in infant brain, the levels will be reported simply as ratios with total choline as the denominator, since this metabolite is expected to vary minimally between gray and white matter. The total gray and white matter content of each voxel will be determined by segmentation of the T1 image, using the FSL FAST algorithm.

Subjects are given research numbers and data are stored behind the Research Network (MRN) firewall and is password-protected. The only links between subjects and their research number is kept on the MRN MR log, which only limited to the BRITE investigators at . The initial data analysis will occur at MRN, and statistical analysis will be performed in the CTSC by Dr.

D. Data Analysis

1. Data management, centralized collecting

The Data Management Center (DMC) is run by the neuroinformatics core of The in) has extensive experience in the design, conduct and analysis of single and multi-centered clinical trials. This includes the Clinical Imaging Consortium, which generates data from more than 400 human research volunteers across four sites (University of , The University University of and ;niversity) that have had comprehensive neuroimaging (MR spectroscopy, fMR, DTI), genetic, clinical, socio-demographic, and neuropsychological assessments. The neuroinformatics core is responsible for all aspects of data management for this project including data security, querying, reporting, analyzing, summarizing, and archiving. Other ongoing projects that the euroinformatics core participates in are the Biomedical Informatics Research Network (BIRN), as well as the National Alliance for Medical Imaging Computing. The neuroinformatics team at is directed by Mr. who will direct the DMC for the BRITE study. Our MRI physicists (are also based at the facility; because they are responsible for all MR image analysis (which will include imaging done at PCMC, UC and , this facilitates analysis and management of all clinical and imaging data. Issues related to treatment assignments and outcomes will be maintained as confidential, masked information at the DMC, and will not be disclosed to other investigators.

2. Hypotheses and Statistical Analyses

Hypothesis 1a: *Children born VLBW who received ESAs will perform significantly better at both assessment points on measures of executive function and emotional development than placebo/controls.* This hypothesis will be analyzed using a one-way ANOVA comparing the tests of neurodevelopment (BSID III cognitive, language and social emotional scales and measures of executive function) between the 3 design groups (Epo, Darbe, Control) at the 30-36 month age level. Initial analyses will be performed on the three original groups, and if results are similar between the two treatment groups, they will be combined as a single ESA "treatment" group and compared with the placebo/control group. The sample size in the control group will be increased by including the ancillary control group, provided preliminary analyses verify that this group is similar in gestational age, birth weight and gender to the original control group. Post-hoc pair-wise comparison will be done by the Fisher's least significant difference method. If one of the ESA treatment groups (either Epo or Darbepoetin) is found to be superior, the treatment groups will remain separated for further analysis.

Hypothesis 1b: *Children born VLBW who received ESAs will demonstrate a positive correlation at both assessment points between peak serum Epo concentrations measured during the original hospitalization and neurodevelopmental outcome.* We will use measured peak Epo concentrations (log transformed to give nearly normally distributed values) treated as a continuous predictor, of each neurodevelopmental test measure in a regression analysis for the pooled data of ESA treatment groups. We anticipate Epo concentrations (prior to log-transformation) will range 400-3000 mU/mL in ESA treated infants. The analysis may have more power in that it will deal with overlap in the measured Epo values among the groups. The analyses are the same at 48-60 months, except that BSID III and executive function testing will be replaced by WIPPSI, DECA and age appropriate measures of executive function mentioned in the testing section.

Hypothesis 2a *Children born VLBW who received ESAs will demonstrate increased regional brain volumes as determined by morphometric MR analysis, improved white matter organization as determined by DTI, and improved neurometabolic profile on magnetic resonance spectroscopy compared to placebo/controls.* This analysis of volumetric measurements will be completed using repeat measures ANOVA with region and the two time points (30-36 months and 48-60 months) as repeated factors, and the 2 groups (ESA-treated versus control) as a grouping factor. If there are significant differences, post-hoc testing will be done with paired or unpaired t-tests as appropriate.

Hypothesis 2b *In children born VLBW who received ESAs, a correlation exists between peak serum Epo concentrations measured during the original hospitalization and neuroimaging parameters.* We will use measured Epo concentrations (performed during the initial hospital stay) as a continuous variable and as a replacement for the grouping factor in the above analysis, evaluating only ESA treated infants. Similarly, repeated measured ANOVA will be used to test changes over time and differences between groups in the MRI measures (volume, NAA:choline ratio, lactate:choline ratio, white matter anisotropy, etc).

Hypothesis 3 will be completed using correlations with results of the neurodevelopmental assessments at each assessment point and the MRI measures of: regional brain volumes (*3a: regional brain volumes as determined by morphometric MR analysis will correlate with neurodevelopmental outcome at both assessment points*); frontal lobe fractional anisotropy as determined by DTI (*3b: increased frontal lobe fractional anisotropy as determined by DTI will show a significant correlation with measures of executive function at both assessment points*); and N-acetylaspartic acid, lactate and choline in the basal ganglia, frontal lobe and anterior cingulate gyrus (*3c: regional brain volumes as determined by morphometric MR analysis will correlate with neurodevelopmental outcome at both assessment points*).

3. Power Analysis

Hypothesis 1a: From our preliminary data in preterm infants <1250 grams, we anticipate the MDI will be 83.4±15.4 (SD). Our sample size of 25 per group is adequate to detect a 12.7 point difference in MDI between either treatment group and our control group with 80% power and α=0.05. The difference of 12.7 is reasonable because it is twice the error of the test.[141] (ref-Jean?). When ESA treatment groups are combined to give 50 subjects, the sample size is adequate to detect an 8.9 point difference. Our total sample size of 100 preterm infants is adequate to detect a correlation as low as 0.28.

Hypothesis 1b: Our sample size of 50 per group is adequate to detect a significant correlation of 0.39 with 80% power and α=0.05.

Hypothesis 2a: Thompson and colleagues[140] (2007) reported that the orbitofrontal volume (mL) was 4.7±2.8 (SD) for preterm infants similar to our control subjects (that did receive ESAs), and a larger SD of 4.2 mL in term infants. Our sample of 50 preterm control and 50 preterm infants treated is adequate to detect a 2.1 mL difference between these two groups with 80% power and α=0.05. This may be reasonable since Thompson and colleagues[140] reported a slightly larger difference of 2.2 mL that was significant between the term and preterm infants he studied. However, we do not necessarily expect the improvement in volume to be this large, but we might have a smaller SD (approximately 3.5) in the treated group. Then our detectable difference is 1.8 mL.

Using recently published data[107] by Miller and colleagues where the NAA:choline ratio was 10% less in affected infants, we determined that the basic standard deviation is 11%, for the information available. Our sample size of 50 per group is adequate to detect a 6.4% difference with 80% power and α=0.05.

Hypothesis 2b: A sample size of 50 per group is adequate to detect a significant correlation of 0.39 with 80% power and α=0.05.

Hypothesis 3: A sample size of 50 per group is adequate to detect a significant correlation of 0.28 with 80% power and α=0.05.

E. Organizational Plan

Although is the Principal Investigator for this R01 proposal, the following leadership plan has been devised to ensure communication and collaboration between the investigators at each of the other sites and the primary site

Dr. is the Principal Investigator for this project. She has worked together with [and previously. In addition, she is the PI for the Thrasher Study, which

has been active at all three sites since June, 2006. Thus, a considerable strength of this study is that it combines the rich and diverse clinical base of three major metropolitan areas ( ) while bringing a team together who have already demonstrated an ability to work effectively. As PI, will be responsible for: 1) the overall scientific, technical, and clinical conduct of the study, 2) development of protocols for interventions and outcomes assessment in collaboration with the other study investigators, 3) oversight of participant enrollment, retention, and safety at the participating clinical sites in the areas 4) leadership of the steering committee, 5) assuring the integrity of the study and its conclusions, and 6) along with the other study investigators timely publications and disseminations of our results.

As PI of the BRITE Study, will have the overall responsibility of the data, overall budget management and fiscal responsibility for the clinical trial, and communication with the Data Safety Monitoring Board. Dr. is medical director of the study and addresses medical questions that may arise in any of the three sites. The BRITE Study will be managed by a steering committee. This committee will be responsible for the overall management including oversight of subject recruitment, execution of the trial, ethical conduct of the trial and publications. The steering committee will include the principal investigato co-Investigators , the site psychologists and he Manager of the Data Management and Analysis Center, Mr. ., and the Biostatistician, Dr Monthly to twice monthly phone conferences are held to discuss general study issues and specifically to review data management as well as to ensure ethical study conduct. Drs communicate via weekly phone calls or emails. We will convene an investigators meeting once per year.

Investigators share their research results with all study investigators and each other, and work together to discuss any changes in study policy. A publication policy will be established based on relative scientific contributions of coauthors.

If a PI moves to a new institution, attempts will be made to transfer the relevant portion of the grant to the new institution. In the event that a PI cannot carry out his/her duties, a new PI will be recruited as a replacement at one of the participating institutions.

F. Time Table

March-December 2008	Obtain IRB approval of the BRITE Study at each site Continue contacting Thrasher Study subjects for 18-22 month follow-up Enroll Thrasher Study subjects into BRITE Study when IRB approved
January-June 2009 year 1	Enroll BRITE Study subjects; enroll ancillary control subjects Perform MR Studies and Developmental Testing on first subjects First BRITE Study Investigators' Meeting
July-December 2009	Enroll Subjects; MR and Developmental Testing continues BRITE Study DSMB meeting Renew BRITE Study at each site Thrasher Study Results (hospital phase) available Begin analysis for hypothesis 3 (comparing developmental testing with MR imaging; can be performed regardless of initial study arm)
January-June 2010 year 2	Enroll Subjects; MR and Developmental Testing continues Continue analysis for hypothesis 3
July-December 2010	Enrollment complete; MR and Developmental Testing continues BRITE Study DSMB meeting Renew BRITE Study at each site Data analysis continues
January-June 2011 year 3	Enroll Subjects; MR and Developmental Testing continues Thrasher Study Results (follow-up phase) available Present at National Pediatric Research Meeting Data analysis continues

July-December 2011	MR and Developmental Testing continues BRITE Study DSMB meeting Renew BRITE Study at each site
January-June 2012 year 4	MR and Developmental Testing near completion Present at National Pediatric Research Meeting Data Analysis; begin manuscript preparation
July-December 2012	MR and Developmental Testing complete Complete Data Analysis; manuscript preparation

8. Protection of Human Subjects

Provide approximate number of subjects to be enrolled locally & study wide
100 infants will be enrolled in the study

Describe the subject recruitment strategies you will use for each group of subjects. State how potential subjects will be identified and how initial contact or notification be made.
The CTSC research nurses involved in this study will identify possible candidates. G-CRC nurses are responsible for collecting general patient information through

Upon meeting inclusion requirements, an informed consent will be signed and placed in the patient's medical record. A written progress note stating enrollment will also be made in patient's chart. There are no modifications or waivers from the HRRC on the consent form. The research nurses will not be caregivers for any of the infants. The PI will not be a caregiver for any infants enrolled at outpatients.

Describe steps that will be taken to protect subject privacy throughout the research (consenting and research procedures, follow-up) Only the CTSC research nurses involved in the study. Only the research nurse will have access to the link between code numbers and direct identifiers for infants already enrolled in the Thrasher Study. This information will be kept in a locked filing cabinet in a locked office. Direct identifiers (names and contact information) will be maintained in order to contact participants should information affecting safety and participation in the study be determined following enrollment. Parents will be approached in private and all attempts will be made to hold conversations in areas where they cannot be overheard

Explain what steps you will take during the recruitment process to minimize potential coercion or the appearance of coercion.
The parent(s) or legal guardian of the infant will be asked to provide informed consent. No incentives will be given to the parents/legal/guardians or study nurses for recruitment and retention of infant(s).It will be explained to parents that participation is voluntary, they can withdraw their consent at any time without penalty, and that there are alternatives to participation in this study.

If any vulnerable subject populations are to be included in this study, list additional protective measures to be taken with these subjects. For populations whose decision making capacity may be in question include how an individual's capacity to consent is determined – both before and during the study.
Parents are introduced to the study when their infant is in Special Baby Clinic. If there are any stressful events occuring for the infant the parents are not approached. The parents are given information about the study, and any questions they have are answered by the research nurse. They are generally contacted again within 24 hours to see if they have further questions. If they agree to the study the consent is signed by the parent and the research nurse. If they need more time then the research nurses identify a time in the future to contact the parents. This period of time may be as long as 2 days.

Describe any potential risks (including risks to an identifiable group), including physical, psychological, social, economic, or legal, as well as those that might arise due to a breach of confidentiality, identifying both their seriousness and likelihood. Discuss alternative treatments where appropriate. Identify circumstances for terminating the study.
A potential risk to infants enrolled in this study is loss of privacy during review of medical records. Each subject will be assigned an anonymous identification number. There are no alternatives to participation—the only alternative is to not participate. Parents or legal guardians may terminate involvement in this study at any time by notifying a member of the research team. In addition, the investigator may discover conditions arising during the study that could prompt considerations that the study should be halted. Any discovery of unexpected, serious, or unacceptable risk to the infants enrolled in the study will result in discontinuation of the study.

Describe procedures for protecting against or minimizing likelihood of identified risks. Include any procedures that will be used to maintain confidentiality as applicable.
All information will be kept confidential, in a locked file inside a locked room (research nurses' office) at the CTSC.

Describe the expected benefits, including any possible direct benefit (such as alleviating a condition or providing a better understanding of a participant's condition) and any benefits to society (such as learning the answer to an important question). Note: payment to subjects is not a benefit.
Study participants in the BRITE study will not directly benefit, but the knowledge gained may benefit future preterm infants if ESA therapy can prevent or decrease injury to the brain.
Explain why the risks are reasonable in relation to the anticipated benefits to subjects and the importance of the knowledge that may reasonably be expected to result.
The administration of Epo or darbepoetin should improve a preterm infant's ability to generate new red cells, thereby decreasing or even eliminating the need for erythrocyte transfusions. The benefit of once a week dosing of darbepoetin is decreased discomfort in preterm infants. If beneficial for neurodevelopmental outcomes, preterm infants will benefit from administration of these growth factors.

List any costs to the subjects (or their third party payers), including any charges for study drug/device, procedures, or visits; explain who will be responsible for cost of treatment for adverse events.
No costs associated with the study will be passed on to the family (labs, study drug, follow up visit) Transfusions and other NICU clinical care are not covered by the study.

Describe any payments to subjècts, explaining amount, timing of payments, and if they are prorated. Substantiate that proposed payments are reasonable and commensurate with the expected benefits of the subject and that payments are fair and appropriate. Substantiate that payments do not constitute (or appear to constitute) undue pressure, influence, or coercion to participate in the research study.
No incentives will be given to the parents/legal/guardians, investigators, or study nurses for recruitment and retention of infants. Travel reimbursement will be provided for families who do not qualify for SAFERIDE sponsored travel support) in order return to ;r neurodevelopmental follow up.
Reimbursement for time

Characteristics (inclusion criteria) which subjects must have to be in this study
Infants will be eligible for study if they were previously enrolled in the Thrasher Study, or if the are born prematurely with birth weight 500-1,250 grams, their hematocrit is ≤55%, they are enrolled within 48 hours of birth, they are expected to survive greater than 72 hours, they did not receive Epo or Darbepoetin, and they do not have hemorrhagic or hemolytic disease. Infants with minor anomalies such as isolated cleft lip, club foot, PDA, or isolated VSD will not be excluded from the study. An informed consent document must be signed by a parent or guardian.

Characteristics (exclusion criteria) which exclude subjects who are otherwise eligible from this study:
Infants will be ineligible for study if they are already receiving Epo or are enrolled in an Epo study, if they have evidence of disseminated intravascular coagulation, if clinical seizures are present, if congenital thrombotic disease is suspected, or if they have systolic blood pressures >100 mm Hg (while not on pressor support). Infants with major congenital anomalies such as trisomy 13 or 18 will be excluded, as will infants with major neurologic anomalies such as meningomyelocele.

Location where will the study procedures be performed:
The portion of the study will be performed at the :nstitute and at the outpatient CTSC. This multicentered study will also be performed at the Brain Imaging Center and the Pediatric CTSC at the University and at ' Children's Medical Center in

Provide a detailed timeline that describes the consent process from study introduction, discussion, to signing of the consent form.
Parents are introduced to the study when their infant is seen in Special Baby Clinic. If there are any stressful events occuring for the infant the parents are not approached. The parents are given information about the study, and any questions they have are answered by the research nurse. The parents are offered the opportunity to discuss the study with the Neonatologist in Special Baby Clinic (not the PI) as well. They are generally contacted again within 24 hours to see if they have further questions. If they agree to the study the

consent is signed by the parent and the research nurse. If they need more time then the research nurses identify a time in the future to contact the parents. This period of time may be as long as 12 months

Describe how you will provide the prospective subject or their representative sufficient opportunity and time to consider whether or not to participate.
The parents are always asked if it is a good time for them to learn about the study, and are never approached if there are stressful events occurring for their infant. They are given time to think about the study and ask questions. The research nurses are available to answer questions until the parents are satisfied that they would like their infant to participate in the study, or that they are not interested in their infant participating.

Describe how you will minimize the possibility of coercion or undue influence.
The parents are only approached if they state that it is a good time for them to learn about the study. The research nurses have the primary responsibility of approaching parents.

Describe how you will ensure the information given to the subject or their representative will be understood.
The parents are asked if they understand the study, and are given multiple opportunites to ask questions of the research nurses. The research nurse signing the consent form with the parent will not do so until they are confident that the parent completely understands the study, including risks and benefits.

How the consent process will be documented in the research and/or medical record.
A copy of the consent form is placed in the child's chart, and a note is written by the research nurse documenting the consent process and enrollment into the BRITE study.

Potential Benefits: Study participants in the BRITE study will not directly benefit, but the knowledge gained may benefit future preterm infants if ESA therapy can prevent or decrease injury to the brain. If ESAs prove to be neuroprotective, future preterm infants will benefit with better long-term neurodevelopmental outcomes. The neurodevelopmental outcome for preterm infants has not improved significantly over the last 20 years, even though more preterm infants are surviving. These infants are often quite compromised, and require lifelong medical attention in addition to special education programs in schools. ESAs have been extensively tested, and used commonly to treat anemia in preterm infants. ESAs show promise as neuroprotective agents that are both effective and safe. We need such agents to improve the health and well being of the most vulnerable infants.

Importance of Knowledge Gained: There is currently no treatment available to improve neurodevelopmental outcome for VLBW infants. This study will provide developmental and structural evidence that ESAs improve the long term outcome for this at-risk population.

DSMP
The DSMP currently being used in the Thrasher Study is included in the appendix. All DSMPs for studies requesting CTSC support are reviewed carefully by the Research Subject Advocate, . The DSMP for the BRITE Study will include a Data and Safety Monitoring Board (DSMB), as this is a multicentered study. The DSMB will be made up of a Neonatologist, Neuroradiologist, and a Developmental Specialist independent from the study. In addition a biostatistician from either the CTSC or the CTSC will be a member of the DSMB. Yearly teleconference meetings will occur to review patient safety and any adverse events or serious adverse events. This is a moderate risk study because of its multi-site nature, however the procedures proposed are low risk.

9. Inclusion of Women and Minorities

Inclusion of Minorities:

Based on current ethnicity at each site, our anticipated enrollment in the Thrasher Study is as follows:

Gender and Ethnic Distribution,

	Am. Indian or Alaskan Native	Asian/Pacific Islander	Black	Hispanic	White	Total
50% Female	**1**	**0**	**1**	**7**	**8**	**17**
50% Male	**2**	**0**	**0**	**7**	**8**	**17**
Total	**3**	**0**	**1**	**14**	**16**	**34**
Expected	**10%**	**1%**	**3%**	**40%**	**46%**	**100%**

Gender and Ethnic Distribution,

	Am. Indian or Alaskan Native	Asian/Pacific	Black	Hispanic	White	Total
50% Female	**1**	**0**	**0**	**2**	**14**	**17**
50% Male	**0**	**1**	**0**	**2**	**14**	**17**
Total	**1**	**1**	**0**	**4**	**28**	**34**
Expected	**0.5%**	**1.5%**	**0.5%**	**12.5%**	**84%**	**100%**

Gender and Ethnic Distribution,

	Am. Indian or Alaskan Native	Asian/Pacific	Black	Hispanic	White	Total
50% Female	**1**	**1**	**1**	**2**	**13**	**17**
50% Male	**0**	**1**	**2**	**2**	**12**	**17**
Total	**1**	**2**	**3**	**4**	**25**	**34**
Expected	**2%**	**4%**	**8%**	**13%**	**73%**	**100%**

The actual gender and ethnic distribution of the 61 subjects currently enrolled is as follows:

	Am. Indian or Alaskan Native	**Asian/Pacific Islander**	**Black**	**Hispanic**	**White**	**Total**
Female	**3**	**0**	**0**	**13**	**11**	**27**
Male	**2**	**0**	**2**	**18**	**12**	**34**
Total (%)	**5 (8%)**	**0**	**2 (3%)**	**31 (51%)**	**23 (38%)**	**61**

We anticipate continuing to enroll similar percentages of all minorities. Enrollment for the BRITE study is open to all minorities.

10. Targeted/Planned Enrollment Table

Total Planned Enrollment: 100

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	20	20	40
Not Hispanic or Latino	28	28	60
Ethnic Category: Total of All Subjects *	50	50	100
Racial Categories			
American Indian/Alaska Native	5	5	10
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	1	0	1
Black or African American	2	1	3
White	42	42	84
Racial Categories: Total of All Subjects *	50	50	100

11. Inclusion of Children

This proposal is a specific to the Pediatric population. Only children between the ages of 2 and 5 will be enrolled. The BRITE Study therefore fulfills the NIH policy for Inclusion of Children in that children must be included in all clinical research, conducted or supported by the NIH. The involvement of children as subjects in this research proposal is in compliance with all applicable subparts of 45 CFR Part 46.

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PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

● New ○ Resubmission ○ Renewal ○ Continuation ○ Revision

Federal Identifier: N/A

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ○ No ○

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ○ No ○

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

○Yes ●No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance, where applicable, provide an explanation and attach below.

Explanation:

Attachments

CertificationExplanation_attDataGroup0

File Name	Mime Type