PI: **AMBALAVANAN, NAMASIVAYAM**	Title: STOP BPD	
Received: 01/16/2015	FOA: HL15-024	Council: 08/2015
Competition ID: FORMS-C	FOA Title: DEFINITION OF RESILIENCE AND PRE-SYMPTOMATIC DISEASE IN LUNG HEALTH AND DISEASE (R01)	
1 R01 HL129907-01	Dual:	Accession Number: 3778718
IPF: 1288803	Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM	
Former Number:	Department: Pediatrics- Neonatology	
IRG/SRG: ZHL1 SRC (99)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: Year 2: Year 3:	Animals: N Humans: Y Clinical Trial: N Current HS Code: 20 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Waldemar Carlo	The University of Alabama at Birmingham	Co-Investigator
Kui Zhang	The University of Alabama at Birmingham	Co-Investigator
Ranjit Kumar	The University of Alabama at Birmingham	Co-Investigator
Namasivayam Ambalavanan	The University of Alabama at Birmingham	PD/PI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 063690705

Legal Name*: The University of Alabama at Birmingham
Department: Office of Sponsored Programs
Division:
Street1*: 1720 2nd Ave. South, AB 1170
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111

Person to be contacted on matters involving this application
Prefix: First Name*: Stephanie Middle Name: Last Name*: May Suffix:
Position/Title: Grants and Contracts Officer
Street1*: 1720 2nd Ave. South, AB 1170
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 205-934-5266 Fax Number: 205-975-5977 Email: stephmay@uab.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1636005396A6

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

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ○ Resubmission
○ Renewal ○ Continuation ○ Revision

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
93.838
TITLE: Lung Diseases Research

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
STOP BPD

12. PROPOSED PROJECT
Start Date* Ending Date*
09/01/2015 08/31/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
AL-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Namasivayam Middle Name: Last Name*: Ambalavanan Suffix:
Position/Title: Professor
Organization Name*: The University of Alabama at Birmingham
Department: Pediatrics- Neonatology
Division: School of Medicine
Street1*: 1720 2nd Ave. South, WIC
Street2: 176F Suite 9380
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 205-934-4680 Fax Number: 205-934-3100 Email*: nambalavanan@peds.uab.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$	
b. Total Non-Federal Funds*		$0.00
c. Total Federal & Non-Federal Funds*	$	
d. Estimated Program Income*		$0.00

16.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

17. 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.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Ms. First Name*: Lynn Middle Name: Last Name*: Stedman Suffix: MBA
Position/Title*: Director, OSP
Organization Name*: The University of Alabama at Birmingham
Department: Office of Sponsored Programs
Division:
Street1*: 1720 2nd Ave. South, AB 1170
Street2:
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 352940111
Phone Number*: 205-934-5266 Fax Number: 205-975-5977 Email*: osp@uab.edu

Signature of Authorized Representative*
Stephanie May

Date Signed*
01/16/2015

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1243-CoverLetter_ResilGrant_Jan2015.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The University of Alabama at Birmingham
Duns Number:	0636907050000
Street1*:	1720 2nd Ave. South, AB 1170
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	352940111
Project/Performance Site Congressional District*:	AL-007

File Name

Additional Location(s)

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ● No

If YES, check appropriate exemption number: — 1 — 2 — 3 — 4 — 5 — 6

If NO, is the IRB review Pending? ● **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number 00005960

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 - positive or negative - 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. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1238-Project Summary.pdf
8. Project Narrative*	1239-ProjectNarrative.pdf
9. Bibliography & References Cited	1240-BibliographyJan152015.pdf
10.Facilities & Other Resources	1241-Facilities_NoEqpt_ResilGrant.pdf
11.Equipment	1242-Major Equipment_ResilGrant.pdf

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

Project Summary

Bronchopulmonary dysplasia (BPD) is a common morbidity in extremely low birth weight (ELBW) infants. Our research group has considerable expertise in translational research on BPD. We have: (a) developed models using only limited clinical information to predict BPD or death by postnatal age or respiratory illness severity in ELBW infants, (b) prospectively evaluated pulmonary hypertension in BPD and (c) identified biomarkers associated with BPD/death in a cohort of 1067 ELBW infants using multiple clinical variables and 25 cytokines in blood. More recently, we have made novel observations on the genetic basis of BPD by genome-wide analysis in 751 ELBW infants. In additional ongoing studies, we have identified novel proteomic biomarkers of BPD, and have determined alterations in the airway microbiome in BPD.

The overall objective of "**STOP BPD**" (**Signature of Top Omic Profiles in BPD**) is to prospectively define and validate clinical and "omic" signatures associated with resilience against, or risk for development of BPD. To address this objective, we will build upon our recent studies on genomics, proteomics, respiratory microbiome, and model development in BPD. We will evaluate a prospective cohort (Generic Database or GDB cohort of the NICHD Neonatal Research Network) of 300 preterm infants <29 weeks gestation born in 2015-2017 (n=213 in 2013 alone, with 80%+ enrollment) by the following **Specific Aims**:

Specific Aim 1- Development and validation of a personalized genomic risk/resilience score for BPD and severe BPD using a combination of genome-wide expression analysis and targeted SNP profiling in blood collected within 72h of birth

Specific Aim 2 - (a) Development and validation of a personalized urinary proteomic risk/resilience score for BPD and severe BPD measured in the first postnatal week

(b) Development and validation of a personalized plasma proteomic and cytokine risk/resilience score for BPD and severe BPD measured within 72h of birth

Specific Aim 3 - Development and validation of a personalized airway microbiome risk/resilience score for BPD and severe BPD using tracheal aspirates collected in the first postnatal week

Specific Aim 4 - Development of a combined "Omic" scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model

We will develop and determine the accuracy of the various models in the Development cohort (n=150), and validate them in the Validation cohort (n=150). These novel models can be used to define the target population for future interventions, assess efficacy of specific interventions, develop a "lab on chip", and support future studies on the biology of BPD.

Project Narrative:

Bronchopulmonary dysplasia (BPD) is a common respiratory disorder in very preterm infants, characterized by impaired lung development, and associated with long-term respiratory complications. In this study, we will evaluate 300 extremely preterm infants to determine alterations in gene expression, protein amounts, or microbial flora in the airway that are associated with resilience (resistance to development of severe BPD, even when considered to be at high risk due to clinical risk factors) or predisposition (higher rate of developing severe BPD even if not initially considered at high risk).

FACILITIES AT UNIVERSITY OF ALABAMA AT BIRMINGHAM:

DR. AMBALAVANAN

CLINICAL:

Dr. Ambalavanan works as an Attending Physician at the Regional Neonatal Intensive Care Unit (RNICU) at UAB, and is Director of the Division of Neonatal Research. The unit now has 120 beds, consisting of 50 intensive care beds, and 70 intermediate nursery beds used for convalescing and chronically ill infants. In 2013, there were 213 admissions of extremely low birth weight infants, of whom 18% died, and 31% developed BPD. He also works as an Attending Physician at the Children's Hospital of Alabama NICU, a 48-bed facility in the 350-bed Children's Hospital of Alabama.

LABORATORY:

Clinical Laboratory: Suite 9380, Women and Infants Center: 225 sq.ft

Clinical laboratory space is located within the Division of Neonatology office space adjacent to the office for the research coordinator, equipped with counter space, refrigerated Eppendorf Centrifuge (5415R), -80^{o}C freezer; -20^{o}C freezer; 2-4^{o}C refrigerator.

Basic Research Laboratory:

Dr. Ambalavanan has laboratory space totaling 1534 sq.ft.; there are two adjacent offices totaling 314 sq. ft. All areas are contiguous and located on the sixth floor of Volker Hall, a major research facility on the UAB main campus. Dr. Ambalavanan founded and received support for a new Translational Research in Normal and Disordered Development (TReNDD) Program, which focuses on disorders of lung development and other organ systems during postnatal development. The TReNDD Program has core facilities for Molecular Analysis (flow cytometry, multiplex PCR, and multi-spectral imaging), cell and tissue culture, and Small Animal Physiology (flexiVent, MouseOx, and cardiovascular monitoring).

ANIMAL:

The animal facility is located in the basement of Volker Hall, in the same building as the Dr. Ambalavanan's lab. This facility has full time animal caretakers and facilities for short-term and long-term care under the direction of full-time veterinarians. Two 200 sq.ft rooms are available for Dr. Ambalavanan's hypoxia chamber, liquid nitrogen cylinder, oxygen tanks, and other equipment. The total space on campus for housing of animals is more than 100,000 sq.ft. The program has been AAALAC accredited since 1971.

COMPUTER:

Eight Dell personal computers running the Windows operating system are used for data storage and analysis. SigmaPlot v12 is used for statistical analysis and graphical display of data. The computer system is interfaced with the Department of Pediatrics server and provides access to Medline, Genbank, email, and the internet. Licenses for Ingenuity Pathway Analysis (IPA), JMP, Metamorph, and Nuance multispectral imaging software are available.

OFFICE:

Supporting offices with secretarial support measuring 172, 71, and 71 sq.ft. are adjacent to the laboratories. The Division of Neonatology financial and main offices are located in the Women and Infants' Center, 2 blocks from the laboratories.

OTHER: UAB has multiple fully-equipped core facilities, three of which will be used in this project: (1) The Heflin Center for Genomic Sciences that will be used in Aim 1 for the Genomic analyses (see letter of support from Dr. Crowley and Dr. Crossman), (2) The UAB Mass Spectrometry / Proteomics (MSP) Shared Facility to be used in Aim 2 for the Proteomic analyses (see letter of support from Dr. Mobley) and (3) The Microbiome/Bioinformatics/Gnotobiotic Animal Core to be used in Aim 3 for the airway microbiome analyses (see letter of support from Dr. Morrow).

UAB maintains several common campus-wide core facilities for oligonucleotide and peptide synthesis, monoclonal antibody production, GC/MS for protein analysis, cell sorting, protein and DNA sequencing, transgenic mice generation, and electron microscopic analysis. The UAB Multidisciplinary Molecular Interaction Core is equipped with Biacore T100 system which can be used for studies on protein-ligand interaction under a fee-for-service. The Core has extensive experience on the analyses of intra-molecular interactions in microbiological and immunological applications. The UAB Mass Spectrometry and Proteomics Shared Facility is equipped with multiple types of liquid chromatography and mass spectrometry systems including Waters Acquity UPLC system and Applied Biosystems API-3200/400 mass spectrophotometers. The UAB Transgenic Mouse Facility has extensive experience on establishment of

transgenic mice and gene targeting. The UAB Fermentation Facility offers custom fermentation services for bacterial and animal cell cultures and the production of recombinant proteins. They can provide products in volumes up to 400 liters under BSL2 containment. UAB also maintains a glasswork, machine, and electronic shops for production of specialized equipment not commercially available.

ENVIRONMENT

The University of Alabama at Birmingham (UAB)

UAB is an autonomous unit of the University of Alabama System. UAB, an urban university and major academic health center, occupies over 80 square blocks near downtown Birmingham and is composed of six schools in the Medical Center (Schools of Public Health, Medicine, Dentistry, Nursing, Optometry and Health Related professionals) and seven other schools including the Graduate School. The University includes 2 major research library facilities and substantial computing capacity. As one of three universities composing the University of Alabama System, UAB is committed to excellence in research, teaching, service and community outreach. UAB currently ranks among the top 15 percent of U.S. colleges and universities by *The Princeton Review*; attracts over $400 million annually in external research funding and ranks consistently in the top 25 nationally in funding from the National Institutes of Health; has been named among the Top 5 Best Places to Work in Academia by *The Scientist* in 2008; and, among 96 public and private universities (and the only Alabama university) is classified as an institution of “very high research activity” by the Carnegie Foundation. UAB’s growth as a world-renowned research university and medical center has driven the social, cultural, and economic revival of Birmingham. Consequently, the Carnegie Foundation recognizes UAB not only in its highest tier for research activity, but also in its highest tier for community engagement, making UAB one of a select few universities nationally to achieve both classifications.

MAJOR EQUIPMENT AT UNIVERSITY OF ALABAMA AT BIRMINGHAM:

DR. AMBALAVANAN

Main Lab: Room 643, Volker Hall: 1244 sq.ft.

-80^{o}C freezer x 3; -20^{o}C freezers x 2; 2-4^{o}C refrigerator; Bellco Model 7741 controlled atmosphere chamber, Eppendorf Model 5414 microcentrifuge, refrigerated Eppendorf Centrifuge (5415R), Picodrop DNA/RNA analyzer, Thermo Scientific Microm HM 550 Cryostat, Leica Vibratome VT1000P, Nikon Labophot microscope, Reichert Microstar IV fluorescence microscope, Denley WellScan microplate reader, Mettler Model H54 precision balance, Beckman DU-20 spectrophotometer, Bio-Rad Model 1000/500 power supply and assorted Bio-Rad gel apparatus, Perkin-Elmer PCR apparatus, Beckman J2-21 centrifuge with 2 rotors, IEC Contra-8R centrifuge, tissue homogenizer, and assorted water baths, platform rockers and dessicators, all necessary instruments and supplies for animal surgery; gas cylinder and other storage, assorted pH meters, balances, water baths, stir plates, etc. A darkroom, cold room, and autoclave facility are adjacent to the Main Lab.

Tissue Culture Room: Rm. 647, Volker Hall: 225 Sq.ft.

2-person Baker SG-600 Sterigard Type A Class II biological safety cabinet, Baker Model EG4252 EdgeGard laminar flow hood, SterilchemGard Model 4TX Biological Safety Cabinet, Queue Model 2720,2721 double chamber cell culture incubators, Queue Model QWJ2710ABA Cellstar cell culture incubator, Powers Scientific refrigerator/incubator, Dynac II centrifuge, Nikon TMS inverted microscope, Bausch and Lomb phase contrast microscope

Translational Research in Normal and Disordered Development or TReNDD Program

Image Analysis System: Room 651b: 65 sq.ft. Nikon Eclipse TE2000 microscope equipped with QiCAM fast imaging cooled CCD camera attached to a Pentium IV workstation with Metamorph v.6.2r4. Brightfield and fluorescence imaging (UV, green, and blue excitation wavelengths are available), as well as a multispectral imaging camera and image analysis system (Nuance system, CRi).

Laser Capture Microdissection System: Room 655: 65 sq.ft. The ArcturusXT™ LCM instrument has a combination of a gentle IR laser and a powerful UV laser that work in conjunction to efficiently isolate cells without changing morphology or integrity of the tissue. The IR laser captures cells of interest, and the UV laser microdissects captured cells.

flexiVent System: Room 651a: 65 sq.ft. flexiVent system (SciReq, Montreal, Canada), MouseOx (Starr Life Sciences, Oakmont, PA). It is equipped with a module 1, which is suitable for evaluating pulmonary function in small animals 10g-45g using the forced oscillation technique. The flexiVent system is an integrated system for mechanical ventilation, data acquisition, and analysis. It also has an Accessory Controller (XC) which extends the standard flexiVent system by four auxiliary channel inputs and four digital input/output ports. An Aeroneb is also integrated into the system, for methacholine and other nebulizations. Vital signs (heart rate, invasive blood pressure, and temperature) probes are also available. Additional facilities in this laboratory include a mouse oximeter (MouseOx) (STARR Life Sciences, Oakmont, PA), and equipment for mouse intubation and mouse restraining tubes.

IX/228 Data acquisition system and Scisense ADVantage Pressure Volume Measurement system (iWorx, Dover, NH) with 1.2F pressure-volume catheters and LabScribe2 software for measurement of cardiac output, pressures, and other hemodynamic data in mice.

Flow cytometer: Room 643. The Beckman Coulter Cell Lab Quanta SC MPL flow cytometer is available with 3-color, Coulter volume and side scatter analysis.

Flexcell FX-5000 tension system: Room 651a: A computer-regulated bioreactor (Flexcell International Corporation, Hillsborough, NC) that uses vacuum pressure to apply cyclic or static strain to cells cultured on flexible-bottomed culture plates.

OTHER:

UAB has multiple fully-equipped core facilities, three of which will be used in this project: (1) The Heflin Center for Genomic Sciences that will be used in Aim 1 for the Genomic analyses (see letter of support from Dr. Crowley and Dr. Crossman), (2) The UAB Mass Spectrometry / Proteomics (MSP) Shared Facility to be used in Aim 2 for the Proteomic analyses (see letter of support from Dr. Mobley) and (3) The Microbiome/Bioinformatics/Gnotobiotic Animal Core to be used in Aim 3 for the airway microbiome analyses (see letter of support from Dr. Morrow).

UAB maintains several other common campus-wide core facilities for oligonucleotide and peptide synthesis, monoclonal antibody production, GC/MS for protein analysis, cell sorting, protein and DNA sequencing, transgenic mice generation, and electron microscopic analysis. UAB also maintains a glasswork, machine, and electronic shops for production of specialized equipment not commercially available.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

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

Position/Title*: Professor
Organization Name*: The University of Alabama at Birmingham
Department: Pediatrics- Neonatology
Division: School of Medicine
Street1*: 1720 2nd Ave. South, WIC
Street2: 176F Suite 9380
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352940111

Phone Number*: 205-934-4680 Fax Number: 205-934-3100 E-Mail*: nambalavanan@peds.uab.edu

Credential, e.g., agency login: AMBALA

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

Degree Type: MBBS Degree Year: 1988

	File Name
Attach Biographical Sketch*:	1234-Ambal_Biosketch_Jan2015_Clinical.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT11812912 Funding Opportunity Number: RFA-HL-15-024 . Received Date: 2015-01-16T14:25:01.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Waldemar Middle Name A Last Name*: Carlo Suffix:

Position/Title*: Professor
Organization Name*: The University of Alabama at Birmingham
Department: Pediatrics- Neonatology
Division: School of Medicine
Street1*: 1720 2nd Ave. South, WIC
Street2: 176F Suite 9380
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352940111

Phone Number*: 205-934-4680 Fax Number: 205-934-3100 E-Mail*: wcarlo@peds.uab.edu

Credential, e.g., agency login: wacarlo

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

Degree Type: MD Degree Year: 1977

	File Name
Attach Biographical Sketch*:	1235-Carlo_Biosketch_Jan2015_Clinical.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

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

Position/Title*: Associate Professor
Organization Name*: The University of Alabama at Birmingham
Department: Biostatistics
Division:
Street1*: 1665 University Blvd
Street2: Ryals Bldg 327H
City*: Birmingham
County:
State*: AL: Alabama
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 352940111

Phone Number*: 205-996-4094 Fax Number: E-Mail*: kzhang@uab.edu

Credential, e.g., agency login: KUIZHANG

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

Degree Type: PhD Degree Year: 1999

	File Name
Attach Biographical Sketch*:	1236-KuiZhang_Biosketch_Jan2015.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person

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

Position/Title*:	Research Associate
Organization Name*:	The University of Alabama at Birmingham
Department:	Bioinformatics
Division:	
Street1*:	1720 7th Ave S, Suite 175
Street2:	
City*:	Birmingham
County:	
State*:	AL: Alabama
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	352940111

Phone Number*: 205-934-2508 Fax Number: E-Mail*: rkumar@uab.edu

Credential, e.g., agency login: RANKUMAR

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

Degree Type: PhD Degree Year: 2010

	File Name
Attach Biographical Sketch*:	1237-RanjitKumar_Biosketch_Jan2015.pdf
Attach Current & Pending Support:	

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Namasivayam Ambalavanan, M.D.	Professor of Pediatrics, Molecular and Cellular Pathology, Cell, Developmental, and Integrative Biology
eRA COMMONS USER NAME (credential, e.g., agency login) AMBALA	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
JIPMER, Pondicherry, India	M.B.,B.S.	1988	Medicine
PGIMER, Chandigarh, India	M.D. (Pediatrics)	1993	Pediatrics
University of Alabama at Birmingham, Birmingham, AL	Fellowship + Residency (PL-3 year)	1997	Pediatrics, Neonatology

A. Personal Statement

As Director of the Translational Research in Normal and Disordered Development (TReNDD) Program and the Division of Neonatal Research at the University of Alabama at Birmingham, I lead a basic science laboratory in addition to managing and directing single center and multicenter observational and interventional clinical studies. The University of Alabama at Birmingham is a regional perinatal center with approximately 350 VLBW infant births per year, and our center is a member of the NICHD Neonatal Research Network (I am alternate center PI), the Global Network, and the MFMU Network. The focus of my research is on biomarkers and prediction of outcome, and mechanisms of lung injury and development. I have recently published on genomic analyses in BPD, cytokine alterations in BPD, intercenter variations in BPD, and prediction models of BPD and respiratory failure in BPD. I also lead one of the four research centers in the LungMAP project funded by the NIH. In brief, I have a demonstrated record of successful and productive multicenter clinical, translational, and basic science research projects in the field of bronchopulmonary dysplasia, and am uniquely suited to be the Principal Investigator on this grant application.

B. Positions and Honors

Professional Experience

1993 - 1997	Fellow, Division of Neonatology, University of Alabama at Birmingham (UAB), Birmingham, AL, USA
1997-2000:	Pediatrician/Neonatologist, Jefferson Clinic P.C., Birmingham, AL (medically underserved area for adjustment of immigration status)
10/2000-9/2007:	Assistant Professor, Department of Pediatrics, UAB, Birmingham, AL
10/2007-9/2010:	Associate Professor, Department of Pediatrics, UAB, Birmingham, AL (Tenured)
10/2010-present:	Professor, Department of Pediatrics, UAB, Birmingham, AL (Tenured)
2007-2009:	Co-Director, TReNDD (Translational Research in Normal and Disordered Development) Program, UAB
2003-present:	Associate Director, Division of Neonatology, Department of Pediatrics, UAB
9/2009-present:	Director, TReNDD Program, *and* Director, Division of Neonatal Research, UAB

Honors and Awards

President, Southern Society for Pediatric Research (SSPR) 2005-2006
Young Investigator Award (Basic Science), Southern Society for Pediatric Research 2000
Grant Reviewer: NIH (ZRG1 SBIB V90; ZRG1 SBIB V82; ZRG1 SBIB-V55; ZRG1 CVRS G (03); LIRR (ad hoc)); AHA 2008→present; AAAS, 2007; Raine Medical Research Foundation (Ad hoc), 2005; PSI Foundation, Canada, 2003; Yale CreFF, 2002
Editorial Board: Pediatric Research, Am J Physiol Lung Cell Mol Physiol, Am J Perinatol
Best Doctors in America, 2005-present
Participant in Clinical Research Training Program (CRTP; NIH K30 award to UAB) 2000-2002
Highest score in the United States in the American Board of Pediatrics Certification examination (score of 770; 1996) and in the Neonatal-Perinatal medicine subspecialty certification examination (800; 1997)

Medical School: Endowment prizes (first in class) in Medicine, Pediatrics, Cardiology, and Radiology,1988

Society Memberships

American Pediatric Society; Society for Pediatric Research; American Thoracic Society; American Academy of Pediatrics; American Physiological Society; Southern Society for Pediatric Research

C. Selected Relevant Peer-reviewed Publications (15 recent from 170+ Medline-indexed publications)

1. **Ambalavanan N**, Cotten CM, Page GP, Carlo WA, Murray JC, Bhattacharya S, Mariani TJ, Cuna AC, Faye-Petersen OM, Kelly D, Higgins RD; Genomics and Cytokine Subcommittees of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Integrated Genomic Analyses in Bronchopulmonary Dysplasia. ***J Pediatr.*** 2014 Nov 6. [Epub ahead of print] PubMed PMID: 25449221. (PMC Journal in process)
2. Cuna A, Halloran B, Faye-Petersen O, Kelly D, Crossman DK, Cui X, Pandit K, Kaminski N, Bhattacharya S, Ahmad A, Mariani TJ, **Ambalavanan N**. Alterations in Gene Expression and DNA Methylation During Murine and Human Lung Alveolar Septation. ***Am J Respir Cell Mol Biol.*** 2014 Nov 11. [Epub ahead of print] PubMed PMID: 25387348. (PMC Journal in process)
3. **Ambalavanan N**, Carlo WA, Wrage LA, Das A, Laughon M, Cotten CM, Kennedy KA, Laptook AR, Shankaran S, Walsh MC, Higgins RD; For the SUPPORT Study Group of the NICHD Neonatal Research Network. PaCO2 in Surfactant, Positive Pressure, and Oxygenation Randomised Trial (SUPPORT). ***Arch Dis Child Fetal Neonatal Ed.*** 2014 Nov 25. [Epub ahead of print] PubMed PMID: 25425651. (PMC Journal in process)
4. Kelleher J, Bhat R, Salas AA, Addis D, Mills EC, Mallick H, Tripathi A, Pruitt EP, Roane C, McNair T, Owen J, **Ambalavanan N**, Carlo WA. Oronasopharyngeal suction versus wiping of the mouth and nose at birth: a randomised equivalency trial. ***Lancet***. 2013 Jul 27;382(9889):326-30. PubMed PMID: 23739521
5. Schulz MH, Pandit KV, Lino Cardenas CL, **Ambalavanan N**, Kaminski N, Bar-Joseph Z. Reconstructing dynamic microRNA-regulated interaction networks. ***Proc Natl Acad Sci U S A.*** 2013 Sep 24;110(39):15686-91. PMID:23986498. PMCID:PMC3785769
6. Askenazi DJ, Koralkar R, Hundley HE, Montesanti A, Parwar P, Sonjara S, **Ambalavanan N**. Urine biomarkers predict acute kidney injury in newborns. ***J Pediatr***. 2012 Aug;161(2):270-5.e1. PubMed PMID: 22424940. PubMed Central PMCID: PMC3598122
7. **Ambalavanan N**, Carlo WA, Tyson JE, Langer JC, Walsh MC, Parikh NA, Das A, Van Meurs KP, Shankaran S, Stoll BJ, Higgins RD; for the Generic Database; Follow-Up Subcommittees of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Outcome Trajectories in Extremely Preterm Infants. ***Pediatrics***. 2012 Jul;130(1):e115-e125. PubMed PMID: 22689874; PubMed Central PMCID: PMC3382921.
8. Askenazi DJ, Montesanti A, Hunley H, Koralkar R, Pawar P, Shuaib F, Liwo A, Devarajan P, **Ambalavanan N**. Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants**.** ***J Pediatr***. 2011 Dec;159(6):907-12.e1. PubMed PMID: 21784446.
9. **Ambalavanan N**, Carlo WA, McDonald SA, Yao Q, Das A, Higgins RD; Generic Database and Follow-up Subcommittees of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Identification of extremely premature infants at high risk of rehospitalization. ***Pediatrics***. 2011 Nov;128(5):e1216-25. PubMed PMID: 22007016; PubMed Central PMCID: PMC3208965.
10. Askenazi DJ, Koralkar R, Levitan EB, Goldstein SL, Devarajan P, Khandrika S, Mehta RL, **Ambalavanan N**. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. ***Pediatr Res.*** 2011 Sep;70(3):302-6. PubMed PMID: 21646940; PubMed Central PMCID: PMC3152663.
11. **Ambalavanan N**, Walsh M, Bobashev G, Das A, Levine B, Carlo WA, Higgins RD; NICHD Neonatal Research Network. Intercenter differences in bronchopulmonary dysplasia or death among very low birth weight infants. ***Pediatrics***. 2011 Jan;127(1):e106-16. PubMed PMID: 21149431; PubMed Central PMCID: PMC3010091.
12. SUPPORT Study Group of the Eunice Kennedy Shriver NICHD Neonatal Research Network, Carlo WA, Finer NN, Walsh MC, Rich W, Gantz MG, Laptook AR, Yoder BA, Faix RG, Das A, Poole WK, Schibler K, Newman NS, **Ambalavanan N**, Frantz ID 3rd, Piazza AJ, Sánchez PJ, Morris BH, Laroia N, Phelps DL, Poindexter BB, Cotten CM, Van Meurs KP, Duara S, Narendran V, Sood BG, O'Shea TM, Bell EF, Ehrenkranz RA, Watterberg KL, Higgins RD. Target ranges of oxygen saturation in extremely preterm

infants. ***N Engl J Med.*** 2010 May 27;362(21):1959-69. PubMed PMID: 20472937; PubMed Central PMCID: PMC2891970.
13. McKee LA, Fabres J, Howard G, Peralta-Carcelen M, Carlo WA, **Ambalavanan N**. PaCO2 and neurodevelopment in extremely low birth weight infants. ***J Pediatr.*** 2009 Aug;155(2):217-21.e1. PubMed PMID: 19447409.
14. **Ambalavanan N**, Carlo WA, D'Angio CT, McDonald SA, Das A, Schendel D, Thorsen P, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Cytokines associated with bronchopulmonary dysplasia or death in extremely low birth weight infants. ***Pediatrics***. 2009 Apr;123(4):1132-41. PubMed PMID: 19336372; PubMed Central PMCID: PMC2903210.
15. **Ambalavanan N**, Carlo WA, Shankaran S, Bann CM, Emrich SL, Higgins RD, Tyson JE, O'Shea TM, Laptook AR, Ehrenkranz RA, Donovan EF, Walsh MC, Goldberg RN, Das A; National Institute of Child Health and Human Development Neonatal Research Network. Predicting outcomes of neonates diagnosed with hypoxemic-ischemic encephalopathy. ***Pediatrics***. 2006 Nov;118(5):2084-93. PubMed PMID: 17079582.

D. Research Support

Active:

1) U01 HL122626 (Corresponding PI: Ambalavanan) 6/15/14-4/30/19

"Alveolar DevMAP"

NIH/NHLBI Annual direct costs: $636,650

The overall objective of "Alveolar DevMAP" in response to RFA-HL-14-008 Molecular Atlas of Lung Development - Research Center (RC) (U01) is to generate a compendium of the dynamic and regional changes in epigenetic marks, microRNA, mRNA and proteins that happen during alveolar septation, and use this compendium to generate a dynamic temporal regulatory model of normal alveolar septation.

2) R01 HD067126 (PI: Rose Viscardi) Ambalavanan (Site PI) 12/1/10-11/30/15

NIH/NICHD

Azithromycin to prevent BPD in *Ureaplasma*-infected preterms: A multi-dose PK and efficacy trial

This proposal will 1) characterize the multiple-dose pharmacokinetics (PK), tolerability, safety, and biologic effects of IV azithromycin (AZI) in mechanically ventilated preterm neonates born 24-28 weeks gestation at high risk for Ureaplasma spp. respiratory tract colonization and subsequent development of BPD; 2) conduct a multicenter, randomized, double-blind, placebo-controlled Phase IIb clinical trial to determine the safety and microbiological efficacy of a multiple dose course of intravenous AZI to eradicate respiratory tract Ureaplasma infection that might lead to physiologic BPD in preterm neonates; and 3) compare the pulmonary outcomes at 36 wk postmenstrual age and 6 months adjusted age in infants treated with AZI vs placebo.

3) R01 HD066982 (PI: A. Catharine Ross) Ambalavanan (Co-Investigator) 8/20/10- 7/31/2015

NIH/NICHD

Vitamin A supplementation and Retinol metabolism in the Neonatal Period

Dr. Ambalavanan's role is to perform experiments in Aim 4: To determine whether vitamin A (VA) and VA+ retinoic acid (RA) will reduce inflammation, increase the alveolar surface area, promote vascular remodeling, and improve measures of lung function such as compliance in newborn mice exposed to hyperoxic conditions, 85% O2 vs. 21% O2 (room air), as control

4) U10 HD34216 (PI: Wally Carlo) Ambalavanan (Co-Investigator) 04/01/96 – 03/31/2016

NIH/ NICHD

Multi-center Network of Neonatal Intensive Care Units

The major goals of this project are to work with the NICHD and the Steering Committee to prioritize, plan, implement, analyze, interpret, and report a series of randomized and observational studies.

Completed:

1) R01 HL092906 Ambalavanan (PI) 07/15/2008 - 6/30/2014

NIH / NHLBI

Transforming Growth Factor -Beta Mediates Effects of Hypoxia in Newborn Lung

2) R01 HD059140 (PI: Ardythe Morrow) Ambalavanan (Co-Investigator) 12/01/2008-11/30/2013
NIH/ NICHD
Novel genetic and salivary glycan biomarkers for risk of NEC in ELBW infants

3) K08 HD046513 Ambalavanan (PI) 4/1/2004 – 3/31/2009
NIH/NICHD K08 Mentored Clinical Scientist Development Award
MMP-2 in Neonatal Hypoxic Pulmonary Vascular Remodeling

4) KPRI Senior Investigator Award (PI: Ambalavanan) 02/22/12-1/30/2014
Kaul Pediatric Research Institute
Vitamin D Supplementation for Extremely Preterm Infants

5) ATS PH-06-006 Ambalavanan (PI) 1/1/2007 – 12/31/2008
ATS/PHA ATS/PHA Research Grant
Regulation of TGF-Beta in Neonatal Hypoxia Induced Pulmonary Vascular Remodeling

6) R03 HD054420-01 Ambalavanan (PI) 3/1/2007 – 2/27/2009
NIH/ NICHD
C-Reactive Protein in Extremely Low Birth Weight Neonates

7) Ferzli (PI) (Role: Mentor/Sponsor) 7/01/2005-06/30/2007
Pulmonary Hypertension Association PHA #0526041H
Lung Assist Device in Neonatal Porcine Respiratory Failure: Effect on Pulmonary Histopathology and Lung Mechanics

8) Ambalavanan (PI) 1/01/2006-12/31/2007
Children's Center for Research and Innovation
Transforming Growth Factor β (TGF-b) and Neonatal Lung Development in Hypoxia

9) 5U01 ES015676-04 (PI: Sadis Matalon) Ambalavanan (Co-Investigator) 9/29/2006-5/31/2011
NIH/NIEHS
Prevention and treatment of chlorine gas induced injury to the pulmonary system
.
10) R01 HL092906-02S1 Ambalavanan (PI) 7/15/2009 –5/31/2010
NIH / NHLBI
Transforming Growth Factor -Beta Mediates Effects of Hypoxia in Newborn Lung

11) NA09OAR4170199 (PI: Sadis Matalon) Ambalavanan (Project 4 leader) 8/1/2009 – 7/31/2011
NOAA
Nanoparticle induced injury to adult and developing lungs

12) F31HL102910 (PI: Masheika James) Ambalavanan (Sponsor) 06/01/2010 – 05/30/2012
NIH/NHLBI
Retinoids and Hyperoxic Lung Injury

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Waldemar A. Carlo, M.D.	Professor and Division Director
eRA COMMONS USER NAME (credential, e.g., agency login) wacarlo	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Univ. of Puerto Rico, Mayaguez, PR	B.S.	05/73	Biology
Univ. of Puerto Rico Med Sci Campus, San Juan, PR	M.D.	05/77	Medicine
Univ Children's Hospital, PR Med Ctr, San Juan, PR	Residency	06/80	Pediatrics
Rainbow Babies & Child Hospital, Cleveland, Ohio	Fellowship	06/82	Neonatology

A. Personal Statement

I have extensive experience in clinical research including the design, implementation, data analysis, and reporting of neonatal and childhood research performed in developing countries and in the US including the First Breath Trial, the BRAIN-HIT Trial, and the SUPPORT Trial. I have also done experimental and epidemiological research. I lead clinical sites of two NIH-funded networks including the Global Network for Women's and Children's Health Research and the Neonatal Research Network. I have successfully implemented two large scale multicenter studies in 6 developing countries that enrolled over 190,000 babies and resulted in large and significant reduction in neonatal and perinatal mortality. I have developed a high frequency ventilator, a flow interrupter to perform pulmonary function testing, and a low-cost oxygen air blender. I am focused on reducing mortality and major morbidities during early childhood in the US and developing countries funded by the NIH. I was the PI for the NICHD NRN Cytokine study, the samples from which initiated the Genomics studies of the NRN. For this "STOP BPD" study, as center PI for the NICHD NRN and the GDB cohort study, I will assist Dr. Ambalavanan with patient enrollment in this study, and with the evaluation of clinical and "Omic" models for resilience and predisposition to severe BPD/death.

B. Positions and Honors

1982 - 1989	Assistant Prof of Pediatrics, Case Western Reserve School of Med, Cleveland, Ohio
1986 - 1990	Associate Director, NICU, Rainbow Babies & Children's Hospital, Cleveland, Ohio
1989 - 1990	Associate Prof of Pediatrics, Case Western Reserve School of Med, Cleveland, Ohio
1991 – present	Professor of Pediatrics, Director of Neonatology, University of Alabama at Birmingham
1994 - present	Selected, Best Doctors in America
1995 – present	PI, NICHD Neonatal Research Network Grant
1998 – 2003	Co-Chairperson Neonatal Resuscitation Program, American Academy of Pediatrics
2000 – 2003	NICHD Maternal & Child Health Research Subcommittee (Study Section)
2000 – present	Edwin M. Dixon Endowed Chair in Neonatology
2001	University of Alabama Hospital Award of Excellence
2003 – 2007	NICHD Pediatrics Subcommittee (Study Section)
2003 – present	PI, NICHD Global Network for Women's and Children's Health Grant
2004	Chairman's Award Department of Pediatrics (highest Department award), UAB
2004	Friend of the Housestaff Award (highest award by the pediatric residents), UAB
2007	University of Alabama School of Medicine Argus Society Award for Excellence in Teaching
2009	University of Alabama School of Medicine Dean's Award for Excellence in Clinical Scholarship
2011	Children's of Alabama, Centennial Award
2012	University of Alabama at Birmingham, Presidential Award 2012 Award for Diversity
2012	American Academy of Pediatrics, 2012 Virginia Apgar Award
2013	Joseph Butterfield Lecture 'How to Save One Million Perinatal Lives Per Year: Helping Babies Breathe and Essential newborn Care Training Programs

C. Selected Peer-reviewed Publications (15 selected from >300 peer-reviewed publications)

1) Ambalavanan N, Cotten CM, Page GP, **Carlo WA**, Murray JC, Bhattacharya S, Mariani TJ, Cuna AC, Faye-Petersen OM, Kelly D, Higgins RD; Genomics and Cytokine Subcommittees of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Integrated Genomic Analyses in Bronchopulmonary Dysplasia. ***J Pediatr.*** 2014 Nov 6. [Epub ahead of print] PubMed PMID: 25449221.(PMC Journal in process)

2) Ambalavanan N, **Carlo WA**, Wrage LA, Das A, Laughon M, Cotten CM, Kennedy KA, Laptook AR, Shankaran S, Walsh MC, Higgins RD; For the SUPPORT Study Group of the NICHD Neonatal Research Network. PaCO2 in Surfactant, Positive Pressure, and Oxygenation Randomised Trial (SUPPORT). ***Arch Dis Child Fetal Neonatal Ed.*** 2014 Nov 25. [Epub ahead of print] PubMed PMID: 25425651.(PMC Journal in process)

3) Kelleher J, Bhat R, Salas AA, Addis D, Mills EC, Mallick H, Tripathi A, Pruitt EP, Roane C, McNair T, Owen J, Ambalavanan N, **Carlo WA**. Oronasopharyngeal suction versus wiping of the mouth and nose at birth: a randomised equivalency trial. ***Lancet.*** 2013 Jul 27;382(9889):326-30. PubMed PMID: 23739521.

4) Ambalavanan N, **Carlo WA**, Tyson JE, Langer JC, Walsh MC, Parikh NA, Das A, Van Meurs KP, Shankaran S, Stoll BJ, Higgins RD; Generic Database; Subcommittees of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Outcome trajectories in extremely preterm infants. ***Pediatrics.*** 2012 Jul;130(1):e115-25. PubMed PMID: 22689874; PubMed Central PMCID: PMC3382921.

5) **Carlo WA**, McDonald SA, Fanaroff AA, Vohr BR, Stoll BJ, Ehrenkranz RA, Andrews WW, Wallace D, Das A, Bell EF, Walsh MC, Laptook AR, Shankaran S, Poindexter BB, Hale EC, Newman NS, Davis AS, Schibler K, Kennedy KA, Sánchez PJ, Van Meurs KP, Goldberg RN, Watterberg KL, Faix RG, Frantz ID 3rd, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22 to 25 weeks' gestation. ***JAMA***. 2011 Dec 7;306(21):2348-58. PubMed PMID: 22147379; PubMed Central PMCID: PMC3565238.

6) Moorman JR, **Carlo WA**, Kattwinkel J, Schelonka RL, Porcelli PJ, Navarrete CT, Bancalari E, Aschner JL, Whit Walker M, Perez JA, Palmer C, Stukenborg GJ, Lake DE, Michael O'Shea T. Mortality reduction by heart rate characteristic monitoring in very low birth weight neonates: a randomized trial. ***J Pediatr.*** 2011 Dec;159(6):900-6.e1. PubMed PMID: 21864846; PubMed Central PMCID: PMC3215822.

7) **Carlo WA**, McDonald SA, Tyson JE, Stoll BJ, Ehrenkranz RA, Shankaran S,Goldberg RN, Das A, Schendel D, Thorsen P, Skogstrand K, Hougaard DM, Oh W, Laptook AR, Duara S, Fanaroff AA, Donovan EF, Korones SB, Stevenson DK, Papile LA, Finer NN, O'Shea TM, Poindexter BB, Wright LL, Ambalavanan N, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Cytokines and neurodevelopmental outcomes in extremely low birth weight infants***. J Pediatr.*** 2011 Dec;159(6):919-25.e3. PubMed PMID: 21798559; PubMed Central PMCID: PMC3215787.

8) **Carlo WA**, Finer NN, Walsh MC, Rich W, Gantz MG, Laptook AR, Yoder BA, Faix RG, Das A, Poole WK, Schibler K, Newman NS, Ambalavanan N, Frantz ID 3rd, Piazza AJ, Sánchez PJ, Morris BH, Laroia N, Phelps DL, Poindexter BB, Cotten CM, Van Meurs KP, Duara S, Narendran V, Sood BG, O'Shea TM, Bell EF, Ehrenkranz RA, Watterberg KL, Higgins RD. Target ranges of oxygen saturation in extremely preterm infants. ***N Engl J Med.*** 2010 May 27;362(21):1959-69. PubMed PMID: 20472937; PubMed Central PMCID: PMC2891970.

9) Finer NN, **Carlo WA**, Walsh MC, Rich W, Gantz MG, Laptook AR, Yoder BA, Faix RG, Das A, Poole WK, Donovan EF, Newman NS, Ambalavanan N, Frantz ID 3rd, Buchter S, Sánchez PJ, Kennedy KA, Laroia N, Poindexter BB, Cotten CM, Van Meurs KP, Duara S, Narendran V, Sood BG, O'Shea TM, Bell EF, Bhandari V, Watterberg KL, Higgins RD. Early CPAP versus surfactant in extremely preterm infants. ***N Engl J Med.*** 2010 May 27;362(21):1970-9. PubMed PMID: 20472939; PubMed Central PMCID: PMC3071534.

10) **Carlo WA**, Goudar SS, Jehan I, Chomba E, Tshefu A, Garces A, Parida S, Althabe F, McClure EM, Derman RJ, Goldenberg RL, Bose C, Krebs NF, Panigrahi P, Buekens P, Chakraborty H, Hartwell TD, Wright LL; First Breath Study Group. Newborn-care training and perinatal mortality in developing countries. ***N Engl J Med***. 2010 Feb 18;362(7):614-23. PubMed PMID: 20164485; PubMed Central PMCID: PMC3565382.

11) Ambalavanan N, **Carlo WA**, D'Angio CT, McDonald SA, Das A, Schendel D, Thorsen P, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Cytokines associated with bronchopulmonary dysplasia or death in extremely low birth weight infants. ***Pediatrics***. 2009 Apr;123(4):1132-41. PubMed PMID: 19336372; PubMed Central PMCID: PMC2903210.
12) Ambalavanan N, Van Meurs KP, Perritt R, **Carlo WA**, Ehrenkranz RA, Stevenson DK, Lemons JA, Poole WK, Higgins RD; NICHD Neonatal Research Network, Bethesda, MD. Predictors of death or bronchopulmonary dysplasia in preterm infants with respiratory failure. ***J Perinatol.*** 2008 Jun;28(6):420-6. PubMed PMID: 18337740; PubMed Central PMCID: PMC2776028.
13) Ambalavanan N, **Carlo WA**, Shankaran S, Bann CM, Emrich SL, Higgins RD, Tyson JE, O'Shea TM, Laptook AR, Ehrenkranz RA, Donovan EF, Walsh MC, Goldberg RN, Das A; National Institute of Child Health and Human Development Neonatal Research Network. Predicting outcomes of neonates diagnosed with hypoxemic-ischemic encephalopathy. ***Pediatrics***. 2006 Nov;118(5):2084-93. PubMed PMID: 17079582.
14) Boulet SL, Alexander GR, Salihu HM, Kirby RS, **Carlo WA**. Fetal growth risk curves: defining levels of fetal growth restriction by neonatal death risk. ***Am J Obstet Gynecol.*** 2006 Dec;195(6):1571-7. Epub 2006 Jun 12. PubMed PMID: 16769013.
15) **Carlo WA**, Stark AR, Wright LL, Tyson JE, Papile LA, Shankaran S, Donovan EF, Oh W, Bauer CR, Saha S, Poole WK, Stoll B. Minimal ventilation to prevent bronchopulmonary dysplasia in extremely-low-birth-weight infants. ***J Pediatr.*** 2002 Sep;141(3):370-4. PubMed PMID: 12219057.

D. Research Support

Active:

2 U10 HD034216 (Carlo) 05/06/96 – 03/31/16 1.5 calendar (12.5%)
NIH $273,886 year 17
Cooperative Multicenter Neonatal Research Network
The major goals of this project are to work with the NICHD and the Steering Committee to prioritize, plan, implement, analyze, interpret, and report a series of randomized and observational studies.

1 U10 HD078437 (Carlo) 05/03/2013 – 04/30/18 1.2 calendar (10%)
NIH $602,300 year 1
Heart Rate Detection during Resuscitation to Reduce Early Neonatal Mortality
The major goals of this project are to evaluate the effectiveness of early identification and innovative but simple antibiotic regimens to treat young infants with suspected serious bacterial infection in low and middle income countries. This project will continue to establish an innovative and flexible research network that will be responsive to the most critical existing and emerging health needs and public health problems of women and children, globally. Investigators will work among women and children, primarily in developing countries.

<u>Completed Research Support</u> (In last three years, partial list)

5 U01 HD043464 (Carlo) 09/26/03 – 04/30/13
NIH
Global Network for Women's and Children's Health Research: Community Based Interventions to Reduce Mortality from Neonatal Infection
The major goals of this project are to evaluate the effectiveness of early identification and innovative but simple antibiotic regimens to treat young infants with suspected serious bacterial infection in low and middle income countries. This project will continue to establish an innovative and flexible research network that will be responsive to the most critical existing and emerging health needs and public health problems of women and children, globally. Investigators will work among women and children, primarily in developing countries.

1 R01 NS053865 (Ment) 06/01/07 – 05/31/13
NIH/Pass through Yale University
Gene Targets for Intraventricular Hemorrhage (IVH)
Role: Site PI (Carlo)
For very low birth weight infants IVH is attributable to a combination of environmental and genetic factors. This project seeks to identify those alleles and haplotypes which provide either susceptibility or protection for IVH.

R01 HD053055 (Carlo) 09/29/06 – 07/31/13
NICHD
Brain Research to Ameliorate Impaired Neurodevelopment – Home-Based Intervention
The major goals of this project are to identify infants at risk for neurodevelopmental disorders and to implement an innovative intervention trial in India, Pakistan, and Zambia.

1 R01 HD048562 (Moorman) 06/01/05 – 05/31/10
NIH/ Pass through University of Virginia
Impact of Neonatal Heart Rate Characteristics Monitoring
Role: Site PI (Carlo)
The major goal of this project is to compare outcomes of infants when heart rate characteristics index information is displayed to health care personnel in the NICU.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kui Zhang	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) KUIZHANG	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Beijing University, P. R. China	B.Sc.	1990-1994	Probability and Statistics
Beijing University, P. R. China	Ph.D.	1994-1999	Probability and Statistics
Yale University, USA	Postdoctoral	1999-2001	Statistical Genetics
University of Southern California, USA	Postdoctoral	2001-2003	Statistical Genetics

A. Personal Statement

This R01 grant application aims to prospectively define and validate clinical and "omic" signatures associated with resilience against, or risk for development of bronchopulmonary dysplasia (BPD), with the goal of informing the development of primary prevention strategies for BPD. The major research activities involve discovery of genes, urinary proteomic, plasma proteomic, cytokine, and microbiome profiles that are associated with resilience/predisposition to severe BPD and development of a combined "Omic" scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model to predict BPD outcomes. The application involves the analysis of gene expression data, proteomic data, microbiome data and the use of statistical model to construct a score system for predicting BPD outcomes. As a co-investigator and statistical geneticist, I will offer expertise in statistical analysis including bioinformatics and biostatistics, facilitate high-dimensional analysis of gene expression, proteomic, and microbiome, and develop appropriate statistical models. I have gained a solid training in statistics and have been working on methodological development in statistical genetics for over fifteen years. As a PI or co-investigator of several NIH-funded projects, I have developed a number of novel statistical methods, including methods for the analysis of gene expression and proteomic data. I have also collaborated with several investigators, to apply our methods and other available methods in their real data analysis. Thus, my experiences with successful and productive projects covering research areas highly relevant to the proposed studies have prepared me well to serve as a co-investigator for this project.

B. Positions and Honors (List in chronological order previous positions, concluding with the present position. List any honors. Include present membership on any Federal Government public advisory committee)

Professional Experience

1994-1997	Teaching Assistant, Department of Probability and Statistics, Beijing University
1997-1998	Instructor, College of Applied Science and Arts, Beijing University
1999-2001	Postdoctoral Associate, Department of Epidemiology and Public Health, Yale University School of Medicine
2001-2003	Postdoctoral Associate, Program of Molecular and Computational Biology, Department of Biological Sciences, University of Southern California
2003-2008	Research Assistant Professor, Section on Statistical Genetics, Department of Biostatistics, University of Alabama at Birmingham
2008-Present	Associate Professor, Section on Statistical Genetics, Department of Biostatistics, University of Alabama at Birmingham

Other Experience and Professional Membership

2000-Present	Member, American Society of Human Genetics

Honors

1991-1993	The First Class Guang Hua Scholarship, Beijing University
1993	The Excellent Students Scholarship of Beijing City, Beijing University
1995	Dong Shi Dong Fang Scholarship, Beijing University

1996	Jiu Zhang Suan Shu Scholarship, Beijing University
2008	The Science Unbound Foundation 2007 Best Paper Award for Statistical Genetics Research at University of Alabama at Birmingham
2014	The Science Unbound Foundation 2013 Best Paper Award for Statistical Genetics Research at University of Alabama at Birmingham

C. Selected Peer-reviewed Publications

1) Wu J, Chen GB, Zhi D, Liu N, **Zhang K**. A hidden Markov model for haplotype inference for present-absent data of clustered genes using identified haplotypes and haplotype patterns. ***Front Genet.*** 2014 Aug 12;5:267. PubMed PMID: 25161663; PubMed Central PMCID: PMC4129397.
2) Nguyen TL, Grizzle WE, **Zhang K**, Hameed O, Siegal GP, Wei S. Syndecan-1 overexpression is associated with nonluminal subtypes and poor prognosis in advanced breast cancer. ***Am J Clin Pathol.*** 2013 Oct;140(4):468-74. PubMed PMID: 24045542.
3) **Zhang K**, Zhi D. Joint haplotype phasing and genotype calling of multiple individuals using haplotype informative reads. ***Bioinformatics.*** 2013 Oct 1;29(19):2427-34. PubMed PMID: 23943637; PubMed Central PMCID: PMC3777110.
4) Rao W, Ma Y, Ma L, Zhao J, Li Q, Gu W, **Zhang K**, Bond VC, Song Q. High-resolution whole-genome haplotyping using limited seed data. ***Nat Methods.*** 2013 Jan;10(1):6-7. PubMed PMID: 23269372; PubMed Central PMCID: PMC3835542.
5) Sanders YY, Ambalavanan N, Halloran B, Zhang X, Liu H, Crossman DK, Bray M, **Zhang K**, Thannickal VJ, Hagood JS. Altered DNA methylation profile in idiopathic pulmonary fibrosis. ***Am J Respir Crit Care Med.*** 2012 Sep 15;186(6):525-35. PubMed PMID: 22700861; PubMed Central PMCID: PMC3480526.
6) Zhi D, Wu J, Liu N, **Zhang K**. Genotype calling from next-generation sequencing data using haplotype information of reads. ***Bioinformatics.*** 2012 Apr 1;28(7):938-46. PubMed PMID: 22285565; PubMed Central PMCID: PMC3493122.
7) Steg AD, Bevis KS, Katre AA, Ziebarth A, Dobbin ZC, Alvarez RD, **Zhang K**, Conner M, Landen CN. Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. ***Clin Cancer Res.*** 2012 Feb 1;18(3):869-81. PubMed PMID: 22142828; PubMed Central PMCID: PMC3271164.
8) Azrad M, **Zhang K**, Vollmer RT, Madden J, Polascik TJ, Snyder DC, Ruffin MT, Moul JW, Brenner D, Hardy RW, Demark-Wahnefried W. Prostatic alpha-linolenic acid (ALA) is positively associated with aggressive prostate cancer: a relationship which may depend on genetic variation in ALA metabolism. ***PLoS One***. 2012;7(12):e53104. Epub 2012 Dec 28. PubMed PMID: 23285256; PubMed Central PMCID: PMC3532426.
9) Gao L, Fang Z, **Zhang K**, Zhi D, Cui X. Length bias correction for RNA-seq data in gene set analyses. ***Bioinformatics***. 2011 Mar 1;27(5):662-9. PubMed PMID: 21252076; PubMed Central PMCID: PMC3042188.
10) Wu X, Patki A, Lara-Castro C, Cui X, **Zhang K**, Walton RG, Osier MV, Gadbury GL, Allison DB, Martin M, Garvey WT. Genes and biochemical pathways in human skeletal muscle affecting resting energy expenditure and fuel partitioning. ***J Appl Physiol*** (1985). 2011 Mar;110(3):746-55. PubMed PMID: 21109598; PubMed Central PMCID: PMC3070475.
11) Pasche B, Wisinski KB, Sadim M, Kaklamani V, Pennison MJ, Zeng Q, Bellam N, Zimmerman J, Yi N, **Zhang K**, Baron J, Stram DO, Hayes MG. Constitutively decreased TGFBR1 allelic expression is a common finding in colorectal cancer and is associated with three TGFBR1 SNPs. ***J Exp Clin Cancer Res.*** 2010 May 25;29:57. PubMed PMID: 20500843; PubMed Central PMCID: PMC2890549.
12) Grunda JM, Nabors LB, Palmer CA, Chhieng DC, Steg A, Mikkelsen T, Diasio RB, **Zhang K**, Allison D, Grizzle WE, Wang W, Gillespie GY, Johnson MR. Increased expression of thymidylate synthetase (TS), ubiquitin specific protease 10 (USP10)and survivin is associated with poor survival in glioblastoma multiforme (GBM). ***J Neurooncol.*** 2006 Dec;80(3):261-74. PubMed PMID: 16773218.
13) Deng M, **Zhang K**, Mehta S, Chen T, Sun F. Prediction of protein function using protein-protein interaction data. ***J Comput Biol.*** 2003;10(6):947-60. PubMed PMID: 14980019.
14) **Zhang K**, Deng M, Chen T, Waterman MS, Sun F. A dynamic programming algorithm for haplotype block partitioning. ***Proc Natl Acad Sci U S A.*** 2002 May 28;99(11):7335-9. PubMed PMID: 12032283; PubMed Central PMCID: PMC124231.
15) **Zhang K**, Zhao H. Assessing reliability of gene clusters from gene expression data. ***Funct Integr Genomics.*** 2000 Nov;1(3):156-73. PubMed PMID: 11793234.

D. Research Support

Ongoing Research Support

NIH/NHGRI-R01-HG008115-01 (Zhang, Yu, and Zhi) 09/10/2015-06/30/2017
Next-Generation Bioinformatics for Next-Generation Sequencing
To develop an integrative and novel analytical framework that can significantly improve the sensitivity and accuracy of rare variant discovery and haplotype phasing, and harmonize multiple datasets within and across genomics studies.
Role: Principal Investigator

NIH/NIGMS-R01-GM-081488 (Liu) 04/01/2008-03/31/2015
Genome Wide Haplotype Association Analysis.
To develop novel statistical and computational methods and software tools for the analysis of haplotypes in mapping of complex human disease genes, especially in the presence of missing genotypes and genotyping errors and with large number of markers.
Role: Co-Investigator

NIH/NIAID/Emory University R01-AI-064060 (Hunter) 04/01/2010- 03/31/2015
CTL and HIV Polymorphisms in Heterosexual Transmission
The major goal of this project is to understand the role that cytotoxic T lymphocyte (CTL) escape plays in HIV transmission and disease pathogenesis.
Role: Co-Investigator

Completed Research Support

NIH/NCI R01CA106168 (Kaslow) 07/01/2004-06/30/2009
Chromosome 6p21-24 Markers in HIV-Related Kaposi Sarcoma
To search beyond the reported associations of *HLA* class II alleles with HIV-KS for alternative genetic determinants within and telomeric to the *HLA* complex.
Role: Co-Investigator

NIH/NIAID HHSN266200400068C (Kaslow) 09/01/2004-08/31/2009
Population Genetics Analysis Program: Immunity to Vaccines/Infections
To identify host genetic characteristics that determine and predict the variability in antibody responses and adverse reactions to anthrax vaccine (AVA).
Role: Co-Investigator

NIH/NIAID/Emory University R01-AI064060 (Kaslow) 02/15/2005 - 1/31/2009
CTL and HIV Polymorphisms in Heterosexual Transmission
To study CTL and HIV Polymorphisms in Heterosexual Transmission.
Role: Co-Investigator

NIH/NIGMS R01GM74913 (Zhang) 07/01/2006-06/30/2012
Haplotype Analysis in Linkage Disequilibrium Mapping.
To develop association methods based on haplotypes for mapping genes that are responsible for complex human diseases.
Role: Principal Investigator

NIH/NIGMS-R01-GM-073766 (Gao) 07/01/2007-06/30/2012
Haplotyping and QTL Mapping in Pedigrees with Missing Data.
The major goal is to develop haplotyping and IBD probability estimation methods for large pedigrees with large numbers of loci and with missing marker data.
Role: Co-Investigator

NIH/NHGRI-R13-HG-004593 (Zhang) 09/01/2007-08/31/2008
Haplotype analysis of population and pedigree data in association studies.
To organize a scientific meeting to discuss the haplotype analysis in association studies
Role: Principal Investigator

NIH/NIAID R01-AI-071906 (Kaslow) 05/01/2008 – 04/30/2014
Host Genetic Epidemiology in HIV-1-Discordant African Couples and Other Cohorts
The overall goal of this project is to investigate the influence of polymorphism in genes regulating two major pathways in the pathogenesis of HIV/AIDS among HIV-1 discordant African couples.
Role: Co-Investigator

NIH/NIDA R01DA025095 (Lou) 07/01/2008-04/10/2014
Detection of multifactor interactions with application to nicotine dependence
The major goal of this project is to develop new method (GMDR) and computer software for identifying gene-gene and gene-environment interactions underlying complex diseases and to detect interactive susceptibility loci or genes for nicotine dependence in cigarette smokers.
Role: Co-Investigator

NIH/NICHD/NIEHS R01-HD-064398 (Aissani) 04/01/2010 – 03/31/2014
Genetic Determinants of Uterine Fibroids in African-American and Caucasian Women
The major goal of this project is to dissect the genetic architecture of a subregion of human chromosome 1q43 associated with rare familial syndromes of uterine leiomyomas (fibroids) to evaluate its effects on the population risk.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Kumar, Ranjit	Research Associate, Bioinformatics
eRA COMMONS USER NAME (credential, e.g., agency login) RANKUMAR	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Indira Gandhi National Open University, New Delhi, India	BIT	08/2003	Computer Science
Institute of Bioinformatics and applied Biotechnology, Bangalore, India	P.G. Diploma	04/2005	Bioinformatics
Mississippi State University, Mississippi	Ph.D.	12/2010	Bioinformatics

A. Personal Statement

My research interests are focused towards understanding biological complexity by utilizing modern technologies including those encompassing genomics, transcriptomics and proteomics. This includes development of new data analysis methods, pipelines, databases and data mining techniques. My educational background covered both computer science and bioinformatics, which provided me with the necessary skills required for this profession. Having a bachelor's degree in computer science helps me to deal with large high-throughput biological datasets programmatically, whereas my master's and PhD degree in Bioinformatics made me aware of biological concepts and analytical skills. During my PhD (title: Development of computational tools and resources for Systems Biology of bacterial pathogens) I was involved in development of several tools including TAAPP (tiling array data analysis pipeline) and a proteomics data analysis workflow. During my PhD, I was involved in many projects related to high throughput data analysis including microarrays, tiling arrays and next generation sequencing. I performed microarray analysis to characterize the transcriptional profile during a host defense response. I was also involved in whole genome transcriptome analysis of a human pathogen (*Streptococcus pneumoniae*) using tiling arrays that lead to identification of around 50 novel small RNAs and operon structures. In another study we used next generation sequencing technology "RNA-Seq" to describe the transcriptome map of the bacterial pathogen "*Histophilus somni* 2336" which helped to identify 95 novel small RNAs and 38 novel proteins. I developed a database, HPIDB (host pathogen interaction database), and assisted in developing an agricultural database (Agbase) at my university. Following my Ph.D., I started focusing on translational research related to human diseases. I joined the Cancer Institute at the University of Mississippi Medical Center as a Bioinformatician. There my project was to perform high throughput "omics" data analysis to identify genetic variants in breast cancer at the population level and their association with clinical information. I moved to the University of Alabama at Birmingham in Oct 2011 as a Research Associate in Bioinformatics where I am working on the analysis of next-gen sequence data derived from transcriptomics, epigenetics and microbiome studies. As part of my current responsibilities, I serve as the main data analyst for the university-wide microbiome and metagenomics initiative, developing new methods and pipelines for data analysis and data mining. I am involved in many different projects covering variation of the microbiome in response to different biological conditions and disease, involving the microbiota of the human gut, vaginal tract, oral cavity, as well as the mouse gut.

B. Positions and Honors

Positions and Employment

2005 - 2006 Research Assistant, Indian Institute of Science, Bangalore, India.
2010 - 2011 Bioinformatician, Cancer institute, University of Mississippi Medical Center, Jackson, USA
2011 – Present Research Associate in Bioinformatics, University of Alabama at Birmingham, USA

Other Experience and Professional Memberships

Member, International Society for Computational Biology (ISCB)
Member, American Society for Microbiology (ASM)
Member, MidSouth Computational Biology and Bioinformatics Society (MCBIOS)
Member, Bioinformatics organization - Bioinformatics.org
Member, Honor Society “Phi kappa Phi”

C. Selected Peer-reviewed Publications (* Equal first author)

1. Stoll ML, **Kumar R**, Morrow CD, Lefkowitz EJ, Cui X, Genin A, Cron RQ, Elson CO. Altered microbiota associated with abnormal humoral immune responses to commensal organisms in enthesitis-related arthritis. ***Arthritis Res Ther***. 2014 Nov 30;16(6):486. PMID 25434931. PMCID: PMC4272554.
2. Muzny CA, Sunesara IR, Griswold ME, **Kumar R**, Lefkowitz EJ, Mena LA, Schwebke JR, Martin DH, Swiatlo E. Association between BVAB1 and high Nugent scores among women with bacterial vaginosis. ***Diagn Microbiol Infect Dis.*** 2014. PMID: 25262105.
3. **Kumar R**, Eipers P, Little RB, Crowley M, Crossman DK, Lefkowitz EJ, Morrow CD. Getting started with microbiome analysis: sample acquisition to bioinformatics. ***Curr Protoc Hum Genet.*** 2014 Jul 14;82:18.8.1-18.8.29. PMID: 25042718.
4. Muzny CA, Sunesara IR, **Kumar R**, Mena LA, Griswold ME, Martin DH, Lefkowitz EJ, Schwebke JR. Characterization of the vaginal microbiota among sexual risk behavior groups of women with bacterial vaginosis. ***PLoS One.*** 2013 Nov 13;8(11):e80254. PMCID: PMC3827412.
5. Lazrak A, Fu L, Bali V, Bartoszewski R, Rab A, Havasi V, Keiles S, Kappes J, **Kumar R**, Lefkowitz E, Sorscher EJ, Matalon S, Collawn JF, Bebok Z. The silent codon change I507-ATC->ATT contributes to the severity of the ΔF508 CFTR channel dysfunction. ***FASEB J.*** 2013 Nov;27(11):4630-45. PMID: 23907436. PMCID: PMC4046180.
6. Hicks C, **Kumar R**, Pannuti A, Backus K, Brown A, Monico J, Miele L. An Integrative Genomics Approach for Associating GWAS Information with Triple-Negative Breast Cancer. ***Cancer Inform.*** 2013;12:1-20. PMID: 23423317. PMCID: PMC3565545.
7. **Kumar R**, Lawrence ML, Watt J, Cooksey AM, Burgess SC, Nanduri B. RNA-Seq Based Transcriptional Map of Bovine Respiratory Disease Pathogen "*Histophilus somni 2336*". ***PLoS One.*** 2012; 7(1):e29435. PMID: 22276113. PMCID: PMC3262788.
8. Hicks C, **Kumar R**, Pannuti A, Miele L. Integrative Analysis of Response to Tamoxifen Treatment in ER-Positive Breast Cancer Using GWAS Information and Transcription Profiling. ***Breast Cancer (Auckl).*** 2012; 6:47-66. PMID: 22399860. PMCID: PMC3292850.
9. Reddy JS, **Kumar R**, Watt JM, Lawrence ML, Burgess SC, Nanduri B. Transcriptome profile of a bovine respiratory disease pathogen: *Mannheimia haemolytica PHL213*. ***BMC Bioinformatics.*** 2012 Sep;13 Suppl 15:S4. PMID: 23046475. PMCID: PMC3439734.
10. **Kumar R**, Burgess SC, Lawrence ML, Nanduri B. TAAPP: Tiling Array Analysis Pipeline for Prokaryotes. ***Genomics Proteomics Bioinformatics.*** 2011 Apr;9(1-2):56-62. PMID: 21641563.
11. **Kumar R**, Nanduri B. HPIDB--a unified resource for host-pathogen interactions. ***BMC Bioinformatics.*** 2010 Oct 7;11 Suppl 6:S16. PMID: 20946599. PMCID: PMC3026363.
12. **Kumar R**, Shah P, Swiatlo E, Burgess SC, Lawrence ML, Nanduri B. Identification of novel non-coding small RNAs from *Streptococcus pneumoniae* TIGR4 using high-resolution genome tiling arrays. ***BMC Genomics.*** 2010 Jun 3;11:350. PMID: 20525227. PMCID: PMC2887815.
13. Buza TJ, **Kumar R**, Gresham CR, Burgess SC, McCarthy FM. Facilitating functional annotation of chicken microarray data. ***BMC Bioinformatics.*** 2009 Oct 8;10 Suppl 11:S2. PMID: 19811685. PMCID: PMC3226191.
14. Pendarvis K, **Kumar R***, Burgess SC, Nanduri B. An automated proteomic data analysis workflow for mass spectrometry. ***BMC Bioinformatics.*** 2009 Oct 8;10 Suppl 11:S17. PMID: 19811682. PMCID: PMC3226188.
15. Pavithra SR, **Kumar R***, Tatu U. Systems analysis of chaperone networks in the malarial parasite Plasmodium falciparum. ***PLoS Comput Biol.*** 2007 Sep;3(9):1701-15. PMID: 17941702. PMCID: PMC1976336.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

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

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

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)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. 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

7. 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*:

PHS 398 Modular Budget

OMB Number: 0925-0001

Budget Period: 1

Start Date: 09/01/2015 **End Date:** 08/31/2016

A. Direct Costs

Funds Requested ($)

Direct Cost less Consortium F&A*

Consortium F&A

Total Direct Costs*

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	**Funds Requested ($)**
1.	MTDC	47.00		
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS Steven Zuraf (301) 492-4855

Indirect Cost Rate Agreement Date 09/25/2014

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

PHS 398 Modular Budget

Budget Period: 2

Start Date: 09/01/2016 **End Date:** 08/31/2017

A. Direct Costs

Funds Requested ($)

Direct Cost less Consortium F&A*

Consortium F&A

Total Direct Costs*

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	**Funds Requested ($)**
1.	MTDC	47.00		
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS Steven Zuraf (301) 492-4855

Indirect Cost Rate Agreement Date 09/25/2014

Total Indirect Costs

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($)

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

PHS 398 Modular Budget

Budget Period: 3

Start Date: 09/01/2017 **End Date:** 08/31/2018

A. Direct Costs **Funds Requested ($)**

Direct Cost less Consortium F&A*

Consortium F&A

Total Direct Costs*

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	**Funds Requested ($)**
1.	MTDC	47.00		
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) DHHS Steven Zuraf (301) 492-4855

Indirect Cost Rate Agreement Date 09/25/2014 **Total Indirect Costs**

C. Total Direct and Indirect Costs (A + B) **Funds Requested ($)**

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium F&A for Entire Project Period ($)
Section A, Total Consortium F&A for Entire Project Period ($)
Section A, Total Direct Costs for Entire Project Period ($)
Section B, Total Indirect Costs for Entire Project Period ($)
Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period ($)

2. Budget Justifications

Personnel Justification 1244-Budget justification_Jan12_2015.pdf
Consortium Justification
Additional Narrative Justification

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

TITLE

STOP BPD

PERSONNEL at University of Alabama at Birmingham

Ambalavanan, Namasivayam, MD	1.8	calendar months
Carlo, Waldemar A, MD	0.6	calendar months
Zhang, Kui PhD	0.6	calendar months
Kumar, Ranjit PhD	0.6	calendar months
Technical personnel:		
Olave, Nelida PhD	8.0	calendar months
Research Coordinator (to be named)	8.0	calendar months

Namasivayam Ambalavanan MD, Principal Investigator (PI), will devote 1.8 calendar months of his effort to this project. Dr. Ambalavanan will be responsible for the design of all experiments and the overall planning, coordination and conduct of the program. He will take responsibility for quality control, processing and analysis of all data and preparation of experimental results for publication. He will supervise Dr Nelida Olave (Research Associate) and the Research Coordinator, and closely work with Dr. Carlo and Dr. Zhang. Dr. Ambalavanan has been working in the Regional NICU as an Attending Physician since 2000, and has led several single-center randomized trials and observational studies, in addition to multicenter projects within the NICHD Neonatal Research Network (for which he is the alternate center PI). He will be responsible for submitting all necessary documents to NIH including annual progress reports.

Waldemar A. Carlo MD, Co-Investigator, will devote 0.6 calendar months of his time to the project. Dr. Carlo is the Director of the Division of Neonatology at UAB, and is the center PI for the NICHD Neonatal Research Network. He has designed and led multiple single-center and multicenter randomized clinical trials and observational studies. He will provide the PI with assistance on patient enrollment, and with development of the prediction models and their validation.

Kui Zhang PhD, Co-Investigator, will devote 0.6 calendar months of his time to the project during Years 1 and 2, with increased effort during Year 3 (6 calendar months) when the data from the “Omic” analyses become available. He is an Associate Professor (Tenured) in the Section of Statistical Genetics in the Department of Biostatistics, and will develop the models for resilience or predisposition using the genomic, proteomic, and airway microbiomic data, and validate them in the validation dataset. He will also develop the integrated model (Aim 4) combining the data from all “Omic” models with the clinical data.

Ranjit Kumar PhD, Co-investigator, will devote 0.6 calendar months of his time to the project during Years 1 and 2, with increased effort during Year 3 (1.2 calendar months) when the data from the “Omic” analyses become available. He will be in charge of bioinformatic analyses of the airway microbiome data.

Nelida Olave PhD, Research Associate, will devote 8 calendar months of her time to the project. Dr. Olave is very experienced with molecular and cell biology techniques, and will be in charge of initial sample processing and isolation of RNA and DNA for the genomic, proteomic, and microbiomic studies.

Research Coordinator (to be named), will devote 8 calendar months of his/her time to the project. The research coordinator will be in charge of patient enrollment, data collection, and assist with regulatory paperwork (IRB) and other record-keeping. The Research Coordinator will collect the samples (blood, urine, tracheal aspirates) for the genomic, proteomic, and microbiomic studies, and provide them to Dr. Nelida Olave, the Research Associate.

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	1245-SpAims_Jan15_2015.pdf
3. Research Strategy*	1246-ResearchStrategyJan152015.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	1247-Protection of Human Subjects_Jan2015.pdf
6. Inclusion of Women and Minorities	1248-Inclusion of Women and Minorities_Jan2015.pdf
7. Inclusion of Children	1249-Inclusion of Children_Jan2015.pdf
Other Research Plan Sections	
8. Vertebrate Animals	
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	1250-CombinedSupportLettersResilienceGrant.pdf
13. Resource Sharing Plan(s)	1251-Resource Sharing_Jan2015.pdf
Appendix (if applicable)	
14. Appendix	

Tracking Number: GRANT11812912

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

Specific Aims

Bronchopulmonary dysplasia (BPD), common in extremely low birth weight (ELBW) infants, is characterized by an impairment of alveolar development and varying degrees of inflammation, fibrosis, and vascular remodeling. Our research group has considerable expertise in translational research on BPD. We have: (a) developed models using only limited clinical information to predict BPD or death by postnatal age (1) or respiratory illness severity (2) in ELBW infants, (b) prospectively determined the incidence and course of pulmonary hypertension, a complication and major risk factor for worse outcome in BPD (3), and (c) identified biomarkers associated with BPD/death in a cohort of 1067 ELBW infants using multiple clinical variables and 25 cytokines in blood collected within 4 h of birth and on days 3, 7, 14, and 21 (4). In the cytokine study, BPD/death was associated with higher concentrations of interleukins (IL)-1β, -6, -8, -10, and interferon (IFN)-γ and lower IL-17, RANTES, and tumor necrosis factor (TNF)-α (4). Models including cytokines were more predictive than those with only clinical variables (r^2 0.43 vs. 0.38) (4).

More recently, we have made novel observations on the genetic basis of BPD by genome-wide analysis (GWAS) in 751 extremely low birth weight infants (5). Genome-wide association and gene set analysis was performed for BPD or death, severe BPD or death, and severe BPD in survivors. Specific targets were then validated using gene expression in BPD lung tissue and in mouse models. Multiple SNPs in adenosine deaminase (ADARB2) and CD44 were just below $p<10^{-6}$. The pathway with lowest false discovery rate (FDR) was miR-219 targets (p=1.41E-08, FDR 9.5E-05) for BPD/death, and Phosphorous Oxygen Lyase Activity for both severe BPD/death (p=5.68E-08, FDR 0.00019) and severe BPD in survivors (p=3.91E-08, FDR 0.00013). Gene expression analysis confirmed significantly increased miR-219 and CD44 in BPD lung tissue (5). In another recent study, we identified alterations in gene expression and DNA methylation during murine and human lung alveolar septation and development of BPD (6).

In additional ongoing studies, we have identified novel proteomic biomarkers in urine that predict subsequent development of BPD, and have determined alterations in the airway microbiome (marked increase in proteobacteria or actinobacteria, and reduction in firmicutes) associated with BPD.

The **overall objective of "STOP BPD"** (**S**ignature of **T**op **O**mic **P**rofiles in **BPD**) our response to **RFA-HL-15-024 Definition of Resilience and Pre-Symptomatic Disease in Lung Health and Disease (R01)** is to use our existing large cohort (>200/yr, the largest number of extremely preterm infants in any center of the NICHD Neonatal Research Network in 2013) of extremely preterm infants to **prospectively define and validate clinical and "omic" signatures associated with resilience against, or risk for development of BPD, with the goal of informing the development of primary prevention strategies for BPD.** To address this objective, we will build upon our recent studies on cytokines, genomics, proteomics, respiratory microbiome, and model development in BPD.

We will address the objective by the following **Specific Aims** in a prospective cohort (Generic Database or GDB cohort of the NICHD Neonatal Research Network) of 300 preterm infants <29 weeks gestation we expect to be born in 2015-2017 (n=213 in 2013 alone, with 80%+ enrollment in observational studies) in the NICHD Neonatal Research Network:

Specific Aim 1- Development and validation of a personalized genomic risk/resilience score for BPD and severe BPD using a combination of genome-wide expression analysis and targeted SNP profiling in blood collected within 72h of birth

Specific Aim 2 - (a) Development and validation of a personalized urinary proteomic risk/resilience score for BPD and severe BPD measured in the first postnatal week

(b) Development and validation of a personalized plasma proteomic and cytokine risk/resilience score for BPD and severe BPD measured within 72h of birth

Specific Aim 3 - Development and validation of a personalized airway microbiome risk/resilience score for BPD and severe BPD using tracheal aspirates collected in the first postnatal week

Specific Aim 4 - Development of a combined "Omic" scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model

Over the course of this study, we will develop and determine the predictive accuracy of the various personalized risk/resilience scores in the Development cohort (n=150; enrolled in Year 1), and evaluate correlation of these scores with each other as well as with concurrent clinical variables. The final models will be validated in the Validation cohort (n=150; enrolled in Year 2). These novel models can be used to define the target population for future interventions, assess efficacy of specific interventions, develop a "lab on chip", and support future studies on the biology of BPD.

(a) Significance:

Importance of the problem:

Bronchopulmonary dysplasia (BPD) in premature infants is characterized by impaired lung development (7, 8), and leads to persistent long-term abnormalities in lung function in survivors (9). BPD ranks among the top three chronic respiratory diseases of childhood along with asthma and cystic fibrosis with a cost burden in the United States of over $2.4 billion per year.

The knowledge gap:

The NIH consensus definition for BPD in infants <32 week GA (defined at 36 weeks post-menstrual age [PMA] or discharge, whichever comes first) categorizes the severity of BPD as *mild* if infants are treated with FiO_2>0.21 for at least 28 days and are breathing room air at 36 weeks PMA or discharge, *moderate BPD* if on FiO2<0.30, and as *severe BPD* if on >FiO2 0.30 and/or positive pressure (PPV or CPAP) (10). The "need for oxygen" is more accurately defined by the "physiologic definition" of BPD (11-13). However, these definitions are purely operational definitions, which do not indicate the nature of lung disease or the contributing pathophysiology. Lung pathology in BPD is variable, as some infants with BPD have severe pulmonary hypertension (3, 14), while others have marked tracheobronchomalacia (15), and many have patchy atelectasis or cystic lung parenchymal lesions (16), superimposed on a variable magnitude of impaired alveolarization, dysmorphic microvasculature, and variable interstitial cellularity and fibroproliferation (8).

It has increasingly become evident that severe BPD is a different entity from mild or moderate BPD, both in terms of clinical operational definition as well as in terms of genetic predisposition (5). We have shown that genetic predisposition to BPD is very different by race/ethnicity, indicating that biologic pathways (and resulting biomarkers of risk or resilience) contributing to BPD in different infants are probably very dissimilar (5). Therefore, it is likely that "BPD" is not a single entity, nor even a spectrum of disease resulting from a single pathophysiologic process, but a combination of several chronic lung diseases characterized by a common "at-risk population" of infants in the saccular stage of lung development with varying magnitudes of impairment of alveolar septation, fibrosis, and abnormal vascular development and remodeling. To paraphrase Leo Tolstoy's quote from Anna Karenina ("All happy families are alike; each unhappy family is unhappy in its own way"), all normally developed preterm lungs are alike; each BPD lung is abnormal in its own way. The corollary is that the clinical predictors and biomarkers of each of these sub-phenotypes of BPD is different, depending upon the pathophysiology. An individual "personalized" approach is therefore essential to determine the pulmonary outcome of an extremely preterm infant.

We have previously identified risk factors and developed a web-based estimator (https://neonatal.rti.org/index.cfm?fuseaction=BPDCalculator.start) using readily available clinical information to predict risk of BPD or death, with a good area under the curve (c statistic) of 0.793 on day 1 (1). In this study, we plan to identify markers of resilience (infants who do not develop BPD despite a high predicted risk of severe BPD of >70% on day 1) and predisposition (infants who develop BPD despite a low predicted risk of severe BPD or <30% on day 1).

It is important to identify the predictor variables (clinical and biomarker) associated with predisposition versus resiliency in infants for the different subphenotypes of BPD. Due to the heterogeneity in BPD and its pathogenesis, integration of molecular, cellular, and organ information from multiple modes of investigation (clinical variables and multiple "omic" profiles) is required to recognize pre-symptomatic disease states, determine points of disease origin, and identify opportunities for intervention.

Therefore, the overall objective of "STOP BPD" (**S**ignature of **T**op **O**mic **P**rofiles in **BPD**) is to prospectively define and validate clinical and "omic" signatures associated with resilience against, or predisposition to BPD, with the goal of informing the development of primary prevention strategies for BPD.

(b) Innovation:

Innovative aspects of our proposal include:

(1) Combination of unbiased "Omic" and targeted known biomarker approaches with standardized validated clinical data collection forms to identify "resilience" and "predisposition" factors for BPD

(2) Use of multiple unbiased "Omic" approaches (genomic, proteomic – blood and urine, microbiomic), for each of which we have good preliminary data and expertise. The availability of parallel information about alterations in genomic, proteomic, and microbiomic markers will allow data integration and generation of comprehensive prediction models.

(3) Use of separate Development and Validation cohorts, with the possibility of scale-up

(c) Approach:

General Methods:

A. Subjects

300 ELBW infants 401-1000 grams inclusive birth weight and/or $22^{0/7}$ to $28^{6/7}$ (<29 w weeks inclusive completed weeks of gestation, for whom a decision has been made to provide full resuscitation, will be enrolled over a two year period (Years 1 and 2 of project), and followed-up to initial hospital discharge (or death, if prior to discharge). These infants are being enrolled prospectively in the NICHD Neonatal Research Network (NRN) Generic Database (GDB; "Survey of Morbidity and Mortality among High Risk Preterm Infants"; Manual of Operations Apr 1, 2011, last revised May 10, 2013). We have been members of the NICHD NRN without interruption since 1996 (5U10HD034216-19; PI Waldemar Carlo; FOA RFA-HD-10-003). Our center (UAB) has had the highest or second highest patient enrollment in 12 of 13 randomized controlled trials completed during the last three five-year cycles of the NRN as well as the highest number of infants followed at 18-22 months.

B. Inclusion/Exclusion Criteria

This study will enroll ELBW neonates in our Regional NICU (RNICU) at UAB as follows:

Inclusion:

- Inborn infants weighing 401-1,000 grams on admission and/or $22^{0/7}$ to $28^{6/7}$ (<29 weeks) inclusive completed weeks of gestation,
- Infants eligible for full care and resuscitation as necessary, and surviving beyond 12 h of age
- Enrollment at <72 hours post-natal age
- Informed consent from parent/guardian

Exclusion

- Refusal or withdrawal of consent
- Major congenital malformations (e.g., not including patent ductus arteriosus, small hernia)

C. Recruitment and Screening

Infants will be recruited for this study by approaching one or both parents soon after birth of the infant. All admissions of ELBW infants to the RNICU will be screened twice daily (morning and evening) by a study coordinator to ensure that eligible patients can be enrolled. We have 5 full-time study coordinators that routinely work every day including weekends in-house for our ongoing clinical studies. The study coordinator will maintain a screening log of eligible and potentially eligible patients by checking with the adjacent Maternal-Fetal Medicine/OB unit.

D. Enrollment /Sample size feasibility

Parents will be approached for informed consent by a study coordinator (neonatal fellows or faculty currently serve as a back-up) soon after admission of the infant to the RNICU.

In 2013, our Regional NICU (RNICU) had 213 extremely preterm infants enrolled in GDB, of whom 204 (96%) survived >12 h. The birth weight of these infants was 770±244 g (mean ±SD; median = 730g). These extremely preterm infants are selected for inclusion as they are the infants at highest risk for RDS and BPD. Infants <401 g or $22^{0/7}$w will not be included as their mortality rate is extremely high (>95%). Our consent rate in recent years has been 80-85% for observational studies such as this project.

Therefore, we estimate that of the approximately 400 infants who are eligible in the first two years of the study (200 per year x 2 yrs), we should be able to enroll 300 infants (approx. 75% consent rate, a conservative estimate). Currently (using our recent 2013 GDB data), at 36w PMA, our rate of BPD is 31% (physiologic definition) and 35% (traditional definition), and the status of infants is 6% discharged, 75% still in hospital, <1% transferred, and 18% died. We can enroll for up to 6 additional months if enrollment is slow, as analysis is rapid. Assuming our clinical outcomes remain similar, it is estimated that of the 300 enrolled infants at 36w PMA approximately 90 (80-100) will have BPD, and 54 (45-65) would have died.

E. Data and sample collection

Much of the data collection for this study is currently already being collected as infants in this study are included for the GDB. Standardized data collection using well defined variables (as defined in the GDB Manual of Operations) by trained research coordinators has been in progress in this population for over 20 years. Additional respiratory data such as we did for the SUPPORT Trial (17, 18) will be collected.

Data to be collected:

Infants in the study will have careful phenotyping of clinical characteristics which will expand upon the data collection done for the NRN GDB, including antenatal (maternal) variables (e.g. gestational age, clinical and histological chorioamnionitis features, duration of rupture of membranes and labor, antimicrobial

use, tocolytic use, antenatal steroids, magnesium sulfate and other medications), intrapartum (e.g. mode of delivery, Apgar scores, resuscitation required), and postnatal variables soon after birth (e.g. birth weight, sex, weight and length percentiles), and at different time points (e.g. ventilatory support, medications, fluid volumes infused, nutritional details, and complications of prematurity such as sepsis, suspected sepsis, intraventricular hemorrhage, PDA, necrotizing enterocolitis etc). Respiratory support will be characterized in detail, with details of ventilatory settings (peak, mean, and end-expiratory pressures), FiO_2, with corresponding oxygenation index, PaO_2, $PaCO_2$, pH, HCO_3 also recorded twice a day for the first two weeks, then daily thereafter (at time point closest to 12 noon) until discharge/death. BPD will be defined according to the NIH consensus definition(10) as well as the physiological definition(11).

Case Definitions and Outcomes:

We developed a web-based estimator using readily available clinical information to predict risk of BPD or death (https://neonatal.rti.org/index.cfm?fuseaction=BPDCalculator.start), with a good area under the curve of 0.793 on day 1 (1). In this study, we plan to identify markers of resilience (infants who do not develop severe BPD despite a high predicted risk of severe BPD of >70% on day 1) and predisposition (infants who develop severe BPD despite a low predicted risk of severe BPD of <30% on day 1).

The two major analyses that we will be doing are evaluation of:

1) Resilience: comparing infants who do survive without severe BPD despite a high predicted risk of severe BPD or death of >70% on day 1 (defined as "**resilient**" infants), to those who develop severe BPD or die with the same magnitude of risk (defined as "**non-resilient**" infants)
2) Predisposition: comparing infants who develop severe BPD or die despite a low predicted risk of severe BPD of <30% on day 1 (defined as "**predisposed**" infants), to those who do not develop BPD with the same magnitude of risk (defined as "**non-predisposed**" infants)

Therefore, this study will not be a simple evaluation of "omic" associations with or risk factors for severe BPD, mild/moderate BPD etc, but an evaluation of "resilience" or "predisposition" to these outcomes. Also, it is important to note that death is a competing outcome for BPD (infants who die early cannot develop BPD), and hence the competing outcome is included in the case definition.

We plan to do the clinical data collection and "Omic" analyses on all enrolled infants. Once the initial analyses for severe BPD have been completed, we will then evaluate resilience/predisposition for sub-phenotypes of BPD (BPD with pulmonary hypertension confirmed on echocardiography at 28 day screening or later vs. BPD with no pulmonary hypertension (3, 14); BPD with severe tracheomalacia identified on bronchoscopy at 3-6 months age vs. BPD with no tracheobronchomalacia) (15).

Samples to be collected and analysis to be performed on samples:

The samples to be collected from the infants are as follows:

Whole blood: Blood from all infants will be collected on day 1-3. 0.3 ml will be collected with EDTA anticoagulant, at the time of routine sampling. Blood samples will be centrifuged (1,000g x 15 minutes), plasma and cell pellets will be separated, and the plasma frozen at -80^{o}C and subsequently analyzed for cytokines and plasma proteomics (Aim 2), while the cell pellet used for genomic analysis (Aim 1).

Isolation of RNA and DNA: The cell pellet from blood will be used for automated RNA and DNA isolation on the QIAcube (Qiagen) using the AllPrep DNA/RNA Mini Kit (Cat# 80204, Qiagen). DNA or RNA concentration is measured by NanoDrop Spectrophotometer (NanoDrop ND-1000; Thermoscientific, Waltham, USA), and quality determined by 2100 Bioanalyzer (Agilent, Santa Clara, USA).

Tracheal aspirates: Tracheal aspirate samples (from intubated infants during routine suctioning) will be collected on day 1-3, and if still intubated, on day 28. Tracheal aspirates will be centrifuged (1,000g x 15 minutes), and the cell pellet and supernatant frozen separately. Microbiome analysis in Aim 3 will be done on supernatant.

Clinical management: Medical interventions and ventilator management will be based upon clinician discretion. Evidence and consensus based guidelines are followed for uniformity in practice. No changes in management are required for this observational cohort study.

Outline of Experiments in Specific Aims

Specific Aim 1- Development and validation of a personalized genomic risk/resilience score for BPD and severe BPD using a combination of genome-wide expression analysis and targeted SNP profiling in blood collected within 72h of birth

Background, Rationale, and Preliminary Data:

Genetic factors account for 53% of the variance in risk for BPD, after controlling for covariates in twin studies (19). Targeted candidate gene analyses suggest single nucleotide polymorphisms (SNPs) in certain surfactant proteins and cytokines and related molecules are associated with BPD (20, 21). A genome-wide association study (GWAS) by Hadchouel et al.(22) identified the SPOCK2 gene as associated with BPD, but Wang et al.(23) did not find any SNPs associated with BPD. We recently published an integrated genomics analysis of BPD (5). We conducted a genome-wide scan on 1.2 million genotyped SNPs, and an additional 7 million imputed SNPs, using a DNA repository of extremely low birth weight infants. GWAS and gene set analysis was performed for BPD or death, severe BPD or death, and severe BPD in survivors. Specific targets were validated using gene expression in BPD lung tissue and in mouse models. Of 751 infants analyzed, 428 developed BPD or died. No SNPs achieved genome-wide significance ($p<10^{-8}$) although multiple SNPs in adenosine deaminase (ADARB2), CD44, and other genes were just below $p<10^{-6}$. Of approximately 8000 pathways, 75 were significant at False Discovery Rate (FDR) <0.1 and p<0.001 for BPD/death, 95 for severe BPD/death, and 90 for severe BPD in survivors (**Figure 1**). The pathway with lowest FDR was miR-219 targets (p=1.41E-08, FDR 9.5E-05) for BPD/death and Phosphorous Oxygen Lyase Activity (includes adenylate and guanylate cyclases) for both severe BPD/death (p=5.68E-08, FDR 0.00019) and severe BPD in survivors (p=3.91E-08, FDR 0.00013). Gene expression analysis confirmed significantly increased miR-219 and CD44 in BPD. Therefore, pathway analyses confirmed involvement of known pathways of lung development and repair (CD44, Phosphorus Oxygen Lyase Activity) and indicated novel molecules and pathways (ADARB2, Targets of miR-219) involved in genetic predisposition to BPD.

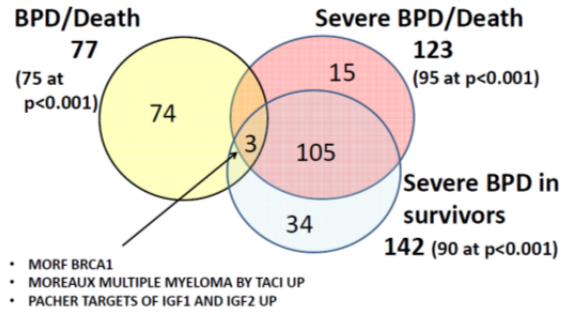

Figure 1: Pathways at FDR<0.1. Venn diagram indicating number of pathways significant at FDR<0.1 and overlap for outcomes of BPD/death, severe BPD/death, and severe BPD in survivors.

Important findings from this study were that severe BPD or death are associated with pathways distinct from mild/moderate BPD, suggesting that they have a different pathophysiologic basis, and that much variation is present in genetic predisposition to BPD by race/ethnicity (**Table 1**). The large differences in pathways by race/ethnicity suggest that although the clinical phenotype of BPD may be similar, the underlying genetic predisposition may differ significantly. This result also suggests that potential therapies may need to be specifically targeted at pathways or genes that are found to be involved, and therefore suggests a role of "personalized genomics" in BPD.

All infants			White infants			Black infants		
Pathway	*P* value	FDR	Pathway	*P* value	FDR	Pathway	*P* value	FDR
GACAATC,MIR-219	1.41E-08	9.52E-05	module_320	1.82E-49	1.23E-45	RODRIGUES_THYROID_CARCINOMA_DN	7.80E-07	0.005262
NUCLEAR_UBIQUITIN_LIGASE_COMPLEX	2.16E-07	0.00073	GNF2_BUB1	2.29E-41	7.73E-38	TOMLINS_METASTASIS_DN	4.91E-06	0.007876
YRCCAKNNGNCGC_UNKNOWN	7.11E-07	0.0016	GNF2_TTK	1.03E-31	2.32E-28	ATAAGCT,MIR-21	5.14E-06	0.007876
VSE2F_Q2	9.88E-07	0.001668	GNF2_SMC2L1	1.38E-31	2.33E-28	KEGG_CELL_ADHESION_MOLECULES_CAMS	2.39E-06	0.007876
NEURON_PROJECTION	1.37E-05	0.015893	GNF2_HMMR	4.80E-24	6.48E-21	module_349	5.83E-06	0.007876
VSGABP_B	1.41E-05	0.015893	GNF2_ESPL1	8.12E-23	9.14E-20	BEGUM_TARGETS_OF_PAX3_FOX01_FUSION_UP	8.48E-06	0.008177
TOMLINS_METASTASIS_DN	1.66E-05	0.015965	GNF2_CENPE	1.23E-22	1.19E-19	CHEOK_RESPONSE_TO_MERCAPTOPURINE_AND_HD_MTX_UP	8.15E-06	0.008177
NUCLEOBASENUCLEOSIDENUCLEOTIDE_ KINASE_ACTIVITY	2.74E-05	0.018801	GNF2_CDC20	5.47E-22	4.61E-19	AMIT_EGF_RESPONSE_480_HELA	1.00E-05	0.008437
VSE2F_Q4	3.27E-05	0.018801	module_244	1.14E-21	8.55E-19	CELLULAR_MACROMOLECULE_METABOLIC_PROCESS	1.98E-05	0.014845
RUIZ_TNC_TARGETS_DN	2.99E-05	0.018801	GNF2_CKS1B	3.48E-20	2.35E-17	KENNY_CTNNB1_TARGETS_DN	2.26E-05	0.015232
JAEGER_METASTASIS_UP	3.34E-05	0.018801	MORI_LARGE_PRE_BII_LYMPHOCYTE_UP	2.86E-19	1.76E-16	PROTEIN_UBIQUITINATION	2.94E-05	0.017542
SUGAR_BINDING	2.71E-05	0.018801	GNF2_CKS2	7.66E-19	4.31E-16	CELLULAR_PROTEIN_METABOLIC_PROCESS	3.27E-05	0.017542

Table 1: Biological pathways associated with BPD or death classified by race, compared to survivors without BPD. Pathways are from the annotated gene sets of the molecular signatures database at the Broad Institute (http://www.broadinstitute.org/gsea/msigdb/index.jsp) are listed in order of increasing false discovery rate (FDR). Only the top 12 pathways are shown for All infants, White infants, and Black infants.

It is important to determine if there are early changes in gene expression profile in peripheral blood associated with development of severe BPD. To date, studies have evaluated changes in gene expression profile in **(a)** autopsy lung tissue of infants with BPD as compared to control infants without BPD (total n=28, of whom 11 developed BPD) (24), **(b)** in umbilical cord tissue (total n=54, of whom 20 developed BPD) (25), and **(c)** in peripheral blood (n=111, of whom 68 developed BPD)(26). Pietrzyk et al.(26) evaluated gene expression profiles in the peripheral blood of 111 preterm infants, and found that overall 2086 genes were differentially expressed on postnatal day 5. Based on pathway analysis, the cell cycle pathway was up-

regulated in BPD, and the most down-regulated pathway was the T cell receptor signaling pathway (26). While this study did not specifically evaluate severe BPD, a monotonic trend in expression of many genes between the BPD severity groups was observed (26). However, these studies did not evaluate for "resilience" (infants who did not develop BPD although at high predicted risk soon after birth) or "predisposition" (infants who developed BPD although initially at low risk).

Analysis:

Blood genome-wide expression analysis:

Gene expression from peripheral blood will be evaluated on the Illumina Human HT-12 v4 bead chip using established workflows at UAB's Heflin center for Genomic Science (see Letter of Support). This provides genome-wide transcriptional coverage of well-characterized genes, gene candidates, and splice variants, delivering high-throughput processing of 12 samples per BeadChip. Each array on the HumanHT-12 v4 Expression BeadChip targets more than 47,000 probes derived from the National Center for Biotechnology Information Reference Sequence (NCBI) RefSeq Release 38 (November 7, 2009) and other sources. With the Human HT-12 BeadChip, expression information can be easily incorporated into Infinium assay-based GWAS or methylations studies. The RNA required is 50-100 ng, for a dynamic range of $\geq$ 3 logs, and precision of $\leq$1.35 fold.

Blood targeted SNP profiling:

We will analyze SNPs that we have observed in our recent study of genomics in BPD(5), by pyrosequencing using established workflows at UAB's Heflin center for Genomic Science (see Letter of Support). These SNPs are in ADARB2 (rs59582957, chr10:1488099, chr10:1488186, chr10:1488126, chr10:1488099), CD44 (chr11:35165510, chr11:35167447), GALNTL6 (rs2610201, rs2653824), WDR45L (rs8082435), NSMC4A (chr10:123726948), NUAK1 (rs1427793), KCNH7 (rs2653829), GRIP1 (rs1504316), and the following intergenic SNPs (rs17119652, rs1504316, rs57481375).

Statistical analysis for identification of differentially expressed genes. The differentially expressed genes will be identified and used in the subsequent development and validation of a personalized genomic risk/resilience score severe BPD/death and severe BPD in survivors.

Statistical team. Statistical support will be provided by Dr. Kui Zhang from the Section on Statistical Genetics (SSG: http://www.soph.uab.edu/statgenetics/) in the Department of Biostatistics at UAB. Dr. Kui Zhang has extensive experience and expertise in design and analysis of gene expression studies, and will serve as the primary statistician on this project.

Statistical methods. Preprocessing will be done using Illumina's GenomeStudio software by robust multiple-array average (RMA) normalization. Quality control will be performed by Principal Component Analysis (PCA), Relative Log Expression (RLE) and Normalized Unscaled Standard Error (NUSE) plots. Specific statistical and filtering approaches will be determined by Dr. Kui Zhang. The false discovery rate (FDR) with the Benjamini-Hochberg procedure will be calculated and used for multiple testing correction. Two-dimensional cluster maps with standard coloring convention will be constructed with GenomeStudio software, with gene trees and condition trees based on the Pearson similarity algorithm.

To identify differentially expressed genes across different groups, we will use a linear model with the gene expression as the response and groups as the predictors. Since some factors, such as birth weight, gestational age, race/ethnicity, gender, Apgar score, delivery room intubation, surfactant therapy, prenatal steroids, PDA, PDA ligation, length of mechanical ventilation etc., may be different across the different categories of outcome (e.g. resilient vs. non-resilient; predisposed vs. non-predisposed), they are also included in the linear model. The genes with FDR of 0.01 or less and with fold change greater than 2 will be used in ontology and pathway analysis and development and validation of a personalized genomic risk/resilience score for BPD and severe BPD.

Ontology analyses will be performed with D.A.V.I.D (the Database for Annotation, Visualization and Integrated Discovery) (http://david.abcc.ncifcrf.gov/) which provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large lists of genes. Canonical pathway analysis will be done with the Ingenuity Systems Pathways Knowledge base (Ingenuity Systems, Redwood City, CA) (http://www.ingenuity.com/), which provides a tool for discovery of pathways within the differentially expressed genes. The KEGG (Kyoto Encyclopedia of Genes and Genomes; www.genome.jp/kegg) and Biocarta pathways (www.biocarta.com) will be selected for pathway analysis.

Power and sample size analysis. Of the 150 newborns in either the development or validation cohort, we anticipate 30% (45) will develop BPD, of whom approximately 2/3rd (~30) will have severe BPD. We anticipate that 50 of 150 infants will be predicted to have severe BPD (based on preliminary studies with

the web-based estimator), but only 15 will develop severe BPD, i.e., 35 infants will be resilient, and 15 non-resilient. On the other hand, of the remaining 100 of 150 who are not predicted to develop severe BPD, 15 will develop severe BPD i.e. 15 infants will be predisposed, and the remaining 85 non-predisposed. If we further consider that the total number of probe sets on the single microarray is 47,000 and a gene with FDR of 0.01 or less and fold-change of 2 or greater is considered as differentially expressed, with normal distribution of normalized gene expression values, and no interaction among genes, and a variance of 0.6 log-transformed gene expression levels from our preliminary results, our study will have at least 90% power for detecting differentially expressed genes (between resilient and non-resilient, or between predisposed and non-predisposed; if at least 11 infants are in each group) if significant gene expression difference was defined as fold expression change equal or greater than 2.0.

Validation: To validate results obtained by microarray, we will use real-time RT-PCR. A total of 40 cDNA samples (10 patients with resilience, 10 with non-resilience, 10 with predisposition and 10 with no predisposition) remaining after microarray analysis, will be used for validation. Samples will be randomly selected from the groups. From each sample 100ng of cDNA will be used for the TaqMan Gene Expression Assay using appropriate TaqMan probes (Life Technologies, Carlsbad, USA). Each sample will be analyzed in duplicate. Average between the expression of endogenous controls (housekeeping genes: GAPDH and 18S) will be used for determination of the relative expression levels using $\Delta\Delta$Ct calculation. For the validation procedure 20 genes will be randomly selected from the group of genes differentially expressed by microarray ($\geq \pm 2$ fold change) and from the genes without significant differences in expression.

Anticipated Results, Potential Problems, and Alternative Strategies:

We anticipate that the genome-wide expression analysis will indicate multiple genome-level perturbations involving hundreds of genes and multiple pathways in infants who are resilient for severe BPD (with or without including the competing outcome of death), and different genes/pathways in infants who are predisposed for severe BPD, as in our recent study (5). These genes and pathways will probably include several biologically relevant gene ontologies and canonical pathways, such as those involved in inflammation and response to injury, developmental processes, and pathways such as the TGF-β signaling pathway and matrix metalloproteinases. We anticipate that there will be substantial overlap between these genes and pathways, and those identified in our recent integrated genomic analysis of BPD that was primarily whole genome SNP-based and not expression-based (5). A potential problem is of the inclusion of the competing outcome of death, as while the infants at highest risk of severe BPD are also at the highest risk of death, not all deaths are related to respiratory issues. We will do additional analyses of resilience and predisposition in all enrolled infants but excluding non-respiratory deaths, and also analyze similarly only in survivors to 36 weeks post-menstrual age (when the diagnosis of severe BPD is made).

We anticipate that our targeted SNP-based analysis will confirm increases in minor allele frequency (MAF) in infants who are predisposed to worse outcome, and reductions of MAF in those who are resilient. A limitation of our approach is that while whole genome expression and targeted SNP methods are being used, we are not performing genome-wide SNP analysis as done in our recent publication (5) or by other investigators (22, 23). Repeating the genome-wide SNP analysis is probably not necessary in this study as the sample size would be smaller in this single center study, and additional data would only be incremental. Another limitation is that we are not evaluating epigenetic changes in DNA (e.g. DNA methylation), as we recently reported in BPD (6). Comprehensive analysis of DNA methylation is beyond the budgetary scope of this project, although we will archive samples and apply for future funding. Yet another issue is that we are evaluating gene expression in peripheral blood, rather than from lung cells. The gene expression profiles of peripheral blood, bronchoalveolar lavage (BALF) or tracheal aspirates, and lung homogenates in animal models of lung injury are all quite different from each other. However, the purpose of this study is not to obtain mechanistic insight into the pathophysiology of lung injury but to correctly classify resilience or predisposition using peripheral blood, and hence this is not a true limitation.

Specific Aim 2 – (a) Development and validation of a personalized urinary proteomic risk/resilience score for BPD and severe BPD measured within 72h of birth

(b) Development and validation of a personalized plasma proteomic risk/resilience score for BPD and severe BPD measured in the first postnatal week

Background, Rationale, and Preliminary Data:

Given the inherent complexity of BPD and patient heterogeneity, a better understanding of the mechanisms underlying BPD requires a global or systems approach aimed at identifying a proteomic

signature as opposed to reductionist or highly narrow approach. A major knowledge gap is identification of biomarkers in an unbiased manner. It is not feasible to directly examine the lungs in preterm infants, but an indirect evaluation can be performed by evaluating the proteomic signature of urine (readily available in relatively large quantities from all infants) and peripheral blood (readily available, but with the disadvantages of being available in smaller quantities, and requiring heel sticks or arterial/venous sampling), which may indicate proteins either released by or acting on the injured and remodeling lung. In this aim, we will perform an unbiased proteomic evaluation of urine and plasma collected soon after birth from extremely preterm infants, and additionally evaluate a panel of cytokines (below threshold limits of detection by proteomic analysis), in order to develop and validate a resilience/predisposition score for severe BPD. This aim is a highly innovative component of the proposal, and has the potential to identify new mediators and markers of BPD and its sequelae. The genomic and proteomic analyses are complementary, and may serve to validate the molecules identified as targets by the other analysis

Urine Studies: We have completed a preliminary study (manuscript being prepared for submission) that identified urinary proteomic markers of BPD (**Figure 2**). This study involved Phase 1 (discovery) and Phase 2 (validation) in preterm infants with gestational age ≤28 weeks and birth weight ≤1000 grams. For the discovery cohort, infants at a post-menstrual age (PMA) of 36 weeks with no, mild, moderate and severe BPD were included (n=4-5/category). Urine samples were collected at 36 w PMA. For the validation

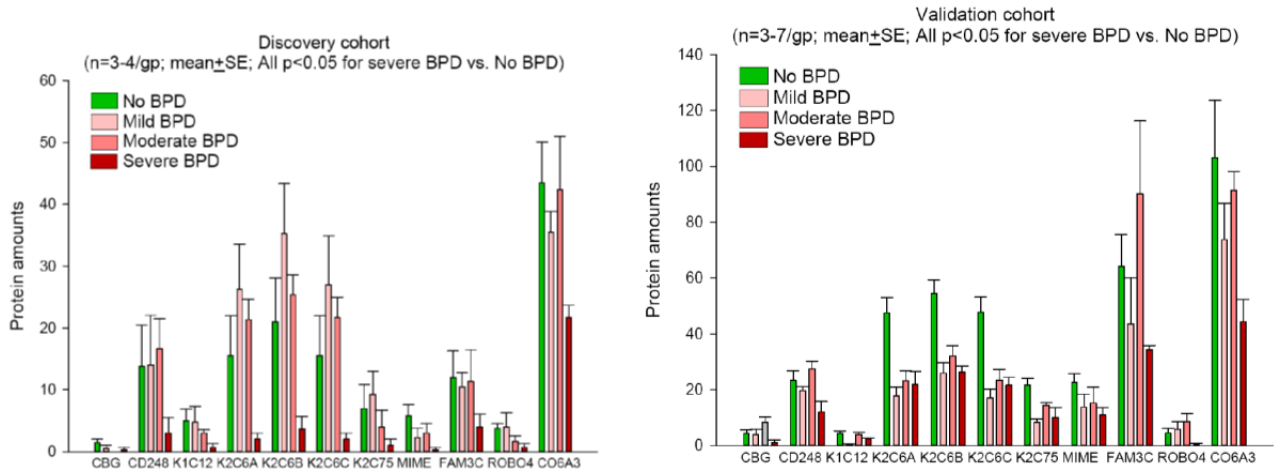

Figure 2: Proteomic biomarkers of BPD in ELBW infants in Discovery cohort (Left panel) and Validation cohort (Right panel).

cohort, urine samples were collected at one month (28-35 days) of age and infants were followed to 36 w PMA, at which time they were evaluated and classified as either no, mild, moderate, or severe BPD (n=3-7 /category). Urine samples were desalted, concentrated, quantified, separated on 1D gel, enzymatically digested, solid phase extracted, and subjected to LC-ESI-MS/MS followed by bioinformatic analyses. In the discovery cohort, 15 proteins were identified as significantly different. Of these, 11 proteins were confirmed to be different in the validation cohort. Of the 11 proteins that were validated, four were type II cytoskeletal keratins (K2C6A, K2C6B, K26C, and K2C75). All validated proteins were in higher concentration in urine of infants without BPD compared to infants with severe BPD.

Blood Studies: To date, there have been no publications of unbiased proteomic analyses of blood (plasma/serum) in BPD. We developed multi-variable logistic regression models for the outcome of BPD and/or death at 36w post-menstrual age using clinical and cytokine data from the first 28 days in a cohort of 1067 extremely low birth weight infants. 25 cytokines were measured from whole blood spots collected within 4 h of birth and on days 3, 7, 14, and 21 (4). Stepwise regression using peak values of the 25 cytokines and 15 clinical variables identified variables associated with BPD/death. Multi-variable logistic regression was done for BPD /death using variables selected by stepwise regression. Similar analyses were also done using average cytokine values from days 0-21, days 0-3, and from days 14-21. Of 1062 infants with available data, 606 infants developed BPD or died. Combining results from all models, BPD/death was associated with higher concentrations of interleukins-1β, -6, -8, -10, and interferon-γ and lower interleukin-17, RANTES, and tumor necrosis factor-α. Compared to models with only clinical variables, addition of cytokine data improved predictive ability by a statistically significant but clinically modest magnitude. The overall pattern of cytokines suggests BPD/death may be associated with impairment in the transition from

the innate immune response mediated by neutrophils to the adaptive immune response mediated by T-lymphocytes.

Methods:

Urine collection:

Urine samples will be collected following enrollment, using the “cotton ball in diaper” method that we have developed and used successfully in previous studies (27).

Urine processing:

Urine samples will be analyzed according to established workflow and pipelines (as in Preliminary Data and (28)) at the UAB MS/Proteomics Shared Facility by Dr. Jim Mobley (see Letter of Support). 1 mL per sample will be cleaned up with StrataX, concentrated down in 100mM Ambc using 3k cut-off spin columns, and quantified with BCA protein assay kit. Samples will be separated on 10 percent Bis-tris gel, and stained overnight with colloidal coomassie. In-gel trypsin digestion will be set up, and the digested samples then subjected to LC-ESI-MS/MS analysis. The LCMS analysis and database searching will first be completed. We will filter the protein list by high confidence filters and trim the data to proteins with a confidence interval of 97-99% and 2 unique peptides per protein (in our preliminary study, from ~2500 proteins down to ~1000 proteins). We then filter this list based on commonality or the number of times a specific protein met the above criteria within a clinical group (which in our preliminary data was 75% for groups of 4 patients, and 100% for groups of 3 patients). These criteria will have to be met at least once in either the control or severe groups to go forward for the statistical analysis. The remaining proteins significantly changed by way of fold change cut off at +/- 2.0 and passing nonparametric statistical tests will then by analyzed by Systems Biology analysis in GeneGo's MetaCore.

Blood collection:

As mentioned in General Methods, 0.3 ml of whole blood collected with EDTA will be centrifuged (1,000g x 15 minutes) and plasma and cell pellets will be separated. The plasma will be frozen at -80^{o}C and subsequently analyzed for proteomics (cell pellet will be used for genomic analyses). Samples with excessive turbidity (visually assessed) or hemolysis (absorption at 540nm) will be excluded.

Plasma cytokine estimation:

As in Preliminary data, we will analyze using the Milliplex MAP magnetic bead-based premixed 41 plex multianalyte panel (HCYTMAG-60K-PX41, EMD Millipre) and Milliplex Analyst 5.1 software on a Luminex 200 system (EMD Millipore). This panel measures 41 analytes in 25 µl of plasma, with 87-107% accuracy and a standard range of 3.2-10,000 pg/mL.

Plasma proteomic processing:

Plasma samples will initially be analyzed according to an established workflow with upgrades to the instrumentation and software pipelines previously published (29) at the UAB MS/Proteomics Shared Facility by Dr. Jim Mobley (see Letter of Support). Briefly, we will assay plasma for total protein content and then perform reverse phase extraction and mass analysis by matrix assisted laser desorption time of flight (MALDI-TOF) mass spectrometry (MS) (29). Freshly prepared protein extracts will be immediately mixed 1:1 with 50% saturated matrix consisting of sinapic acid in 50:50 CH_3CN:1% TFA. The dried droplet method will be used to crystallize 1 µl of the matrix/sample mixture on a ground steal 384 well probe (Bruker Daltronics; Billerica, MA). Protein mass profiles will be obtained in automatic mode using a Bruker Daltronics Ultraflex III with FlexControl 3.3.64, and 1000 replicate spectra will be summed and saved using FlexAnalysis 3.3 build 64 within Compass for Flex Series 1.3 (Bruker Daltronics). The mass window will be set to acquire data from 2.0 to 20.0 kDa with a 150 nanosecond delayed extraction time. Patient plasma will be extracted and analyzed in triplicate and averaged prior to statistical analysis. Any sample that presents with a portion of the triplicate spectra exhibiting error above 25% will be re-analyzed. On the day of the experiment external and internal calibration will be carried out with a combination of external protein standards, and common serum proteins to that encompass the MW range of interest, respectively. We have found that average instrumental error was 15.7%, experimental error was 18.3% and estimated population error was 24.1%. All post-acquisition data processing are carried out together using Refiner MS (Genedata Expressionist, Basel, Switzerland), and will include baseline subtraction, normalization, peak (feature) picking, alignment, and exporting to an Excel spreadsheet (30). The resulting data matrix are standardized such that all data are set between 0 and 1.0 by taking the difference between each sample intensity (S_X) and the lowest intensity within a group (S_L) for the range ($S_H - S_L$) using the equation, $(S_X - S_L)/(S_H - S_L)$ (29). This technique places equal emphasis on all signal intensities, whether high or low, and is especially useful when minimally and highly expressed proteins are important for diagnosis.

Evaluation of the development (training) set for detection of outcomes ("resilience", "predisposition" etc) using multivariate analysis will then be performed using Statistica (Dell): The training set will first be analyzed by calculating the relative class separation matrices from the standardized data matrix containing all protein spectra (31). Weight values are then calculated for each MW value and sorted according to the highest statistical relevance between the patient groups. The weight value (W) is a statistically derived function that approaches significance as the distance between the means (μ) for each group increases and the SD (σ) decreases using the formula, $W = (\mu_1 - \mu_2)/(\sigma_1 + \sigma_2)$. The most significant protein peaks are then used as the top weighted data points (*m/z* intensities). Top weighted data points, which maximally differentiate individuals (e.g. resilience vs. non-resilience), will be obtained from the combined set of 30 mass spectra (15 patients with resilience and 15 non-resilient patients) randomly pulled from the original set of all spectra. A series of data sets containing the most significantly changed features to the least significantly changed will be further analyzed with multivariate analysis as a means of determining the optimal number of top weighted points required to completely separate the spectra of patients with resilience from those of non-resilience (or predisposition vs. non-predisposition). K-means and hierarchical cluster analysis (HCA) will be carried out using Euclidean distance matrices formed from the standardized data matrix using Statistica. The data set with the least number of mis-clustered patient samples will then be used as the diagnostic standard. Final feature intensities will be analyzed by Cluster and TreeView analysis freeware (Michael Eisen, Stanford, California). Protein peaks found to be outcome specific (e.g. resilience) by statistical analysis will be QC checked to determine that high weighted data points are in fact real and not artifacts associated with noise or other unforeseen factors.

Class prediction by weighted voting scheme will be performed on blinded spectra using the optimized training set as the diagnostic standard (31). The optimized training set will also be tested along with the unknowns but separately by compiling the entire standardized data matrix minus the sample being tested (n −1), thus allowing each known sample to be treated as an unknown (n-way cross validation). The most significant features with diagnostic efficiency will be identified as previously described using a Top-Down-Directed (TDD) approach (32).

Anticipated Results, Potential Problems, and Alternative Strategies:

We anticipate confirming and validating the changes in the urinary proteomic biomarkers and cytokines associated with BPD that we have identified (Preliminary Data). We anticipate that these alterations will indicate infants "predisposed" for BPD (e.g. increased IL-8 and IL-10, decreased TREM-1 and RANTES in plasma; reduced urinary K2C6A, K2C6B, K26C, and K2C75), and those who are "resilient". In addition, we also anticipate identifying novel plasma proteomic biomarkers of resilience or predisposition.

A limitation is that many proteins shift between post-translationally modified (PTMs) forms without changing in amount and are not picked up with standard proteomic methods, but analysis of PTMs is beyond the budgetary scope of this project. Other methods will also be applied as needed, including a highly quantitative isobaric chemical tagging approach using commercially available kits, such as TMT-6plex (Tandem Mass Tag Reagents; Thermos). This approach is combined with a 2D multidimensional protein identification technology (MudPIT) using a nano-liquid chromatography (nLC) system in-line with an LTQ Velos Pro Orbitrap (Thermo) (33). This will be applied as an alternative approach, and will be run on a subset of samples to determine if additional peptides can be identified, and as needed for validation.

A more general limitation of the proteomic approaches proposed are that these are "discovery" methods, and not hypothesis-driven (even though we have potential urinary candidate biomarkers). It is possible, despite the stringent statistical methods used, that some relationships may be by chance. Therefore, our approach is to identify these proteins (develop these models) in one set of patients, and then test the hypothesis that these markers are associated with the worse outcome through validation experiments carried out in a different set of patients.

Specific Aim 3 - Development and validation of a personalized airway microbiome risk/resilience score for BPD and severe BPD using tracheal aspirates collected in the first postnatal week.

Background, Rationale, and Preliminary Data:

The microbiome is becoming increasingly recognized as a contributor to normal as well as abnormal developmental and metabolic processes. While initially thought to be sterile, there is much recent evidence that the airways harbor a microbiome, and that different phyla dominate diseased as compared to healthy lungs, in disorders such as asthma, COPD, and cystic fibrosis (34, 35). However, it is not currently known if airway microbiota directly contribute to onset and progression of disease. There is some evidence that

airway colonization with pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* in neonates is associated with increased pneumonia and bronchiolitis in early life(36).

Lohmann et al.(37) evaluated the microbiome in 25 preterm infants using 16S rRNA amplification and sequencing of bacterial DNA from tracheal aspirates. It was found that Acinetobacter was the predominant genus in the airways of all infants at birth(37). 10 of 25 infants developed BPD and showed reduced bacterial diversity at birth. No differences were detected in bacterial diversity, cytokines, lipopolysaccharide, and lipoteichoic acid (gram+ bacterial product) from infants with and without exposure to chorioamnionitis, suggesting chorioamnionitis was not a major influence on the airway microbiome (37). This study indicates airways of premature infants are not sterile at birth. Reduced diversity of the microbiome may be an important factor in the development of BPD and is not associated with differences in inflammatory mediators (37). However, this study had a very limited sample size, and did not determine differences in microbiome by severity of BPD.

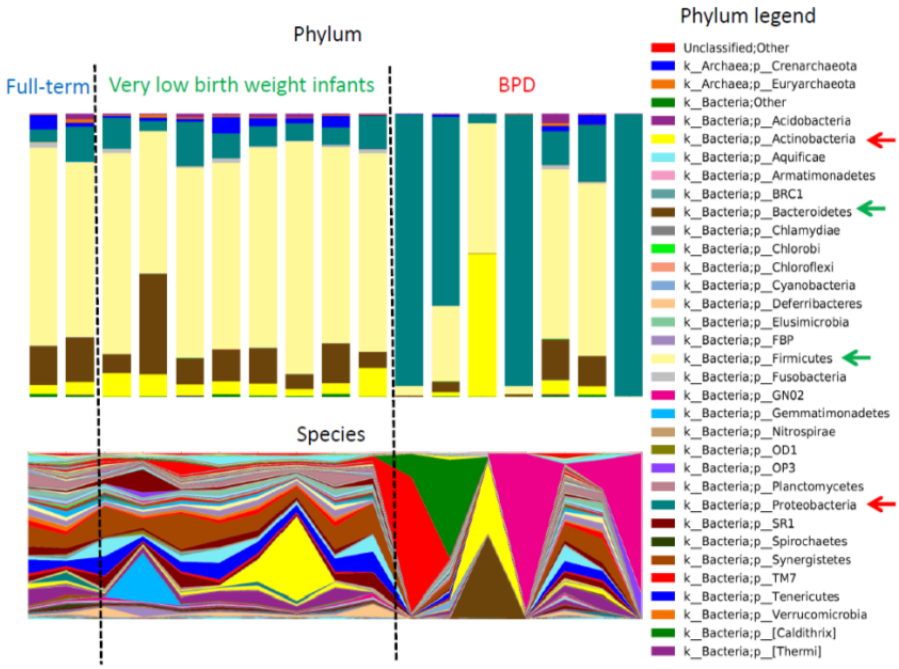

Figure 3: The airway microbiome is similar soon after birth in full-term and very low birth weight preterm infants, but infants with BPD have a very different microbiome, characterized by an increase in phyla Proteobacteria or Actinobacteria, and a decrease in Bacteroidetes and/or Firmicutes (upper panel). The lower panel shows that there is a reduction in bacterial diversity in infants with BPD (each color represents a different bacterial species). Each individual infant is a column.

We have performed preliminary analysis of the airway microbiome in BPD, by 16S RNA sequencing on tracheal aspirates from full-term controls, extremely preterm infants soon after birth, and preterm infants with established BPD (**Figure 3**). We found that the microbiota of both full-term and extremely preterm infants soon after birth was similar, with a predominance of Firmicutes, and fewer Bacteroidetes, Proteobacteria, or Actinobacteria. In contrast, many infants with BPD had greater Proteobacteria or Actinobacteria, and fewer Bacteroidetes and/or Firmicutes, with a reduction in bacterial diversity indicated by fewer bacterial species within these phyla (**Figure 3**).

Methods:

We will do airway microbiome analysis on tracheal aspirates using established workflows at the UAB Microbiome Resource, directed by Dr. Casey Morrow (see letter of support). Dr. Morrow's laboratory uses Fecal DNA isolation kit (Zymo Research (catalog # D6010) for all DNA isolation. The isolated DNA is quantitated prior to PCR(38). Barcoded PCR amplification of the V4 region of the rRNA gene (39) is accomplished using degenerate primers originally from Caparoso et al.(40), and modified as described by Kumar et al.(38) for use on the MiSeq. The PCR is carried out under conditions described by Kozich et al.(41) and Kumar et al.(38). PCR products are resolved on agarose gels, the DNA isolated, purified using Qiagen kits and quantitated. The products are sequenced using Nextgen sequencing (MiSeq platform) by the UAB Heflin Center Genomics Core Facility. The procedures used for MiSeq analysis of the amplified V4 PCR products can be found in Kumar et. al.(38). The MiSeq is a single flowcell, single lane instrument that can generate approximately 9Gb of sequence data from a paired end 250bp run.

The raw sequence data is transported to Dr. Elliot Lefkowitz and Dr. Ranjit Kumar (Bioinformatics). To support the analysis of data derived from the large-scale sequencing of the 16S ribosomal RNA genes to support microbiome studies, we have established an analytical pipeline similar to that used by Rob Knight from the University of Colorado (40, 42), based on the QIIME tool suite (43). The first step is to analyze the quality of raw dataset using FASTQC and then filter the high quality data using the FASTX toolset. Then a

combination of tools within the QIIME pipeline are utilized for clustering reads into operational taxonomic units (uclust), taxa assignment (RDP classifier using the Greengenes 16S rDNA database (44-46), and as necessary alignment and phylogenetic inference using PyNAST (47) and Fasttree (48). These procedures allow us to quantitatively assess the microbiome population down to the genus, and frequently species level. For comparative analyses, tools such as UniFrac (49) are used to assess the overall differences between microbiome populations of different samples by principal coordinates analysis. As needed, other analytical packages such as Mothur (50) and Genboree (51) are utilized to extend the analytical pipeline.

Anticipated Results, Potential Problems, and Alternative Strategies:

We anticipate confirming our preliminary findings that there is an initial predominance of Firmicutes, which would transition to greater Proteobacteria or Actinobacteria, and fewer Bacteroidetes and/or Firmicutes in the infants who are “predisposed”, while infants who are “resilient” will not demonstrate these changes. We also expect that infants who are “predisposed” will have a greater reduction in bacterial diversity indicated by fewer bacterial species, as compared to infants who are “resilient”.

One limitation is that some preterm infants are not intubated (maintained on CPAP), and we will not be able to evaluate the airway microbiome in these infants (the nasopharyngeal microbiome is not the same as the tracheal microbiome). At our center, 70% of ELBW infants are intubated on day 1 (210 infants of 300 enrolled). We have been able to analyze >80% of collected tracheal aspirates. Therefore, we expect to evaluate 160 samples. This sample size is adequate to demonstrate a 40% difference between groups at 80% power for a given species/phylum, if the standard deviation within a group is two-third of the mean. Another limitation is that while we are evaluating the microbiome (which includes Ureaplasma in Tenericutes), we are not evaluating viruses that may contribute to respiratory deterioration (52). As “omic” approaches are not suitable for viruses (lack of a common sequence analogous to the 16S bacterial sequence), specific primers are needed for each viral species requiring “viral respiratory panels” that can identify only a limited number of viruses. We will archive the tracheal aspirates for future viral and fungal analyses beyond the budgetary scope of this project, but for future funding will be sought.

As an alternative/complementary microbiome profiling strategy, we are planning to develop a “lab on chip” (LOC) approach using a length heterogeneity polymerase chain reaction (LH-PCR) method as described by Bjerketorp (53), in which the LH-PCR profile analyses are based on the inherent variation in sequence lengths of undigested 16S rRNA genes amplified from different bacterial species (54). In LH-PCR, the fluorescent dye incorporated into the gel matrix of commercially available chips is used to stain and size the separated DNA molecules by comparison to standards. The LOC would enable rapid microbiome analyses not feasible with current batch processing of samples.

Specific Aim 4 – Development of a combined “Omic” scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model

Background, Rationale, and Preliminary Data:

Our web-based estimator (https://neonatal.rti.org/index.cfm?fuseaction=BPDCalculator.start) provides an estimate of the risk of severe BPD or BPD in individual infants using readily available clinical information (1). This risk estimate will then be used to identify infants who are “resilient”, “non-resilient”, “predisposed”, and “non-predisposed” as defined earlier in Aim 1. In this project, we will first identify markers of resilience and predisposition using genomic markers (Aim 1), urinary and serum proteomic markers (Aim 2), and airway microbiomic markers (Aim 3). Then, we will develop a combined “Omic” scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model. This model will be developed in the development dataset and validated in the validation dataset.

In recent years, we have developed several innovative prediction models and scoring systems for neonatal outcomes. We have developed scoring systems and classification and regression tree (CART) models for prediction of death/disability in infants diagnosed with hypoxic-ischemic encephalopathy (55), “outcome trajectories” using a web-based estimator that enables dynamic modeling of the changing probability of individual outcome over time in extremely preterm infants (56), predictors of death or BPD in preterm infants with respiratory failure (2), predictors of neurodevelopment (https://neonatal.rti.org/OTEstimator/index.cfm?fuseaction=OTEstimator.start) using CART models (57), mortality using logistic regression and neural networks (58, 59).

In this Aim, we will develop scoring systems and CART models (similar to our models for hypoxic-ischemic encephalopathy (55)) to predict resilience and predisposition.

Methods:

Scoring systems and CART models will be developed for "resilience" and for "predisposition" to severe BPD or death, with the following variables (available in the first 3 days) considered for inclusion:

(a) Clinical variables (day 1 variables from the web-based BPD estimator): Gestational age (weeks), Birth weight (g), Sex, Race/Ethnicity – White/Hispanic/Black, Respiratory support (no support, nasal cannula or hood, CPAP, conventional ventilator, or high frequency ventilator), oxygen concentration (maximum FiO_2). Additional clinical variables from days 1-3 (from all collected variables – General Methods) will be included if they are selected by stepwise regression models at $p<0.2$.

(b) Genomic/Proteomic/Microbiomic variables: The components of the genomic (or proteomic or microbiomic) signature that maximally differentiate between the "resilient" and "non-resilient" infants (or between the "predisposed" and "non-predisposed" infants) will be selected for inclusion. Similar to our preliminary urinary proteomic data or blood cytokine data, we anticipate that 5-10 variables will be selected from each of the "Omic" approaches.

We plan to develop scoring systems on infants in the development set using just clinical variables as well as using clinical variables in combination with the "Omic" variables, similar to our approach for infants diagnosed with hypoxic-ischemic encephalopathy (55), by initial multivariable logistic regression analysis in SAS using the included clinical and "Omic" variables, followed by creation of scoring systems, weighting each predictor in proportion to its odds ratio in the regression equation (55). We will also develop CART models using SPSS Decision Tree (IBM), by recursive partitioning and automatic selection of optimal cutoff points for variables (e.g. for gene expression value, protein amount, or % expression of bacterial phylum) (55). Optimal CART models will be identified by 10-fold cross-validation.

The scoring systems and CART models will then be validated (correct classification rates, sensitivity, specificity, positive & negative predictive values, AUC of ROC) on Validation dataset as described (55).

Anticipated Results, Potential Problems, and Alternative Strategies:

We anticipate that the addition of our "Omic" variables will improve prediction accuracy for "resilience" or "predisposition" from approximately AUC of ROC of 0.60 (0.50= no better than chance, 1.0 = perfect prediction) using just clinical variables, to 0.75 using one of the "Omic" models (either genomic or proteomic or airway microbiomic), to 0.85 using two "Omic" models (e.g. genomic + urinary/plasma proteomic) to 0.90 using all clinical and "Omic" model variables. At present, there is insufficient data to indicate if one of the "Omic" methods would prove superior to the others, or to indicate the magnitude of improvement with each method, although our preliminary studies suggest we should be able to achieve an improvement in AUC of about 0.25 with an initial method, with a declining increment with additional method.

A potential problem in modeling is that the included variables are not truly "independent" and have some degree of collinearity. However, we have not found this to be a major issue in prior studies. We will develop parsimonious models to have the best accuracy with the least number of variables. A limitation is that while the models may be excellent at predicting resilience or predisposition, this does not imply causation or suggest that the variable (e.g. gene or protein the expression of which is important) is a therapeutic target. Basic science studies will be required to investigate these potential modulators of resilience or predisposition.

Another potential problem in modeling is the large number of variables and the relatively small sample size (35 resilient vs. 15 non-resilient, 15 predisposed vs. 85 non-predisposed), which decreases the reliability of estimates of parameters in the models. We will explore shrinkage based methods enabling reliable parameter estimation even when the number of variables is greater than the number of samples. Specifically, we will use an R package, BGLR (Bayesian Generalized Linear Regression), developed by Dr. Zhang's colleague Dr. Campos (60). There are several advantages of this package: (1) the methods are based on generalized linear models; and (2) several different types of shrinkage methods are implemented.

Timeline and Milestones:

These Aims are ambitious, but our center has a large number of extremely preterm infants being enrolled in the GDB, the Methods for each of the Aims are established with workflows and pipelines (see Support Letters), and investigators have the necessary expertise. We anticipate that the initial 150 infants for the Development dataset will be enrolled by end-Yr 1 (assuming 75% of eligible 200 infants will be enrolled), and the remaining 150 infants will be enrolled by end-Yr 2. If enrollment is slow for any reason, we will be able to extend enrollment by 3-4 months for either cohort. We anticipate that this project will result in at least 4 high-impact publications (one from each Aim), and multiple publications resulting from secondary analyses and archived samples.

Protection of Human Subjects

1. Risks to the Subjects

Human Subjects Involvement and Characteristics

We propose to study 300 extremely low birth weight (ELBW) infants admitted to the Regional Neonatal Intensive Care Unit (RNICU) at the University of Alabama at Birmingham (UAB) hospital in Birmingham, AL. These infants are critically ill, and have a high mortality (20% overall in the UAB RNICU) and morbidity (about one in three survivors will have a major handicap).

This study will enroll ELBW neonates in our Regional NICU (RNICU) as follows:

Inclusion:

- Inborn infants weighing 401-1,000 grams on admission and/or $22^{0/7}$ to $28^{6/7}$ (<29 weeks) inclusive completed weeks of gestation,
- Infants eligible for full care and resuscitation as necessary, and surviving beyond 12 h of age
- Enrollment at <72 hours post-natal age
- Informed consent from parent/guardian

Exclusion

- Refusal or withdrawal of consent
- Major congenital malformations (e.g., not including patent ductus arteriosus, small hernia)

The samples to be collected from the infants are as follows:

Whole blood: Blood from all infants will be collected on day 1-3. 0.3 ml will be collected with EDTA anticoagulant, at the time of routine sampling. Blood samples will be centrifuged (1,000g x 15 minutes), plasma and cell pellets will be separated, and the plasma frozen at -80^{o}C and subsequently analyzed for cytokines and plasma proteomics (Aim 2), while the cell pellet used for genomic analysis (Aim 1).

Isolation of RNA and DNA: The cell pellet from blood will be used for automated RNA and DNA isolation on the QIAcube (Qiagen) using the AllPrep DNA/RNA Mini Kit (Cat# 80204, Qiagen). DNA or RNA concentration is measured by NanoDrop Spectrophotometer (NanoDrop ND-1000; Thermoscientific, Waltham, USA), and quality determined by 2100 Bioanalyzer (Agilent, Santa Clara, USA).

Tracheal aspirates: Tracheal aspirate samples (from intubated infants during routine suctioning) will be collected on day 1-3. Tracheal aspirates will be centrifuged (1,000g x 15 minutes), and the cell pellet and supernatant frozen separately. Microbiome analysis in Aim 3 will be done on supernatant.

Clinical management: Medical interventions and ventilator management will be conventional and based upon the clinical discretion during the hospital course. No changes in management are required for this observational cohort study. These infants are at high risk for bronchopulmonary dysplasia and other complications of prematurity, but enrollment in this study which is strictly observational poses no additional risk or changes in clinical management.

There are many reasons why we propose studying extremely low birth weight infants:

(i) These infants are most at risk for bronchopulmonary dysplasia or death (a competing outcome for BPD, as infants who die early cannot develop BPD), with approximately 1 in 3 infants either developing BPD or dying. Studies in older or larger infants are therefore not considered as they are not at high risk for BPD and the risk of mortality is also lower. These very preterm infants are also the ones at highest risk of respiratory sequelae in early infancy.

(ii) biomarkers to be investigated in this study may have much prognostic significance in this vulnerable population.

(iii) There is a biologic rationale in that BPD is a multifactorial disorder, and systems biology or global approaches using genomic or proteomic approaches are needed to identify novel biomarkers and mechanisms of disease, as multiple approaches evaluating single pathways or key molecules have not been successful.

Sources of materials

We will obtain blood samples at the same time as clinically indicated tests and tracheal aspirate samples following clinically indicated tracheal suctioning. We will also prospectively collect clinical data on patient characteristics, illness severity, and outcomes from the medical records of the enrolled infants using electronic templates.

Potential risks

For risks using pediatric subjects, for which there is no promise of direct benefit, it is imperative that risk be minimal. Current technology utilizing very small samples of blood enable us to use relatively small blood samples or tracheal aspirate samples for analysis of biomarkers. The volume of blood collected (0.3 ml) is also small. We will collect blood or tracheal aspirates during the time of clinically indicated blood draws (e.g. during sampling for blood gases or electrolytes) or suctioning, respectively, to minimize pain and discomfort.

We will also collect clinical data on patient characteristics, illness severity, and outcomes from the medical records of the enrolled infants. Results and their linked clinical data will then be assigned an unique identifying code and anonymized to minimize risks to confidentiality. Only the PI and research coordinator will have access to patient data before anonymization. No patient identifiers will therefore remain in the final data set.

There is no financial risk from participation in this study.

Our laboratory genomic, proteomic, and microbiomic analyses are not certified for use in human neonates for diagnosis or treatment, and are proposed in order to confirm current hypotheses or generate new ones. We do not know how to use the information medically and there is a risk if such information were made available and misinterpreted. A danger inherent in analysis of risk factors or genetic testing is the identification of markers predictive of later poor outcome. This can result in psychological harm (to parents or other caregivers), discrimination, or other adverse consequences. Anonymization of the data set after data collection, and before analysis for genomic or proteomic markers or gene polymorphisms, should protect against this risk.

2. Adequacy of protection against risks

Recruitment and Informed Consent

We plan to prospectively study 300 consecutive ELBW infants surviving beyond 12 h of age at our Regional Neonatal Intensive Care Unit (RNICU) over a 2-year period (total duration of study including analyses of samples is three years). All data will be prospectively collected and anonymized using a code number to link the clinical data and collected samples, before genomic or proteomic analysis. Institutional Review Board (IRB) approval will be obtained before initiation of the study.

Protection against risk

All remnant blood and tracheal aspirates will be collected during clinically indicated blood sampling or suctioning, respectively. The volume of blood being collected (0.3 ml on day 1-3) is relatively small (for a 600 g infant, about 0.5 ml/kg at birth as blood volume in ELBW infants is 90 ml/kg).

Informed consent will be obtained from parents by trained research coordinators who have many years of experience in obtaining informed consent from parents of very preterm and critically ill newborn infants.

Our laboratory genomic, proteomic, and microbiomic analyses are not certified for use in neonates for diagnosis or treatment, and analyses will also be performed after collection of the clinical data. We also do not know at the current time how best to apply the findings for purposes of medical care. The data set will also be anonymized without any patient identifiers. Only the PI and research nurse will have access to patient data before anonymization. We therefore do not plan to share individual test results with the subjects or their families, except in aggregate form after publication.

3. Potential benefits of the proposed research to the subjects and others

There is no direct benefit to study participants from participating in this study. Genomic, proteomic, and microbiomic analyses and prediction models will be done on stored samples, after most enrolled infants are already discharged/dead. Based on previous discussions, parents of premature infants who die or develop BPD are strongly interested in knowing why (as a group) they were affected and in contributing to future prevention. The results of this study will be published in the peer-reviewed literature and then deposited in PubMed Central to make the manuscript freely available, which may benefit other investigators in the field as well as interested parents and family.

In the event that we identify biomarkers in blood or tracheal aspirates, or certain gene polymorphisms, are predictors of death/BPD, then these may prove to be important prognostic markers that may be helpful in risk-stratifying infants for possible interventions.

4. Importance of the knowledge to be gained

At the conclusion of this study, we should have answered several questions about the role of pathways important in resilience or predisposition to severe BPD, a disease of considerable public health importance. We will also have identified a genomic, proteomic, and airway microbiomic profile associated with resilience or

predisposition to BPD. The identification of novel biomarkers or novel BPD/respiratory sequelae pathways by genomic or proteomic analysis may lead to the development of new treatment strategies

Inclusion of Women

The proportion of female to male infants is expected to be approximately 1:1 in the population of ELBW infants to be studied.

Inclusion of Minorities

Extremely low birth weight infants will be recruited independent of ethnicity or race because death/BPD in ELBW infants occurs in all races. Our study composition will reflect the epidemiology of extreme prematurity in the state of Alabama, with approximately 51% being Black, 47% White, and the remaining Hispanic/Other.

Inclusion of Women and Minorities

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OMB Number: 0925-0002

Planned Enrollment Report

Study Title: STOP BPD

Domestic/Foreign: Domestic

Comments: 300 preterm infants 401-1000 grams inclusive birth weight and/or 22 weeks 0 days to 28 weeks 6 days (<29 w weeks inclusive completed weeks of gestation), for whom a decision has been made to provide full resuscitation, will be enrolled, and followed-up to initial hospital discharge (or death, if prior to discharge).

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	**0**
Asian	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	77	76	0	0	**153**
White	70	71	3	3	**147**
More than One Race	0	0	0	0	**0**
Total	**147**	**147**	**3**	**3**	**300**

Study 1 of 1

Funding Opportunity Number: RFA-HL-15-024. Received Date: 2015-01-16T14:25:01.000-05:00

Inclusion of Children

We are investigating genomic, proteomic, and airway microbiomic biomarkers in relation to resilience or predisposition to bronchopulmonary dysplasia (BPD). BPD is a respiratory disorder that occurs in premature infants following respiratory distress syndrome, and thus only very premature infants will be studied. We plan to study extremely low birth weight (ELBW) infants as this is the population of very premature infants at highest risk for BPD. BPD is extremely uncommon in larger (> 1 kg birth weight) preterm infants and does not occur in older children or adults. Death is a competing outcome for BPD, as infants who die soon after birth cannot develop BPD. The outcomes studied are therefore death or severe BPD by 36 weeks' post-menstrual age.

The PI and Dr. Waldemar Carlo (Co-Inv) are board-certified in Pediatrics and Neonatal-Perinatal medicine, and have been on the faculty of the University of Alabama at Birmingham Department of Pediatrics for fifteen and twenty-five years, respectively, and have performed over 50 studies involving critically ill infants.

The involvement of children as subjects in research will be in compliance with all applicable subparts of 45 CFR Part 46 as well as with other pertinent Federal laws and regulations.

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Heflin Center for Genomic Sciences

January 8, 2015

Namasivayam Ambalavanan MD
Division of Neonatology,
Professor, Departments of Pediatrics, Molecular and Cellular Pathology, and Cell, Developmental, and Integrative Biology
Neonatal-Perinatal Medicine Fellowship Training Program Director
University of Alabama at Birmingham
Birmingham, AL 35294

Dear Ambal,

The Heflin Center Genomics Core facility has the genetic technologies you require for your R01 grant application entitled "STOP BPD (Signature of Top Omic Profiles in BPD)" to the NIH. For the expression studies you have outlined we will run the Illumina Human HT-12 expression arrays. The core runs and maintains the Tecan Evo liquid handling robot and the Illumina iScan system to process the expression arrays. Analysis of the expression microarrays will be done in GenomeStudio initially and further analysis will be done using packages from BioConductor. Pathway analysis can also be done on significantly expressed genes using Ingenuity Pathway Analysis (IPA). In addition to the expression studies the core will perform the genotyping analysis on the selected SNPs by Pyrosequencing. The core utilizes the Biotage (now Qiagen) HSQ 96 pyrosequencing instrument that allows for batching up to 10 96-well plates for genotyping analysis.

We look forward to working with you on this project.

Sincerely,

Michael R. Crowley, Ph.D
Director, Heflin Center Genomics Core Facility

David K. Crossman, Ph.D
Director of Bioinformatics

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UAB SCHOOL OF MEDICINE
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University of Alabama at Birmingham
176F Suite 9380, 619 South 19th Street
Birmingham, AL 35249-7335
Ph: (205) 934 4680, Email: ambal@uab.edu

Date: January 2nd, 2015

RE: Support letter for the project entitled "STOP BPD (Signature of Top Omic Profiles in BPD)"

Dear Dr. Ambalavanan,

Dear Dr. Ambalavanan,

I look forward to expanding our current collaborations in your RO1 proposal entitled "STOP BPD (Signature of Top Omic Profiles in BPD)" where you have proposed to evaluate genomic, proteomic, and microbiomic profiles in BPD. The proteomic component of this proposal makes sense, as it is a follow-up to our previous work carried out on neonatal urine specimens. I understand that in this new study, your group will be collecting urine specimens to further validate/confirm our previous biomarkers for BPD. The mass spectrometry-proteomics applications will be carried out by my group within the UAB CCC MS/ Proteomics (MSP) Shared Facility (SF) where high resolution mass spectrometry will be complemented with a host of the latest in informatics applications to carry out quantification, statistical & systems analysis, interpretation, and reporting. Finally, all data will be uploaded to our laboratory informatics system as it is produced and evaluated by myself.

As you know, I have over 15 years of mass spectrometry experience specifically in the area of proteomics. As director of the MSP-SF, I have established state-of-the-art mass spectrometry systems, including a Velos Orbitrap Pro, two LTQXL's with CID/ETD, an Ultraflex III MALDI TOF/TOF, a 6530 QTOF, and a 7700 ICP-MS. These instruments are very well equipped with the additional of nano-LC systems, software tools that include MASCOT, SEQUEST, Proteome Software (Scaffold, Scaffold PTM and Q+), ProteoIQ (NuSep), Skyline, and Genedata (Refiner MS) to offer the very best outcomes for a wide range of -omics based procedures. Our Genedata server also houses a vast array of putative time and mass tag MS, and assigned MS/MS peptide libraries where Refiner MS is capable of quickly aligning, assigning, and quantify individual peptide species. Finally, I have site licenses to both IPA (Inginuity), and Metacore (Genego) to generate the highest level in systems biology analysis. This level of informatics combined with the latest in high resolution LCMS technologies will provide excellent assistance to your proposed project. Additionally, as discussed we have a Ph.D./ M.D. level statistician/ systems biologist that collaborates with our facility to assist when necessary.

Again, I look forward to working with you and your group to support this project, best of luck!

Sincerely,

01-02-2015

James A. Mobley, PhD
Assistant Professor, Surgery, Chemistry, Preventive Medicine, Toxicology & Pharmacology
Director, The UAB Bioanalytical and Mass Spectrometry Shared Facility

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THE UNIVERSITY OF
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December 17, 2014

Namasivayam Ambalavanan MD
Division of Neonatology,
Professor, Department of Pediatrics,
176F Suite 9380, Women and Infants Center
619 South 19th Street
Birmingham, AL 35249-7335

Dear Dr. Ambalavanan,

I am writing to indicate my great enthusiasm for the experiments in your proposal **"STOP BPD."**

In your current application, we would be happy to continue our collaboration with you for microbiome analysis of tracheal aspirate specimens. We will follow our protocols described in a soon to be published in *Current Protocols in Human Genetics* **"Getting Started with Microbiome Analysis: Sample Acquisition to Bioinformatics"** by Ranjit Kumar, Peter Eipers, Rebecca B. Little, Michael Crowley, David K. Crossman, Elliot J. Lefkowitz and Casey D. Morrow.

As **Director of the UAB Microbiome Resource**, we will also help with the analysis and data interpretation required for this project. UAB has made a commitment to become a leader in microbiome related studies. To provide the complete spectrum of analytical capabilities necessary to fully support microbiome analysis, the UAB Microbiome Resource has been established. From a recent strategic planning process, the UAB Comprehensive Cancer Center and UAB School of Medicine have provided funds to **subsidize** the Microbiome Resource to ***off set*** most of the cost for analysis, making this resource available to investigators.

The Microbiome Resource manages the samples to allow the large scale sequencing of up to 100 samples per run that reduces the cost per microbiome (MiSeq run length reads of 250 base pairs of the V4 rRNA gene, 100,000 read average per sample). My laboratory provides reagents for DNA preparation to analyze human fecal DNA is prepared for the samples using kits obtained from Zymo Research (catalog # D6010). Specific bar coded PCR primers are used to prepare the 16S rDNA region sequencing library Caparoso *et al.* (Caporaso, J. G., C. L. Lauber, et al. (2011). "Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample." Proc Natl Acad Sci U S A 108: 4516-4522) as modified by Kozich *et al.* for MiSeq (Kozich, J.J., Westcott, S. L., Baxter, N.T., Highlander, S.K. and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:17 5112-5120). PCR products are purified by gel electrophoresis and quantitated. The DNA is sequenced using the NextGen Sequencing platforms available in the UAB Heflin Center for Genomics (**Dr. Michael Crowley**). Following completion of the sequencing run, the data is placed on the high-capacity storage fabric of the high-performance computing platform

maintained by UAB Research Computing. The data is then directly available for processing by the Bioinformatics Group, directed by **Dr. Elliot Lefkowitz**. To support the analysis of the 16S ribosomal RNA genes, we have established an analytical pipeline similar to that used by Rob Knight from the University of Colorado and based on the QIIME tool suite. For data Collection, analysis, integration, and reporting, we have established an analytical pipeline for routine processing of the 16S sequence reads, along with a web site that collects data from each scientific project, consolidate that data into a web-based visualization template, and provides investigator access to the data on our protected intranet site (**developed by Dr. Ranjit Kumar**). These include a bioinformatics resource framework for sequence data, data annotation, and analysis previously developed by the Lefkowitz group to support the NIH-NIAID Viral Bioinformatics Resource Center (http://www.vbrc.org). For information derived from NextGen Sequencing technologies, including microbiome analysis, we utilize the Galaxy analytical framework for both the storage and analysis of data. Galaxy provides a wide variety of analytical tools in its basic configuration as well as the capability to add new tools as needed. Galaxy also provides the ability to create automated workflows that can be reused by multiple investigators and multiple datasets to rerun complicated analytical pipelines on a wide variety of datasets. To visualize data stored in Galaxy, we use the Broad Integrated Genomics Viewer (IGV: http://www.broadinstitute.org/igv/) to view assembled genomic sequences.

Based on the services we can offer we can easily help you in the proposed study. Therefore, in summary, I am happy to offer you the services of our resource and I look forward to working together with you on this exciting and very important project.

Sincerely,

Casey

Casey D. Morrow, Ph.D.
Professor
Department of Cell, Developmental and Integrative Biology
Director, UAB Microbiome Resource
University of Alabama at Birmingham

Resource Sharing Plan

A. Tools (Devices, Monitors, Probes, Research tools, Reagents, Model Organisms)
Tools will be made available, in accordance with the NIH Data Sharing Policy (http://grants.nih.gov/grants/policy/data_sharing), to all researchers in both the private and public sector free or for a nominal charge and with minimal restriction.

B. Data Sharing (Results, Tables, Graphs, Raw Data, Analyses)
In accordance with NIH Data Sharing Policy, we will look to share data at the earliest opportunities throughout this research project, subject to intellectual property aspects. Results will be made available to the community at large. For example, complete gene expression data will be contributed to the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/).

C. Knowledge and Results (Abstracts, Papers, Publications, Patent Applications)
As a means of sharing knowledge, NIH encourages grantees to arrange for publication of NIH-supported original research in primary scientific journals. The awardees therefore will strive to publish their findings in a timely manner and acknowledge that the research was supported by the NIH. The investigators have published their data and results in numerous publications and worldwide scientific meetings over the last ten years and they intend to continue to share data at the earliest opportunities throughout this research project. In particular:

- Results will be written up and sent for publication in relevant journals such as Nature, Science, Journal of Clinical Investigation, American Journal of Respiratory and Critical Care Medicine, Pediatrics, or Journal of Pediatrics.
- The PIs will seek to present publishable results at scientific conferences such as the annual conference of the American Thoracic Society, Pediatric Academic Societies' etc.

SUMMARY STATEMENT

PROGRAM CONTACT:
CAROL BLAISDELL
301-435-0222
blaisdellcj@nhlbi.nih.gov

(Privileged Communication) ***Release Date:*** **07/21/2015**

Application Number: **1 R01 HL129907-01**

Principal Investigator

AMBALAVANAN, NAMASIVAYAM MBBS, MD

Applicant Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Review Group: **ZHL1 CSR-H (S1)**
National Heart, Lung, and Blood Institute Special Emphasis Panel
NHLBI Lung Primary Prevention Review

Meeting Date: **07/01/2015** ***RFA/PA:*** **HL15-024**
Council: **AUG 2015** ***PCC:*** **LLLBCN**
Requested Start: **09/01/2015**

Project Title: **STOP BPD**

SRG Action: **Impact Score: 29**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **30-Human subjects involved - Certified, no SRG concerns**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**
Gender: **1A-Both genders, scientifically acceptable**
Minority: **1A-Minorities and non-minorities, scientifically acceptable**
Children: **2A-Only Children, scientifically acceptable**
Clinical Research - not NIH-defined Phase III Trial

Project Year	Direct Costs Requested	Estimated Total Cost
1		
2		
3		
TOTAL		

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

RESUME AND SUMMARY OF DISCUSSION: The applicant proposes to identify genomic, proteomic and microbiomic factors as biomarkers of resilience to or risk for the development of bronchopulmonary dysplasia (BPD) in infants enrolled in the NICHD Neonatal Research Network. The proposed research is of high public health relevance in that BPD is the most frequent complications of premature births associated with the significant morbidity and mortality and long term pulmonary consequences into adulthood. The comprehensive approach integrating genomic signatures in blood, proteomic profiling in urine and plasma, and the airway microbiome with computational modeling for estimation of the disease risk is innovative. There were concerns that the relatively small sample size proposed to represent the resilience or predisposed group may not be sufficient to generate reproducible omic signature for a disease that is multifactorial and complex. In addition, it is not clear that the outcome of the studies would provide better predictive models than current clinical data such as birth weight, gestational age etc. The applicant is a senior investigator who has extensive experience in basic and clinical research in the field of BPD. His established role in the NICHD network provides unique strengths. The investigator team is excellent providing complementary expertise needed for the project. The environment is outstanding. Overall, this is a very strong application.

DESCRIPTION (provided by applicant): Bronchopulmonary dysplasia (BPD) is a common morbidity in extremely low birth weight (ELBW) infants. Our research group has considerable expertise in translational research on BPD. We have: (a) developed models using only limited clinical information to predict BPD or death by postnatal age or respiratory illness severity in ELBW infants, (b) prospectively evaluated pulmonary hypertension in BPD and (c) identified biomarkers associated with BPD/death in a cohort of 1067 ELBW infants using multiple clinical variables and 25 cytokines in blood. More recently, we have made novel observations on the genetic basis of BPD by genome-wide analysis in 751 ELBW infants. In additional ongoing studies, we have identified novel proteomic biomarkers of BPD, and have determined alterations in the airway microbiome in BPD. The overall objective of “STOP BPD” (Signature of Top Omic Profiles in BPD) is to prospectively define and validate clinical and “omic” signatures associated with resilience against, or risk for development of BPD. To address this objective, we will build upon our recent studies on genomics, proteomics, respiratory microbiome, and model development in BPD. We will evaluate a prospective cohort (Generic Database or GDB cohort of the NICHD Neonatal Research Network) of 300 preterm infants <29 weeks gestation born in 2015-2017 (n=213 in 2013 alone, with 80%+ enrollment) by the following Specific Aims: Specific Aim 1- Development and validation of a personalized genomic risk/resilience score for BPD and severe BPD using a combination of genome-wide expression analysis and targeted SNP profiling in blood collected within 72h of birth Specific Aim 2 - (a) Development and validation of a personalized urinary proteomic risk/resilience score for BPD and severe BPD measured in the first postnatal week (b) Development and validation of a personalized plasma proteomic and cytokine risk/resilience score for BPD and severe BPD measured within 72h of birth Specific Aim 3 - Development and validation of a personalized airway microbiome risk/resilience score for BPD and severe BPD using tracheal aspirates collected in the first postnatal week Specific Aim 4 - Development of a combined “Omic” scoring system combining the genomic, proteomic, and microbiomic scores with the clinical model We will develop and determine the accuracy of the various models in the Development cohort (n=150), and validate them in the Validation cohort (n=150). These novel models can be used to define the target population for future interventions, assess efficacy of specific interventions, develop a “lab on chip”, and support future studies on the biology of BPD.

PUBLIC HEALTH RELEVANCE: Bronchopulmonary dysplasia (BPD) is a common respiratory disorder in very preterm infants, characterized by impaired lung development, and associated with long-term respiratory complications. In this study, we will evaluate 300 extremely preterm infants to determine alterations in gene expression, protein amounts, or microbial flora in the airway that are associated with resilience (resistance to development of severe BPD, even when considered to be at high risk due to

clinical risk factors) or predisposition (higher rate of developing severe BPD even if not initially considered at high risk). (End of Abstract)

CRITIQUE 1:

Significance:
Investigator(s):
Innovation:
Approach:
Environment:

OVERALL IMPACT:

The proposed research seeks to identify genomic, proteomic and microbiomic factors as markers of resilience in infants who despite being born premature do not develop bronchopulmonary dysplasia. The project is based on previous data from the investigators and now will combine different omic signatures to improve prediction and identify possible resilience factors. The potential impact of a better understanding of the pathogenic mechanisms of BPD is very high because it may lead to future strategies for prediction and prevention of this serious complication of prematurity. The few weaknesses of the application are relatively minor and can be easily addressed by the investigators.

Significance:
Strengths

- Bronchopulmonary dysplasia is one of the most frequent complications of premature births and is associated with significant morbidity and mortality and long term pulmonary consequences that can persist into adulthood.
- The investigators plan to identify omic profiles that may allow the early prediction of BPD. This would identify populations at high risk that may be included in prospective trials.
- Results may also provide insight into the mechanisms for resilience in those infants that although born at low gestation do not develop this complication.
- Accurate prediction tools may open the possibility for novel preventive interventions in the future.

Weaknesses

- Although most aims are based on previous data implicating specific mechanisms of disease, the proposal is mainly exploratory in a disease that is multifactorial and extremely complex.

Investigator(s):
Strengths

- Dr. Namasivayam Ambalavanan, the PI, is a neonatal clinician scientist who has worked and published important data on the pathogenesis and epidemiology of BPD.
- PI combines expertise in basic laboratory research with clinical responsibilities in the neonatal intensive care unit.
- PI has published important papers in the area of this proposal and is widely recognized for his expertise in lung development and injury.
- PI has a strong track record in multicenter clinical translational and basic science research in the area of BPD and has been extramurally funded for his work in this area.
- Dr. Waldemar Carlo is the Director of the neonatal program at UAB and a world renowned neonatologist with a strong record in research in neonatal lung diseases and respiratory care of the premature infant. He also has multiple publications in this area and a long track record of NIH funding.

- Dr. Kui Zhang is an Associate Professor in the section of statistical genetics, department of biostatistics at UAB and will be responsible for the statistical analysis of gene expression, proteomic and microbiome and the development of the statistical models.
- He has been working in this area for more than 15 years and also has a strong track record of publications and extramural funding.
- Dr. Kumar Ranjit is a Research Associate in bioinformatics at UAB and will be the main data analyst of the microbiome from the tracheal aspirates an area in which he has special expertise. He has authored many publications in this area. No extramural support is listed.

Weaknesses

- No weaknesses are identified in the team of researchers.

Innovation:
Strengths

- This is a proposal that is at the forefront of BPD research, a complex multifactorial disease where the normal process of lung development is altered by various interactive mechanisms of lung injury.
- This research can lead to a better understanding of some of the factors that decrease the propensity of preterm infants to develop this condition and in this way uncover effective preventive strategies.
- The investigators will focus on three major areas of interest where there is limited knowledge and they are likely to yield novel information that may improve prediction and prevention of BPD.
- Some of the underlying mechanisms that are responsible for deranged lung development may also be relevant in the developmental alterations of other organ systems in premature infants and therefore the results may have implications beyond the respiratory system.

Weaknesses

- There are no significant weaknesses in its innovative approach.

Approach:
Strengths

- In response to the RFA the study will not only evaluate the association between specific omics and the development of BPD but will also evaluate resilience mechanisms for the outcomes BPD or death.
- To achieve this objective the investigators will include infants born under 29 weeks of gestation that are those at higher risk for developing BPD.
- The investigators plan to utilize a cohort of premature infants who are available because of their enrolment in the NICHD Neonatal Network that collects the clinical information and some of the specimens that will be utilized for the omic studies in this proposal.
- Because all of these infants are included in the generic database of the Neonatal Research Network there will be detailed phenotyping of their clinical characteristics allowing the correlation between the results of the omics studies and the clinical course of these infants.
- The biological samples necessary for the omics analyses are readily available because most are obtained as part of the clinical management of these infants.
- The study does not require any intervention or management different from the routine care that these infants receive.
- This detailed information will allow the identification of factors that may predict the development of different BPD severities. This is important because the implications for future lung function and respiratory course is closely dependent on the severity of the pulmonary damage during the neonatal period.

- Infants who develop BPD are frequently treated with broad spectrum antibiotics and because they are exposed to prolonged endotracheal intubation they are colonized with pathogens quite distinct from the normal microbiota. This coupled with the well-established role of infection and inflammation in the pathogenesis of BPD makes this a very promising area of investigation.
- While most of the data available until now is from regular endotracheal bacterial cultures the investigators will perform airway microbiome analysis using established workflows that have been used at UAB microbiome resource.
- The combination of the results from the different omic studies will add strength to the proposal and allow the investigators to combine the scoring system that include the genomic, proteomic and microbiomic results with the clinical phenotypes

Weaknesses

- Because of the relatively small sample size proposed and the multiplicity of factors that will be evaluated it is possible that some of the potential associations may be missed.
- Because BPD and death are competing outcomes they have to be considered together but there are many non-pulmonary causes of death that are not easy to define. The omic factors that may contribute to BPD are not necessarily the same ones that may influence the risk of death.
- The microbiota studies in tracheal fluid will be limited only to infants who are intubated. Although these are the ones who are at higher risk of BPD, present clinical practice has limited considerably the number of babies who are intubated versus those who receive non-invasive respiratory support.
- The personalized airway microbiome risk/resilience score will be based on tracheal aspirate collected in the first postnatal week. It is likely that the microbiome changes considerably during the following weeks of life in babies who are intubated and exposed to antimicrobials for long periods of time. It would be interesting to expand this observation to a longer period of time in infants who remain intubated.
- There are significant issues with BPD definition that apply to all researchers in this area that will need to be addressed by the investigators in more detail during the study.

Environment:
Strengths

- UAB has excellent facilities that assure the proper conduct of this study.
- The investigators have well established collaboration in the work required for this application including the clinical, basic research and the data analysis activities.

Weaknesses

- No weaknesses identified

Protections for Human Subjects:
Acceptable Risks and/or Adequate Protections

- Besides a small amount of additional blood necessary for the genomic studies there are no significant additional risks from participating in this study.
- The study will not require modifications of routine clinical care and samples will be obtained at the same time as those for standard care.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Inclusion of Women, Minorities and Children and not IRB Exemption #4.
Sex/Gender: Distribution justified scientifically
Race/Ethnicity: Distribution justified scientifically
Inclusion/Exclusion of Children under 21: Including ages < 21 justified scientifically

- Female and male infants will be included in the study.
- Infants of all races will be recruited independent of their racial or ethnic background.
- All subjects included in the study will be children.

Vertebrate Animals:
- Not Applicable (No Vertebrate Animals)

Biohazards:
- Not Applicable (No Biohazards)

Budget and Period of Support:
Recommended budget modifications or possible overlap identified:

CRITIQUE 2:

Significance:
Investigator(s):
Innovation:
Approach:
Environment:

OVERALL IMPACT:

This is an excellent proposal from a team of neonatologists and scientific investigators with outstanding track records in performing biomarker studies in clinical cohorts. Strengths include a high likelihood of success in completing the study and generating high impact publications in the field, the use of multiple omic platforms, and experience with development mathematical models that predict disease outcomes. Among the minor potential weaknesses include the potential that the end products do not appear focused on better understanding mechanisms of disease or the avoidance of disease. More emphasis on discovering basis of resilience might be beneficial. This could be important especially in focusing proteomic analysis on subsets of peptides (i.e. what markers are higher in patients that do not develop BPD or are absent in patients with BPD?). Not clear how preliminary and published data have shed new insight into BPD pathogenesis or potential treatment, although this is a minor weakness inherent to discovery based studies.

Significance:

Strengths
- Very high prevalence of BPD in extremely preterm infants.
- No available therapies for prevention or treatment.
- Large populations of preterm infants do not develop BPD, emphasizing the existence of a resilient population.
- Despite many proposed genes and factors, the molecular and cellular mechanisms of disease remain unclear.
- Animal models may not accurately portray human disease, making human trials such as this proposal that much more important.

Weaknesses
- The proposal does not provide compelling argument for how the data obtained in this project will lead to better treatment or development of new therapies, although this is a minor weakness and inherent to these types of discovery studies.

Investigator(s):
Strengths

- The investigative team is outstanding. The PI and collaborators are leaders in the field of performing biomarker studies on preterm patient populations.
- Established role within the NICHD network provides unique strengths.
- Strong track record of performing these types of studies and publishing results.
- Diverse group of collaborators and consultants with expertise in various omic technologies and informatics pipelines.
- Unique experience and qualifications in developing complex mathematical models that predict disease outcomes.

Weaknesses

- None noted.

Innovation:
Strengths

- Incorporates current state-of-the-art omic technologies into a multi-modal treatment of BPD in preterm infants.
- Goal is to incorporate many different types of high content data into models and systems based algorithms that can identify and predict patients at high and low likelihood of developing disease.

Weaknesses

- Not clear that the outcome of these studies will provide better predictive models than current clinical data (birth weight, gestational age, FiO2, requirement for mechanical ventilation past 1 week, etc).
- Uses current state-of-the-art omic approaches but does not include innovation or technological advances. This is considered a minor weakness as overall aim of the project is to use current technologies, not develop new ones.

Approach:
Strengths

- Prospective cohort.
- Track record supports the likelihood of success.
- Strong disease phenotyping.
- The use of multiple platforms and modalities increases chances of new insight into disease.

Weaknesses

- The timing of various samples appears somewhat random and not necessarily connected to rigorous time points in disease progression. Should samples instead be obtained at time points related to when resilient patients show significant improvement in clinical course compared to susceptible patients?
- Multiple samples from each patient may be more beneficial, even if they are pooled.
- Little consideration of cellular sources of patient material and how disease resilience or susceptibility might contribute to variability. For example, with the various cell pellets obtained for gene expression contain different cell populations? How will the investigators determine this? What is the cellular source of the various peptides measured in urine? Since confounding variables could contribute to these differences, at least an in depth consideration of the data is warranted.

Environment:

Strengths

- Outstanding clinical and investigative environment for performing these studies.
- Appropriate collaborators with excellent expertise are included.

Weaknesses

- Collaborators and/or consultants from outside the institution are not included (minor weakness).

Protections for Human Subjects:

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Inclusion of Women, Minorities and Children and not IRB Exemption #4.
Sex/Gender: Distribution justified scientifically
Race/Ethnicity: Distribution justified scientifically
Inclusion/Exclusion of Children under 21: including ages <21 justified scientifically
Proposal involves only children < 21 years of age. Appropriate distribution of sex and ethnicity included.

Budget and Period of Support:
Recommended budget modifications or possible overlap identified:

CRITIQUE 3:

Significance:
Investigator(s):
Innovation:
Approach:
Environment:

OVERALL IMPACT:

This proposal seeks to enroll 300 early low birth weight (ELBW) infants and prospectively define “omic” signatures in blood, plasma/urine, and airway microbiome that collectively will be used to predict resilience or disposition to bronchopulmonary dysplasia (BPD). The comprehensive integration of genomic signatures in blood, proteomics in urine/plasma, and the airway microbiome with a web-based estimator of risk is considered innovative. The PI and the investigative team are highly regarded and productive in studying chronic lung disease of the neonate. However, the number of infants predicted to represent the resilience or predisposed group is too small to identify a reproducible omic signature for a disease that is widely considered to be multifactorial in nature. Preliminary data describing the small number of SNPs detected in blood of infants with severe BPD and the wide variability in urinary proteins or microbiome do not alleviate this concern. The need to continue identifying SNPs when the investigator has already identified some associated with disease is puzzling. It is not clear where this project heads if resilience or susceptibility factors are not identified. These concerns reduce enthusiasm for the proposal.

Significance:
Strengths

- The study is important because it focuses on a significant health concern of infants born preterm.

Weaknesses

- No major weaknesses noticed.

Investigator(s):
Strengths

- The investigative team has the expertise needed to conduct the studies.
- The PI, Dr. Ambalavanan is a Professor of Pediatrics at UAB. He has extensive experience leading single center clinical trials and using mouse models to study neonatal chronic lung disease. He is a productive and highly regarded investigator.
- Dr. Carlo is Division Chief of Neonatology and the site PI for the Neonatal Network. He will help the PI with patient enrollment and development of the predication model in Aim 4.
- Drs. Zhang and Kumar will be responsible for the "omic" analysis of the data. Both individuals have the required expertise needed to help the PI.
- Additional support is requested for a Research Associate who will process the samples and a Research Coordinator who will be in charge of patient enrollment
- Publications show PI and co-PIs have worked together.

Weaknesses

- No major weaknesses noticed.

Innovation:
Strengths

- Proposal compares specific molecular outcomes against a web based computer program that predicts risk of developing BPD.

Weaknesses

- Methods used to generate molecular signatures are not overly innovative.
- Proposed studies do not leverage the PI's own published data correlating SNPs with severity of BPD.

Approach:
Strengths

- Comprehensive analysis of genes in blood, proteins in plasma/urine, and the airway microbiome increases chance of detecting something that correlates with risk of respiratory disease.
- The number of infants born preterm at Birmingham is sufficiently high that enrolling 300 for the study should not be a problem.
- The first 150 infants are used to identify biomarkers while the second group of 150 infants is used for validation.
- The PI has published SNP evidence of genetic susceptibility to severe BPD (related to Aim 1) and provided preliminary evidence suggesting that urinary proteins (Aim 2) and airway microbiome (Aim 3) might also be different.
- The PI proposes to define BPD by the NIH consensus and the physiologic definition, which are two of the most commonly accepted definitions.

Weaknesses

- Generating different "omic" signatures in different compartments, while a potential strength, is also a weakness because it dilutes the power of finding something in common.
- The identification of 3 SNPs correlating severity of BPD is commendable but has not yet proven reproducible. This knowledge is also not used to strengthen the approach.
- Graph in figure 2 shows levels of urinary proteins in infants with severe, modest, mild or no BPD. It is not clear why the trends reported in the validation graph do not correlate with the trends shown in the discovery graph. The small sample size in both graphs increases concern that these trends will not be reproduced with more samples.

- Power analyses predict 50 of 150 infants will have severe disease, but only 15 will be predisposed while 35 will have resilience factors that prevent disease. In the other 100 infants, 15 will be predisposed and 85 will be resilient. It is unlikely 30 infants is sufficient to successfully generate a reproducible three tiered “omic” in blood, urine, and airway that predicts predisposition to BPD. As a comparison, the web based project used nearly 4000 individuals and in the end confirmed gestational age at birth is still the best predicator of risk.
- It is not clear why statistical studies assume there is no interaction between genes.
- Infants are enrolled for 2 years through the NICHD sponsored NRN at UAB that ends in 2016. How enrollment is affected if this grant is not renewed was not mentioned.

Environment:
Strengths

- The research environment at UAB is outstanding.

Weaknesses

- No major weakness noticed.

Protections for Human Subjects:
Acceptable Risks and/or Adequate Protections

- Approximately 300 preterm infants less than 29 weeks gestation are enrolled. Protection from risk and benefits of the study are adequately described.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

- Not Applicable (No Clinical Trials)

Inclusion of Women, Minorities and Children and not IRB Exemption #4.

Sex/Gender: Distribution justified scientifically
Race/Ethnicity: Distribution justified scientifically
Inclusion/Exclusion of Children under 21: Including ages < 21 justified scientifically

- Study design only includes infants born less than 29 weeks gestation. Infants are recruited independent of sex/gender or race/ethnicity. Enrollment is expected to correlate with the population in Birmingham AL.

Vertebrate Animals:

- Not Applicable (No Vertebrate Animals)

Biohazards:
Acceptable

- Investigators are reminded to advise workers of the hazards associated with handling human samples.

Resource Sharing Plans:

- Acceptable

Budget and Period of Support:
Recommend as Requested
Recommended budget modifications or possible overlap identified:

- A modular budget of $250,000 per year is requested and is appropriate for the work being proposed.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

VERTEBRATE ANIMAL (Resume): NOT APPLICABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

National Heart, Lung, and Blood Institute Special Emphasis Panel
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
NHLBI Lung Primary Prevention Review
ZHL1 CSR-H (S1)
July 01, 2015

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.