# Biomarker Phenotyping of Pediatric Sepsis and Multiple Organ Failure (PHENOMS)
# CPCCRN Protocol Number 047

Collaborative Pediatric Critical Care Research Network
*Eunice Kennedy Shriver* National Institute for Child Health and Human Development (NICHD)

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PROTOCOL TITLE:

Biomarker Phenotyping of Pediatric Sepsis and Multiple Organ Failure

Short Title: PHENOMS
CPCCRN Protocol Number: 047

Lead Investigator and Author:
Joseph A. Carcillo, MD
University of Pittsburgh

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*I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.*

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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**Abstract**

Severe sepsis remains the leading killer of children worldwide. Most children dying from sepsis in the US do so with Multiple Organ Failure (MOF). [1-20] Sepsis induced MOF is a poorly understood syndrome for which treatment is directed to organ dysfunction rather than inflammation biomarker responses. In contrast, rheumatologic multiple system organ disease is considered to be a spectrum of inflammation pathobiology phenotypes that improve with use of specific therapies directed to normalizing inflammation biomarkers such as C-reactive protein (CRP) and Ferritin. Our central hypothesis is that improved understanding of sepsis induced MOF might occur if we consider the syndrome to include a unique spectrum of inflammation pathobiology phenotypes related to coagulation and immune responses with predisposing genetic and environmental risk factors. [21-44] Three phenotypes have been characterized using animal models, human clinical pathological correlates, genetic profiles, and biomarker responses to pathobiology targeted therapies. [41-169] Children with Thrombocytopenia Associated MOF have reduced ADAMTS13 activity which causes an inability to resolve von Willebrand Factor: Platelet clots leading to a thrombotic microangiopathy which can be reversed with plasma exchange therapy. Children with Immunoparalysis / Lymphoid Depletion syndrome have lymphoid organ depletion and reduced innate immune function with an inability to kill bacterial or fungal infections. These children are identified by a decreased ex vivo whole blood TNF -$\alpha$ response to endotoxin which can be recovered with immune modulation or immune suppressant tapering. Children with Sequential MOF (respiratory followed by liver failure) have perturbed Natural Killer / Cytotoxic T Lymphocyte function and an inability to induce virus, cancer, or activated immune cell death. Viral infections in these children cause lymphoproliferation, hemophagocytosis, and sFasL mediated liver injury which can respond to varied treatment strategies.

This is an observational cohort study in 400 severe sepsis patients recruited from the national, ethnically and geographically diverse *Eunice Kennedy Shriver* National Institute of Child Health and Development (NICHD) Collaborative Pediatric Critical Care Research Network (CPCCRN) to test the hypotheses that children with these phenotypes have; 1) increased mortality, 2) predisposing genotype and environmental risk factors, and 3) increased CRP and Ferritin levels that correlate with clinical outcome. If patients' clinical outcomes are related to a spectrum of inflammation pathobiology and increased systemic inflammation biomarkers then this will inform a paradigm shift supporting future study of the use of phenotype specific therapies, directed to normalizing CRP and Ferritin levels in children with severe sepsis induced MOF.

# 1 Study Design Summary

## 1.1 Specific Aims

This study has the following Specific Aims:

**Specific Aim 1.** Determine the incidence and outcomes of thrombocytopenia associated MOF, immunoparalysis / lymphoid depletion associated MOF, and the sequential MOF phenotypes in children with severe sepsis.

**Specific Aim 2.** Determine the relative contribution of genetic and environmental risk factors to the development of each of the three sepsis phenotypes of multiple organ failure.

**Specific Aim 3.** Measure CRP and/or ferritin levels, as markers of systemic inflammation, and determine whether increased inflammation is increased in children with one of the three phenotypes compared to children who do not develop a MOF phenotype, and whether specific levels of these biomarkers are associated with increased mortality risk.

## 1.2 Phenotype Definitions

**Thrombocytopenia associated multiple organ failure (TAMOF)** is defined by three organ failures, new onset thrombocytopenia, increased lactate dehydrogenase (LDH), renal dysfunction, and ADAMTS13 activity $< 57\%$ of normal.

**Immunoparalysis / lymphoid depletion associated multiple organ failure** is defined by an *ex vivo* whole blood endotoxin-stimulated TNF-$\alpha$ response $< 200$ pg/mL after three days.

**Sequential multiple organ failure** is defined by respiratory failure followed by liver dysfunction with sFasL level $> 200$ ng/mL.

## 1.3 Hypotheses

The study hypotheses are:

**Hypothesis 1.** Mortality will be higher in children with one of these three phenotypes compared to children without these phenotypes.

**Hypothesis 2a.** Children with thrombocytopenia associated MOF will have a higher rate of ADAMTS13 mutations and/or antecedent liver failure, increased free hemoglobin levels or cytokine levels, compared to MOF patients without thrombocytopenia associated MOF.

**Hypothesis 2b.** Children with immunoparalysis / lymphoid depletion associated MOF will have primary relatives with a lower whole blood ex vivo TNF-$\alpha$ and higher IL-10 response to endotoxin, as well as a higher rate of clinical exposure to immune suppressant / chemotherapies compared to MOF patients without immunoparalysis or lymphoid depletion.

**Hypothesis 2c.** Children with sequential MOF will have a greater rate of perforin signaling mutations, a higher incidence of solid organ transplantation (post-transplant lymphoproliferative disease), and a higher incidence of leukemias (primary Hemophagocytic Lympho Histiocytosis) compared to MOF patients without sequential MOF.

**Hypothesis 3.** Patients with MOF and the inflammation phenotypes will have increased CRP and Ferritin levels compared to children with MOF without these phenotypes. In addition, reduction of CRP to $< 4$ mg/L and Ferritin to $< 1000$ ng/mL will be associated with survival, adequate source control, and the use of phenotype-specific therapies.

## 1.4 Inclusion Criteria

- Age $\geq$ 44 weeks gestation and $<$ 18 years; AND
- Anticipated to have an indwelling arterial or venous catheter for blood sampling for at least one study day; AND
- At least one organ failure; AND
- Systemic inflammatory response syndrome (SIRS) (at least 2 of 4 criteria); AND
- Suspicion of sepsis or infection.

## 1.5 Exclusion Criteria

- Lack of commitment to aggressive intensive care as indicated by do not resuscitate orders or other limitations of care.

### 1.6 Anticipated Subject Accrual

The PHENOMS study will enroll 400 subjects, or up to 80 subjects per site. Screening will occur on Monday and Thursday of each week, and all eligible subjects identified by screening will be approached for permission to participate in the study, until the site reaches maximum enrollment. In order to assure balance of subject enrollment across the entire calendar year, 20 subjects may be enrolled from the beginning of seasonal quarters of the year that will be defined in the Manual of Operations.

For example, sites will begin screening on the first Monday of the first seasonal quarter; during that quarter, screening is done every Monday and Thursday and a total of 10 subjects are enrolled. In the subsequent calendar year, the site will screen and enroll subjects in that same seasonal quarter. The target enrollment is 20 subjects per seasonal quarter. Enrollment may be accomplished over four years, but if adequate numbers of eligible subjects are identified by screening, sites should enroll subjects as quickly as possible. Thus, it is possible that up to 80 subjects per site will be enrolled in the first year; we anticipate that enrollment will require up to three to four years because the eligibility criteria are quite restrictive.

## 2 Rationale and Background

This study approaches severe sepsis with multiple organ failure with an approach similar to the current paradigm used in pediatric rheumatologic diseases. In past decades, conceptualization of multi-system organ inflammation in pediatric rheumatologic diseases has changed from that of one syndrome called "connective tissue disease" to a spectrum of inflammation pathobiology-specific phenotypes including systemic juvenile idiopathic arthritis, systemic lupus erythematosis, and others. These phenotypes are considered distinctive, even though they not uncommonly have shared characteristics. These connective tissue disease phenotypes have been characterized on the basis of several criteria including characteristic animal models, clinical-pathological correlates with accompanying clinical and biomarker criteria, some typical genotype and primary relative characteristics, and favorable biomarker responses to phenotype specific therapies. Clinical inflammation biomarkers used to direct therapies include C-reactive protein (e.g., systemic rheumatoid arthritis, giant cell arteritis, systemic lupus erythematosis with serositis) and Ferritin (e.g., adult onset Still's disease, macrophage activation syndrome). Conceptualizing connective tissue disease as a spectrum of phenotypes has led to the development of improvements in care for these patients through development of specific pathobiology targeted therapies.

Sepsis-induced MOF is presently considered a singular entity in adults and children. In this study, we will characterize inflammation pathobiology phenotypes to improve our understanding and potentially outcomes in sepsis induced MOF in children. A similar paradigm shift in conceptualization of pediatric sepsis induced MOF could some day lead to improvement in care for the more common and burdensome pediatric multiple system organ inflammation sepsis syndrome possibly with implementation of therapeutic strategies directed to reducing CRP and Ferritin levels.

In this study, we will use the innovative approach of characterizing inflammation pathobiology phenotypes to improve understanding and potentially outcomes in sepsis-induced MOF. In this regard, upon demonstrating that greater inflammation was associated with increased and persistent MOF, Carcillo and colleagues next investigated clinico-pathological correlates. Reviewing a decade of autopsies from their PICU, it was observed that children who died from sepsis induced MOF had evidence of thrombosis and bleeding (50%), and uneradicated infection (75%). They reasoned that if the phenotype approach was going to be important, then it would likely link inflammation to aberrant coagulation and immune function pathobiology. In the past decade they have investigated three such phenotypes in pediatric sepsis induced MOF. They have pursued inflammation pathobiology induced aberrant coagulation (related to decreased ADAMTS 13 activity, formerly known as von Willebrand Factor vWF cleaving protease ) in the Thrombocytopenia associated MOF phenotype,[41,45-50] and aberrant immune function in the Immunoparalysis / Lymphoid depletion associated MOF phenotypes (predominant TH2 milieu) [51-53] and the Sequential MOF lympho-proliferative phenotype (decreased NK/CTL function and perforin signaling activity, with increased sFasL levels).[42,54] Over the last decade, they have accumulated evidence that children with these three MOF phenotypes have identifiable clinico-pathologic correlates with accompanying clinical and biomarker criteria, increased risk of developing secondary infection and death, and favorable biomarker and outcome responses to phenotype specific therapies directed to reversing specific pathobiology.

# 3 Study Design and Workflow

This is a prospective observational cohort study in which enrolled subjects with severe sepsis will be characterized in five groups:

1. Severe sepsis without MOF (< 2 organ failures),
2. Severe sepsis with MOF but without any of the three phenotypes,
3. Thrombocytopenia Associated MOF,
4. Immunoparalysis / Lymphoid Depletion associated MOF, and

5. Sequential MOF.

## 3.1 Definitions

### 3.1.1 General Definitions

**Severe sepsis:** Sepsis with organ failure. Sepsis is defined by the following:

- Suspicion of sepsis or infection; AND
- Systemic inflammatory response syndrome (SIRS) (at least 2 of 4 criteria):
  1. Hypothermia (temperature < 36.0° C) or fever (temperature > 38.5° C);
  2. Leukocytosis (>12,000) or neutropenia (<4,000) or >10% immature neutrophils;
  3. Heart rate $>90^{th}$ percentile for age in absence of stimulation;
  4. Respiratory rate $>90^{th}$ percentile for age OR hyperventilation to $P_aO_2$ <32 torr OR mechanical ventilation requirement unrelated to drug administration.

**Infection:** Culture, PCR or antigen proven bacterial, fungal, protozoal, or viral infection.

**Primary infection:** Infection within three days (72 hours) of admission to PICU.

**Secondary infection:** Infection after the first three days (72 hours) in the PICU.

**Effective removal of infection nidus:** Clinical care team states that the infection nidus has definitely been removed.

**Effective control of inflammation source:** Clinical care team states that the source of inflammation (e.g., rejection, graft versus host, pancreatitis, necrotic tissue, etc.) has definitely been removed.

**Multiple organ failure:** Two or more organ failures.

**Macrophage Activation Syndrome (MAS):** Hepatobiliary and hematologic organ failure with Ferritin > 500. MAS may be superimposed on any of the phenotypes.

### 3.1.2 Organ Failure Definitions

Organ failure definitions are:

**Cardiovascular failure:** Requirement for inotrope or vasopressor infusion

**Pulmonary failure:** Requirement for mechanical ventilator and PaO2/FiO2 ratio < 300

**Hepatic failure:** ALT > 100 and either bilirubin> 1 or INR > 1.5

**Renal failure:** Creatinine > 1 with oliguria

**Hematologic failure:** Platelet count < 100,000 and INR > 1.5

**Central nervous system failure:** Glasgow coma score < 12 in absence of sedatives

### 3.1.3 Phenotype Definitions

**Thrombocytopenia associated multiple organ failure (TAMOF)** is defined by three organ failures, new onset thrombocytopenia, increased lactate dehydrogenase (LDH), renal dysfunction, and ADAMTS13 activity < 57% of normal.

**Immunoparalysis / lymphoid depletion associated multiple organ failure** is defined by an *ex vivo* whole blood endotoxin-stimulated TNF-$\alpha$ response < 200 pg/mL after three days.

**Sequential multiple organ failure** is defined by respiratory failure followed by liver dysfunction with sFasL level > 200 ng/mL.

In addition to the three phenotypes of interest, three additional definitions are important because this study will collect data concerning these concepts:

**Primary Hemophagocytic Lympho-Histiocytosis (HLH) Syndrome.** Five of 8 criteria including: fever, two line cytopenia, splenomegaly, hyperferritinemia > 500, hypofibrinogenemia, hypertriglyceridemia, high sCD25, bone marrow hemophagocytosis [54,139] in a child < 2 years old or with consanguinity or primary CNS disease (seizures or encephalopathy) or history of a relative dying in childhood of fever related disease. Confirmed by homozygous mutations for 9q21.3-22, *PRF* 1, *UNC 13D*, *STX* 11, *STXBP*-2, *RAB27A*, *CHS1/ LYST*, *AP3B1*.[141-145]

**Macrophage Activation Syndrome (MAS) / Secondary HLH Syndrome.** Five of 8 criteria including: fever, two line cytopenia, splenomegaly, hyperferritinemia > 500, hypofibrinogenemia, hypertriglyceridemia, high sCD25, bone marrow hemophagocytosis [54,139] in a child > 2 years old, without consanguinity, without primary CNS disease (seizures or encephalopathy), and without a history of a relative dying in childhood of fever related disease and without homozygous mutations for 9q21.3-22, *PRF* 1, *UNC 13D*, *STX* 11, *STXBP*-2, *RAB27A*, *CHS1/ LYST*, *AP3B1*.[141-145]

**Use of phenotype specific therapies.** Phenotype specific therapies include G-CSF for neutropenia and GM-CSF for neutropenia and immune paralysis, tapering immune suppressants by 50% or more for immune paralysis, intensive plasma exchange for TAMOF, solumedrol/IVIG/Anakinra/biologics for TAMOF associated with MAS/secondary HLH, Rituximab and immune suppressant withdrawal for post-transplant lymphoproliferative disease related Sequential Multiple Organ failure, and HLH-94 protocols including dexamethasone and chemotherapy for primary HLH associated with Sequential Multiple Organ failure phenotype.



## 3.2 Study Workflow

### 3.2.1 Subject Screening

Screening will be conducted twice weekly (i.e., Monday and Thursday) during each seasonal quarter as defined in the Manual of Operations.

During the first year, each site may enroll up to 20 subjects in each seasonal quarter. If a site does not enroll 20 subjects in a specific seasonal quarter in the first year, then screening for that seasonal quarter should be continued in the second year until 20 subjects have been accrued for that seasonal quarter. It may require three or four years to complete the enrollment of 20 subjects for each seasonal quarter. The total target enrollment for each site is up to 80 subjects (20 from each seasonal quarter), and the total study target enrollment is 400 subjects.

On each day of screening, the research coordinator or site investigator will evaluate all patients currently in the PICU and CICU to evaluate eligibility for enrollment. All eligible subjects should be recorded and their parents should be approached for permission to participate in the study. If the number of eligible subjects would lead to enrollment exceeding 20 subjects for the seasonal quarter, all of them should be approached for enrollment to avoid enrollment bias. Thus it is possible that there will be enrollment beyond 20 subjects in a seasonal quarter at a site if there are too many eligible subjects on the last day of screening for the seasonal quarter. It is important that on any day of screening, *all eligible subjects* are approached for enrollment, even if it leads to a total enrollment exceeding 20 subjects for the seasonal quarter.

### 3.2.2 Informed Consent Summary

When an eligible subject has been identified by the screening process, the parents or legal guardians should be approached to obtain permission for participation in the study. The permission will be a layered permission to allow inflammation phenotyping of the subject, and optionally, genotyping the subject for genotypes potentially related to severe sepsis induced multiple organ failure syndrome. When applicable, the subject's assent will also be obtained in a similar layered manner.

In addition, consent will be requested from a primary relative, biological mother or father, for one blood sample to measure whole blood *ex vivo* TNF-$\alpha$ and IL-10 response to LPS.

Table 1: Summary of PHENOMS Study Procedures.

| | Mon | Thurs | Mon | Thurs | Mon | Thurs | Mon | Thurs |
|---|---|---|---|---|---|---|---|---|
| Draw Blood (5.7 mLs) | X | X | X | X | X | X | X | X |
| Collect Clinical Data | X | X | X | X | X | X | X | X |
| Genotype x 1 only | X | (X) | | | | | | |
| Biomarkers | X | X | X | X | X | X | X | X |
| C-reactive protein | X | X | X | X | X | X | X | X |
| ADAMTS13 | X | X | X | X | X | X | X | X |
| TNF alpha / IL-10 | X | X | X | X | X | X | X | X |
| Ferritin | X | X | X | X | X | X | X | X |
| sCD163, sFASL | X | X | X | X | X | X | X | X |
| Free hemoglobin | X | X | X | X | X | X | X | X |

### 3.2.3 Schedule of Study Procedures

Clinical characteristics will be recorded at enrollment and twice weekly (Table 1). The only intervention in the cohort study is blood drawing through an existing central venous line or arterial line one time for genotype analyses, and twice weekly for measurement of biomarkers (5.7 mL) while in the ICU for up to 28 days. Biomarkers and genotypes will be batch analyzed, and results will not be available to the clinical care team. Batch analyses will be performed every 6 months at the University of Pittsburgh clinical laboratory (Ferritin, CRP, ADAMTS 13 activity and send out ADAMTS 13 and perforin genotypes)

and research laboratory (cytokine and free hemoglobin analyses).

If a primary relative (biological mother or father) has consented to provide blood, one 3 mL sample will be obtained for analysis of whole blood *ex vivo* TNF-$\alpha$ and IL-10 response to LPS.

### 3.2.4 Blood Sample Procedures

Blood sampling will occur when there is an existing central venous or arterial line available. 5.7 mL blood will be drawn twice weekly from subjects while in the ICU for up to 28 days. Collected samples will be de-identified and labeled with a unique subject study ID number and will be batch shipped to the University of Pittsburgh central laboratory and Washington University in St. Louis.

**Whole blood ex vivo response to LPS.** Three mL whole blood samples will be obtained with heparin containing tubes from enrolled subjects twice weekly to evaluate immune response and once (3 mL) from a biological parent, mother or father, (if consent has been obtained for this). Within 30 to 60 minutes of obtaining the blood, site personnel will place 50 microliters of whole blood in an Eppendorf tube with culture media and a given amount of endotoxin (provided by the site PI)[53] and will incubate this sample for 4 hours at 37° C. The remainder of the blood (just under 3 mL) will be centrifuged and the plasma supernatant will be stored at -70° C for batch shipment to the University of Pittsburgh for cytokine analyses (see Plasma samples below). The remaining heparinized blood pellet will be added to a glycerol tube and stored at -70° C for batch shipment to Washington University in St. Louis. The incubated pink media Eppendorf tube will then be centrifuged and the supernatant frozen at -70° C (the pellet will be discarded as waste). Samples will be stored at the clinical site and batch shipped to the University of Pittsburgh where *ex vivo* endotoxin stimulated TNF-$\alpha$ and IL-10 production will be measured by ELISA.[53]

**Plasma samples.** 2.7 mL of whole blood will be collected in citrate tubes and will be centrifuged and the plasma frozen at the site at -70 ° C. Samples will be batch shipped to the University of Pittsburgh for later analysis of cytokines, free hemoglobin, Ferritin, CRP, and ADAMTS 13 activity.

**Genotype samples.** The remaining blood pellet obtained from the citrate containing tube after removal of the supernatant (see above) will be frozen at -70° C and batch shipped to the University of Pittsburgh for genotyping (x1). The plasma supernatant

sample should also be frozen at -70° C and batch shipped to Pittsburgh (see Plasma samples above). Salivary sample kits may be provided in the future, and should be used to obtain genotype samples from the subject. These will be used to validate the use of salivary sampling in critically ill children. These samples will be batch shipped to the University of Pittsburgh for subsequent genotyping.

# 4 Data Collection and Procedures

Data elements for this study are described in detail in the following sections. Note that specific questions and choice sets described in the following sections may not represent the final data collection forms built in the electronic data capture system. Minor changes to choice sets of these data elements may be made and will not be regarded as protocol changes, but if substantively new data elements are added to the study, this protocol will be amended and submitted to the Institutional Review Board (IRB) for amended approval.

## 4.1 Screening and Eligibility Data Elements

The following data elements are collected on all screened and eligible subjects. Waiver of informed consent is requested for Screening and Eligibility data elements only. If parents do not provide permission for their child to be enrolled in the study, no additional data will be recorded.

*Demographic Summary:*

- Birthdate
- Gender
- Race
- Ethnicity

*Eligibility:*

- Date of screening
- Date of onset of sepsis induced organ failure
- SIRS criteria met (at least 2 of 4):
  - Hypothermia ($< 36$ ° C) or fever ($> 38.5$ ° C);
  - Leukocytosis ($> 12,000$) or neutropenia ($< 4,000$) or $> 10\%$ immature neutrophils;
  - Heart rate $>$ 90th percentile for age in absence of stimulation;

  - Respiratory rate > 90th percentile for age OR hyperventilation to $PaCO_2$ <32 torr OR mechanical ventilation requirement unrelated to drug administration.
- Nature of infection (suspected or documented)
- Is the infection considered to be nosocomial by the clinical team? (Y/N)
- Type of organism (suspected or documented):
  - Bacterial
  - Fungal
  - Viral
  - Protozoal
- Parent(s) or legal guardian(s) approached for parental permission? (Y/N)
  If no, indicate reason.
- Did parent(s) or legal guardian(s) give permission for the subject to be enrolled? (Y/N)
  If no, indicate reason.
- If permission was obtained, did parent(s) or legal guardian(s) give permission for subject genotyping? (Y/N)
  If no, indicate reason.
- Did a biological parent (mother or father) consent for one-time whole blood sampling? (Y/N)
  If no and the reason is known, indicate reason.

## 4.2 Study Entry Data Elements

*Hospitalization Summary:*

- Date and time of hospital admission
- Date and time of PICU admission

*Baseline Clinical Data:*

- Date of enrollment
- Was the subject immunocompromised? (Y/N)
- Did the subject have any of the following:
  - Congenital immunodeficiency (Y/N)
  - Bone marrow or stem cell transplantation (Y/N)
  - Graft versus host disease (Y/N/NA)
  - Solid organ transplantation (Y/N)
    If yes, specify type of transplant
  - Rejection (Y/N/NA)
  - Malnutrition, severe (Y/N)

  - Malignancy (Y/N)
    If yes, specify type of malignancy
  - Chemotherapy or radiotherapy within last 3 months (Y/N)
  - Human immunodeficiency virus (Y/N)
  - Rheumatologic disease (Y/N)
  - Neutropenia (ANC <1000) (Y/N)
  - Sickle Cell Disease (Y/N)
  - Systemic steroid use, chronic or acute (Y/N)
  - Asplenia or s/p splenectomy (Y/N)
  - Other immunosuppression (Y/N)
    If yes, specify

*ICU Admission Diagnoses:*

- Primary diagnosis as determined at admission
- Secondary diagnoses as determined at admission
- Other Diagnoses:
  - Leukemia (Y/N)
  - Hemolytic anemia (Y/N)
  - Rheumatological disease (Y/N)
  - Inflammatory bowel disease (Y/N)
  - Immunologic renal disease (Y/N)
  - Chromosomal abnormality (not hereditary condition) (Y/N)
  - Metabolic disease (Y/N)
  - Diabetes (Y/N)
  - Cardiovascular disease - congenital (Y/N)
  - Trauma (Y/N)
  - Short gut syndrome (Y/N)
  - Post-operative/surgery (Y/N)
  - Liver disease (Y/N)

*PRISM III:*

- Total PRISM Score for PICU admission
- PRISM Score components

Data about the infections and antibiotics from the time of admission are to be entered on the microbiology and antibiotic log forms described later.

## 4.3 Daily Data Elements

The day of enrollment is Day 0 (onset of sepsis induced organ failure). Each day is defined as midnight (00:00) through 23:59. Daily data elements will be recorded on a daily basis, including Day 0, until PICU discharge, death, or Day 28, whichever occurs first.

- Study date
- Study day number
- Organ Failure Index (number of organ failures)
- Organ failures:
  - Cardiovascular (Y/N)
  - Pulmonary (Y/N)
  - Renal (Y/N)
  - Hematologic (Y/N)
  - Hepatic (Y/N)
  - CNS (Y/N)
- Infection nidus effectively removed (Y/N/Unknown/NA)
- Inflammation source effectively removed (Y/N/Unknown/NA)
- Mechanical ventilation? (Y/N)
- ECMO? (Y/N)
- Nitric oxide? (Y/N)
- Continuous renal replacement therapy (CRRT)? (Y/N)
- Immune suppressant tapered by 50% (Y/N)
- Plasma exchange started or continued (Y/N)
- Did subject receive blood transfusion? (Y/N)
  - PRB? (Y/N)
  - Platelets? (Y/N)
- Suspected Thrombocytopenia Associated MOF? (Y/N)
- Suspected Sequential Multiple Organ Failure? (Y/N)
- Suspected Macrophage Activation Syndrome / Secondary HLH? (Y/N)
- Suspected Primary HLH (Y/N)
- Suspected Post Transplant Lymphoproliferative Disease? (Y/N)
- Suspected organ rejection (Y/N)
- Suspected graft vs host disease (Y/N)
- Suspected pancreatitis (Y/N)
- Hepatomegaly? (Y/N/Unknown)
- Splenomegaly? (Y/N/Unknown/NA)
- Are all known infections covered by sensitive antibiotics on this day? (Y/N)

The following labs are often obtained as part of the clinical care for patients who are eligible for this study. If these labs were not obtained for clinical care, DO NOT sample them for purposes of this study.

*Single Daily Laboratory Data:*

- Lowest absolute lymphocyte count
- Lowest absolute neutrophil count
- Lowest hemoglobin
- Lowest platelet count
- Highest INR
- Highest prothrombin time (PT)
- Highest creatinine
- Highest total bilirubin
- Highest amylase
- Highest lipase
- Highest alanine transaminase (ALT)
- Highest lactate dehydrogenase (LDH)
- Highest triglycerides

## 4.4 Study Log Forms

*Microbiology Results Log:*

- Date and time microbiology specimen obtained
- Sample site:
  - Abscess
  - Blood
  - Bronchial brush
  - Bronchoalveolar lavage
  - Nasopharyngeal
  - Pleural fluid
  - Peritoneal fluid
  - Skin
  - Spinal fluid
  - Sputum
  - Stool/Rectal
  - Surgical Site
  - Urine

  - Vascular catheter
  - Wound (non-surgical)
  - Other (no specify)
- Test type:
  - Culture
  - PCR
  - Other
- Test result (negative, positive, contaminant)
- If test result positive, upload report including sensitivities.

*Atypical Laboratory Log:*

- CRP
- Ferritin
- Free hemoglobin
- Bone marrow (upload results)
- Genotyping (upload results)
- sCD25 (upload results)
- NK cells activity (upload results)

*Immune Medication Administration Log:*

This form will record steroids, other immunosuppressive drugs, chemotherapy, and immune-modulating drugs (systemic corticosteroids, hydrocortisone, methylprednisolone, dexamethasone, Primary HLH directed chemotherapy, G-CSF, GM-CSF, IVIG, etoposide, Rituximab, Tocilizumab, Infliximab, Anakinra, Dacilizumab, Basilixumab, etc.).

- Name of drug
- Start date
- Stop date

*Antimicrobial Administration Log:*

This form will record systemic antibiotic, antifungal or antiviral administration only. Topical agents to skin, eye, wounds, etc. should not be recorded.

- Name of antimicrobial
- Start date
- Stop date

*Biosampling Log:*

- Blood draw date and time
- Biological parent one-time blood draw date and time

### 4.5 Study Exit Data Elements

*Hospitalization Summary:*

- Date and time of PICU discharge
- Date and time of hospital discharge
- Vital status at PICU and hospital discharge (alive, dead, or alive, but transferred to another ICU)
- Vital status at 28 days post-onset of sepsis induced organ failure (alive or dead)
- Date and time of death (if applicable)
- Autopsy obtained (Y/N/NA)
- If autopsy obtained, upload report.

## 5 Data Analysis

This is a prospective observational cohort study in which enrolled subjects with severe sepsis will be characterized in five groups:

1. Severe sepsis without MOF (< 2 organ failures),
2. Severe sepsis with MOF but without any of the three phenotypes,
3. Thrombocytopenia Associated MOF,
4. Immunoparalysis / Lymphoid Depletion associated MOF, and
5. Sequential MOF.

### 5.1 Endpoints

- Severe Sepsis without Multiple Organ Failure
- Multiple Organ Failure without Phenotypes
- Multiple Organ Failure with Phenotypes
- Immune paralysis associated Multiple Organ Failure
- Thrombocytopenia associated Multiple Organ Failure
- Sequential Multiple Organ Failure
- Genotypes related to low ADAMTS13
- Genotypes related to Inflammation
- Genotypes related to Hyperferritinemia including

  - Genotypes related to Perforin
  - Genotypes related to Iron Metabolism
- Genotypes related to Immune Function
- Cytokine multiplex including interferon gamma, TNF-$\alpha$, IL-10, sFasL, sCD25, sCD163
- Free hemoglobin
- Whole blood ex vivo TNF-$\alpha$ and IL-10 response to LPS in primary relative and child
- Environmental risk factors related to Multiple Organ Failure with Phenotypes
- C-Reactive Protein levels
- Ferritin levels
- Effective control of inflammation source
- Effective removal of infection nidus
- Secondary Infection
- 28 day, PICU mortality
- Suspected Macrophage Activation / Secondary Hemophagocytic Lympho-Histiocytosis Syndrome
- Suspected Primary Hemophagocytic Lympho Histiocytosis Syndrome
- Use of Phenotype specific therapies
- Clinical Macrophage Activation Syndrome (MAS)

## 5.2 Analysis Overview

The 400 enrolled children will be classified into the following subgroups: 1) Severe sepsis without MOF, 2) Thrombocytopenia associated MOF, 3) Immunoparalysis / Lymphoid Depletion associated MOF, 4) Sequential MOF, and 5) MOF without any of these three phenotypes. Numbers and proportions of children in each category will be reported. The primary and exploratory analyses described below involve many tests of significance. As PHENOMS is a moderately sized study intended to identify key populations and outcomes for subsequent interventional studies, all tests reported will use an 0.05 level of significance. However, all reports will explicitly identify the primary outcome(s) for each hypothesis, as well as describe the total number of tests performed for each hypothesis as well as overall in each report. Analysis strategies for each hypothesis are outlined below; more explicit details will be finalized in a Statistical Analysis Plan.

### Hypothesis 1

Within Specific Aim 1, the key statistical evaluation of Hypothesis 1 will involve comparison of mortality at PICU discharge, hospital discharge, and 28 days between study subgroups.

Mortality rates among children with any of the three MOF phenotypes will be compared to children with severe sepsis without MOF, and to children with MOF without these phenotypes. Two-sided chi-squared tests (asymptotic or exact, depending on cell counts) will be used for these comparisons. Mortality at hospital discharge will be reported as the primary Hypothesis 1 outcome. Risk ratios and 95% confidence intervals for mortality rates will be reported in association with these comparisons. Supportive analyses will examine Kaplan-Meier survival curves for 28 days of follow - up. The distributions of age, race, gender, and the baseline data elements identified in Section 4.2 will be reported for study subgroups by appropriate descriptive statistics. Exploratory multivariable logistic regression modeling will assess influence of patient characteristics on relative mortality risk between subgroups.

### Hypothesis 2a

Among the approximately 60 children tested for ADAMTS13 mutations, the incidence of ADAMTS13 mutations will be reported, and using exact chi-squared methods, compared with the most appropriate normal population rate available at the time of evaluation. If this incidence is significantly higher than in the normal population, and overall study/network resources allow, a sample of matched study patients whose MOF is not TAMOF will also have testing for ADAMTS13 mutations, and rates of mutations will be compared between the phenotypes via chi-squared approaches. Rates of antecedent liver failure will be compared between TAMOF and the other MOF phenotypes using chi-squared methods. If genotype information is indeed available at the end of the study for multiple MOF phenotypes, then multivariable logistic regression with genetic outcomes as well as environmental factors (e.g., liver failure) will be used to quantify "genetic" versus "environmental" relationship to phenotypes.

As multiple datapoints about hemoglobin and cytokine levels will be available for many children, a repeated measures data analysis using a linear mixed model will be implemented to compare these levels between subgroups on different study days, and explore trajectories over time. If these outcomes are substantially skewed and transformation does not adequately remedy departures from normality, the alternative approach employed will be rank-based comparisons at each timepoint and of changes over time.

### Hypothesis 2b

Clinical exposure to immune suppressants and chemotherapies will be compared between children with immunoparalysis/lymphoid depletion associated MOF and other MOF phenotypes using chi-squared approaches. Levels of whole blood ex vivo TNF alpha and

quantified IL - 10 response to endotoxin will be compared, using rank-based comparisons, between primary relatives of children with immunoparalysis/lymphoid depletion associated MOF and primary relative of children in the other four PHENOMS cohorts. Multivariable logistic regression will again be used to assess independent relationships of TNF/IL-10 response and the environmental baseline factors to MOF phenotype.

**Hypothesis 2c**

Rates of solid organ transplantation and leukemias will be compared between children with sequential MOF and the other MOF phenotypes using chi-squared approaches. If rates of perforin signaling mutation among the approximately 25 children with sequential MOF appear significantly higher than in a reference population, and resources allow, a sample of matched study patients whose MOF is not sequential MOF will be assessed for perforin signaling mutation. Rates of such mutations will be compared between the MOF phenotypes via chi-squared approaches, and multivariable logistic regression will be used to assess if perforin signaling mutation shows independent association with sequential MOF after adjustment for environmental factors including solid organ transplantation and leukemia.

**Hypothesis 3**

Levels of cytokines and ferritin will be assessed at initial reading, final reading, and over time, and compared between children with the inflammation phenotypes and children with MOF without these phenotypes. Maximum levels of CRP and ferritin will also be assessed. To test the primary hypothesis (generated by a previous single-center study), rates of PICU mortality will be compared in the PHENOMS cohort using chi-squared approaches for children divided into four subpopulations according to whether their CRP was below 4 mg/L, and/or ferritin levels were below 1000 ng/mL, at entry and at last reading. The primary hypothesis will evaluate whether the patients whose CRP was below 4 mg/L, and/or whose ferritin levels were below 1000 ng/mL at last reading have significantly lower risk of mortality than other patients. Risk ratios for mortality and confidence intervals will be presented for the other three subpopulations versus this presumed "low risk" population. Supportive analyses will use chi-squared testing to assess whether patients who start in a "higher risk" category at first reading but migrate to the "low risk" category have significantly higher survival rates than patients not migrating to this "low risk" category. As availability of (additional) readings is associated with survival per se, these analyses will be descriptive and appropriately account for number of available Core Laboratory assessments. Multivariable logistic regression analysis will determine whether maximum CRP and ferritin levels predict survival after controlling

for MOF and phenotype. While the primary evaluation will use cutpoints as described above, Receiver Operator Characteristics (ROC) curves will also determine the strength of relationship between change in CRP and Ferritin and survival. Exploratory analyses will assess whether source control and appropriate use of phenotype specific therapies (as defined above) is associated with achievement of, and migration to, the "low risk" levels of CRP and ferritin.

## 5.3 Statistical Power Issues

To inform the feasibility of this observational cohort study we have just completed an 18 month study using these same eligibility and exclusion criteria and the same observational cohort design at our center to inform the sample size estimate for the proposed multiple center study and analyses. One hundred children were enrolled. The mortality rate was 8%. Seventy six (76%) of the children developed MOF with an observed mortality rate of 11%. There were no deaths in the cohort of children without MOF. Fifteen children had Thrombocytopenia Associated MOF (mortality 40%), 24 children had Immunoparalysis / Lymphoid Depletion associated MOF (mortality 17%), and 6 children had Sequential MOF (mortality 18%). Children with these MOF phenotypes had an increased mortality rate (8/38 = 22%) compared to the children with MOF without these phenotypes (0/38 = 0%; $p < 0.05$). If this distribution holds in the PHENOMS cohort of 400 children, then we will have approximately 60 patients with Thrombocytopenia associated MOF, 96 patients with Immunoparalysis / Lymphoid Depletion associated MOF, 24 patients with Sequential MOF, 152 MOF patients without any of these phenotypes, and 96 patients with severe sepsis without MOF . We describe below statistical power of the PHENOMS study to address the key hypotheses of each Specific Aim.

**Specific Aim 1.** Determine the incidence and outcomes of thrombocytopenia associated MOF, immunoparalysis / lymphoid depletion associated MOF, and the sequential MOF phenotypes in children with severe sepsis.

For Hypothesis 1, using a two tailed Fisher's exact test with alpha of 0.05, to compare PICU mortality rates between approximately 150 patients with MOF and inflammation phenotypes (assuming mortality of at least 16%) and approximately 150 patients with MOF but no inflammation phenotypes (assuming mortality of at most 5%) power is over 80% for comparing differences between MOF patients with and without the phenotypes. Power is quite high, 95-96%, using the same testing approach to compare MOF patients with the phenotypes to the MOF patients without the phenotypes + patients without MOF, assuming that the patients without MOF have a PICU mortality rate of no more than 2%.

**Specific Aim 2.** Determine the relative contribution of genetic and environmental risk factors to the development of each of the three sepsis phenotypes of multiple organ failure.

Based on our pilot study we expect that 60 of the 400 enrolled patients will have decreased ADAMTS13 activity and will be eligible for genotyping. Patients with a sFasL level > 200 pg/mL will undergo genetic testing for familial HLH disease or its carrier state. Based on our pilot study we expect that 24 of the 400 enrolled patients will have increased sFasL levels. If we see an increased incidence of alleles associated with the disease or its carrier states which is greater than predicted in the normal population then we will perform a case control study with matched patients from other cohorts. One-sided exact tests with alpha level of 0.05 have approximately 90% power to determine that population incidences are significantly higher than 1% if the true incidences are at least 18%($n$=24 patient samples) or 8%($n$=60 patient samples). In addition, we will also test a more complex genetic association by comparing whole blood ex vivo TNF alpha response to endotoxin in primary relatives. Because this testing is far less expensive we will test as many primary relative / child pairs as have primary relatives agree to a blood draw. A previous study demonstrating association of death of a child with meningococcemia to a primary relative having a low TNF alpha / high IL10 ex vivo whole blood response to endotoxin,[133] reported differences of approximately 2000 pg/mL in both TNF and IL-10 production between relatives of survivors and nonsurvivors. Conservatively estimating standard deviations using that study's larger within-group interquartile ranges (3000 for TNF production and 4000 for IL-10 production), and that only 80% of the 400 enrollees' parents agree to sampling, we will have over 95% power to detect differences of the magnitude reported by Westendorp et al.[133] between parents of patients with versus without MOF, as well as in the comparison of patients with inflammatory MOF versus MOF without inflammatory phenotypes. Power to detect within-phenotype differences will be more limited but still over 80% in many instances.

**Specific Aim 3.** Measure CRP and/or ferritin levels, as markers of systemic inflammation, and determine whether increased inflammation is increased in children with one of the three phenotypes compared to children who do not develop a MOF phenotype, and whether specific levels of these biomarkers are associated with increased mortality risk.

In our single center study of 100 patients, PICU mortality was 0% in the "low risk" population of children (43% of the cohort) with CRP below 4 mg/L and ferritin levels below 1000 ng/mL at first reading, and 45% in the 13% of the cohort with CRP and ferritin levels both above these cutpoints. Assuming a similar magnitude of findings,

our cohort of 400 children should provide extremely high power to verify this significant mortality difference. In the single-center study, 42% of the population classified as "at risk" had ferritin levels below 1000 but CRP levels of 4 or above, and PICU mortality in this group was 2%. Our current study, assuming 1% mortality in a "low risk" group of 160 children, will allow us to rule out mortality of more than 7% in this "at risk" group if it is of similar size.

# 6 Data Management

The investigators and study staff are responsible for maintaining a comprehensive and centralized filing system containing all study-related documentation. Study worksheets are to be completed in a neat, legible manner to ensure accurate interpretation of data. Any corrections or changes on the worksheets when made, the original entry should be crossed out using a single line, and must be dated and initialed by the individual making the change. The original entry will not be erased or overwritten.

## 6.1 Electronic Data Capture System

Data from this study will be entered into an electronic data capture (EDC) system used by the Data Coordinating Center (DCC). This system provides secure user access via the Internet, and maintains an audit log for all study events and data. Specimen data will be obtained by research staff at the University of Pittsburgh. The security of these two computer systems is described below.

## 6.2 Data Security (University of Utah)

The DCC is located at the University of Utah in Salt Lake City, Utah. The DCC has a state-of-the-art data center infrastructure with a dedicated secure server facility with racks, inline cooling, uninterruptible power supply, high speed networking, security cameras, firewall protection, and 24/7 systems and security monitoring. The server facility is locked separately from the remainder of the DCC and access to the building is monitored by security personnel year round. The DCC coordinates its network infrastructure and security with the Health Sciences Campus (HSC) Information systems at the University of Utah. This provides the DCC with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2008 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using secure socket layer (SSL) or virtual private

network (VPN) technologies, both of which provide at least 128 bit encryption. The EDC system, eRoom$^{TM}$ (Web-based collaborative workspace), and other web applications use the SSL protocol to transmit data securely over the Internet. Direct access to DCC machines is only available while physically located inside the DCC offices, or via a VPN client. All network traffic is monitored for intrusion attempts, security scans are regularly run against DCC servers, and DCC IT staff are notified of intrusion alerts.

Servers are backed up daily through a dedicated backup server and internal high speed network. Incremental backups occur hourly and nightly. Full system backups occur nightly and weekly with off-site rotations. Security is maintained with Windows 2008 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group level access to databases, tables, and views in Microsoft SQL Server.



## 6.3 Protection of Confidentiality

The investigators and staff of the DCC are fully committed to the security and confidentiality of all data collected for CPCCRN studies. All DCC personnel at the University of Utah have signed confidentiality agreements concerning all data encountered in the center. Violation of these agreements may result in termination from employment at the University of Utah. In addition, all personnel involved with data coordinating center data systems have received Human Subjects Protection and HIPAA education.

The coordinators, reviewers and investigators involved with this study will be required to sign agreements from the DCC that relate to maintenance of passwords, information system security, and data confidentiality.

Blood sample data sent to Pittsburgh will be kept in a locked filing cabinet in an access-controlled facility. In addition, all data will be de-identified prior to sending. Electronic data will be de-identified, and stored in a password protected computer. All data and records generated during this study will be kept confidential, and publications will not enable identification of individual subjects. These efforts will decrease the risk of loss of confidentiality.

## 6.4 Data Analysis, Security, and Confidentiality (CRISMA, University of Pittsburgh)

Data management will follow many of the policies and procedures established in prior multicenter clinical studies and will be coordinated by the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) laboratory. CRISMA is a research center within the Department of Critical Care Medicine. The data management system will be based on a PC platform, a database server (Microsoft Access, SQL Server 7), and Microsoft Visual Basic. Data will be handled by a web-based system that uses Microsoft's Internet Information Server (IIS) (version 6) Active Server Pages with source code in VBScript. A complete backup of the server data is done every day. SSL certification ensures encryption of data between sites and the CRISMA web server.

Following recruitment of subjects meeting all study eligibility criteria, the site Research Coordinator (RC) will enter subject information into the data collection form using the subject's study ID#. Concurrently a subject profile (including initials and sex) will be generated and transmitted to the coordinating center to initiate the cohort tracking system for PHENOMS. The data collected per study procedures will be entered into the data collection form. Each data collection form will be identified by study ID# and enrollment date. Data will be entered by the site RC into a secure web-based data entry system and uploaded to the study database server via a secure internet connection. The CRISMA Center will monitor enrollment and perform routine data edit checks for consistency.

Laboratory data will be maintained in a separate, secure location with access limited only to laboratory personnel. Any identifying data will be maintained separately from clinical, laboratory and follow-up data for added security and confidentiality. University of Pittsburgh staff will run weekly laboratory analyses. If discrepancies are found, University of Pittsburgh staff will directly contact the site investigator in order to resolve any problems.

On a regular basis, the University of Utah DCC will obtain specimen data from CRISMA and the University of Pittsburgh to merge with the other study data.

## 6.5 Data Quality Management and Monitoring

The Data Coordinating Center at the University of Utah monitors CPCCRN studies on behalf of the investigators and the funding agency. The purposes of monitoring include

demonstration of adherence to human subject protection requirements and assurance of high quality study data. Monitoring is done remotely. Remote monitoring involves detailed review of the data entered by the Clinical Center and telephone consultations with the Clinical Center investigator and/or research coordinator to review data quality. This requires uploading de-identified copies of specific parts of the medical record to the DCC staff, who review those materials against the data recorded in the electronic data capture system.

### 6.6 Record Access

The medical record must be made available to authorized representatives of the DCC, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives of the National Institutes of Health, and the Institutional Review Board (IRB) for each study site, if appropriate.

## 7 Protection of Human Subjects

### 7.1 Institutional Review Board (IRB) Approval

Institutional Review Board (IRB) approval will be obtained by the Data Coordinating Center (DCC) and each participating Clinical Center prior to enrolling patients into this study. This approval may be accomplished via a central IRB mechanism if this is available within the Network. The DCC will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

### 7.2 Potential Risks and Benefits

Physical risks associated with participating in this study are related solely to blood sampling. The total amounts of blood required are well below the threshold recommended in NIH guidelines ($< 2$ mL / kg per day). Infants admitted to pediatric intensive care units weigh between 4 -10 kg and older children weigh more than 10 kg. 5.7 mLs of blood will be needed no more than 8 times (46 mL) over 28 days. Over any 7 day period the most blood sampled for research purposes will be 10 mLs total which is less than the NIH recommended $< 2$ mL / kg per day. We will additionally attain a one time 2 mL blood sample by venipuncture from a biological parent if they agree.

There is a minor risk of loss of confidentiality but this is mitigated by security of the network Data Coordinating Center information technology.

There are no other physical risks associated with participating in this study, as this is an observational study and no therapeutic intervention is being tested. The potential benefit to future patients is that more effective strategies for management and treatment of sepsis may be developed, leading to decreased mortality and improved quality of survival following severe sepsis.

There is no immediate direct benefit to subjects enrolled in this study. Since the clinical significance of non-genotype results is not established, these results will not become part of the subject's medical record and will not be provided to the family or their physicians. The two genotype analyses to be performed to rule out congenital TTP and primary HLH will be reviewed by the study PI, and *will be shared* with the site PI and parents if the results demonstrate one of these autosomal recessive disorders because clinical experts believe that care of these patients should be directed to these specific genetic disorders.

## 7.3 Protection against Risks

The risk of loss of confidentiality is mitigated by data security and confidentiality procedures at the DCC that have been described. Dates of birth, death, hospital and PICU admission, hospital and PICU discharge, and dates of follow up evaluation will be recoded into days of age at each milestone prior to data analyses by investigators. During preparation of a public use dataset, required by the National Institutes of Health (NIH), the DCC will de-identify the data set.

## 7.4 Informed consent and assent procedures

Waiver of informed consent and assent is requested for the Screening and Enrollment Data Elements. The justification for waiver of consent is based on the following factors:

- Obtaining informed consent would threaten the scientific validity of analyses related to this phase of study, because it is necessary to account for all patients who present with the eligibility criteria.
- The data elements involve no direct patient or family interaction, no interventions, and no changes in clinical practice.

- The minimal risk of loss of privacy is mitigated by secure data management at the DCC, and analysis datasets will be de-identified.

For additional data collection and participation in the study, parents or legal guardians will be approached to obtain informed consent for blood sampling and data collection. Separate signature consent will be obtained for genotyping. Subjects who are capable of giving consent and who are alert and competent will be asked, following an age-appropriate discussion of risks and benefits, to give assent to the study in a similar layered fashion.

Informed consent will be obtained from a biological parent who consents to provide a blood sample for assessment of immune function.

# 8 Health Insurance Portability and Accountability Act

All study sites have been offered Business Associate Agreements (BAAs) with the University of Utah. Copies of signed BAA are maintained at the DCC.

In accordance with NIH requirements, a public use dataset will be made available after completion of the study. This database will be completely de-identified in accordance with the Health Insurance Portability and Accountability Act (HIPAA).

# 9 Inclusion of Women and Minorities

The gender, ethnic and racial composition of patients enrolled in all CPCCRN studies is a function of the underlying referral population at each Clinical Center selected by the National Institute for Child Health and Human Development (NICHD) to participate in the network. There will be no exclusion of patients based on gender, race, or ethnicity.

# 10 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research

also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

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