The Critical Illness Stress-induced Immune Suppression Prevention Trial

(The CRISIS Prevention Trial)

CPCCRN Protocol Number 003

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

Version 1.18
Version Date: March 4, 2008
Printing Date: March 4, 2008

This protocol is CPCCRN Protocol Number 003, and the lead CPCCRN investigator for this protocol is Joseph Carcillo, M.D., University of Pittsburgh School of Medicine.

The CPCCRN Clinical Centers are the Arkansas Children's Hospital, Children's Hospital of Pittsburgh, Children's National Medical Center, Children's Hospital of Los Angeles, Seattle Children's Hospital, and Children's Hospital of Michigan, and are supported by Cooperative Agreements U10-HD500009, U10-HD049983, U10-HD049981, U10-HD050012, U10-HD049945, and U10-HD050096, respectively, from the National Institute for Child Health and Human Development (NICHD).

This document was prepared by the CPCCRN Data Coordinating Center located at the University of Utah School of Medicine, Salt Lake City, Utah. The document was written and typeset using LaTeX 2_ε. The CPCCRN Data Coordinating Center at the University of Utah is supported by Cooperative Agreement U01-HD049934 from the National Institute for Child Health and Human Development (NICHD).

PROTOCOL TITLE:

The Critical Illness Stress-induced Immune Suppression Prevention Trial

Short Title: The CRISIS Prevention Trial
CPCCRN Protocol Number: 003
IND Number: 74,500
Phase of Study: IIIb

Lead Investigator and Author:
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Protocol Version: 1.18
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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 have read and understood the contents of the Package Insert for metoclopramide, and the information sheets on the nutritional supplements. 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

Despite strict hand washing, sterile technique, and antibiotic-coated catheters, nosocomial infection and sepsis remain the leading acquired causes of morbidity and mortality in critically ill children.[1–7] Subsequent use of antibiotics to treat nosocomial infection and sepsis is considered a major attributable factor in the rise of antibiotic-resistant organisms in this population of children. Presently, "prophylaxis" strategies are used to prevent stress-induced gastrointestinal bleeding; however, no "prophylaxis" strategy is used to prevent stress-induced nosocomial infection and sepsis. When left unopposed, the stress hormone, cortisol, induces lymphocyte apoptosis, lymphopenia, and immune insufficiency.[8–16] Prolactin is the counter-regulatory stress hormone that prevents cortisol-induced apoptosis and immunosuppression.[17–30] Zinc, selenium, and glutamine are also important in maintenance of lymphocyte health.[31–35] Critically ill patients commonly develop hypoprolactinemia secondary to increased central nervous system dopaminergic activity,[36–41] as well as zinc, selenium, and glutamine deficiency caused by increased utilization and decreased supply. Hypoprolactinemia can be prevented by metoclopramide, a dopamine 2 receptor antagonist commonly used as a prokinetic in children,[42–44] and zinc, selenium, and glutamine deficiency can be prevented with enteral supplementation.

This study will use a double-blind randomized controlled trial design to test the hypothesis that daily prophylaxis with metoclopramide, zinc, selenium and glutamine will reduce nosocomial infection and sepsis in critically ill children.

1 Study Summary

1.1 Hypothesis

The hypothesis of this prospective, randomized, double–blind placebo–controlled trial is that daily prophylaxis with intravenous metoclopramide, and enteral zinc, selenium, and glutamine, will reduce nosocomial infection and sepsis in critically ill children.

1.2 Primary Endpoint

The primary endpoint of this study is the time (hours) between admission to the PICU and occurrence of nosocomial infection or clinical sepsis in PICU patients who have endotracheal tubes, central venous catheters, or urinary catheters.

1.3 Secondary Endpoints

Secondary endpoints of this study are:

1. rate of nosocomial infection or clinical sepsis per 100 PICU days;
2. antibiotic-free days;
3. incidence of prolonged lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$7 days);
4. all-cause 28-day mortality rate.

1.4 Patient Eligibility

1.4.1 Inclusion Criteria

During the initial accrual period for this study, prior to the first interim analysis, patients will be eligible for enrollment if they:

- are less than 18 years of age; AND
- are within the first 48 hours of the PICU admission; AND
- have an endotracheal tube, central venous catheter (new or old, tunneled or not tunneled), or Foley catheter; AND
- are anticipated to have an indwelling arterial or venous catheter for blood sampling during the first three days of study enrollment, AND
- are anticipated to have an IV and an enteral feeding tube for administration of study drug.

After the Data Safety Monitoring Board (DSMB) conducts its first interim evaluation, after enrollment of approximately 200 subjects, a decision will be made by the DSMB concerning enrollment of subjects between 40 weeks gestational age and 12 months. If the DSMB approves enrollment of infants after the first interim analysis, then patients will be eligible for enrollment if they:

- are between 40 weeks gestational age and less than 18 years; AND
- are within the first 48 hours of the PICU admission; AND

- have an endotracheal tube, central venous catheter (new or old, tunneled or not tunneled), or Foley catheter; AND
- are anticipated to have an indwelling arterial or venous catheter for blood sampling during the first three days of study enrollment, AND
- are anticipated to have an IV and an enteral feeding tube for administration of study drug.

1.4.2 Exclusion Criteria

During the initial accrual period for this study, prior to the first interim analysis, patients will be ineligible for enrollment if ANY of the following is true or anticipated:

- are less than 1 year age; OR
- are greater than or equal to 18 years of age; OR
- have a known allergy to metoclopramide; OR
- planned removal of endotracheal tube, central venous catheter, AND Foley catheters within 72 hours of study enrollment, or anticipated discharge from the PICU within 72 hours of study enrollment; OR
- suspected intestinal obstruction, OR
- intestinal surgery or bowel disruption, OR
- has other contraindication to enteral administration of drugs or nutrients, OR
- chronic metoclopramide therapy prior to enrollment, OR
- have a known allergy to whey (milk) or soy based products, OR
- discharged from PICU in the previous 28 days, OR
- previously enrolled in this study, OR
- pregnancy, OR
- lack of commitment to aggressive intensive care therapies.

After the Data Safety Monitoring Board (DSMB) conducts its first interim evaluation, after enrollment of approximately 200 subjects, a decision will be made by the DSMB concerning enrollment of subjects between 40 weeks gestational age and 12 months. If the DSMB approves enrollment of infants after the first interim analysis, then patients will be ineligible for enrollment if ANY of the following is true or anticipated:

- are less than 40 weeks gestational age; OR
- are greater than or equal to 18 years of age; OR
- have a known allergy to metoclopramide; OR
- planned removal of endotracheal tube, central venous catheter, AND Foley catheters within 72 hours of study enrollment, or anticipated discharge from the PICU within 72 hours of study enrollment; OR
- anticipated discharge from the PICU within 72 hours of study enrollment, OR
- suspected intestinal obstruction, OR
- intestinal surgery or bowel disruption, OR
- has other contraindication to enteral administration of drugs or nutrients, OR
- chronic metoclopramide therapy prior to enrollment, OR
- have a known allergy to whey (milk) or soy based products, OR
- discharged from PICU in the previous 28 days, OR
- previously enrolled in this study, OR
- pregnancy, OR
- lack of commitment to aggressive intensive care therapies.

1.5 Anticipated Recruitment and Study Duration

It is anticipated that the CPCCRN sites will recruit 25 patients per month (total). Duration of the study will depend on interim analysis of median PICU length of stay and median time to nosocomial infection or sepsis (for final sample size calculation), but is initially scheduled for 24 months (600 patients).

2 Background and Significance

The role of neutropenia as a risk factor for nosocomial sepsis has long been appreciated in part because chemotherapeutic agents are regularly associated with neutropenic sepsis. The AIDS epidemic has increased appreciation for the importance of lymphopenia as another risk factor for nosocomial infection and sepsis. Although this immune deficiency primarily affects CD4 lymphocytes, other primary immune deficiency disorders cause critical reductions in both T-cells and B-cells. An absolute lymphocyte count (total WBC x total % lymphocyte) $\leq$1,200/mm^3 is associated with infection.[45–47] Investigators have now demonstrated that lymphopenia acquired with critical illness is associated with nosocomial sepsis and death. Two groups of investigators (Hotchkiss et al in adults, and Gurevitch et al in newborns) have shown that critically ill patients who die from nosocomial sepsis, have depletion of T-cells and B-cells from thymus, lymph nodes, and spleen.[48–50] Treatment with caspase inhibitors prevents stress-induced apoptosis of lymphocytes, reduces bacteria cell counts, and improves survival in experimental sepsis. Similarly, in an experimental hemorrhagic shock model, treatment with the dopamine 2 receptor antagonist, metoclopramide, maintains prolactin levels, prevents lymphocyte apoptosis, and improves survival with subsequent experimentally induced 'nosocomial' sepsis.[51–53]

A recent study[54] designed to evaluate the importance of lymphopenia and hypoprolactinemia in critical illness–associated nosocomial sepsis observed an association between prolonged lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$7 days) and the development of nosocomial sepsis (multivariate OR = 5.5; 95% CI 1.7–17), lymphoid depletion (multivariate OR = 42.2; 95% CI 3.2–473) and death (multivariate OR = 6.8; 95% CI 1.3 – 34). Increased apoptosis of B-cells, T-cells, and dendritic cells was found in immune cell depleted thymus, lymph nodes and spleens in patients who died with lymphopenia (ALC $\leq$1,000/mm^3) and nosocomial sepsis. In contrast, patients who died without lymphopenia did not develop nosocomial sepsis. They had normal numbers of B-cells, T-cells, and dendritic cells in the thymus, lymph nodes, and spleen. Prolonged hypoprolactinemia was associated with an increased risk of developing prolonged lymphopenia (multivariate OR = 8.3; 95% CI 2.1-33) and lymphoid depletion (multivariate OR = 12.2; 95% CI 2.2-65). This study raises the question whether prevention of stress induced lymphocyte apoptosis could reduce nosocomial infection and sepsis in critically ill children.

The dosage of metoclopramide commonly used for gastrointestinal prokinesis maintains prolactin levels in the high normal range in children. One

randomized control trial examined the ability of metoclopramide to prevent nosocomial pneumonia in adults.[55] Although metoclopramide increased the time to onset of nosocomial pneumonia by 50%, it had no effect on the incidence of nosocomial pneumonia nor outcome in this population. In addition to prolactin, zinc, selenium, and glutamine also prevent stress induced lymphopenia. In several randomized controlled trials, zinc supplementation reduced morbidity and mortality in children with severe pneumonia.[56,57] Because zinc also reduces morbidity in children with diarrhea,[58–60] the World Health Organization now recommends zinc supplementation for all children with the two leading worldwide causes of pediatric sepsis, severe pneumonia or diarrhea. Zinc supplementation reduced infectious disease mortality in small for gestational age infants (rate ratio 0.32; 95% CI 0.12-0.89).[61] A Cochrane Database review of randomized controlled trials found that selenium supplementation reduced the risk of nosocomial sepsis in preterm neonates (relative risk 0.73; 95% CI 0.57-0.93).[62] Glutamine enriched enteral nutrition also reduced serious nosocomial infection in very low birthweight infants in a randomized controlled trial (odds ratio 0.32 95% CI 0.14-0.74).[63] Enteral gutamine also safely maintains TH1 lymphocyte function for bacterial killing.[64,65]

3 Study Hypothesis and Design

The hypothesis of this prospective, randomized, double–blind placebo–controlled trial is that daily prophylaxis with intravenous metoclopramide, and enteral zinc, selenium, and glutamine, will reduce nosocomial infection and sepsis in critically ill children.

This trial will be analyzed as an intention-to-treat study.

4 Study Outcomes

4.1 Primary Endpoint

The primary endpoint of this study is the time (hours) between admission to the PICU and occurrence of nosocomial infection or clinical sepsis in PICU patients who have endotracheal tubes, central venous catheters, or urinary catheters.

4.2 Secondary Endpoints

Secondary endpoints of this study are:

1. rate of nosocomial infection or clinical sepsis per 100 PICU days;
2. antibiotic-free days;
3. incidence of prolonged lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$7 days);
4. all-cause 28-day mortality rate.

4.3 Additional Analyses

We will analyze:

1. absolute lymphocyte count
2. incidence of moderate lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$3 days;

The following laboratory analyses will be conducted on the day of enrollment (Study Day One) and on day 7 (or study exit if discharged prior to day 7), to corroborate the hypothesized biological mechanism behind a positive effect of active study drug on the primary and secondary endpoints of the trial:

1. serum prolactin levels
2. serum zinc levels
3. selenium levels

For children enrolled at the Pittsburgh clinical site only, the following additional laboratory analyses are planned on the day of enrollment (Study Day One) and on days 7, 14, 21, and 28. These studies will be conducted in the clinical laboratory or in the Carcillo research laboratory:

1. subset lymphocyte counts
2. glutathione peroxidase levels
3. gamma globulin levels

4. whole blood TNF response

The following PICU process parameters will be analyzed to corroborate the beneficial health care delivery effects of reducing sepsis in patients receiving active study drug:

1. antibiotic resistant nosocomial infection events
2. PELOD scores (days 1,7,14,21, and 28)[66,67]
3. OFI scores (days 1,7,14,21, and 28)[68]
4. ventilator-free days
5. PICU mortality
6. 28 day mortality

5 Patient Eligibility

5.1 Inclusion Criteria

During the initial accrual period for this study, prior to the first interim analysis, patients will be eligible for enrollment if they:

- are less than 18 years of age; AND
- are within the first 48 hours of the PICU admission; AND
- have an endotracheal tube, central venous catheter (new or old, tunneled or not tunneled), or Foley catheter; AND
- are anticipated to have an indwelling arterial or venous catheter for blood sampling during the first three days of study enrollment, AND
- are anticipated to have an IV and an enteral feeding tube for administration of study drug.

After the Data Safety Monitoring Board (DSMB) conducts its first interim evaluation, after enrollment of approximately 200 subjects, a decision will be made by the DSMB concerning enrollment of subjects between 40 weeks gestational age and 12 months. If the DSMB approves enrollment of infants after the first interim analysis, then patients will be eligible for enrollment if they:

- are between 40 weeks gestational age and less than 18 years; AND
- are within the first 48 hours of the PICU admission; AND
- have an endotracheal tube, central venous catheter (new or old, tunneled or not tunneled), or Foley catheter; AND
- are anticipated to have an indwelling arterial or venous catheter for blood sampling during the first three days of study enrollment, AND
- are anticipated to have an IV and an enteral feeding tube for administration of study drug.

5.2 Exclusion Criteria

During the initial accrual period for this study, prior to the first interim analysis, patients will be ineligible for enrollment if ANY of the following is true or anticipated:

- are less than 1 year age; OR
- are greater than or equal to 18 years of age; OR
- have a known allergy to metoclopramide; OR
- planned removal of endotracheal tube, central venous catheter, AND Foley catheters within 72 hours of study enrollment, or anticipated discharge from the PICU within 72 hours of study enrollment; OR
- suspected intestinal obstruction, OR
- intestinal surgery or bowel disruption, OR
- has other contraindication to enteral administration of drugs or nutrients, OR
- chronic metoclopramide therapy prior to enrollment, OR
- have a known allergy to whey (milk) or soy based products, OR
- discharged from PICU in the previous 28 days, OR
- previously enrolled in this study, OR
- pregnancy, OR

- lack of commitment to aggressive intensive care therapies.

After the Data Safety Monitoring Board (DSMB) conducts its first interim evaluation, after enrollment of approximately 200 subjects, a decision will be made by the DSMB concerning enrollment of subjects between 40 weeks gestational age and 12 months. If the DSMB approves enrollment of infants after the first interim analysis, then patients will be ineligible for enrollment if ANY of the following is true or anticipated:

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- have a known allergy to whey (milk) or soy based products, OR
- discharged from PICU in the previous 28 days, OR
- previously enrolled in this study, OR
- pregnancy, OR
- lack of commitment to aggressive intensive care therapies.

5.3 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. During this study, the Data Coordinating Center (DCC) will monitor patient accrual by race, ethnicity, and gender. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate patient sample contains appropriate gender and minority subsets.

6 Study Methods

6.1 Screening, Enrollment and Randomization

Children will be screened, enrolled with parental permission (and when applicable, assent of the child), and randomized to treatment or placebo within the first 48 hours of PICU admission. The day of enrollment is designated as Study Day 1.

Randomization will be stratified by clinical center and by Immune Compromise Status (immune compromised, or immune competent). The DCC will prepare randomization schedules, using randomized blocks of varying length. Patient randomization will be accomplished using an Internet connection to the DCC, or an interactive telephone service.

6.2 Study Drug and Placebo Administration

Study drug will be obtained by a central pharmacy, and will be sent to each study site. Study drug (metoclopramide, zinc, selenium and glutamine) or placebo will be stored, prepared, and dispensed by the hospital pharmacy/nutrition services in a fashion which is blinded to the care takers, data collectors, and data analysts. The Manual of Operations will include specific instructions for preparing the enteral nutritional supplements.

The first dose of study drug will be administered after sampling blood for baseline laboratory analyses, within 24 hours of study enrollment. In all cases, the first dose of study drug should be administered before the 72nd hour of PICU admission.

Administration of study drug will continue until the earliest of the following three conditions is met:

- Discharge from PICU

- PICU day 28
- Death in PICU

6.2.1 Metoclopramide

Children allocated to the metoclopramide/zinc/selenium/glutamine arm will receive metoclopramide 0.2 mg/kg/dose IV every 12 hours (the dose often selected for facilitation of enteral feeding), and children allocated to the placebo arm will receive an equivalent volume of intravenous saline. The maximum dose will be 10 mg/dose (maximum daily total 20 mg). The study drug will be administered in accordance with the clinical site's policy for IV administration of metoclopramide. In the absence of a clinical site policy for IV administration of metoclopramide, the study drug should be administered over 15 to 30 minutes. If an IV is not available, metoclopramide/placebo will be discontinued.

Dosage should be modified in response to the degree of renal impairment. This should be done according to the practice of the intensive care unit at each clinical site. One suggested dosing adjustment in renal impairment of adults and children is displayed in Table 1.

Table 1: Adjustment of Dose of Metoclopramide for Renal Impairment

Estimated Creatinine Clearance	Study Drug Dose Adjustment
40 to 50 ml/minute	75% of recommended dose
10 to 40 ml/minute	50% of recommended dose
$<$ 10 ml/minute	25% of recommended dose

6.2.2 Zinc, Selenium, and Glutamine

Intravenous preparations of zinc and selenium have been chosen to facilitate proper dosing, but these drugs will be administered *enterally*. Children allocated to the metoclopramide/zinc/selenium/glutamine arm will receive one enteral dose daily of zinc chloride (10 mg/day elemental zinc for infants $\leq$1 year of age, and 20 mg /day elemental zinc for patients $>$1 year of age). They will also receive one enteral dose daily of selenium (40 μg for infants $<$ 8 months of age, 60 μg for infants 8 to 12 months of age, 90 μg for children 1-3 years, 150 μg for children 4-8 years, 280 μg for children 9 to 13 years, and 400 μg for children $>$ 13 years).

Zinc absorption is reduced when given with foods or drugs containing high amounts of calcium or phosphate. Therefore, antacids should not be administered within 1 hour (prior or after) of study drug administration.

Children allocated to the metoclopramide/zinc/selenium/glutamine arm will also receive one enteral dose daily of glutamine (0.3 gm/kg/day). Children allocated to the placebo group will receive one enteral dose daily of whey-protein placebo. The study drug will be administered via an enteric feeding tube (nasogastric, NG; nasoduodenal, ND; nasojejunal, NJ; gastrostomy, GT), clamping the feeding tube for 1 hour after study drug administration. The morning dose of zinc, selenium and glutamine will be administered after administration of intravenous study drug (metoclopramide or placebo).

In the event that the child has multiple enteral tubes, the study drug should be administered by the most distal site. For example, if a child has an NJ and NG tube, the drug should be administered through the NJ tube.

If the child does not have an enteral feeding tube, administration of all enteral supplements will be discontinued.

6.3 Drug Administration Time Window

Drug administration within 60 minutes prior to or 60 minutes after the desired time will be considered within the protocol requirements, and will not be considered a protocol violation.

6.4 Subject Withdrawal

Every effort must be made by the investigator to keep the subjects in the study. However, subjects may be discontinued from study medication prior to completion of the study for any of the following reasons:

1. Adverse event requiring discontinuation
2. Failure to tolerate study medication
3. Development of an exclusion criterion (e.g. bowel obstruction)
4. Other

All patients withdrawn from the study medication must have a reason for withdrawal recorded in TrialDB including the circumstances leading to withdrawal. All adverse events leading to withdrawal of study medication must be fully documented and followed up as appropriate. To ensure that all

withdrawals due to adverse events are correctly identified, "Adverse Event" should only be marked as the reason for withdrawal on the "Final Status" page for those patients for whom an adverse event was considered to have been the direct cause of the patient withdrawing from study medication. This is particularly important when adverse events are on-going at the time of withdrawal, but the reason for withdrawal is not related to the adverse event.

Study site personnel will attempt to follow the progress of every patient admitted to the study through to study completion. If a patient is discharged from the hospital or otherwise unable to complete the study as planned, a reasonable effort should be made to contact the parent or guardian to ascertain the disposition of the patient. If a patient does not complete the study for any reason (including Investigator discretion), the reason and circumstances for the patient's early termination must be fully documented.

All study drug administration will be discontinued if the clinical provider withdraws the subject due to an adverse event or the subject's parents withdraw permission for the subject to continue in the study. In these examples, the medical course of the subject will continue to be reviewed for adverse events until discharge from the hospital, or for 28 days following enrollment because this study is designed as an intention-to-treat study.

7 Data Management

Clinical data will be collected at the time of enrollment, daily as scheduled below, and at the time of hospital discharge or death. Daily collection will occur up to five days after the last day of study drug administration, unless hospital discharge or death occur earlier.

This protocol includes a detailed list of data elements to be collected, and if data elements are added or subtracted from this, a protocol amendment will be produced. However, specific choice sets for these data may be amended without being considered a change to this protocol. The choice sets that are listed in the protocol are intended to help the reader completely understand the intent of the data element and to assist implementation of the electronic data collection system by the CPCCRN DCC.

Data will be entered into an electronic data collection system to be designed and implemented by the CPCCRN DCC. The Study Coordinator may choose to print hard copy forms to use as worksheets. If used, the paper worksheets should be retained at the clinical center in a locked file cabinet within a locked office until the study is complete and all CPCCRN

publications have been accomplished.

Site monitoring visits will be performed on a regular basis by staff from the DCC, to ensure that all regulatory requirements are being met and to monitor the quality of the data collected. Records of IRB approvals will be examined. During site monitoring visits by DCC or National Institute for Child Health and Human Development (NICHD) staff, paper worksheets and original source documents will also be inspected. The primary criterion for data element verification is identification in the source document, which is generally the medical record.

7.1 Definitions

Nosocomial: defined as occurrence 48 hours after admission until 5 days after discharge from the PICU

Clinical sepsis: must meet at least one of the following two criteria:

1. Criterion 1
   - patient has at least *one* of the following clinical signs or symptoms with no other recognized cause: fever ($\geq$ 38 °C), hypotension (systolic BP $\leq$90 mm Hg), or oliguria ($\leq$20 cc/hr); AND
   - blood culture *not* done or *no* organisms or antigen detected in blood; AND
   - no apparent infection at another site; AND
   - physician institutes treatment for sepsis.
2. Criterion 2
   - patient $\leq$1 year of age has at least *one* of the following clinical signs or symptoms with no other recognized cause: fever ($\geq$ 38 °C), hypothermia ($\leq$ 37 °C), apnea, or bradycardia; AND
   - blood culture *not* done or *no* organisms or antigen detected in blood; AND
   - no apparent infection at another site; AND
   - physician institutes treatment for sepsis.

Infection: microbiologically (culture, antigen, PCR, or antibody) proven infection in a patient with fever, hypothermia, chills, or hypotension

Lymphopenia: absolute lymphocyte count $\leq 1000/mm^3$

Prolonged lymphopenia: lymphopenia occuring for ≥7 consecutive PICU days

Moderate lymphopenia: lymphopenia occuring for ≥3 consecutive PICU days

PICU day: calendar day during any part of which the child is an inpatient in the PICU

Ventilated day: PICU day during any part of which the child requires mechanical ventilation support greater than chronic baseline requirements (out of the hospital), provided by endotracheal tube, tracheostomy, or face mask

Resistant organism: defined according to CDC criteria; e.g. MRSA, ESBL, VRE, Fluconazole resistant candida/fungus etc, and/or the presence of MICs which are ≥1 for all antibiotics listed in the susceptibility chart.

7.2 Study Entry Data Elements

The following data elements will be obtained and recorded when a patient is enrolled into the study:

1. Study Code ID
2. Clinical Center ID
3. Date of Enrollment
4. Date and Time of Admission to Hospital
5. Date and Time of Admission to PICU
6. Gender
7. Race

   American Indian or Alaska Native A person having origins in any of the original peoples of North and South America, including Central America, and who maintains tribal affiliation or community attachment.

Asian A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

Black or African American A person having origins in any of the black racial groups of Africa.

Native Hawaiian or Other Pacific Islander A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

Other (provide text) Should provide text description.

Stated as Unknown Explicitly stated as unknown.

8. Ethnicity
    - Hispanic or Latino
    - Not Hispanic or Latino
    - Stated as Unknown
9. Date of Birth
10. Height (cm)
11. Weight (kg)
12. Patient History
    - History of present illness
    - Review of systems
    - Past medical history
    - Physical examination
13. Admission Pediatric Risk of Mortality III (PRISM III) Score[69]
14. Chronic Ventilator Support (Yes or No)
15. Immune Status
    - Immune Competent
    - Immunocompromised

  - Bone Marrow Transplant Recipient
  - Other Organ Transplant Recipient
  - Cancer Patient
  - Human Immunodeficiency Virus (HIV)
  - Other (provide text)

16. Primary Diagnostic Category (Select One)

    - Asthma (reactive airway disease)
    - Cancer
    - Cardiac arrest w/in 24 hours (closed chest massage)
    - Chromosomal abnormality (not hereditary condition)
    - Diabetes
    - Drug overdose (e.g. ingestion, toxicity)
    - Gastroesophageal reflux
    - Cardiovascular disease - acquired
    - Cardiovascular disease - congenital
    - HIV infection
    - Hypoxic-ischemic encephalopathy (acute, not static)
    - Medical device malfunction
    - Meningitis
    - Pneumonia / bronchiolitis
    - Seizures (includes complications of seizure therapy)
    - Sepsis
    - Shock
    - Suicide attempt (includes intentional drug overdose)
    - Transplant
    - Trauma
    - Other Diagnosis

17. Secondary Diagnostic Category (Select One)

    - Asthma (reactive airway disease)
    - Cancer
    - Cardiac arrest w/in 24 hours (closed chest massage)

- Chromosomal abnormality (not hereditary condition)
- Diabetes
- Drug overdose (e.g. ingestion, toxicity)
- Gastroesophageal reflux
- Cardiovascular disease - acquired
- Cardiovascular disease - congenital
- HIV infection
- Hypoxic-ischemic encephalopathy (acute, not static)
- Medical device malfunction
- Meningitis
- Pneumonia / bronchiolitis
- Seizures (includes complications of seizure therapy)
- Sepsis
- Shock
- Suicide attempt (includes intentional drug overdose)
- Transplant
- Trauma
- Other Diagnosis

18. Chronic Diagnoses (Select Multiple)

- Bronchopulmonary dysplasia (BPD)
- Cancer
- Cerebral palsy
- Chromosomal abnormality (not hereditary condition)
- Congenital heart disease
- Diabetes
- HIV infection
- Hydrocephalus
- Intraventricular hemorrhage (from perinatal period)
- Mental retardation
- Meningomyelocele / spina bifida
- Short gut syndrome

- Static encephalopathy
- Transplant
- Other Diagnosis

19. Postoperative Surgical Status (Select one)

    - Not postoperative
    - Postoperative
        - Cardiac surgery
        - Neurosurgery
        - Transplant surgery
        - Trauma surgery
        - Other surgery

20. Infection (Select One)

    - No infection
    - Existing infection at time of study entry
        - Date of diagnostic specimen
        - Type(s) of specimen(s)
        - Name(s) of organism(s)
        - Organism(s) sensitivity (send report)
        - Presumed Site of Infection (Select One)
          Defined as the clinician's best assessment. Indicate *Unknown* only if the clinicians are unable to make a reasonable assessment.
            * Urinary tract
            * Surgical wound
            * Pneumonia
            * LRTI other than pneumonia
            * Bloodstream (primary)
            * Bone or joint
            * CNS
            * Cardiovascular
            * ENT
            * GI tract
            * Reproductive tract

        * Skin or soft tissue
        * Systemic
        * Unknown

21. Septic (Select One)
    Note that there must be antibiotic therapy for the diagnosis of sepsis. If no antibiotic therapy is present, then the answer to this question should be "No sepsis".

    - No sepsis
    - Existing sepsis at time of study entry
        - Date of diagnosis of sepsis
        - Presumed Site of Infection (Select One)
          Defined as the clinician's best assessment. Indicate *Unknown* only if the clinicians are unable to make a reasonable assessment.
            * Urinary tract
            * Surgical wound
            * Pneumonia
            * LRTI other than pneumonia
            * Bloodstream (primary)
            * Bone or joint
            * CNS
            * Cardiovascular
            * ENT
            * GI tract
            * Reproductive tract
            * Skin or soft tissue
            * Systemic
            * Unknown

22. Antibiotic Therapy (Select One)

    - None
    - On therapy at time of study entry
        - Name(s) of drug(s)
        - Start date of drug(s)

23. Endotracheal Tube (Select One)
    - Not present
    - Present
      - Date of insertion
      - Time of insertion
24. Central Venous Catheter (Select One)
    - Not present
    - Present
      - Date of insertion
      - Time of insertion
25. Urinary Catheter (Select One)
    - Not present
    - Present
      - Date of insertion
      - Time of insertion

7.3 Daily Data Elements

Study Day One is the day of enrollment. Each day is defined as midnight (00:00) to 23:59. The following data will be recorded on a daily basis, including on Study Day One:

1. Date
2. Study Day Number
3. Time of administration of enteral study drugs
4. Time of administration of parenteral study drug
5. Lowest Absolute Lymphocyte Count (units) if clinically obtained
6. Lowest Absolute Neutrophil Count (units) if clinically obtained
7. Highest Glucose Concentration (mg/dL) if clinically obtained
8. Infection (Select One)

- No infection
- Continued infection from previous day
- New infection noted today
  - Date of diagnostic specimen
  - Type(s) of specimen(s)
  - Name(s) of organism(s)
  - Organism(s) sensitivity (send report)
  - Presumed Site of Infection (Select One)
    Defined as the clinician's best assessment. Indicate *Unknown* only if the clinicians are unable to make a reasonable assessment.
    * Urinary tract
    * Surgical wound
    * Pneumonia
    * LRTI other than pneumonia
    * Bloodstream (primary)
    * Bone or joint
    * CNS
    * Cardiovascular
    * ENT
    * GI tract
    * Reproductive tract
    * Skin or soft tissue
    * Systemic
    * Unknown

9. Septic (Select One)
   Note that there must be antibiotic therapy for the diagnosis of sepsis. If all antibiotics were discontinued in previous study days, then the answer to this question should be "No sepsis".

   - No sepsis
   - Continued sepsis from previous day
   - New sepsis diagnosed today
     - Presumed Site of Infection (Select One)
       Defined as the clinician's best assessment. Indicate *Unknown* only if the clinicians are unable to make a reasonable assessment.

    * Urinary tract
    * Surgical wound
    * Pneumonia
    * LRTI other than pneumonia
    * Bloodstream (primary)
    * Bone or joint
    * CNS
    * Cardiovascular
    * ENT
    * GI tract
    * Reproductive tract
    * Skin or soft tissue
    * Systemic
    * Unknown

10. Antibiotic Therapy (Select One)

    - None
    - No change from previous day
    - New antibiotic therapy instituted
      - Name(s) of drug(s)
      - Start date of drug(s)
    - Existing antibiotic therapy discontinued
      - Name(s) of drug(s)
      - Stop date of drug(s)

11. Endotracheal Tube (Select One)

    - Not present during this day
    - No change from previous day
    - Removed during this day
      - Date of removal
      - Time of removal
    - Inserted or reinserted during this day
      - Date of insertion
      - Time of insertion

12. Central Venous Catheter (Select One)
    - Not present during this day
    - No change from previous day
    - Removed during this day
      - Date of removal
      - Time of removal
    - Inserted or changed during this day
      - Date of insertion or change
      - Time of insertion or change
13. Urinary Catheter (Select One)
    - Not present during this day
    - No change from previous day
    - Removed during this day
      - Date of removal
      - Time of removal
    - Inserted or changed during this day
      - Date of insertion or change
      - Time of insertion or change
14. Additional Invasive Therapies or Catheters Present During Study Day(Select One or More)
    - None
    - Arterial catheter
    - Tracheostomy
    - Chest tube
    - Open Mediastinum
    - ECMO
    - Hemodialysis
    - Peritoneal dialysis
    - LVAD or BIVAD
    - ICP monitor

- Epidural catheter
- Other (specify)

15. Steroids (Yes or No)
    - Name(s) of systemic steroid(s) administered
    - Total daily dose of steroid(s) administered
16. Calcineurin Inhibitors (Yes or No)
    Defined as tacrolimus, sirolimus, or cyclosporine A
17. Other Immunosuppressant Therapy (Yes or No)
    Includes antilymphocyte globulin, chemotherapeutic agents, etc. but excludes steroids or calcineurin inhibitors.
18. Total Parenteral Nutrition (TPN) (Yes or No)
    Defined as receiving at least three hours of infusion during the study day.
19. Enteral Feeding (Yes or No)
    Defined as any attempt to feed enterally during the study day.
20. Dopamine (Yes or No)
    Defined as receiving at least three hours of dopamine infusion (any dose) during the study day.
21. Metoclopramide Received (Yes or No)
    Metoclopramide may be received as a single dose during placement of feeding tubes without protocol violation. If patient is placed on regular metoclopramide therapy, study drug will be permanently discontinued, and a protocol violation will be noted.
22. Supplemental Zinc Received (Yes or No)
    Defined as explicit prescription for supplemental zinc. Routine zinc content of TPN solution is not sufficient for this question to be answered *Yes*.
23. TPN Contains Zinc (Yes or No)
    Defined as standard zinc concentration for TPN solution.
24. Supplemental Selenium Received (Yes or No)
    Defined as explicit prescription for supplemental selenium. Routine selenium content of TPN solution is not sufficient for this question to be answered *Yes*.

25. TPN Contains Selenium (Yes or No)
    Defined as standard selenium concentration for TPN solution.

26. Supplemental Glutamine received (Yes or No)
    Defined as explicit prescription for supplemental glutamine. Routine glutamine content of TPN solution is not sufficient for this question to be answered *Yes*.

27. TPN Contains Glutamine (Yes or No)
    Defined as standard glutamine concentration for TPN solution.

28. Intravenous gamma globulin (IVIG) (Yes or No)
    If infusion crosses midnight, only indicate *Yes* on study day when infusion was started.

29. Granulocyte colony–stimulating factor (G-CSF) (Yes or No)

30. Granulocyte–monocyte colony–stimulating factor (GM-CSF) (Yes or No)

31. Ventilatory Support (Yes or No)
    Defined as support beyond chronic baseline support by endotracheal tube, tracheostomy, or non-invasive ventilatory support provided by ventilator.

32. Organ Failure Index (OFI) (Only on days 1,7,14,21, and 28)

33. Pediatric Logistic Organ Dysfunction (PELOD) Score (Only on days 1,7,14,21, and 28)

34. Patient in PICU at end of study day (Yes or No)

    - Yes
    - No
        - Date and time of discharge from PICU

35. Alive at end of study day(Yes or No)

    - Yes
    - No
        - Date and time of death

7.4 Clinical Laboratory Sampling

Pregnancy is a contraindication for participation in this study. All female patients of child-bearing potential must have a negative urine pregnancy test prior to enrollment.

Safety Laboratory Values For safety monitoring in this study, the following tests will be obtained and recorded twice in the first week, then once per week thereafter:

1. Complete blood count
2. Serum creatinine
3. Aspartate aminotransferase (AST)
4. Alanine aminotransferase (ALT)
5. Alkaline phosphatase
6. Total bilirubin

These laboratory tests will frequently be part of the clinical care of patients who are eligible for this study. If this is not the case, then sampling for these tests should be coordinated with blood draws already being conducted for normal clinical care, in order to minimize blood wastage. If there is no venous catheter or arterial line for obtaining these monitoring laboratory tests, and the tests are not otherwise clinically indicated, the study drug should be discontinued (all study drugs). No venipuncture should be performed solely for the purpose of laboratory testing in this study.

In addition, clinical staff should recognize the potential risk of hyperammonemia from administration of enteral nitrogen (glutamine), and if subjects show any signs of toxic encephalopathy, serum ammonia measurements should be obtained. Metoclopramide has been associated with methemoglobinemia, and if subjects show signs of methemoglobinemia, such as oxygen desaturation, methemoglobin levels should be obtained.

7.5 Blood Sampling Schedule

Blood sampling will occur when there is an existing arterial or venous line for blood sampling. Samples will be obtained on the day of enrollment (Study Day One), and on day 7 (or study exit if discharged prior to day 7). The sample on study day one should be drawn prior to study drug administration.

The sample on day 7 or study exit should be drawn between 1 – 4 hours after metoclopramide is given.

Samples will be appropriately processed for storage or transport to the laboratory assigned by the CPCCRN DCC to do the following analyses:

1. Prolactin level
2. Zinc concentration
3. Selenium concentration

At the Pittsburgh clinical site only, the following samples will be obtained on the day of enrollment (Study Day One), and on days 7, 14, 21, and 28 for the following studies in the clinical laboratory or the Carcillo research laboratory:

1. Glutathione peroxidase levels
2. Lymphocyte subsets
3. Gamma globulin levels
4. Lymphocyte apoptosis
5. Whole blood TNF response

The results of these laboratory analyses will not be provided to clinicians caring for the patients, and certain analyses may not be accomplished until long after the patient has exited the study.

7.6 Study Exit Data Elements

The following data will be recorded when the patient exits the study:

1. PICU Survival (Yes, No, Not applicable)
   Did the patient leave the PICU alive prior to Study Day 28? If patient remains alive but is still in the PICU, the answer is *Not applicable*.
2. Hospital Survival (Yes, No, Not applicable)
   Was the patient discharged from the hospital alive prior to Study Day 28? If patient remains alive but is still in the hospital or PICU, the answer is *Not applicable*.

3. Survival to 28 Days (Yes, No)
   Is the patient alive in PICU or hospital on Study Day 28? If patient has been discharged from the hospital prior to Study Day 28, was the family successfully contacted regarding patient status on Day 28?

4. Study completion details, such as whether the patient completed the study as planned, summaries of protocol violations, etc.

For example, if a child has not yet been discharged from the PICU on day 35, there can be no judgment about PICU survival, nor about hospital survival. Hence, these two data elements would be coded as "Not Applicable" because the child has neither left the PICU nor the hospital. However, the child has survived to 28 days, and the answer to the third data element would be "Yes".

For another example, if the child is discharged from the PICU on day 11 and then from the hospital on day 15, then the answers to the first two data elements would be "Yes". After checking medical records and if no death information is found, a follow-up phone call should be made to the family ascertaining how the child is doing. The answer to the third data element should be "Yes" or "No". Should the family report that the child has died, the caller should request date of death.

Figure 1 on the next page summarizes the measurements to be obtained in this study.

Assessment	Screening	Day 1 Day of Enrollment	Day 2-6	Day 7	Day 8-13	Day 14	Day 15-20	Day 21	Day 22-27	Day 28	Exit
Inclusion/Exclusion	X										
Demographics	X										
Informed Consent	X										
Patient History	X										
Randomization		X[1]									
Labs at all sites: Absolute Lymphocytes[2], Absolute Neutrophils[2], Glucose Concentration[2], CBC w/Diff[2], Chemistry[3]		X	X	X	X	X	X	X	X	X	
Serum Prolactin[4]; Zinc[4]; Selenium[4]		X		X							
Additional labs Pittsburgh site only: Glutathione Peroxidase, Lymphocyte subsets, Gamma globulin, Lymphocyte apoptosis, Whole Blood TNF response		X		X		X		X		X	
PELOD Score		X		X		X		X		X	
Organ Failure Index (OFI)		X		X		X		X		X	
Study Drug Administration[5]		X[6]	X	X	X	X	X	X	X	X	
Daily Assessments: Infection Status, Sepsis Status, Antibiotic Therapy, Endotracheal Tube Status, Central Venous Catheter Status, Urinary Catheter Status, Additional invasive therapies or catheters, ventilory support, PICU/mortality status @ end of study day		X	X	X	X	X	X	X	X	X	
Adverse Events		X	X	X	X	X	X	X	X	X	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	
Final Patient Status: PICU Survival, Hospital Survival, Survival to 28 days											X

1) Children will be screened, enrolled, and randomized within the first 48 hours of PICU admission
2) Absolute Lymphocytes, Absolute Neutrophils, and Glucose levels collected daily starting at Day 1
3) CBC w/ differential, Serum creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase and Total bilirubin collected twice in the first week, then once per week thereafter, or more frequently in accordance with local PICU standard of care
4) Processed for storage and transport per Mayo Clinical Trials protocol
5) (+) or (–) 60 minutes of the designated time
6) 1st dose of study drug will be administered after baseline labs drawn and within 24 hrs of study enrollment but no later than the 72nd hour of PICU admission

Figure 1: Summary of Study Assessments

8 Data Analysis

8.1 Primary Endpoint

The primary endpoint of this study is the time (hours) between admission to the PICU and occurrence of nosocomial infection or clinical sepsis in PICU patients who have endotracheal tubes, central venous catheters, or urinary catheters.

8.2 Secondary Endpoints

Secondary endpoints of this study are:

1. rate of nosocomial infection or clinical sepsis per 100 PICU days;
2. antibiotic-free days;
3. incidence of prolonged lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$7 days);
4. all-cause 28-day mortality rate.

8.3 Additional Analyses

We will analyze:

1. absolute lymphocyte count
2. incidence of moderate lymphopenia (absolute lymphocyte count $\leq$1,000/mm^3 for $\geq$3 days;

The following laboratory analyses will be conducted on the day of enrollment (Study Day One) and on day 7 (or study exit if discharged prior to day 7), to corroborate the hypothesized biological mechanism behind a positive effect of active study drug on the primary and secondary endpoints of the trial:

1. serum prolactin levels
2. serum zinc levels
3. selenium levels

For children enrolled at the Pittsburgh clinical site only, the following additional laboratory analyses are planned on the day of enrollment (Study Day One) and on days 7, 14, 21, and 28. These studies will be conducted in the clinical laboratory or in the Carcillo research laboratory:

1. subset lymphocyte counts
2. glutathione peroxidase levels
3. gamma globulin levels
4. whole blood TNF response

The following PICU process parameters will be analyzed to corroborate the beneficial health care delivery effects of reducing sepsis in patients receiving active study drug:

1. antibiotic resistant nosocomial infection events
2. PELOD scores (days 1,7,14,21, and 28)[66,67]
3. OFI scores (days 1,7,14,21, and 28)[68]
4. ventilator-free days
5. PICU mortality
6. 28 day mortality

8.4 Outline of Analyses

8.4.1 Primary Endpoint

The primary endpoint will be tested with a logrank test (see Section 8.5 on page 42 for details). Median time to infection will also be reported by treatment arm with confidence intervals.

8.4.2 Secondary Endpoints

Rates of infection between the two treatment arms, and cumulative number of nosocomial sepsis episodes per patient over time, will be analyzed using Poisson-type regression analysis approach that is appropriate for the study data. While a standard Poisson model will be used if feasible, assessment of overdispersion will be perfomed. Depending on the extent of observed

overdispersion, a zero-inflated Poisson model or negative binomial model may be more appropriate for the final reported analysis.

Differences in PICU-acquired nosocomial sepsis in the placebo and treatment groups will be further analysed using a Kaplan-Meier plot, relative risk (nosocomial infection, nosocomial sepsis, and combined nosocomial infection or sepsis free survival rates), and true hazard ratios (time to first nosocomial infection or nosocomial sepsis). We will also examine event rates over time calculated using the competing risks method.[70]

Differences in antibiotic free days will be analyzed over 28 days using Kaplan Meier analysis and the logrank test. Patients who are discharged without antibiotics will be considered antibiotic-free from the day of discharge through day 28.

Differences in the incidence of prolonged lymphopenia will be analysed using the χ^2 test or the Fisher exact test if expected cell sizes are small.

Differences in all-cause hospital mortality will be compared using χ^2 test.

8.4.3 Subgroup Analyses

The study has been designed to test the primary hypothesis in the entire study population. However, analyses will also be performed for subgroups of study patients defined by the following:

1. Immune Compromise Status (immune compromised, or immune competent)
2. Postsurgical Status (admitted to PICU after surgical procedure, or not)
3. Gender
4. Race/Ethnicity
5. Clinical Center

Reporting of subgroup analysis results will take the multiplicity of comparisons issue into account. While subgroup-specific event rates will be reported, determination of significance of a subgroup effect will be determined by the (multiplicity-adjusted) significance of the subgroup-by-treatment interaction in the appropriate analysis. It is expected that subgroup findings would lead to potential future confirmatory studies in the appropriate subpopulation(s).

8.4.4 Additional Analyses

Differences in lymphocyte counts, lymphocyte subsets, prolactin levels, zinc levels , selenium levels, glutathione peroxidase levels, gamma globulin levels, whole blood TNF response to LPS, PELOD scores, and OFI scores, within and between groups will be analyzed using the 2 factor repeated measures ANOVA for Ranks if data is non-parametric or 2 factor repeated measures ANOVA if parametric assumptions are met. Use of the linear mixed model will allow analysis of data for patients discharged from the PICU, as well as non-survivors before 28 days and any subjects who may drop out of the analysis before 28 days.

Differences in alive and ventilator free days, alive and PICU free days, alive and antibiotic free days, and alive and resistant organism free days, will be analyzed over 28 days using Kaplan Meier analysis and the logrank test. For all these analyses, patients who have been discharged home will be considered free of the respective parameter from the day of discharge through day 28.

Differences in 28 day and PICU mortality will be compared using χ^2 test.

Exploratory analyses of factors associated with outcomes will employ approaches inlcuding Poisson regression, Cox proportional hazards regression, and appropriate variants of the mixed model. Reports of these analyses will be clearly described as exploratory rather than formal hypothesis testing.

8.5 Power Analysis for Primary Outcome

The primary endpoint of this study is the time (hours) between admission to the PICU and occurrence of nosocomial infection or clinical sepsis in PICU patients who have endotracheal tubes, central venous catheters, or urinary catheters. This endpoint will be tested using a logrank test with a two-sided α =0.05, to compare freedom from nosocomial infection or sepsis (from the time of admission to the PICU until up to five days following discharge from the PICU) between treatment arms.

For comparison of survival curves between treatment arms using the logrank test, the statistical power is directly dependent on the number of events observed. Thus, one approach to designing a study of this type is to enroll and follow subjects until a given number of subjects have had an event. It is desired to have 90% power to detect an inverse hazard rate as small as 1.5 in this study, using a two-sided test with Type I error of 0.05. Using a conservative, nonparametric approach to estimating needed number

of events[71] yields a needed number of 263 subjects with events in the study. As is discussed below, an O'Brien-Fleming type of monitoring scheme will be used by the DSMB. A small increase in sample size of approximately 2% is required to maintain 90% power assuming this monitoring scheme.[72] Thus, this study will plan to enroll patients until a total of 268 subjects with events have been observed.

Clearly, the investigators and the DSMB need to be aware of the approximate number of subjects required for enrollment to achieve the desired number of events. The number of subjects needed to enroll is affected by the median time to infection in the control arm and the number of days of risk of infection. Since the number of patients to be enrolled is dependent on these parameters, it is not possible to accurately project the number of patients needed (to achieve the necessary number of events) at the outset of the study. After 200 subjects have been accrued, the DCC (blinded to treatment arm identity) will calculate the estimated number of subjects remaining to be enrolled and present these data to the DSMB and NICHD prior to the first formal interim analysis. For example, if 100 events were to be observed among the first 200 enrolled, then assuming a continued rate of 1 event among every 2 subjects enrolled, one could estimate that an additional 168*2=336 subjects would remain to be enrolled to achieve the maximum target sample size of 268 events.

The power for the primary outcome is affected by the hypothesized beneficial effect of the study intervention; the *a priori* estimate of the effect size in this trial is an inverse hazard rate of 1.5. (Assuming exponential hazard functions, an inverse hazard rate of 1.5 means the median time to infection is 1.5 times longer in the active drug arm than in the placebo arm.)

For purposes of study planning, Table 2 shows estimated numbers of subjects required to be enrolled to achieve 90% power under various assumptions for each of these parameters. For example, if the median time to infection in the control arm is 6 days (Carcillo estimate) and the median ICU stay is 7 days, then under a true inverse hazard of 1.5, approximately 530 total subjects are required to beenrolled for 90% power (Type II error=0.1). The calculations in this table are intended solely as approximate guidelines (as they will be superseded by the actual event counts in the first 200 enrolled subjects), are not adjusted for interim monitoring, and assume:

- dropout occurs from PICU discharge, death, hospital discharge, or withdrawal from study
- up to 33 days followup for subjects who do not drop out

- all infection and dropout curves are exponential
- two-sided logrank test, α =0.05

Table 2: Total Subjects Required Across Two Arms Under Different Assumptions of Time to Infection, Length of PICU Stay, and True Hazard Rate

Median Time to Infection	Median LOS	Inverse Hazard Rate	80% Power ($\beta = 0.2$)	90% Power ($\beta = 0.1$)
6 days (Carcillo[73])	3 days (LeClerc[74])	1.5	n = 662	n = 886
		2.0	n = 254	n = 340
		2.5 (Maki[75])	n = 160	n = 214
6 days (Carcillo)	5 days	1.5	n = 476	n = 636
		2.0	n = 180	n = 240
		2.5 (Maki)	n = 112	n = 150
6 days (Carcillo)	7 days	1.5	n = 396	n = 530
		2.0	n = 148	n = 198
		2.5 (Maki)	n = 92	n = 124
8 days (Maki)	3 days (LeClerc)	1.5	n = 820	n = 1096
		2.0	n = 316	n = 424
		2.5 (Maki)	n = 200	n = 268
8 days (Maki)	5 days	1.5	n = 570	n = 762
		2.0	n = 218	n = 290
		2.5 (Maki)	n = 136	n = 182
8 days (Maki)	7 days	1.5	n = 464	n = 620
		2.0	n = 176	n = 234
		2.5 (Maki)	n = 110	n = 146
13 days (LeClerc)	3 days (LeClerc)	1.5	n = 1212	n = 1622
		2.0	n = 474	n = 634
		2.5 (Maki)	n = 302	n = 404
13 days (LeClerc)	5 days	1.5	n = 806	n = 1078
		2.0	n = 312	n = 416
		2.5 (Maki)	n = 196	n = 264
13 days (LeClerc)	7 days	1.5	n = 634	n = 850
		2.0	n = 244	n = 326
		2.5 (Maki)	n = 154	n = 204

It is desired to have 90% power for the trial, but it is possible this may require more subjects than can be accommodated by the study budget. In this instance, there would be the option to modify the study to have 80% power by reducing the maximum target number of events (to an estimated 202) prior to the first examination of the outcome data by treatment arm.

If the required enrollment parameters to achieve 80% power also exceed the number of subjects that can be accommodated by the study budget, then continuation of the study would be reassessed by the Steering Committee and the National Institute for Child Health and Human Development (NICHD).

8.6 Interim Analyses and Stopping Rules

This study will be monitored by the Data Safety Monitoring Board (DSMB) appointed by the National Institute for Child Health and Human Development (NICHD). The DSMB will have final jurisdiction regarding frequency of meetings and appropriate formal monitoring boundaries for study stopping in terms of superiority. While boundaries are calculable for futility (lack of treatment effect) simultaneously with superiority, a less formal conditional power approach is more often adopted by DSMBs to address futility issues if this becomes necessary.

The initial estimate of the study accrual period (see Section 9 on the next page) is two years; this discussion assumes that study results will be presented to the DSMB after 33% and 66% of the total number of events have occurred. Thus, there will be two interim analyses, with an additional final analysis of the study data if the study is not terminated early.

As there is the possibility of a "learning curve" early in the study, as well as of some centers beginning enrollment later than others, a conservative O'Brien–Fleming–type boundary will be used for assessing superiority. Under this scheme, assuming that the DSMB meets when one–third and two–thirds of the total final "statistical information" is available, Table 3 shows the actual monitoring boundaries (expressed in terms of p-values), and expected probability of stopping the study (for benefit) at each look, for the trial powered at 80% or at 90%. The probability of stopping is altered between these power levels because larger numbers of patients would be available at the time of each interim analysis if the study is powered at 90%. Probabilities in Table 3 are based on 100,000 simulations using East 3.1 software.

Table 3: Probability of Early Stopping at Interim Analyses

Analysis	2-sided p value	Probability of Stopping, Power = 80% ($\beta = 0.2$)	Probability of Stopping, Power = 90% ($\beta = 0.1$)
First interim	≤ 0.0002	1.9%	3.4%

continued on next page

Table 3: *continued*

Analysis	2-sided p value	Probability of Stopping, Power = 80% ($\beta = 0.2$)	Probability of Stopping, Power = 90% ($\beta = 0.1$)
Second interim	≤ 0.012	39.8%	52.8%
Final	≤ 0.046	58.3%	43.8%

If the treatment effect is at least as large as expected *a priori*, the study has a substantial chance of stopping early due to superiority, despite the conservative stopping boundaries that are prespecified. Also, it should be noted that some "α spending" occurs from interim analyses, and if the study is carried to completion, the final determination of overall significance of the primary endpoint at the α=0.05 level will require using a p level of 0.046 for the final analysis.

9 Accrual Projections and Duration of Study

In 2003, the network clinical centers report 10,550 patient admissions to the PICU. Table 4 shows the distribution of these children by diagnostic categories. In order to estimate potential eligible patients for this study, the following assumptions have been made in the construction of this table:

- trauma patients include gut disruption patients for whom study drug is contraindicated;
- trauma patients include large number of short stay admissions;
- surgery patients have short PICU length of stay;
- cardiac surgery patients will either have short PICU length of stay or are likely to have single ventricle physiology, lowering likelihood of physician acceptance for enrollment;

Table 4: Projected Numbers of Eligible Subject

Diagnostic Category	Per Cent	Patients	Estimated Eligible Rate	Eligible Patients
Trauma	7%	739	10%	74
Surgery	29%	3102	5%	155
Cardiac Surgery	19%	2026	5%	101

continued on next page

Table 4: *continued*

Diagnostic Category	Per Cent	Patients	Estimated Eligible Rate	Eligible Patients
Medical (non-BMT, Ca)	40%	4241	20%	848
Medical (BMT, Ca)	4%	454	20%	91

Assuming 25% successful recruitment from eligible patients, approximately 25 patients would be enrolled per month. If the target enrollment is 600 total patients, this will require 24 months.

10 Human Subjects

All protocols will require that parents or other legally empowered guardians sign an informed consent. When the patient is developmentally able to provide assent, and the condition of the patient allows, then the patient's assent will also be obtained. All protocols will require prior IRB approval before any subject is entered into the study. All study participants or their families will be informed about the objectives of the study and the potential risks. All laboratory specimens, evaluation forms and reports will be identified by a coded number only to maintain patient confidentiality. All records will be kept in a locked/password protected computer. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the FDA, the National Institute for Child Health and Human Development (NICHD), the CPCCRN DCC, or other governmental regulatory bodies.

11 Potential Adverse Reactions and Drug Interactions Involving Metoclopramide

Adverse Reactions. Extrapyramidal reactions occur most frequently in children and young adults and following IV administration of high doses (1.0-2.0 mg/kg), usually within 24-48 hours after starting therapy: hypertension, hypotension, SVT, bradycardia, A-V block, drowsiness, fatigue, restlessness, anxiety, agitation, depression, tardive dyskinesia, dystonia, seizures, hallucinations, neuroleptic malignant syndrome, gynecomastia, amenorrhea, galactorrhea, hyperprolactinemia, constipation, diarrhea, urinary frequency,

impotence, methemoglobinemia, neutropenia, leucopenia, agranulocytosis, porphyria, visual disturbances, hypersensitivity reactions.

Drug Interactions. Decreased cimetidine and digoxin GI absorption; increased cyclopsporine GI absorption; levodopa decreases the metoclopramide effects; increases hypertensive episodes with MAO inhibitors; increased neuromuscular blocking effects of succinylcholine; anticholinergics and narcotic analgesics antagonize GI motility effects of metoclopramide; metoclopramide may increase tacrolimus levels.

12 Adverse Events

12.1 Definition

An adverse event is any untoward medical occurrence experienced by a subject. An event constitutes a disease, a set of related symptoms or signs, or a single symptom or sign.

On each study day, the investigator will evaluate adverse events. Adverse events not previously documented in the study will be recorded in the adverse event record form. The nature of each experience, date and time (where appropriate) of onset, outcome, course (i.e. intermittent or constant), maximum intensity, action taken with respect to study medication and relationship to treatment, should be established.

12.2 Classification of Adverse Events

12.2.1 Assessment of Intensity

Maximum intensity should be assigned to one of the following categories:

Mild: An adverse event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.

Moderate: An adverse event that is sufficiently discomforting to interfere with normal everyday activities.

Severe An adverse event that is incapacitating and prevents normal everyday activities.

12.2.2 Relationship

The suspected relationship between study drug (*any of* metoclopramide, zinc, selenium, or glutamine) and any adverse event will be determined by the investigator using the following criteria:

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject.

Possibly Related: The event follows compatible temporal sequence from the time of drug administration, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of drug administration, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject.

12.2.3 Severity

The severity of clinical adverse events and laboratory abnormalities will be assessed according to the following criteria:

Not Serious: Any event which:

- Results in minimal transient impairment of a body function or damage to a body structure; or
- Does not require any intervention other than monitoring.

Moderately Serious: Any event which:

- Results in moderate transient impairment of a body function or transient damage to a body structure; or
- Requires intervention, such as the administration of medication or a procedure, to prevent permanent impairment of a body function or damage to a body structure

Serious: Any event which:

- Is fatal; or

- Is life-threatening (the patient was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred); or
- Is severely or permanently disabling; or
- Necessitates significant intervention, such as major surgery, to prevent permanent impairment of a body function or permanent damage to a body structure; or
- Requires or prolongs hospitalization (An elective hospitalization for a planned procedure will not be considered an adverse event and reporting is not required); or
- the Principal Investigator considers to be a serious adverse event.

12.2.4 Expectedness of the Event

All adverse events will be evaluated as to whether their occurrence was expected (as described in the protocol or consent forms), or whether it was not expected to occur.

Expected: An event is considered expected if it is known to be associated with the underlying condition and is related to study outcome, and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study subject's clinical state immediately prior to the event. For this protocol, expected events include:

- mortality (unless believed to be drug-related)
- electrolyte abnormalities
- sepsis, infections, or bacteremia
- fever
- renal dysfunction
- liver dysfunction
- wound infection
- reintubation
- hypoxia
- respiratory distress
- pleural effusions
- malrotation

- failure to thrive
- General pediatric problems such as otitis media, reactive airways disease, gastroesophageal reflux, urinary tract infection, upper respiratory infection, croup, bronchiolitis including respiratory syncytial virus infection, pneumonia, gastroenteritis

Unexpected: An event is considered *unexpected* if there are no prior data linking this event with either the condition or intervention under study or an event that occurred unexpectedly in the course of treatment for severe critical illness in the PICU.

12.2.5 Treatment or Action Taken

AEs and SAEs will result in:

- Intervention: Surgery or procedure
- Other Treatment: Medication initiation, change, or discontinuation
- None: No action taken

12.2.6 Outcome

The clinical outcome of the AE or SAE will be characterized as follows:

- Death
- Recovered: the patient returned to baseline status
- Recovered: with sequelae
- Symptoms continue

12.3 Data Collection Procedures for Adverse Events

After patient enrollment, all adverse events, whether expected or unexpected, will be recorded according to the date and time of first occurrence, severity, and their duration, as well as any treatment prescribed. Any medical condition present at the time of enrollment, recorded in the Patient History data element at study entry, which remains unchanged or improves, will not be recorded as an adverse event on the Adverse Events Log. However, worsening of a medical condition that was present at enrollment will be considered a new adverse event and reported. Abnormal laboratory values

that are clinically significant will be recorded on the Adverse Event Form and assessed in terms of severity and relationship to study drug if felt by the investigator to be clinically significant. Laboratory values that are abnormal at study entry and that do not worsen will not be recorded on the Adverse Event Form.

All study subjects, including subjects who withdraw from the study, will be monitored for adverse events for 28 days (if remains an inpatient) or with a followup telephone call to the family if discharged prior to day 28.

12.4 Reporting Procedures

Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. The Study Coordinator must view patient records for possible adverse events throughout the study period. All adverse events will be evaluated by the Principal Investigator, and will be classified as noted in Section 12.2.2 on page 49 . All adverse events occurring within the study period must be reported in the participants electronic case report forms in the electronic data entry system provided by the DCC

Reports of all unexpected serious adverse events will be submitted to the local Institutional Review Board (IRB) and the DCC within one working day of the event. The investigator will then submit a detailed written report to the DCC and the local Institutional Review Board no later than 5 days after the investigator learns of the event. The DCC will report the unexpected serious adverse event to the National Institute for Child Health and Human Development (NICHD) as soon as possible and no later than seven calendar days after learning of the event.

The DCC will report all *serious, unexpected and study-related* adverse events to the Data Safety Monitoring Board (DSMB), by fax or by telephone, within 7 calendar days. A written report will be sent to the DSMB within 15 calendar days and these reports will be sent to clinical site investigators for their submission to their respective Institutional Review Boards. The DSMB will also review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings (interim analyses). The DCC will provide the written summary of the DSMB's periodic review of adverse events to clinical site investigators for submission to their respective Institutional Review Boards in accordance with National Institute for Child Health and Human Development (NICHD) Guidelines.

The DCC will code adverse events using MedDRA terminology, and will report all *serious, unexpected, and study-related* adverse events to the Food

and Drug Administration (FDA). Expedited safety reports that have been sent to the DSMB will also be faxed or telephoned to the FDA within 7 days of the DCC receipt of the information. The DCC will provide a complete written report of these unexpected fatal or life-threatening experiences to the FDA within 15 calendar days in accordance with 21 CFR 312.32.

12.5 Post-Study Adverse Events

All unresolved adverse events at the time of the patient's termination from the study will be followed by the investigators until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained or has stabilized. At the last scheduled contact, the investigator will instruct each parent to report any subsequent event(s) which the parent, or the subject's personal physician, believes might reasonably be related to administration of study drug. Any death or other clinically serious adverse event that may be related to study drug and that occurs at any time after a subject has discontinued or terminated study participation will be reported as in 12.4 on the facing page.

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