GM-CSF for Reversal of Immunoparalysis in Pediatric Sepsis-induced MODS (GRACE-2)

Collaborative Pediatric Critical Care Research Network

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1 Rationale and Background

1.1 Immunologic Phenotypes in Sepsis-induced MODS

Current pediatric sepsis management is largely focused on antibiotics and supportive care including fluid resuscitation and the use of vasoactive drugs. Except for titration of vasoactive support based on hemodynamics, there are no recommended treatment approaches that are personalized to the individual septic child's pathophysiology in the 2020 pediatric Surviving Sepsis guidelines, our field's leading resource for evidence-based pediatric sepsis care.[26] Our network has systematically collected data from hundreds of septic children across multiple pediatric centers clearly showing that children with sepsis-induced MODS have distinct pathophysiologic immune phenotypes that we hypothesize could benefit from personalized care.

The host's immune response to a pathogen is the key driver of organ dysfunction in sepsis. In the 1980s and 1990s, numerous studies in septic adults targeted the blockade or removal of specific pro-inflammatory mediators including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and others, but phase III clinical trials were not successful in reducing sepsis mortality.[1, 2, 7–9, 23] Anti-cytokine therapies and other forms of immunomodulation in sepsis were largely abandoned thereafter until we began to have a clearer understanding of the heterogeneity of the immune response. Immunophenotyping studies, many conducted by CPCCRN member investigators,[5, 6, 11, 12, 18] have shown that children with sepsis-induced MODS often have markedly hypoactive circulating leukocytes and could potentially benefit from an immunostimulatory approach rather than an anti-inflammatory one, whereas others have evidence of severe hyperinflammation that may benefit from anti-cytokine therapies. The prior generation of clinical trials failed to account for these inter-individual variations in immune response, leading many subjects to be treated with what may have been the wrong immunomodulator.

We have developed the laboratory infrastructure to rapidly diagnose these phenotypes, allowing, for the first time, the creation of interventional protocols that are personalized to an individual septic child's immune state.

1.2 Sepsis, MODS and Innate Immune Suppression

Severe sepsis/septic shock remain a major source of pediatric morbidity and mortality worldwide, with the highest rates of adverse outcomes seen in children who develop failure of two or more organs. Children with sepsis-induced multiple organ dysfunction syndrome (MODS) represent a heterogeneous group of patients who have been shown to have several distinct phenotypes of underlying pathophysiology.[5] The most common phenotype, immunoparalysis,

is the result of an exaggerated compensatory anti-inflammatory response. Impairment of the innate immune system is common and measurable in pediatric sepsis. Innate immune cells such as monocytes and neutrophils serve critical functions including migration to sites of infection, phagocytosis of pathogens, promotion of microbial killing, antigen presentation, and production of immunomodulatory cytokines. We have repeatedly shown that severe reduction in the ability of a child's circulating leukocytes to produce pro-inflammatory cytokines occurs commonly in pediatric critical illness, and is strongly associated with increased risks of prolonged organ dysfunction, nosocomial infection, and death.[5, 11, 12, 17, 18, 22, 25]

1.3 Whole Blood *ex vivo* LPS-induced TNFα Production Capacity

Stimulation of whole blood with lipopolysaccharide (LPS) allows for quantification of the innate immune system's responsiveness to a new challenge. LPS stimulation should result in rapid and robust production of the proinflammatory cytokine tumor necrosis factor (TNFα). Severe reductions in the TNFα response have been associated with adverse outcomes including increased risks for prolonged organ dysfunction, nosocomial infection, and death in children with life-threatening infection.[5, 11, 12, 16, 18, 22] We have consistently shown that a TNFα response < 200 pg/ml in our assay identifies the population of patients at highest risk for these adverse outcomes.

The Immune Surveillance Laboratory at The Abigail Wexner Research Institute at Nationwide Children's Hospital, under the direction of Dr. Hall, has been conducting single- and multi-center immune monitoring and modulation studies using the TNF-α response assay since 2007. We use a consistent LPS type with rigorous quality control procedures, small blood volumes appropriate for pediatric studies, a four-hour incubation period (suitable for same-day processing), and TNF-α quantitation on a highly automated, Good Laboratory Practices instrument. The *Immulite 1000* (Siemens, Deerfield, IL) is an automated chemiluminometer that is used to measure hormones and other analytes in the clinical laboratory. We perform quantitation of TNF-α using this instrument under a Research Use Agreement, though these test kits are used clinically in Europe for patient management. The intra-assay coefficient of variation for the TNF-α assay on the *Immulite* is less than 10%. By using the *Immulite 1000* we avoid the need to perform highly operator-dependent assays such as enzyme-linked immunosorbent assays (ELISAs).

1.4 Reversibility of Immunoparalysis Using GM-CSF Therapy

GM-CSF is an endogenous, immunostimulating cytokine produced primarily by TH1 lymphocytes. It is available in recombinant human form (sargramostim, Leukine; Partner Therapeutics, Lexington, MA) and has been FDA-approved for bone marrow reconstitution following bone

marrow transplantation (BMT) since 1991. It has a long track record of safe use in acutely and critically ill patients, including children, with a very low incidence of adverse events, the bulk of which are rate-of-infusion related when given by the IV route.[19–21] Rapid IV administration of GM-CSF (over less than 2 hours) has been associated with respiratory distress, peripheral edema (11% incidence with GM-CSF vs 7% incidence with placebo) and pericardial effusion (4% vs 1%), but these side effects have not been observed with IV infusion durations of > 2 hours or via the subcutaneous (SQ) route.[13]

Author, year	Patient population	GM-CSF regimen	N	Result
Presneill, 2002	Septic adults, placebo-controlled RCT	3 µg/kg/day (~90 µg/m²/day) IV x 5 days	18	Increased neutrophil function and oxygenation
Nierhaus, 2003	Septic adults, uncontrolled case series	5 µg/kg/day (~200 µg/m²/day) SQ x 3 days	9	Increased monocyte HLA-DR expression and TNFα response; 66% survival
Rosenbloom, 2005	Septic adults, placebo-controlled RCT	125 µg/m² IV infused over 3 days	40	Increased monocyte HLA-DR expression, faster resolution of infection
Meisel, 2009	Septic adults, placebo-controlled RCT	4 µg/kg/day (~125 µg/m²/day) SQ x 8 days	38	Increased monocyte HLA-DR expression and TNFα response; improved illness severity
Hall, 2011	Pediatric MODS, open-label RCT	125 µg/m²/day IV x 7 days	14	Increased TNFα response, decreased risk of nosocomial infection

RCT: randomized, controlled trial, IV: intravenous, SQ: subcutaneous, HLA: human leukocyte antigen, TNF: tumor necrosis factor

Table 1: Published evidence for use of GM-CSF for immunomodulation in critically ill adults and children.

Monocyte hyporesponsiveness has been shown to be reversible in vitro through co-culture with GM-CSF.[3, 4, 10, 14, 24] GM-CSF has been used in several small published studies of immunomodulation in critically ill adults and children, summarized in Table 1. The doses used were substantially lower than the FDA-approved dose that is used for bone marrow reconstitution (250 g/m^2/day). In all studies, immune recovery was prompt (within 3 days of initiation of GM-CSF therapy) though they were largely underpowered to detect effects on clinical outcomes. There were no serious adverse events ascribed to GM-CSF in any of the studies. GM-CSF therapy did not result in increased systemic inflammation as measured by plasma levels of the pro-inflammatory cytokines IL-6 or IL-8.

The CPCCRN network has conducted two pilot, open-label, dose-finding trials of IV GM-CSF over the last five years, also covered under IND#112277. The recently completed "GM-CSF for Immunomodulation Following Trauma (GIFT)" study is a dose-escalation study for reversal of critical trauma-induced immune suppression in children. We found that GM-CSF doses < 125 mcg/m^2/day were ineffective at normalizing innate immune function in the first week after injury while a dose of 125 mcg/m^2/day was associated with improvement in innate immune function. Further, we found that relapse of immune suppression was common when a short duration of treatment was used (3 days), and treatment through the end of the first post-injury week was required for lasting effect. Lastly, we found that IV GM-CSF, when administered at a dose of 125 mcg/m^2/day with each dose infusing over at least 6 hours, was not associated with drug-attributable adverse events.

Our group has also studied the effect of IV GM-CSF on immune function in critically ill children with sepsis-induced MODS. In 2011 we reported the results of a single-center, open-label, RCT of IV GM-CSF at a dose of 125 mcg/m^2/day for 7 days vs standard care in a 14-subject cohort of immunoparalyzed children with MODS, most of whom had sepsis as the MODS-inciting event. GM-CSF treatment was associated with significantly faster resolution of immunoparalysis (Figure 1 on the next page, A). No subjects in the GM-CSF-treated group went on to develop nosocomial infection, while all of the subjects in the standard therapy group did.[12] The CPCCRN network recently replicated this approach on its multi-center platform by conducting the "GM-CSF for Reversal of immunopAralysis in pediatriC sEpsis-induced MODS (GRACE) – 1" study. This is a multi-center, open-label, dose- and route-of-administration study that is being carried out in 7 centers across the United States. Thus far, the GRACE–1 study has shown that a GM-CSF dose of 125 mcg/m^2/day given by the IV route for 7 days, with each dose being infused over a minimum of 6 hours, is associated with the safe reversal of immunoparalysis in children with sepsis-induced MODS (Figure 1, B). Subcutaneous administration of GM-CSF is still being evaluated in the GRACE–1 study. The restoration of a normal TNFα response was not associated with the development of systemic inflammation. Rather, plasma biomarkers of inflammation including interleukin (IL)-6, IL-8, and ferritin decreased over time as TNFα response normalized (Figure 1, C).

1.5 Immunoparalysis and MAS/HLH

Immunoparalysis is not the only pathophysiologic phenotype that is seen in children with sepsis-induced MODS.[5] Another phenotype in pediatric sepsis-induced MODS is one biochemically similar to macrophage activation syndrome (MAS) or secondary hemophagocytic lymphohistiocytosis (HLH). This is characterized by very high serum ferritin levels (typically ≥ 2,000 ng/ml), and children with this phenotype may benefit from targeted anti-inflammatory treatment

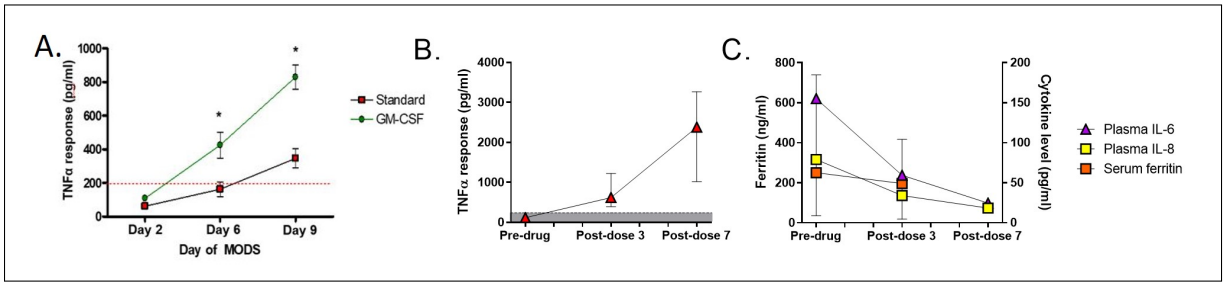

Figure 1: GM-CSF therapy is associated with restoration of innate immune function and reduction of systemic inflammation in immunoparalyzed children with MODS. In a 14-subject RCT, GM-CSF was associated with rapid normalization of the TNFα response (A). Dashed line represents immunoparalysis threshold. In the GRACE-1 study, the same regimen of GM-CSF was associated with prompt resolution of immunoparalysis (N=10) (B) with reduction in systemic inflammation (C). Shaded area represents immunoparalysis threshold. Error bars represent interquartile range.

rather than immunostimulation. We will therefore screen for and exclude children with serum ferritin levels $\geq$ 2,000 ng/ml from receiving GM-CSF. This will also ensure that children with primary HLH, who typically have serum ferritin levels > 10,000 ng/ml and to whom treatment with GM-CSF could potentially be harmful, are prevented from receiving GM-CSF.

2 Study Summary

2.1 GRACE-2 Trial

The "GM-CSF for the Reversal of Immunoparalysis in Pediatric Sepsis-induced MODS (GRACE)-2" study is a prospective, double-blind, randomized controlled trial of the drug GM-CSF vs placebo in children with a TNFα response < 200 pg/ml and a serum ferritin level < 2,000 ng/ml in the setting of sepsis-induced MODS. This allows us to use GM-CSF only in subjects who have immunoparalysis and will minimize the risk of inadvertently giving GM-CSF to a child who has HLH/MAS. The GRACE-2 study will use adaptive sample size determination to use the smallest sample size possible.

Subjects with immunoparalysis and a serum ferritin level < 2,000 ng/ml (an expected 40% of the total cohort) will be assigned to the GRACE-2 trial in which subjects will be randomized to receive GM-CSF at a dose of 125 mcg/m^2/day intravenously x 7 days or placebo. A maximum ferritin level of 2,000 ng/ml to limit inclusion in the GRACE-2 trial was chosen in a conservative

effort to avoid giving GM-CSF to subjects who may have MAS/HLH. An adaptive interim look approach will be used to ensure the smallest possible sample size.[27]

2.2 Longitudinal Immune Phenotyping

All subjects receiving study drug will undergo serial immune phenotyping including quantitation of inflammatory biomarkers and immune function twice weekly for two weeks. This follow-up sampling allows us to 1) quantitate the effects of the study drugs on inflammation and immune function; 2) detect the presence of immunologic relapse in the week after study drug discontinuation; and 3) understand the trajectory of immune function and inflammation, including identification of further immune subphenotypes, over time in all subjects.

2.3 Study Outcomes

Primary Outcome

The primary outcome of the study will be a composite of duration/severity of organ dysfunction and mortality as measured by the cumulative Pediatric Logistic Organ Dysfunction (PELOD)-2 score[15] over 28 days after randomization, with non-survivors being assigned the highest possible PELOD-2 score. On any given day, the minimum score is 0 and maximum score is 33, with lower values indicating less organ dysfunction. This corresponds to a maximum possible 28-day sum of 924. Mortality is incorporated into this analysis, with children who die within 28 days of randomization assigned the worst possible score in a rank-based analysis. Rank based analyses will be performed to account for mortality as well as the fact that for surviving children, a difference in the cumulative PELOD-2 of a specified magnitude may reflect different clinical consequences.

Secondary Outcomes

The two formal secondary efficacy outcomes are:

- Change from baseline status to 3 months after randomization in the Pediatric Quality of Life Inventory (PedsQL) Generic Core or Infant Scales;
- Change from baseline status to 3 months after randomization in the Functional Status Scale (FSS).

In both analyses, children deceased at the 3-month timepoint will be treated as having the worst possible change in outcome at 3 months.

Exploratory Outcomes

Exploratory outcomes include:

- Hospital and 28-day mortality;
- MODS-free days in 28 days following randomization;
- Days free of any organ dysfunction in 28 days following randomization;
- Incidence of nosocomial infection (by CDC criteria) from the time of enrollment through 28 days following randomization;
- Change from baseline to 12-month PedsQL Generic Core or Infant Scales;
- Change from baseline to 12-month FSS score;
- Change from baseline to 3-month and 12-month PedsQL Family Impact Module 2.0 score;
- Change from baseline to 3-month and 12-month Pediatric Evaluation of Disability Inventory – Computer Adaptive Test score;
- Hospital readmissions occurring by 3 and 12 months after randomization.

Safety Outcomes

In the GRACE-2 trial, hyperinflammation will be monitored by measuring systemic (plasma or serum) levels of IL-6, ferritin, and CRP twice weekly during throughout the immune monitoring period (through study day 17 ± 1 or hospital discharge, whichever is earlier).

3 Subject Eligibility, Accrual and Study Duration

3.1 Eligibility criteria.

Eligible participants will be identified by on-site study staff. Inclusion criteria are:

- ≥ 40 weeks corrected gestational age** to < 18 years; AND
- Admission to the PICU or CICU; AND
- Onset of ≥ 2 new organ dysfunctions within the last 3 calendar days (compared to pre-sepsis baseline) as measured by the modified Proulx criteria; AND
- Documented or suspected infection as the MODS inciting event.

** Corrected gestational age will be used in infants ≤ 4 months of age with a history of prematurity. It will be calculated as the current chronological age in weeks + estimated gestational age

at birth (as reported by the parent/guardian and/or medical record review).

Exclusion criteria are:

- Weight <3kg; OR
- Limitation of care order at the time of screening; OR
- Patients at high likelihood of progression to brain death in opinion of the clinical team; OR
- Moribund condition in which the patient is unlikely to survive the next 48 hours in opinion of the clinical team; OR
- History of myeloid leukemia, myelodysplasia, or autoimmune thrombocytopenia; OR
- Current or prior diagnosis of hemophagocytic lymphohistiocytosis or macrophage activation syndrome; OR
- Peripheral white blood cell count < 1,000 cells/mm^3 as the result of myeloablative therapyOR receipt of myeloablative therapy within the previous 14 days; OR
- Known allergy to GM-CSF; OR
- Known pregnancy; OR
- Lactating females; OR
- Receipt of anakinra or GM-CSF within the previous 28 days; OR
- Resolution of MODS by MODS Day 2; OR
- Previous enrollment in the GRACE-2 study.

If all organ failure is resolved on Study Day 1 (the day following immune phenotyping), or if the subject has developed an exclusion criterion since immune phenotyping, the subject's participation in the study will end.

3.2 Subject Accrual and Study Duration

Up to 1095 subjects will be phenotyped and have at least single organ failure on Study Day 1 over a four year period. We estimate that 40% will have immunoparalysis, 50% will have moderate to severe hyperinflammation, 5% will have neither, and 5% will have very severe hyperinflammation (likely MAS or HLH). We anticipate enrolling 400 subjects into the GRACE-2 trial.

4 Study Procedures

4.1 Screening and Enrollment

All patients admitted to the pediatric or cardiac ICU at CPCCRN sites will be evaluated for eligibility for our overall immune phenotyping and immunomodulation study that will feed subjects into the GRACE-2 trial depending on their immune phenotyping results, which will not be known at the time of enrollment. Patients who meet inclusion criteria will be entered into the data capture system and exclusion criteria (if present) will be recorded in that system. If the patient is eligible (no exclusion criteria are present) then the legal guardian(s) will be approached and offered the opportunity for their child to participate in the overall immune phenotyping and immunomodulation study, as well as the interventional trial based on the phenotyping results. Randomization into the interventional trial will only occur after immune phenotyping data are known.

4.2 Immune Phenotyping

We have developed a highly feasible, highly standardized, next-day approach to multi-center immunophenotyping in critically ill children. This includes the measurement of systemic levels of serum ferritin levels on a clinical-grade instrument (the Immulite 1000 automated chemiluminometer [Siemens Healthcare Diagnostics]) using commercially available, FDA-approved test kits. We also use this instrument to quantitate TNFα levels in the stimulated supernatants using commercially available kits that are used clinically in Europe but are available to us under a Research Use Only agreement.

We will evaluate subjects for blood sampling on the second day after the onset of sepsis-induced MODS (MODS Day 2). If MODS has resolved on what would have been MODS Day 2, we will not perform immune testing and subject's participation in the study will be complete. If MODS persists on MODS Day 2, the subject will undergo immune function screening. The subject will undergo an approximately 2 ml blood draw for measurement of whole blood LPS-induced TNFα production capacity (TNFα response) and inflammatory biomarkers including serum ferritin levels. Blood samples will be stimulated on-site within one hour of sample collection using highly standardized LPS stimulation kits provided by the Immune Surveillance Laboratory (ISL) at the Research Institute at Nationwide Children's Hospital. Briefly, aliquots of 50 μL of heparinized whole blood will be stimulated in duplicate, using tubes containing 500 pg/ml of LPS (phenol-extracted from *Salmonella abortus equi*, Alexis Biochemicals). Stimulation tubes will be incubated at 37° C for four hours after which the samples will be immediately centrifuged. LPS-stimulated supernatants, along with

unstimulated serum, will be overnight-shipped on dry ice to the ISL where TNFα production capacity and serum ferritin levels will be measured as early as possible the next day (Study Day 1).

If a subject's TNFα production capacity is < 200 pg/ml and the serum ferritin is < 2,000 ng/ml, the subject will be considered to have immunoparalysis with a very low likelihood of MAS/HLH, and will therefore be eligible to be randomized to receive GM-CSF or placebo. Subjects who have improved to single-organ dysfunction on the day of randomization will continue to receive study drug per-protocol. While the Nationwide Children's site is able to assess subject eligibility on the same day as LPS stimulation (MODS Day 2), GM-CSF administration will be delayed until the next morning (Study Day 1) in order to remain consistent with the dosing schedule at other CPCCRN sites.

In addition to phenotyping on MODS Day 2, subjects will undergo follow-up immune function testing including measurement of the TNFα response and systemic biomarkers on the day after the third dose of study drug, the day after the seventh dose of study drug, and on study days 12±1, and 17±1. These follow-up samples will be processed locally, frozen at -70° C, and batch-shipped to the ISL at the conclusion of the subject's study participation. These samples will not be used to drive additional study drug delivery but will instead be used to understand the relationships between immunologic response to study drug and clinical outcomes.

4.3 GRACE-2 Study Drug Administration

4.3.1 Eligibility to Receive Study Drug Doses

In order to minimize the risk of leukostasis, study drug will not be administered if the subject's total while blood cell (WBC) count is > 50,000 cells/mm^3. If not already ordered by the clinical team, subjects will undergo measurement of a complete blood count (CBC) prior to receiving their first dose of study drug and prior to receiving their fourth dose of study drug. If, on either other of these assessments (or on any clinically-obtained CBC during the study drug treatment period), the subject's total peripheral WBC count is > 50,000 cells/mm^3, the study drug will be held and the CBC will be assessed the following day. Study drug will be resumed if/when the total WBC count drops to ≤ 50,000 cells/mm^3 with the total treatment duration not to exceed 7 days from the day of randomization. A peripheral WBC count of > 50,000 cells/mm^3 was not seen in any subjects in the GIFT or GRACE-1 studies to date.

4.3.2 Intravenous Infusion

The study drug will be infused over a minimum of 6 hours on the day after the qualifying immune testing sample (Study Day 1). The infusion may be interrupted if needed, but must run over a total of no more than 12 hours. Study drug may be given via a peripheral or central IV catheter. The first administered dose of study drug will be initiated as soon as possible after randomization. All subsequent IV doses will be initiated between 4 AM and noon. If the window is missed, study drug should still be started as soon as possible.

If logistical problems with sample shipping result in up to 24 hours of delay in receiving the immune phenotyping sample at the Immune Surveillance Laboratory, the sample is able to be analyzed, and at least single organ dysfunction persists, randomization will be performed on Study Day 2, administration of GM-CSF may be started on Study Day 2, and continued for a total treatment duration not to exceed 7 days after randomization.

4.3.3 Pharmacy Function and Monitoring

The investigational pharmacy at Nationwide Children's Hospital will serve as the central pharmacy for the GRACE-2 study. They will intake GM-CSF from the manufacturer (Partner Therapeutics), document lot number and expiration information, facilitate blinding, and ship study drug to clinical sites' investigational pharmacies. They will also develop source documentation, pharmacy training materials, operating manuals, and study-specific pharmacy procedures as needed. Clinical sites' investigational pharmacies will be responsible for preparing placebo doses, all of which will consist of normal saline infusions of equivalent volume to GM-CSF. The clinical site pharmacy must maintain adequate records of all dispensed study drug. Each pharmacy will be monitored and may be requested to send copies of these documents to the Data Coordinating Center and/or the investigational pharmacy at Nationwide Children's Hospital which will conduct remote audits at regular intervals.

4.4 Randomization

Upon determination of a subject's immunophenotype, Dr. Hall or his designee will notify the Data Coordinating Center and the clinical site investigator of the laboratory results. Subjects who are eligible to undergo randomization in the study will be randomized by the DCC to active or placebo arms. The randomization system or DCC personnel will notify the investigational pharmacy at the clinical site and the site's investigational pharmacy will provide the proper blinded study drug to the subject's bedside for infusion. The DCC will also notify the study's central pharmacy at Nationwide Children's Hospital of the randomization results so that the central pharmacy can track and ensure drug supply.

4.5 Blinding Study Arms

The GRACE-2 trial is a double-blind trial. Subjects will receive GM-CSF or saline placebo intravenously over at least six hours daily for seven days.

4.6 Discontinuation of Study Drug

Study drug may be discontinued if an adverse event occurs that requires discontinuation in the opinion of the clinical or investigative teams, if a contraindication to active drug develops, or if parents request discontinuation of study drug. This does not constitute withdrawal from the study, and all data and sample collections should continue.

4.7 Withdrawal from Study

Parents may withdraw consent for their child to be in the study at any time, but such an occurrence should be very rare. In most instances, if a family or subject indicates that they want to stop being in a study, they usually mean they wish to stop the intervention and/or blood sampling. Efforts should be made to be able to continue all data collection and sampling. In the event that the parents insist on cessation of data collection and sampling, all study procedures and data collection will be discontinued, though we will continue to review study-related data collected prior to the subject's withdrawal from the study.

5 Data Collection

The schedule of activities and data collection is shown in Table 2 on page 21. Data elements to be collected are briefly described below. The described data are not intended to be inclusive, and additional data that are similar in nature may be added to the final study database without revision of this protocol. If there are entirely new areas of data collection added during the study, a protocol amendment will be filed with the sIRB.

5.1 Demographic Data

Demographic data may include birthdate, gender, race, ethnicity, and socioeconomic status.

5.2 Eligibility Data

For all screened patients who meet inclusion criteria, the inclusion and exclusion criteria will be entered, as well as the final eligibility status. Exclusion criteria are recorded to create the

eventual CONSORT diagram as well as to inform decisions about potential protocol revisions if the exclusion criteria are determined to be too restrictive. Changes in exclusion criteria will require sIRB, DSMB and FDA approval before implementation.

5.3 Consent Procedure Data

For all eligible patients, we will record whether the parents were approached for study consent, whether they consented, and date of consent, if applicable. In addition, reasons for not approaching parents of eligible subjects will be recorded, and if parents are willing to express a reason for not consenting, this will also be recorded.

5.4 Baseline Review of Systems

Medical historical data and review of systems will be recorded to provide a baseline against which to evaluate adverse events.

5.5 Baseline Admission Data

Dates of admission to hospital and PICU, reasons for hospital admission, co-morbidities, allergies, height, weight, and physical findings will be recorded.

5.6 PICU/WARD Daily Data

Data concerning the physiologic status, therapies, and significant events will be recorded for each day until 28 days after randomization, hospital discharge, or death, whichever occurs first.

5.7 PELOD-2 Data

Data required for calculation of the daily PELOD-2 score will be collected.

5.8 Immunophenotyping

Data collection will include the time of sampling, relevant times involved in sample processing, temperature maintenance, and shipping details.

5.9 Randomization Procedures

Randomization data will include information provided to the randomization system, confirmation of the phenotype and eligibility for interventional study, information required for stratification

of randomization, and the actual randomization number received from the system and assigned to the subject.

5.10 Drug Administration Data

Drug administration data include time of administration, dose of study drug, and whether there were any adverse reactions noted during the administration.

5.11 Quality of Life Instruments

Instruments to assess aspects of quality of life include the PedsQL generic scales or infant scales, the Functional Status Scale, the PedsQL Family Impact Module 2.0 (FIM) and the Pediatric Evaluation of Disability Inventory - Computer Adaptive Test (PEDI-CAT). These will be collected as early as possible for baseline assessment, and at 3 (allowable interval 3 to 5 months) and 12 months (allowable interval 11 to 13 months) after MODS Day 2.

5.12 Data Logs

Data forms will be created to collect concomitant medications, laboratory data that are not collected on daily forms, and adverse events.

5.13 Discontinuation or Withdrawal from Study

If a subject is discontinued from study drug or withdrawn from study, data concerning the event will be recorded.

Definitions of Study Days																					
Study Day			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 to 28	3 Months	12 Months
MODS Day	0	1	2	Not defined - MODS may resolve by day 3.																	
	Enrollment window																				
Study Drug Day	Not applicable			1	2	3	4	5	6	7	Not applicable										
Demographic Data			X																		
Eligibility Data			X																		
Consent Procedures			X																		
Baseline Review of Systems			X																		
Baseline Admission Data			X																		
PICU/WARD Daily Data			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
PELOD-2 Data			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Immunophenotyping			X				X				X				12 +/- 1d				17 +/- 1d		
Randomization Procedures				X																	
Drug Administration Data				X	X	X	X	X	X	X											
PedsQL	X (Baseline Status)																			X	X
FSS	X (Baseline Status)																			X	X
PedsQL FIM 2.0	X (Baseline Status)																			X	X
PEDI-CAT	X (Baseline Status)																			X	X
Readmissions																				X	X
Concommitant Medications Log			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Laboratory Data Log			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse Events Log*				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Discontinuation of Study Drug*																					
Withdrawal from Study*																					

Table 2: Schedule of study events and data collection.

6 Statistical Summary

6.1 GRACE-2 Randomization and Power Estimation

GRACE-2 trial's "Goldilocks" framework[27] uses the current observed distribution of the primary outcome (sum of PELOD-2 over 28 days, with deaths assigned the worst possible score) for interim decision-making. Prespecified, trial-customized stopping cutpoints are used for declaring significant treatment efficacy, or declaring futility of trial continuation, if compelling evidence from the trial data to date indicate either action. Frequent interim efficacy data review yields potentially earlier trial stopping (for efficacy or futility) than traditional frequentist monitoring schemes.

The assumed placebo distribution for the primary outcome is based on the historic distribution of PELOD-2 over 28 days from our published LAPSE study.[28] GM-CSF is assumed to improve mean sum of PELOD-2 over 28 days among survivors by approximately 20 points. Figure 2 on the next page shows arm-specific histograms and quantiles. Mortality is assumed to decrease from 12% placebo to 9% in the active arm. Under this scenario, simulation-estimated power with 400 randomized patients is 91% to detect a difference between GM-CSF and placebo, using a one-sided Wilcoxon Rank Sum test to compare arms and controlling Type I error at 2.5%. The Statistical Design and Power document has more details on the adaptations and assumptions, as well as our design's complete operating characteristics under this scenario and others considered.

In the GRACE-2 trial, if we do not stop the trial early for efficacy or futility, the final one-sided p-value threshold for a conclusion of success is 0.020.

6.2 Data Analyses

6.2.1 Primary Outcome

The primary outcome is duration and severity of organ dysfunction as quantified by the cumulative Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score (the sum of each day's score) at 28 days from time of randomization. On any given day, the minimum score is 0 and maximum score is 33, with lower values indicating less organ dysfunction. This corresponds to a maximum possible 28-day sum of 924. Mortality is incorporated into this analysis, with children who die within 28 days of randomization assigned the worst possible score in a rank-based analysis. Rank based analyses will be performed to account for mortality as well as the fact that for surviving children, a difference in the cumulative PELOD-2 of a specified magnitude may reflect

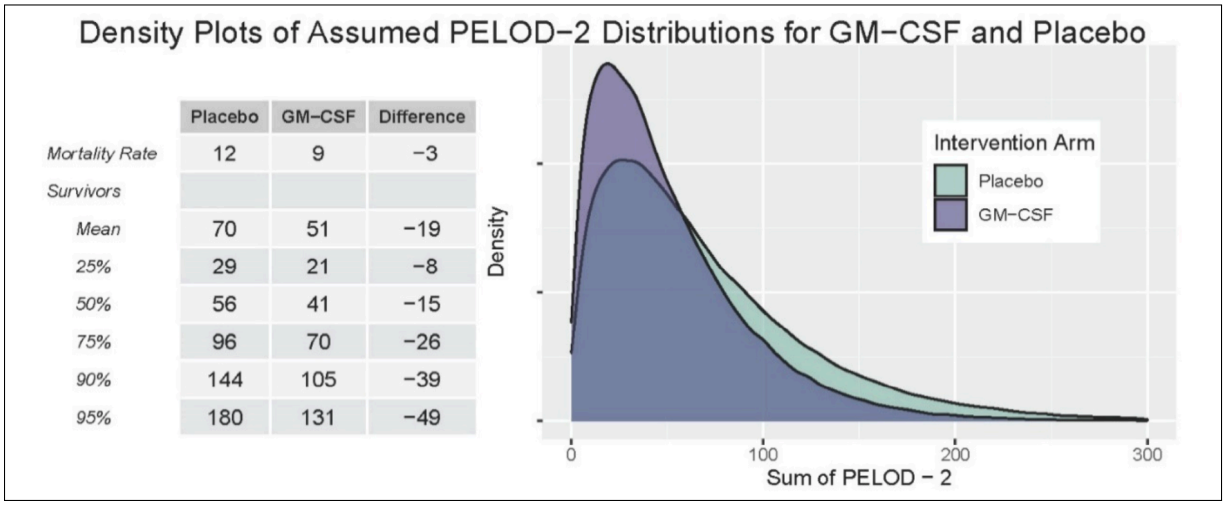

Figure 2: Assumed distributions of GM-CSF and placebo for mortality and sum of PELOD-2 among survivors.

different clinical consequences.

6.2.2 Secondary Outcomes

The analytical plan is designed under the approach that 2.5% Type I Error (one-sided) is to be allocated for the primary efficacy outcome comparison, while an additional (separate) 2.5% Type I Error (one-sided) is allocated for efficacy assessment for a small number of fully prespecified secondary efficacy outcomes.

The two formally designated secondary efficacy outcomes, change in PedsQL from baseline to 3 months and change in Functional Status Score (FSS) from baseline to 3 months, will be evaluated for efficacy using all available data in the enrolled population of children. Significance of each these two outcomes will be evaluated using the same approach as the primary outcome and the ranks will be modeled in order to account for deaths. For the GRACE-2 analyses, we will implement a Bonferroni-Holm stepdown procedure to maximize power to detect a significant effect while limiting the overall Type I error rate to 2.5% for these two comparisons.

6.2.3 Exploratory Outcomes

For the GRACE-2 trial, mortality rates and other binary outcomes will be compared between treatment arms using chi-squared tests, stratified by site. MODS-free and organ-dysfunction-free

days will be compared between treatment arms in a rank-based fashion, accounting for site.

For outcomes evaluated at both 3 and 12 months, we have elected to perform separate analyses at each time-point, using multiple imputation that will incorporate available information from all evaluation timepoints. As change in FSS from baseline is expected to exhibit substantially skewed distributions (with many surviving children returning to baseline status), and since under intention to treat analysis, children who die are treated as having the worst-possible outcome, rank-based analyses will be used.

6.2.4 Safety Outcomes

The primary safety outcome in the GRACE-2 trial (systemic levels of IL-6 and ferritin) will be analyzed separately using linear mixed models predicting the laboratory value with a random effect for site and subject. Arm, time to laboratory draw, and the interaction between arm and time will be used to assess whether the GM-CSF arm has a higher rate of laboratory values over time than the placebo arm.

6.2.5 Subgroup Analyses

Primary, secondary, and exploratory outcome analyses will be repeated by the following subgroups to assess possible heterogeneity in treatment effect:

- Age (< 1 year, 1 year to onset of puberty, post-pubertal)
- Sex
- Race
- Ethnicity
- Infectious organism type (gram positive bacterium, gram negative bacterium, fungus, virus, polymicrobial)
- Site of infection
- Presence of a complex chronic condition
- Presence of baseline immune compromise
- Use of extracorporeal therapies

The outcomes will be re-analyzed using the appropriate statistical methods (e.g., the Wilcoxon Rank sum test will be replaced by rank regression for subgroup effect assessment) and include the main effect of the subgroup and arm and an interaction between the subgroup and arm. If the interaction p-value is significant at the 0.05 level, this indicates evidence the effect of arm differs by subgroup level when predicting the outcome.

6.2.6 Handling of Missing Data

Per the intention-to-treat principle, patients who withdraw from the study or are lost to follow-up will have all available data used in the analysis. Patients whose families withdraw consent to administer the study agent will be encouraged to allow their child to remain in the trial for assessment of outcomes. In the event that a substantial number of patients are withdrawn or lost to follow-up, baseline characteristics and available information on hospital course and later follow-up timepoints will be reviewed and compared to patients not withdrawn or lost, to assess empirically whether these patients differ from those remaining in the study for the scheduled treatment and follow-up time. Missingness for primary, secondary, exploratory, and safety outcomes will be reviewed in aggregate and by site. Reviews will start as soon as enrollment opens and will be regulatory monitored so missing data problems can be addressed early in the study.

We expect to observe nearly 100% of outcomes for the primary analysis. PELOD-2 will be collected daily during the hospital stay, and vital status assessed at 28 days. We expect minimal loss-to-follow-up for visits after discharge. If substantial missingness is observed, we will perform multiple imputation to account for missing data in-hospital as well as during the follow-up visits. We will minimize loss to follow-up by collecting (and using) multiple routes of contact with subjects (e.g. email, cell phone, home phone, address); by using outcome measurement tools that can, together, be completed in 30 minutes; by using local Research Coordinators to do the follow-up contacts (rather than use a non-local number which families may be reluctant to answer); and by providing financial incentives for each follow-up contact.

7 Data Management

7.1 Clinical Site Data Management

Each clinical site will maintain study records in locked filing cabinets. The site will maintain an Essential Documents Binder, which may be in paper or electronic form. Copies of all informed consent documents will be kept on file and be available for site monitoring inspection (on site or remote).

7.2 Data Coordinating Center

7.2.1 Data Center Description

The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for CPCCRN and

a variety of other national research networks. Anchoring these services is a state-of-the-art, energy efficient data center. The data center facility supports more than 3500 users around the country and will provide a secure, reliable, enterprise-wide infrastructure for delivering mission critical DCC systems and services to CPCCRN.

7.2.2 Facility, Hardware, Storage, Data Backup and System Availability

The data center was built using industry standards and energy efficient cooling solutions. The data center is cooled by Liquid Cooling Package (LCP) inline cooling technology, providing the desired efficiency, redundancy and modularity. The LCP utilizes a hot/cold aisle design that allows for even air distribution to minimize hot spots. The data center's electrical power system contains an uninterruptible power supply with a diesel backup generator. The data center is protected with an FM-200 backed fire suppression system, which provides waterless fire suppression without leaving behind residue or particulate. Enhanced security measures are implemented to safeguard the equipment and the data within in it. Security measures are enforced 24 hours a day, seven days a week, 365 days a year by a combination of on-premise security guards, University police officers, and video surveillance.

The data center has a virtualized environment. This environment consists of more than 415 virtual servers across 25 host servers. Virtualization provides key advantages: (1) High availability - in the event of hardware failure, virtual machines automatically restart on healthy resources, minimizing impact to end-users; (2) Flexible infrastructure - compute and storage is seamlessly scaled as current needs change; (3) Rapid deployment - new resources are provisioned on-demand.

Production servers running mission critical applications are clustered and configured for failover events across multiple clusters. Entire servers are backed-up to a dedicated infrastructure. The backup repositories are encrypted-at-rest using AES-256. The storage area networking applications, clusters, and switch-to-switch links are highly redundant. Incremental backups of data occur Monday through Friday. A full data backup occurs weekly. Full backups are sent off-site on a weekly basis to a secure secondary location. The data center currently manages over 125 terabytes of data.

Information systems are available 24/7/365 unless a scheduled maintenance period or mitigation of an unexpected event is required. Critical systems availability has exceeded 99.9% for the past 5 years.

7.2.3 Security, Support, Encryption, and Confidentiality

The data center coordinates the network infrastructure and security with University Information Technology (UIT) at the University of Utah. This provides us with robust firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Centralized authentication and communication over public networks are encrypted using transport layer security (TLS) and/or virtual private network (VPN) technologies. Direct access to data center machines is only available while on premise or via a VPN client.

All network traffic is monitored for intrusion attempts. Security scans are regularly run against data center servers and IT staff are notified of intrusion alerts. Security is maintained with Windows user/group domain-level security. Users are required to change their passwords every 90 days. All files are protected at user/group levels and database security is handled in a similar manner with group-level access to databases, tables, and views. Finally, all laptops used by faculty and staff in the DCC are whole-disk encrypted.

The data center uses monitoring tools to continuously monitor applications and servers. Environmental and network systems are also monitored to ensure up-time. System Administrators are on-call 24/7/365 to respond to urgent events.

All personnel involved with the DCC have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act education. We require all users to sign specific agreements concerning security, confidentiality, and use of our information systems before access is provided.

7.3 Electronic Data Capture System

The Data Coordinating Center (DCC) will develop an electronic data capture system for this study. Data will be entered by each clinical site, and data quality will be monitored at the DCC. The DCC will use an electronic discrepancy management system to notify sites of inconsistent or erroneous data entry, which will be corrected by the clinical site. The discrepancy management system maintains an audit trail of all discrepancy resolution.

8 Study Site Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data

quality in previous Collaborative Pediatric Critical Care Research Network studies, and we will utilize this process to ensure excellent quality data in the proposed study. The DCC utilizes risk-based methodology to identify and correct problems that may arise at sites. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitor's review of data in the electronic medical record.

8.1 Site Monitoring Plan

A supplemental study-specific risk-based monitoring plan, separate from the protocol, will be completed which outlines specific criteria for monitoring. This plan may include the number of planned site visits, criteria for focused visits, additional visits or remote monitoring, a plan for chart review and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found.

8.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of an in-person or virtual site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

8.3 Remote Monitoring

The DCC may supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with

the Clinical Center investigator and/or research coordinator to review safety and data quality. This may require uploading copies of medical records, patient study files, regulatory documentation, or other source documents for the monitor to review. Alternatively, other methods, such as remotely viewing source documentation, may be utilized. This helps assure protocol compliance and accurate data collection. The DCC may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

8.4 Pharmacy Monitoring

This trial will use the investigational pharmacy at Nationwide Children's Hospital as a central pharmacy. Each clinical site pharmacy must maintain adequate records of all dispensed study drug. Each pharmacy will be monitored and may be requested to send copies of these documents to the DCC and/or the investigational pharmacy at Nationwide Children's Hospital which will conduct remote audits at regular intervals.

8.5 Record Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the Data Coordinating Center, 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 (when applicable) of the Food and Drug Administration (FDA), NIH, other Federal funders or study sponsors, and the Institutional Review Board (IRB) for each study site.

9 Protection of Human Subjects

9.1 Risks to Human Subjects

9.1.1 Human Subjects Involvement and Characteristics

Subject Population to be Studied Participating sites will enroll infants, children and adolescent patients who are admitted to a Pediatric or Cardiac Intensive Care Unit with sepsis-induced multiple organ dysfunction syndrome (MODS). The goal is to determine if personalized immunomodulation is an effective strategy to reduce mortality and morbidity from sepsis-induced MODS. All subjects in this study will be less than 18 years of age and $\geq$ 40 weeks corrected

gestational age. The inclusion and exclusion criteria are listed in Section 3 on page 13.

Subjects enrolled in sepsis-induced MODS trials face significant mortality and morbidity from the sepsis-induced MODS, and the study drug (GM-CSF) is associated with known risks. These risks will be specified in the parental / guardian permission forms and will be explained thoroughly when permission is obtained by the site investigator or designee.

Collaborating Sites Performing the Research All of the collaborating sites are tertiary or quaternary pediatric intensive care units in large academic health centers or children's hospitals. The patients who are eligible for participation in the trials are critically ill with sepsis-induced multiple organ dysfunction syndrome (MODS), and require 24 hour care by pediatric critical care physicians, nurses, respiratory therapists, and other clinical staff specialized in pediatric critical care.

9.1.2 Study Procedures and Materials

Study Procedures Patients will be screened by site research staff, and if eligible, their parents/guardians will be approached for permission to participate in the study. Patients whose parents or legal representative give permission will have 1 - 3 ml of blood (adjusted for body weight) drawn on MODS Day 2 for measurement of whole blood LPS-induced TNFα production capacity and inflammatory biomarkers, including serum ferritin levels. Blood samples will be stimulated on-site within one hour of sample collection using highly standardized LPS stimulation kits provided by the Immune Surveillance Laboratory at the Abigail Wexner Research Institute at Nationwide Children's Hospital. The incubation period is 4 hours. After stimulation, samples will immediately centrifuged and all supernatants will be overnight-shipped on dry ice to Nationwide, where TNFα will be quantitated in the LPS-stimulated supernatants along with serum ferritin levels the next morning. The results will be double checked to assure correct immunophenotype assignment, verifying eligibility for participation in GRACE-2.

Study Materials Sources of research material will include data collected specifically for the study, as well as data that are normally found in the subject's medical record. These data will be collected during the acute hospitalization during which the subject is enrolled, at three months, and at 12 months following randomization. Data will be recorded on worksheets at each clinical site, and then entered into a computerized data system maintained by the DCC. The final analysis data sets will be de-identified by the DCC.

Study Resource Sharing Parental permission forms will include language explaining that de-identified study data will be made available for other research, and that residual biological samples will be stored at a biorepository after research in this study is completed.

9.1.3 Potential Risks of Study Participation

GM-CSF. GM-CSF is FDA-approved for the reconstitution of bone marrow following chemotherapy and bone marrow transplantation for certain malignancies and has a long track record of safe use in adults and children. The incidence of adverse events such as fever, chills, bone pain, dyspnea, tachycardia, and hemodynamic instability was no different between GM-CSF and placebo-treated groups in controlled adult BMT studies. Rapid IV administration of GM-CSF (over ≤ 2 hours) has been associated with peripheral edema (11% incidence with GM-CSF vs 7% incidence with placebo) and pericardial effusion (4% vs 1%), but this appears to be related to the rate of infusion. Out of an abundance of caution, we infuse GM-CSF over at least 6 hours. We are unaware of these side effects being reported with slow IV infusion. Also, the GRACE-2 trial will use a daily dose that is 1/2 of the FDA-approved dose, based on our pilot work. Among the published small randomized controlled trials in critically ill adults and neonates, there were no GM-CSF related adverse events reported and no evidence that GM-CSF treatment resulted in increased systemic inflammation. In the original dose-finding GRACE study and the GIFT study, GM-CSF administration has not been associated with increased systemic inflammation nor other significant adverse events. A risk of hypersensitivity/allergic reaction exists with any drug.

Biological Sampling. Another potential risk to enrolled subjects in this study is a low red blood cell count or anemia. Any blood sampling can contribute to anemia. While many children with critical illness go on to require a blood transfusion, we will keep the total blood drawn for research purposes to < 5.0 ml/kg on MODS Day 2, and 9.5 ml/kg over the entire study period. This volume of blood loss should not significantly increase the risk of an enrolled subject to require a blood transfusion.

Some children enrolled in this study may need to undergo additional venipuncture for the purposes of study sample collection, particularly for later time points when central catheters may have been removed. We will try to coordinate blood sampling with previously ordered phlebotomy but if we are unable to do this we may have to perform venipuncture. While venipuncture can cause pain, bruising, bleeding, and rarely, infection, these risks are generally viewed as modest. Guidance will be provided to sites for truncation of sampling or reduction of blood sample volume such that infants may participate in the immunomodulation study without exceeding the 9.5 ml/kg total blood draw limit.

Loss of Confidentiality. There is a minimal risk of loss of confidentiality for data collected in this study.

9.1.4 Alternatives to Study Participation

Study participation is not required for patients to receive state-of-the-art critical care for sepsis-induced multiple organ dysfunction syndrome. Parents may elect to discontinue study interventions at any time, which will be considered discontinuation of study drug. Data collection and blood sampling will continue unless parents wish to discontinue all study participation. Data that have been collected up to that point will be retained in the study, and only safety data will be collected through Study Day 28 or ICU discharge, whichever occurs first.

9.2 Adequacy of Protection Against Risks

9.2.1 Parental Permission, Informed Consent and Assent

Waiver of Consent For Screening Data Collection Waiver of consent is requested for collection of demographic data, eligibility data, and details of the consent procedures. Our justification for waiver of consent for these observational data is based on the following factors:

1. The scientific validity of the study is dependent on non-biased enrollment of eligible patients, and these data are required to properly create the trial CONSORT diagram, as well as to ensure that eligible patients were not excluded based on race, ethnicity, or gender.
2. Collection of these screening data will not require additional patient or parent contact.
3. The minimal risk of loss of privacy is mitigated by secure data management at the DCC, and analysis datasets will be de-identified.

Parental Permission Subjects who are eligible for this study are under 18 years of age, and written permission will be required for participation. After determining that a subject is eligible, the site investigator or designee will approach the parent or legal guardian to offer participation for their child in the study. Single parent permission is permitted under 45 CFR §46.405. The parent or legal guardian will be informed about the objectives of the study and the potential risks and benefits of their child's participation. If the parent or legal guardian refuses permission for their child to participate, then all clinical management will continue to be provided by the clinical ICU staff in accordance with institutional practice and judgment.

Child Assent Subjects who are eligible for this study will be critically ill, and child assent is typically not possible at the time of study enrollment. However, during follow up after discharge from the ICU, issues about assent become applicable. Children who are capable of giving assent and who are alert and competent, may be asked, following an age-appropriate discussion of risks and benefits, to give assent to the study for collection of follow up information at 3 and 12 months. Assent will be waived if the child is too young, has a severely reduced mental age, decreased level of consciousness, psychological problems, or other legitimate reasons as judged by the single IRB and the institution's specific Human Research Protection program policies.

Subject Consent If a subject attains the age of 18 years during the study intervention period while critically ill, it will not be possible to obtain informed consent from the subject. During the follow up after discharge from the ICU, 18-year-old subjects who are alert and competent and capable of giving consent will be asked for informed consent at the 3 or 12 month follow up appointment (depending on when the subject reached 18 years of age). Subject consent will be waived if the subject has a severely reduced mental age, decreased level of consciousness, psychological problems, or other legitimate reasons as judged by the single IRB and the institution's specific Human Research Protection program policies.

9.2.2 Institutional Review Board and Human Research Protection

The Institutional Review Board (IRB) at the University of Utah will serve as the single IRB. The Utah IRB has extensive experience with single IRB implementation due to our participation in the NCATS funded Trial Innovation Center, which has implemented over 35 sIRB studies. No human subjects research activities will be conducted at any CPCCRN site prior to sIRB approval at the University of Utah.

In addition to sIRB activities, each institution has Human Research Protection activities that will be completed prior to site activation. These include conflict of interest, assuring competence of investigators, impact on hospital services, etc., and are individualized to each site.

9.2.3 Protections Against Risk

Study Drug Risk All patients in this study have sepsis-induced MODS and will be receiving antibiotic therapy to treat the underlying infection. They will be in critical care units and closely monitored for changes in infection status or laboratory abnormalities, which will be recorded as adverse events (see Section 10.2 on page 35).

GM-CSF will be given at a low dose (1/2 of the FDA-approved dose), for a short duration (7 days), and with a conservative infusion rate (over 6 hours). Accordingly, we do not expect

to see rate-of-infusion-related adverse events nor do we expect to generate severe leukocytosis (white blood count > 50,000 cells/mm^2) or exacerbate systemic inflammation. We have not seen serum ferritin levels increase above 2,000 ng/ml during treatment with GM-CSF in our prior dose-finding work and we have consistently seen a reduction in systemic pro-inflammatory cytokine levels in the setting of GM-CSF-induced reversal of immunoparalysis.

Phlebotomy Risks The risk of anemia is minimized by minimizing the volume of blood samples taken and by reducing the sampling volume for infants. The risk of phlebotomy is minimized by making use of indwelling catheters when present and by timing sampling with previously scheduled venipuncture if able.

Loss of Confidentiality The minimal risk of loss of privacy is mitigated by the substantial data management resources and security described in Section 7.2.3 on page 27.

9.2.4 Vulnerable Subjects

All subjects enrolled in this study will be children < 18 years of age and this is a vulnerable population. The study will be carried out in pediatric intensive care units staffed by board-certified or board-eligible pediatric critical care physicians, and a pediatric clinical team that is specifically trained and expert in the clinical management of critically ill infants, children and adolescents. All clinical sites in CPCCRN are pediatric intensive care units that normally admit patients within the age group eligible for this study.

Research in children involves special protections under 45 CFR §46 Subpart D "Additional DHHS protections for children involved as subjects in research" and 21 CFR §50 and §56. The study in this protocol is permissible under these regulations as:

- Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects (45 CFR §46.405).

9.3 Potential Benefits of Proposed Research

The goal of this study is to improve outcomes from pediatric sepsis-induced MODS through normalization of immune function with resulting enhancement of infection clearance, improvement in surveillance for new infection, and promotion of tissue healing, all of which we hypothesize will speed resolution of organ failure. For research participants who are allocated to GRACE-2, it is not known if GM-CSF will be effective in reducing organ dysfunction or

mortality. If effective, there is potential for direct benefit to participants in terms of both morbidity and mortality. Knowledge gained from this study may help other children in the future.

9.4 Importance of the Knowledge to be Gained

Multiple organ dysfunction syndrome (MODS) increases mortality from sepsis by over 10-fold and is a leading cause of death and morbidity in critically ill children. Current treatment of sepsis and MODS is largely supportive in nature. The overall hypothesis of this proposal is that using immune phenotyping to personalize immune care for each patient will reduce mortality and morbidity from sepsis-induced MODS. This transformative study will provide highly unique information about whether this personalized approach is beneficial, potentially improving the outcomes of critically ill infants, children and adolescents in the future.

10 Data and Safety Monitoring Plan

10.1 Data Safety Monitoring Board (DSMB)

The study will have a Data Safety Monitoring Board (DSMB) including members who have expertise in pediatric sepsis, pediatric critical care medicine, immunology, biostatistics and/or bioethics. The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim data as applicable. The purpose of the DSMB is to advise the Principal Investigator(s) regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of individual Clinical Center, review of adverse events, and other subject safety issues.

10.2 Adverse Event Reporting

The site investigator is responsible for evaluating all adverse events at their Clinical Center and ensuring that they are properly reported. Adverse events that occur during this study will be recorded. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

10.2.1 Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE). An adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE). A serious adverse event (SAE) for this population is an adverse event that:

- results in death; or
- is life-threatening (immediate danger of death from the event as it occurred); or
- requires new inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

10.2.2 Classification of Adverse Events (Relatedness and Expectedness)

Clinical judgment is required for properly classifying relatedness and expectedness for adverse events. It is not appropriate to classify an event as possibly related if, in the opinion of the clinical investigator, it is clinically unlikely that the event is related. It is impossible to prove a negative, and the FDA expects clinical judgment to be used in assessing relatedness. Similarly, it is not appropriate to classify an event as unexpected because the patient was not anticipated to suffer the event at the time of enrollment into the study, if the event is a known sequelae of the underlying disease process (sepsis-induced MODS) or has been previously noted with study interventions as determined from the package insert or investigator brochure of the study drug.

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. *Relatedness must be assessed by an investigator and may **not** be assessed by a research coordinator.*

Not Related: The event is believed to be 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 a clinically compatible temporal sequence from the time of beginning the assigned study intervention, 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 beginning the assigned study intervention, and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with complications of sepsis-induced MODS and critical illness, nor consistent with adverse events noted in the drug investigator brochures.

Expected: An event is considered expected if it is known to be associated with the underlying condition or is related to the study intervention 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.

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.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status

- Recovered with permanent sequelae
- Symptoms persist

10.2.3 Time Period for Adverse Events

Adverse events will be recorded from the time of randomization and will continue through 28 days after randomization, hospital discharge, or death, whichever occurs earliest.

10.2.4 Data Collection Procedures for Adverse Events

Subjects who have been randomized to study drug will undergo *daily screening* for adverse events from the first day of study drug receipt through post-randomization day 28, hospital discharge, or death, whichever happens first. This screening will include review of all vital signs, all clinically-ordered laboratory data and imaging studies, physical exam findings (as documented in the medical record by the clinical team), and clinical progress and procedure notes since the time of the prior day's screening. A pre-drug problem list will be generated that documents clinical, laboratory, and radiographic abnormalities that are present prior to the start of study drug. Any medical condition or laboratory abnormality that is present prior to randomization, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

After patient randomization all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Abnormal laboratory values that are *clinically significant* will be recorded as adverse events and the site investigator will assess the relationship to the study and expectedness.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center. Adverse event severity will be classified using the Common Terminology for Criteria for Adverse Events (CTCAE) scoring system.

10.2.5 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly related to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours

of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event. After receipt of the complete report, the Data Coordinating Center will report these unanticipated problems to the NICHD Project Officer in an expedited manner (as close to 24 hours as possible). The medical monitor (Section 10.2.6) for the study will assess the report, and reporting to the University of Utah IRB, acting as the single IRB for the study, may be required. In addition, dependent on the nature of the unanticipated problem, all participating institutions may require notification. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NICHD staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigators (Drs. Hall, Zuppa and Mourani) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

10.2.6 Monitoring Serious Adverse Events

A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by DSMB members and NICHD staff. The SAE reporting process may be incorporated into the Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, NICHD staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the NICHD staff and the DSMB chairperson cannot be reached expeditiously, the Data Coordinating Center will notify the study investigators (Drs. Hall, Zuppa and Mourani) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB. The IRB in Utah will be notified, and each site investigator will notify their institutional Human Research Protection program of the trial suspension.

- The death of a randomized subject in whom the cause of death is judged to be probably or definitely related to the study drug.
- The occurrence, in any subject, of a life-threatening SAE whose causal relationship to study drug is judged to be probable or definite.
- Two (2) occurrences of Grade 3 or higher toxicities that are judged to be probably or definitely related to the study drug.
- Two (2) occurrences of a clinically significant Grade 3 or higher laboratory abnormality that are judged to be probably or definitely related to study drug.

After notification of the NICHD Program Official or Project Officer, and the DSMB chairperson, of *serious, unexpected, and study-related* adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigators (Drs. Hall, Zuppa and Mourani) and all clinical investigators, who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings which will occur at least twice annually. The Data Coordinating Center will prepare a Summary Report of Adverse Events, classified with the MedDRA coding system, for the DSMB meetings, including the proportion of subjects experiencing treatment-emergent adverse events. The occurrence of any adverse event will be considered as a dichotomous outcome and compared between arms using a chi-squared test. Cumulative data on treatment-emergent adverse events will be analyzed and reported in the primary study manuscript at the conclusion of the trial.

10.2.7 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study or discharge from the hospital, will be followed by the site investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, and continuity of care with a responsible clinical team has been assured.

10.2.8 Reporting to the Food and Drug Administration

Serious, unexpected and related adverse events will be reported to the FDA in an expedited manner consistent with FDA requirements. The Data Coordinating Center will prepare the

report for submission by the principal investigator, Dr. Hall, who holds the IND.

11 Study Training

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. No site will be activated until all training requirements have been fulfilled by the site investigators and research staff.

A manual of operations will be provided to each investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The Data Coordinating Center, in collaboration with the study investigators (Drs. Hall, Zuppa and Mourani), will be the main contact for study questions.

Each participating clinical site that has not previously participated in CPCCRN studies using the TNFα response assay will undergo one on-site visit prior to enrollment start to insure familiarity with clinical and laboratory procedures required for immune phenotyping. In the event that this is not possible (e.g., due to COVID-19 restrictions), this visit will be conducted virtually, including video documentation of pharmacy and laboratory capabilities.

12 Regulatory Considerations

12.1 Food and Drug Administration

This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration. The clinical investigator at each participating site will complete a Form FDA 1572, “Statement of Investigator.”

12.2 Health Insurance Portability and Accountability Act

Data elements collected include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events.

Data elements for race, ethnicity, and gender are also being collected. These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

For purposes of the DCC handling potential protected health information (PHI) and producing the de–identified research data sets that will be used for analyses, all study sites have been offered a Business Associate Agreement with the University of Utah. Copies of executed Business Associate Agreements are maintained at the DCC.

12.3 Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

12.4 Clinical Trial Registration Requirements

The GRACE-2 trial will be registered at https://clinicaltrials.gov in accordance with Federal regulations.

12.5 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)].

12.6 Public Use Data Set

After subject enrollment and follow up have been completed, the DCC will prepare a final study database for analysis. A releasable database will be produced and completely de-identified in accordance with the definitions provided in the Health insurance Portability and Accountability Act (HIPAA). Namely, all identifiers specified in HIPAA will be recoded in a manner that will make it impossible to deduce or impute the specific identity of any patient. The database will not contain any institutional identifiers.

The DCC will also prepare a data dictionary that provides a concise definition of every data element included in the database. If specific data elements have idiosyncrasies that might affect

interpretation or analysis, this will be discussed in the dictionary document. In accordance with policies determined by the investigators and funding sponsors, the releasable database will be provided to users in electronic form.

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