Targeted Reversal of Inflammation in Pediatric Sepsis-induced MODS (TRIPS)

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.[70] 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, 13, 18, 21, 53] 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,[6, 7, 28, 29, 49] 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 Heterogeneity of the Immune Response to Sepsis

The host's immune response to a pathogen is the key driver of organ dysfunction in sepsis. Immune cells and injured or stressed tissues produce cytokines and chemokines that make the local environment favorable for fighting infection through vasodilation, increased capillary permeability, and recruitment of additional leukocytes. This response is beneficial when limited to the site of infection, but is pathologic when it becomes systemic, resulting in fever, hypovolemia due to

capillary leak, tissue edema, malperfusion, and organ dysfunction. Numerous investigators have identified mediators involved in the Systemic Inflammatory Response Syndrome (SIRS) that can serve as biomarkers of the pro–inflammatory response including IL-1β, IL-6, TNFα, and others, with high serum levels predicting adverse outcomes (Figure 1).[20, 25, 35, 61, 71]

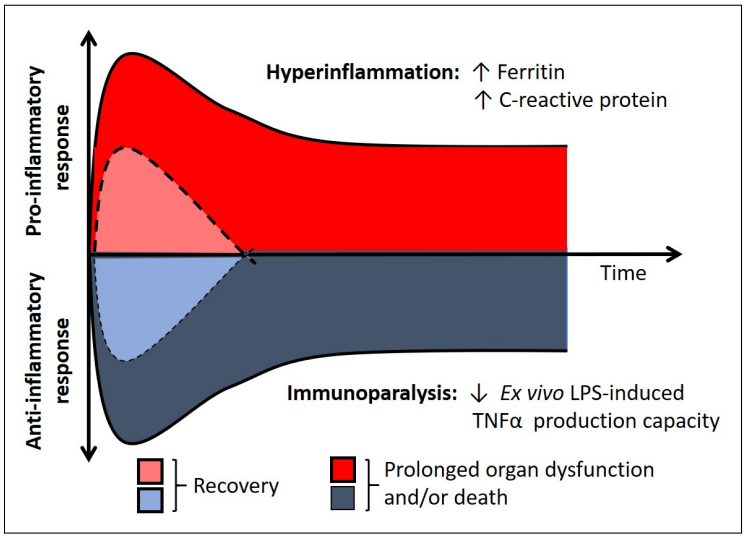

Figure 1: Immune response to pediatric sepsis includes systemic inflammation (SIRS), compensatory anti-inflammatory response syndrome (CARS), or both. When severe, both are associated with adverse outcomes.

1.3 Immunoparalysis

Immunophenotyping studies, many of which were conducted by CPCCRN member investigators, have shown that while some children with sepsis-induced MODS have severe hyperinflammation that may benefit from anti-cytokine therapies, others have markedly hypoactive circulating leukocytes and could potentially benefit from an immunostimulatory approach rather than an anti-inflammatory one.[6, 7, 28, 29, 49] Innate immune suppression in children with sepsis-induced MODS is a manifestation of the compensatory anti-inflammatory response syndrome (CARS) (Figure 1) and, when severe, is termed immunoparalysis. This can be diagnosed in our research laboratory through the measurement of the ability of a subject's whole blood to make the pro-inflammatory cytokine TNFα upon ex vivo stimulation with a highly standardized lipopolysaccharide (LPS) solution. A low TNFα response is characteristic of immune suppression, and a TNFα response < 200 pg/ml is diagnostic of immunoparalysis in our hands. Immunoparalysis, *as defined by our assay*, has been validated in independent cohorts to be associated with adverse outcomes in

critically infected children including those with MODS (mortality: RR 5.8 (2.1-16)[29]), influenza (mortality: AUC: 0.97, p<0.0001;[28] mortality: 9.3% vs 0%, p=0.007[52]), and severe sepsis/septic shock (mortality: RR: 1.98 (1.08-3.61), p<0.05;[6] prolonged organ dysfunction: AUC:0.71, RR: 1.25 (1.1-1.4), p=0.0005[49]). In sum, immunoparalysis is occult, is measurable in the research laboratory, and is associated with prolonged organ dysfunction and death in children with sepsis.[6, 7, 28, 29, 49] Unless immunoparalysis occurs in the setting of very high levels of systemic inflammation, data from our group and others suggest that this population of patients will benefit from treatment with immunostimulatory drugs (e.g. granulocyte macrophage-colony stimulating factor [GM-CSF]).[29, 44, 58] Accordingly, we will use prospective immune function testing to exclude children with immunoparalysis who have mild to moderate inflammation (i.e. a serum ferritin level < 2,000 ng/ml) from the TRIPS trial. Those subjects will be instead entered into a completely distinct clinical trial of immune stimulation with GM-CSF (GRACE-2) that is covered by a separate IND (#112277).

1.4 Inflammatory Biomarker Selection

Assays for pro-inflammatory cytokines are not readily available in most clinical laboratories, rendering them difficult to use for treatment decision-making. Our group has extensive experience using serum ferritin and C-reactive protein (CRP), both of which are easily measured in clinical laboratories around the world, as indicators of the magnitude of the pro-inflammatory response to sepsis. We, in the 401-subject PHENOMS study,[6] found that systemic elevations in ferritin and CRP are common in septic children, with 96% of the cohort having a serum CRP level > 4 mg/dl or a ferritin level > 500 ng/ml. The greatest mortality risk (40%) occurred in those with marked elevations in both biomarkers.

Ferritin is an iron-binding protein that, in sepsis, is released by activated macrophages and other innate immune cells. Systemic ferritin levels are therefore a reflection of the pro-inflammatory innate immune phenotype. While very severe elevations in serum ferritin levels (>10,000 ng/ml) are characteristic of disorders like systemic juvenile idiopathic arthritis (sJIA)-induced macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH),[3] more moderate hyperferritinemia (e.g. levels 500 – 10,000 ng/ml) is frequently seen in children with sepsis-induced MODS.[8]

CRP is an acute phase reactant that is produced by the liver in response to IL-6. CRP has long been used as a marker of systemic inflammation and is increasingly used as a measure of response to therapy for inflammatory conditions.[14, 16, 51, 73] Together, ferritin and CRP represent a parsimonious and immediately clinically translatable panel of biomarkers that identify children with severe systemic inflammation. Despite this, neither ferritin nor CRP is currently used as

part of an evidence-based, personalized approach to pediatric sepsis care.

1.5 Timing of Immunophenotyping

Morbidity and mortality from severe sepsis in children is bimodal. It is the minority of pediatric sepsis deaths in the U.S. (a third or fewer) that occur in the setting of refractory shock within the first 2-3 days of illness.[5, 69] The majority of pediatric sepsis deaths occur over the ensuing days to weeks in the setting of unresolving organ dysfunction.[6, 69] It is this population with persistent sepsis-induced MODS that we are targeting with the TRIPS trial, which targets septic children with MODS on day 2-3 who we believe will benefit from targeted anti-inflammatory therapy. It is the next critical step in this line of research to show that an individualized approach to immunomodulation will improve outcomes in children with sepsis-induced MODS.

The performance of next-day immunophenotyping will allow us to exclude children with immunoparalysis who only have mild to moderate inflammation from being randomized into the TRIPS trial, enhancing its safety profile. Children with immunoparalysis who have serum ferritin levels >2,000 ng/ml will be randomized into the TRIPS trial, since GM-CSF is contraindicated in those patients and hyperinflammation may be contributory to their immunoparalysis.

1.6 Rationale for Anakinra

1.6.1 Potential for Direct Benefit

Anakinra is a recombinant version of the endogenous counter-regulatory cytokine IL-1 receptor antagonist (IL-1ra) which has been FDA-approved since 2001 for the treatment of rheumatoid arthritis and Cryopyrin-Associated Periodic Syndromes (CAPS) including neonatal-onset multisystem inflammatory disease (NOMID) at doses ranging from 1 to 8 mg/ kg/day. Anakinra was, however, initially developed as a sepsis therapeutic, and was evaluated in multiple phase II and III clinical trials in septic adults in the 1990s.[21, 22, 53] Those clinical trials failed to meet their therapeutic goals (mortality reduction) in the cohorts as a whole, though it is likely that the drug effects were diluted by subjects with relatively low severity of illness and/or low levels of inflammation. Secondary analyses of both phase III trials showed strong survival benefits for anakinra in the subset of subjects with ≥1 organ dysfunction (p=0.002),[21, 22] those with higher a priori risk of death (p<0.005),[38] those with higher-pretreatment cytokine levels (aRR: -0.12 [-0.23 – -0.01]),[45] and those with hepatobiliary dysfunction and disseminated intravascular coagulation suggesting an MAS-like phenotype (HR:0.28 [0.11– 0.71], p=0.007).[60] It is noteworthy that both phase III sepsis trials used anakinra by the intravenous route and with a dose well in excess of the FDA-approved dose (48 mg/kg/ day) with no increase in nosocomial infection risk

or other drug-related adverse events.

The TRIPS study will only enroll children with sepsis-induced MODS (the highest severity of illness within the septic population) and we will restrict the use of anakinra to those with high levels of systemic inflammation (serum ferritin 500 –10,000 ng/ml or CRP ≥4 mg/dL). *This enhances the likelihood that children enrolled in the TRIPS trial will derive direct benefit from targeted anti-inflammatory therapy with anakinra.* Further, we will exclude children with immunoparalysis in the setting of mild to moderate inflammation, a population in whom anti-inflammatory therapies may be harmful.

The COVID-19 pandemic has afforded another opportunity to evaluate the use of anakinra in the setting of active infection. While prospective randomized controlled trials are ongoing, multiple non-randomized cohort studies have suggested direct benefit of anakinra when used in hospitalized adults with inflammation due to COVID-19. Three meta-analyses of the use of anakinra in COVID-19 have been done to date and they all demonstrated a reduced mortality risk in anakinra-treated subjects (aOR: 0.32, 95% CI: 0.23 – 0.45, P<0.00001;[4]); (aOR: 0.34, 95% confidence interval [CI], 0.21 – 0.54, P<0.00001;[65]); (aOR: 0.26, 95% CI 0.14 – 0.48, P<0.0001;[54]). There were no differences in the risk of adverse events in these meta-analyses, including liver dysfunction or nosocomial infection. There are currently at least 15 active studies of anakinra for the treatment of COVID-19-related pneumonia and/or cytokine storm in adults or children listed on www.ClinicalTrials.gov.

Anakinra is increasingly used as part of a chemotherapy-sparing approach for the treatment of hyperinflammation due to HLH/MAS in adults and children, including an ongoing clinical trial of anakinra (10 mg/kg/day) in children with MAS (NCT02780583). The literature supports anakinra's safe use as an anti-inflammatory treatment in children with MAS, with doses ranging from 1 – 48 mg/kg/day being associated with resolution of organ dysfunction and/or improvement in disease activity.[12, 37, 55, 57] Recent clinical trials of anakinra in children with MAS and sJIA include NCT02780583 and NCT03265132. We reported the use of anakinra (5 – 10 mg/kg/day) in a series of children with critical illness due to secondary HLH and showed a 67% reduction in CRP levels and a 64% reduction in ferritin levels over a week of anakinra treatment, with survival in 7/8 subjects with no occurrence of nosocomial infection.[57]

Together, these data strongly support the potential for direct benefit of anakinra in critically ill, hyper-inflamed children with sepsis-induced MODS.

1.6.2 Safety of Anakinra

Anakinra has a long history of safe use in acutely and critically ill children and adults. While its long-term use in adults with rheumatoid arthritis has been associated with an increased incidence of severe infections (2% vs < 1%), particularly in patients who were receiving concurrent therapy with TNFα blocking drugs, this risk has not been seen with short-term use. In placebo-controlled studies of long-term treatment of adults with anakinra, 8% of patients receiving anakinra had decreases in total white blood counts of at least one WHO toxicity grade, compared with 2% of placebo patients, though statistically significant reductions in white blood cell counts have not been seen with short-term use. There were no drug-attributable adverse events (including nosocomial infection and leukopenia) reported in the 1994 and 1997 phase II and III clinical trials that enrolled over 1,600 septic adults, despite using a dose of 48 mg/kg/day (six times higher than the upper FDA-approved dose, and three times higher than the highest dose to be evaluated in the TRIPS trial). Anakinra use was not associated with increased risk for adverse events (including nosocomial infection and leukopenia) in any of the three meta-analysis of its use in adults with acute COVID-19 disease to date.[4, 54, 65] In another example of the safe use of extremely high-dose anakinra, no increased risk for adverse events was noted in reports of anakinra use in adults with acute brain injury due to stroke[17] or traumatic injury[31] despite using doses as high as 48 mg/kg/day.

Anakinra was similarly well-tolerated in non-controlled reports of its use for pediatric HLH/MAS at doses ranging from 1 – 20 mg/kg/day.[12, 37, 55, 57] Among a total of 31 reported anakinra-treated subjects, there was one instance of reversible transaminase elevation, and four instances of low white blood cell counts (three of which were associated with concurrent corticosteroid use, and none of which resulted in secondary infections). Anakinra has a strong record of safety in the treatment of neonatal-onset multisystem inflammatory disease (NOMID) for which it is FDA-approved (including in infants) at a dose of 8 mg/kg/day, with no dose-limiting toxicities reported.[26, 50, 62]

We hypothesize that short-duration treatment with anakinra has the potential to safely restore immunologic homeostasis in hyper-inflamed children with sepsis-induced MODS. This includes children with hyperferritinemia without immunoparalysis and those with immunoparalysis in the setting of marked hyperferritinemia, both of whom stand to benefit from targeted reduction in systemic inflammation. In contrast to prior adult studies of anti-cytokine therapy, anakinra will not be given to subjects with immunoparalysis in the setting of mild to moderate inflammation. We expect that this will enhance both the safety and efficacy of our personalized approach to immunomodulation in sepsis-induced MODS.

1.6.3 Biological Rationale for Anakinra Use

Hyperferritinemic sepsis is increasingly recognized as a clinically important entity whose pathophysiology includes (but is not limited to) overactivation of the macrophage's IL-1β-producing "inflammasome" pathway, but likely does not require the degree of immunosuppressive treatment (e.g. etoposide, high-dose glucocorticoids) that is typical for primary HLH.[8] Patients with HLH and MAS often have serum ferritin levels >10,000 ng/ml while patients with hyperferritinemic sepsis typically have ferritin levels below 10,000 ng/ml.[6] Hyperferritinemic sepsis can result in liver injury, disseminated intravascular coagulation, and lymphopenia.

Anakinra can reverse this pathology by reducing inflammation through the induction of a Type 1 interferon response to facilitate resolution of infection. Anakinra has been FDA approved since 2001 for the treatment of rheumatoid arthritis and Cryopyrin-Associated Periodic Syndromes (CAPS) and is increasingly used as part of a chemotherapy-sparing approach for the treatment in HLH/MAS in adults and children, including an ongoing clinical trial of anakinra (10 mg/kg/day) in children with MAS (NCT02780583). The literature already supports anakinra's use as an anti-inflammatory treatment in patients with MAS[59] and sJIA,[66] with recent clinical trials for these indications including NCT02780583 and NCT03265132. Anakinra is also being used in patients with infection-related hyper-inflammation including a recent trial in hyperferritinemic septic adults (8-10 mg/kg/day) (NCT03332225). There are currently at least 15 active studies of anakinra for the treatment of COVID-19-related pneumonia and/or cytokine storm in adults or children listed on www.ClinicalTrials.gov.

We reported the use of anakinra in a series of children with hyperferritinemic sepsis-induced MODS and showed a 67% reduction in CRP levels and a 64% reduction in ferritin levels over a week of anakinra treatment, with survival in 7/8 subjects with no occurrence of nosocomial infection.[57] In post hoc analysis of an adult septic shock trial we found that, compared to placebo, anakinra improved mortality associated with hyperferritinemia, hepatobiliary dysfunction, and disseminated intravascular coagulation.[60] Exome analysis of adult subjects with hyperferritinemic sepsis frequently found monogenic disorders amenable to anakinra therapy.[36] Use of anakinra in this population can reduce inflammation, increase viral immunity, reverse lymphopenia, and resolve immunoparalysis. Figure 2 on the following page shows the effect of anakinra on one of our patients, a child with serum ferritin > 2,000 ng/ml and immunoparalysis in the setting of adenovirus infection. Once the cycle of hyper-inflammation was broken with anakinra, there was prompt recovery of the TNFα response and clearance of the virus, providing rationale for including immunoparalyzed children with ferritin levels of 2,000 – 10,000 ng/ml in the TRIPS trial. Anakinra has also been shown to improve the TNFα response in adults with stroke-induced immune suppression[63] without impairing other aspects of host defense.[43]

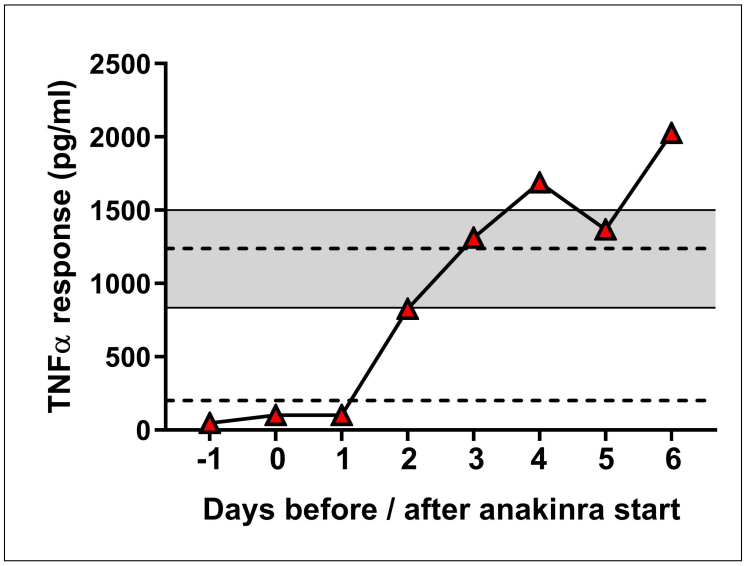

Figure 2: Anakinra was associated with reversal of immunoparalysis and survival in a child with hyperferritinemic MODS. Lower dashed line represents threshold for immunoparalysis; shaded area represents healthy control values.

Anakinra has been shown to reduce systemic inflammation in the absence of hyperferritinemia in conditions including adult heart failure,[67] myocardial infarction,[48] ischemic stroke,[17, 64] subarachnoid hemorrhage,[24] chronic renal failure[33] , traumatic brain injury[31] , and Kawasaki Disease.[39] The majority of studies of anakinra for the treatment of COVID-19-related pneumonia and/or cytokine storm that are currently listed on www.ClinicalTrials.gov require either no specific inflammatory biomarker elevation for inclusion or accept elevations of either ferritin or CRP as evidence of hyperinflammation. Anakinra is also part of the recommended treatment algorithm for hyperinflamed children with severe Multi-system Inflammatory Syndrome in Children (MISC) in consensus guidelines in the US,[32] the UK,[30] and Latin America.[19]

We will quantify plasma levels of IL-1β in all samples from all subjects so that we may, in post hoc analyses, 1) understand if there is a differential treatment effect of anakinra based upon pre-treatment cytokine levels, and 2) identify thresholds and trajectories of IL-1β that predict differential treatment effects, if present.

2 Study Summary

2.1 TRIPS Trial

The "Targeted Reversal of Inflammation in Pediatric Sepsis-induced MODS (TRIPS)" study is a prospective, double-blind, adaptively randomized, placebo-controlled trial of anakinra in children with sepsis-induced MODS and moderate to severe hyperinflammation. The primary objective of the trial is to demonstrate reduced duration/severity of organ dysfunction and mortality in children with sepsis-induced MODS and moderate to severe hyper-inflammation through the use of targeted anti-inflammatory therapy with anakinra. Secondary objectives include understanding the impact of anakinra treatment in this population on short-term health-related quality of life and functional status. Exploratory objectives include understanding the impact of anakinra treatment in this population on longer-term health-related quality of life and discrete effects on mortality and organ dysfunction.

Populations that are allocated to the TRIPS study include children with moderate to severe systemic inflammation (serum ferritin level ≥500 ng/ml or CRP ≥ 4 mg/dl) without immunoparalysis, and children with immunoparalysis whose ferritin level is too high to receive GM-CSF (> 2,000 ng/ml). This approach allows us to avoid use of anti-inflammatory drugs in children whose immune function is critically impaired except for those in whom severe hyperinflammation may be driving their immune suppression.

Subjects with evidence of moderate to severe inflammation (serum CRP level ≥ 4 mg/dl or ferritin level 500 – 10,000 ng/ml) without immunoparalysis and subjects with immunoparalysis and a ferritin level ≥ 2,000 ng/ml are, together, expected to represent 50% of the total cohort that is immunophenotyped. These subjects will be adaptively randomized into the TRIPS trial to receive placebo or anakinra (4, 8, 12, or 16 mg/kg/day X 7 days), as justified in Section 4.3.3 on page 22.

After undergoing immunophenotyping, the following subjects will be randomized into the TRIPS trial:

- Subjects who have moderate to severe systemic inflammation (serum ferritin 500 – 10,000 ng/ml or CRP > 4 mg/dl) *without* immunoparalysis;
- Subjects with immunoparalysis (TNFα response < 200 pg/ml) whose serum ferritin is between 2,000 and 10,000 ng/ml

The following subjects will not be randomized into the TRIPS trial:

- Subjects with immunoparalysis (TNFα response < 200 pg/ml) and a serum ferritin level < 2,000 ng/ml;
- Subjects without immunoparalysis whose serum ferritin level is < 500 ng/ml and whose CRP is < 4 mg/dl;
- Subjects whose serum ferritin level is > 10,000 ng/ml.

These criteria were chosen in order to exclude subjects from the TRIPS trial who may benefit from immunostimulation rather than an anti-inflammatory approach (immunoparalysis with mild to moderate inflammation); those who likely do not require immunomodulation (no immunoparalysis with mild inflammation); and those with MAS/HLH who may be harmed by placebo.

Subjects randomized into the TRIPS trial will receive standard-of-care therapy for sepsis-induced MODS, in addition to study drug, at the discretion of their clinical teams.

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[42] 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 TRIPS trial, the rate of nosocomial infections will be monitored through 28 days after randomization (already an exploratory outcome).

Though not a formal safety outcome, the development of neutropenia is a potential non-infectious treatment-emergent adverse event, though transient neutropenia can occur in the setting of sepsis-induced MODS regardless of study arm assignment. In the event that a subject is found to have a new reduction in absolute neutrophil count (ANC) to <1,000 cells/mm^3 for

two consecutive days while receiving study drug, the drug will be discontinued.

Adverse events will be monitored as described in Section 10.2.6 on page 46. The medical monitor has the authority to suspend enrollment in the event of an unexpected, study-related serious adverse event that is judged to change the risk/benefit of subject participation.

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 anakinra, or *E. coli*-derived products; 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 TRIPS 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 500 subjects into the TRIPS 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 TRIPS 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 and CRP 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 and CRP. 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 and CRP will be measured as early as possible the next day (Study Day 1).

Subjects with evidence of moderate to severe inflammation (serum CRP level $\geq$ 4 mg/dl or ferritin level 500 – 10,000 ng/ml) without immunoparalysis and subjects with immunoparalysis and a ferritin level $\geq$ 2,000 ng/ml are, together, expected to represent 50% of the total cohort that is immunophenotyped. These subjects will be adaptively randomized into the TRIPS trial to receive anakinra or placebo.

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 TRIPS Study Drug Administration

4.3.1 Intravenous Infusion

Anakinra 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 every 12 hours thereafter for a total of 14 doses (unless renal function-related dose reduction is required.)

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 will be analyzed. If at least single organ dysfunction persists, and the subject qualifies for study drug by laboratory analysis, randomization will be performed on Study Day 2 (instead of Study Day 1). Administration of study drug would begin on Study Day 2 and continue for a total of 14 doses (7 days after randomization). Shipping delays of more than 24 hours would result in the subject being withdrawn from the study.

4.3.2 Justification for IV Route of Administration

Anakinra is currently approved for administration by the subcutaneous (SQ) route, though it can also be given by the intravenous (IV) route. All studies of anakinra for the treatment of sepsis to date have been done using the IV route. This is because sepsis and sepsis-induced MODS are characterized by abnormalities in tissue perfusion and edema, both of which dramatically affect drug absorption when given by the SQ route. Pediatric sepsis, in particular, is frequently accompanied by peripheral vasoconstriction that would significantly decrease SQ drug absorption.[11] For the purposes of ensuring similar bioavailability across all study subjects, it will be essential to deliver anakinra via the IV route in the TRIPS trial. Further, patients with sepsis-induced MODS may have coagulopathy that would predispose them to bleeding with SQ injections. Intravenous administration will eliminate these risks, along with risk of SQ injection site reactions which have been commonly reported in adults and children. In addition to the adult sepsis trials, anakinra has been reported to be safely administered by the IV route in children with HLH/MAS,[12, 55, 57] adults with HLH/MAS,,[34, 46] adults with acute stroke,[17] adults with acute traumatic brain injury,[31] and adults with COVID-19.[9, 10, 15, 23, 27, 40, 41, 47, 56]

Utilizing a sparse-sampling approach, we will measure anakinra concentrations by enzyme-linked immunosorbent assay in blood samples of subjects who are randomized to study drug. During each immune function testing, one blood sample per subject will be obtained. We anticipate that this sampling will occur in subjects at various times during the dosing interval. As such, this sparse sampling strategy will provide adequate data for a population pharmacokinetic modeling approach that will describe anakinra pharmacokinetic parameters in critically ill children and evaluate the effects of organ dysfunction and systemic inflammation on drug disposition.

4.3.3 Justification for Anakinra Dosing Strategy

The dose of anakinra that might be effective in improving outcomes in hyper-inflamed children with sepsis-induced MODS is unknown. Doses at or above the highest FDA-approved dose (8mg/kg/day) have been frequently reported to be required for symptom control in children with inflammatory syndromes.[12, 37, 40, 41, 55, 57] In the 1994 and 1997 adult sepsis trials, a dose of 48 mg/kg/day given for three days by continuous infusion was used.[21, 53] Despite its apparent safety in adults, this dose is substantially higher than what has been used in children with HLH/MAS or adults with COVID-19.

In the largest (to date) published study of anakinra in hospitalized, hyper-inflamed adults with COVID-19 (N=392), a dose of 10 mg/kg/day IV divided every 12 hours (infused over 1 hour) was associated with lower mortality compared to contemporaneous controls (hazard ratio 0.45, 95% CI 0.20 – 0.99, p=0.047).[10] This was in agreement with a prior pilot study (N=45) that showed lower mortality in the same population with this dosing strategy (10% vs 40%, p=0.009).[9] No drug-attributable adverse events (including no increase in risk of nosocomial infection) were reported in these studies. The Cavalli studies[9, 10] (10 mg/kg/day) suggested beneficial effect in adults with COVID-19 who had elevations in serum ferritin or CRP levels similar to those targeted in the TRIPS trial. The adult sepsis trials showed similar benefit of anakinra in subjects with severe inflammation, but it is not clear if doses of 48 mg/kg/day will be safe in the pediatric population, nor is it clear that doses that high are necessary to improve outcomes from pediatric sepsis-induced MODS.

The optimal duration of anakinra therapy for hyper-inflamed children with sepsis-induced MODS is similarly unknown. The adult sepsis studies utilized a continuous infusion over 72 hours (for a total of 144 mg/kg). Those studies targeted the acute phase of sepsis (within 24 hours of sepsis onset) whereas the TRIPS trial targets the subacute phase of sepsis-induced MODS. Our prior work with immunomodulation in pediatric MODS suggests that 7 days of treatment is effective in normalizing immune function,[29] and the duration of anakinra treatment reported in adult COVID-19 studies has ranged from 5 days to 14 days (where treatment duration is specified).

The TRIPS trial will therefore adopt an adaptive dosing strategy that includes the FDA-approved dosing range and extends it to include doses that have been associated with benefit in HLH/MAS and COVID-19. Subjects who meet criteria for receiving study drug in the TRIPS trial will be adaptively randomized to receive placebo or anakinra at a dose of 4, 8, 12, or 16 mg/kg/day IV divided every 12 hours (each dose infused over 1 hour) for 7 days. Initial randomization allocation will be skewed toward lower doses, with increasing proportions of enrollments being allocated to higher doses over time if there is a favorable safety profile with

lower doses and a suggestion of benefit with higher doses.

The q12 hour dosing approach was chosen for the following reasons:

- Due to a lower number of available IV lumens in critically ill children (compared to adults), and with a relative lack of drug compatibility data, it would be unfeasible to occupy a dedicated IV line for 7 days. Intermittent dosing removes these barriers.
- We conducted pharmacokinetic simulations on bolus vs infusion dosing regimens, using published pharmacokinetic data (allometrically scaled for pediatrics), and found that IV administration over 1 hour is predicted to result in a C_{max} across our dosing range that is similar to those seen in the adult sepsis trials, but with a similar area under the concentration curve (AUC) to IV bolus dosing (in which C_{max} would be substantially higher). The predicted C_{max} with a 1-hour infusion are similar to those seen in adult studies in which there were no drug-attributable adverse events.

4.3.4 Dose Adjustment for Severe Renal Dysfunction

Anakinra is eliminated through both receptor-based clearance and renal clearance.[72] The package insert recommends a 50% dose reduction (switching from daily to every-other-day dosing) in patients with a creatinine clearance (CrCL)< 30 ml/min. We will therefore plan a 50% dose reduction in subjects with CrCL < 30 ml/min. Our simulations predict that, in our patient population with severe renal dysfunction, adopting a once-daily dosing approach using 50% of the assigned dose, will result in a C_{max} and AUC that are similar to subjects with intact renal function who are receiving the full assigned dose.

4.3.5 Pharmacy Function and Monitoring

The investigational pharmacy at Nationwide Children's Hospital will serve as the central pharmacy for the TRIPS study. They will intake anakinra from the manufacturer (Sobi), 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. 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 TRIPS trial is a double-blind trial. Subjects will receive anakinra or normal saline placebo every 12 hours for seven days. All active drug and placebo doses are intravenous. Because varying doses of anakinra are being evaluated, the drug will be diluted to the equivalent volume of the highest possible dose (16 mg/kg/day) by the investigational pharmacies prior to dispensing in order to maintain blinding. The saline placebo will be similarly dispensed in a volume to match what would be the volume of the 16 mg/kg/day dose of anakinra.

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 1 on page 28. 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 +/- PK			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 1: Schedule of study events and data collection.

6 Statistical Summary

6.1 TRIPS Randomization and Power Estimation

This study will utilize response adaptive randomization which updates allocation proportions throughout the trial to preferentially allocate patients to arms appearing superior based on accumulating data.[68] We will utilize the "Goldilocks" principle of frequent looks at the accumulating data to frequently alter the randomization proportions often and stop when the sample size is "just right".

Response adaptive randomization is used to allocate more patients to an active arm that appears to be superior based on the accumulating data. The proportion of patients allocated to placebo will be fixed throughout enrollment (described in more detail below). Once response adaptive randomization begins, patients will be randomized in block sizes of 30 among the four doses of anakinra (4 mg/kg/day, 8 mg/kg/day, 12 mg/kg/day, 16 mg/kg/day). The blocks will be re-defined after each 30-patient interval starting at 180, based on the current estimates of the E_{max} dose response curve. All slots in the block will be allocated adaptively. The proportion will be determined based on simulation-based operating characteristics. At each interim, we will calculate *pr(ED85)* for each arm using the currently available outcome data. To avoid assigning patients to an arm with a minimal chance of being the best dose, any allocation probability less than 0.05 is set to zero at that interim and the resulting probability is reallocated among the remaining arms. In this manner, an anakinra dose may be temporarily dropped but may be re-introduced if the response adaptive randomization probability increases at subsequent interims. The placebo dose will never be dropped.

Throughout the study, 50% of patients will be allocated to placebo regardless of what happens in the response adaptive randomization. This proportion will be chosen based on the optimization of the operating characteristics in simulations. In order to establish an initial safety profile, a larger proportion of patients will be initially allocated to lower anakinra doses (i.e., 4 mg/kg/day and 8 mg/kg/day) compared to the higher doses (i.e., 12 mg/kg/day and 16 mg/kg/day). After the first 180 patients are enrolled and a full safety review by the DSMB of each dose, the randomization probabilities will be statistically updated based on the response adaptive randomization described above. The goal is to allocate more subjects to the efficacious arms while minimizing the allocations to higher doses when lower doses are almost equivalent in efficacy. The allocations will be updated based on the collective study data. Allocation proportions will not be stratified by site nor any other clinical variable. Additional details can be found in the Statistical Analysis Plan.

We have studied the immunology of pediatric sepsis-induced MODS in single- and multi-center populations and have found the incidence of immunoparalysis to be 48-53% in children who have sepsis-induced MODS using the Proulx criteria. This includes the CPCCRN network's GRACE-1 study (48%), Drs. Hall and Zuppa's multi-center ongoing PARADIGM study (50% to date), and Dr. Hall's recent 102-patient single-center cohort of children with severe sepsis (53%). Among the 401 children with severe sepsis/septic shock in CPCCRN's recently-completed multi-center PHENOMS study (289 of whom had sepsis-induced MODS), 96% of the cohort had a CRP > 4 mg/dl and/or a ferritin level > 500 ng/ml. Of those, 5% would not qualify for the TRIPS trial by virtue of very severe hyperferritinemia. We anticipate that approximately 50% of immunophenotyped patients will qualify for entry into the TRIPS trial. We plan to enroll up to 1095 patients into the overall study in order to accrue 500 patients into the TRIPS trial.

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 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. Different non-informative priors

for the TRIPS analysis may be used to reflect the different scales.

6.2.3 Exploratory Outcomes

For the TRIPS trial, mortality rates, and other binary outcomes, will be compared between the placebo and active arms using an E_{max} dose-response model with a logit link function. In particular, the model for the primary outcome will be transformed such that the probability of the event occurring is defined:

$$P_d = \frac{e^{\theta_d}}{1 + e^{\theta_d}}$$

The remaining parameters and priors are the same except the variance parameter is excluded due to the natural relationship between the mean and variance with the logit link. MODS-free and organ-dysfunction-free days will be compared in a rank-based fashion similar to the primary outcome.

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 TRIPS trial (rate of nosocomial infection) will be compared between arms using Poisson regression using Generalized Estimating Equations with arm as the primary predictor and number of days of exposure as the offset. Nosocomial infection rates will also be summarized using the pooled number of infections per 1000 ICU days. Specific thresholds and/or patterns of systemic inflammatory biomarker elevation and nosocomial infection rates that would merit a pause in enrollment or study stoppage will be developed with the DSMB a priori and will be included in the DSMB charter.

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
- Immune subgroup (TRIPS trial)
  - Hyperinflammation without immunoparalysis
  - Ferritin > 2,000ng/ml with immunoparalysis

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. En-

vironmental 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 ≥ 40 weeks corrected gestational age. The inclusion and exclusion criteria are listed in Section 3 on page 18.

Subjects enrolled in sepsis-induced MODS trials face significant mortality and morbidity from the sepsis-induced MODS, and the study drug (anakinra) 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 and CRP. 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 and CRP the next morning. The results will be double checked to assure correct immunophenotype assignment, verifying eligibility for participation in TRIPS.

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

Anakinra. Anakinra is FDA-approved for the treatment of rheumatoid arthritis (RA) and cryopyrin-associated pediatric syndromes (PDA). Since anakinra will be given by the IV route in the TRIPS trial, we will not have the risk of injection sites reactions which occur in up to 71% of subcutaneous injection recipients. Anakinra has been associated with an increased incidence of serious infections (2%) vs. placebo (< 1%) in clinical trials of long-term use in RA. The other most serious adverse reaction with prolonged use in RA was neutropenia, particularly when used in combination with TNF blocking agents. We do not expect to see these adverse events in this study given the short duration of treatment (7 days). Hypersensitivity reactions, including anaphylactic reactions and angioedema, have been reported rarely.

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 43).

Anakinra is highly specific for a single cytokine signaling pathway (IL-1β), leaving other signaling pathways for ongoing host defense. While increased infection risk is possible with prolonged use of anakinra, we are using it for a very short period of time (7 days) and are restricting its use to subjects with documented hyperinflammation. We are also excluding children with severely impaired innate immune function (immunoparalysis) in the setting of mild to moderate inflammation, as well as those who have severe leukopenia due to myeloablative therapy. We will not be starting study drug until at least 3 days following the onset of sepsis, during which subjects will have been receiving antimicrobial therapy. It is therefore highly unlikely that we will be giving anakinra to children with uncontrolled infection. Increased nosocomial infection risk has not been seen in adult studies of sepsis, stroke, TBI, or COVID-19 in which the cumulative dose of anakinra was up to three times higher than the highest dose to be used in the TRIPS trial.

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 34.

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 TRIPS, it is not known if anakinra 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.

Criteria for halting enrollment in the TRIPS trial pending a formal safety review include:

- 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 Individual Subject Stopping Rules for TRIPS Trial

The administration of study drug will be discontinued in individual subjects if they develop:

- A new, unexpected, serious adverse event that is determined to be probably related to study drug.
- A new reduction in absolute neutrophil count to < 1,000 cells/mm^3 on two consecutive days while receiving study drug.

10.2.8 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.

10.2.9 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 TRIPS 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.

13 References

14 Bibliography

[1] E. Abraham, A. Anzueto, G. Gutierrez, S. Tessler, G. San Pedro, R. Wunderink, A. Dal Nogare, S. Nasraway, S. Berman, R. Cooney, H. Levy, R. Baughman, M. Rumbak, R. B. Light, L. Poole, R. Allred, J. Constant, J. Pennington, and S. Porter. Double-blind

randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. *Lancet*, 351(9107):929–33, 1998.

[2] E. Abraham, M. P. Glauser, T. Butler, J. Garbino, D. Gelmont, P. F. Laterre, K. Kudsk, H. A. Bruining, C. Otto, E. Tobin, C. Zwingelstein, W. Lesslauer, and A. Leighton. p55 Tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. a randomized controlled multicenter trial. Ro 45-2081 Study Group. *JAMA*, 277(19):1531–8, 1997.

[3] C. E. Allen, X. Yu, C. A. Kozinetz, and K. L. McClain. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*, 50(6):1227–35, 2008.

[4] F. Barkas, S. F. Ntekouan, M. Kosmidou, E. Liberopoulos, A. Liontos, and H. Milionis. Anakinra in hospitalized non-intubated patients with Coronavirus Disease 2019: a systematic review and meta-analysis. *Rheumatology (Oxford)*, 2021.

[5] J. P. Burns, D. E. Sellers, E. C. Meyer, M. Lewis-Newby, and R. D. Truog. Epidemiology of death in the PICU at five U.S. teaching hospitals. *Crit Care Med*, 42(9):2101–8, 2014.

[6] J. A. Carcillo, R. A. Berg, D. Wessel, M. Pollack, K. Meert, M. Hall, C. Newth, J. C. Lin, A. Doctor, T. Shanley, T. Cornell, R. E. Harrison, A. F. Zuppa, R. W. Reeder, R. Banks, J. A. Kellum, R. Holubkov, D. A. Notterman, J. M. Dean, H. Eunice Kennedy Shriver National Institute of Child, and N. Human Development Collaborative Pediatric Critical Care Research. A multicenter network assessment of three inflammation phenotypes in pediatric sepsis-induced multiple organ failure. *Pediatr Crit Care Med*, 20(12):1137–1146, December 2019.

[7] J. A. Carcillo, E. S. Halstead, M. W. Hall, T. C. Nguyen, R. Reeder, R. Aneja, B. Shakoory, D. Simon, H. Eunice Kennedy Shriver National Institute of Child, and I. Human Development Collaborative Pediatric Critical Care Research Network. Three hypothetical inflammation pathobiology phenotypes and pediatric sepsis-induced multiple organ failure outcome. *Pediatr Crit Care Med*, 18(6):513–523, 2017.

[8] J. A. Carcillo, K. K. Kernan, C. M. Horvat, D. W. Simon, and R. K. Aneja. Why and how is hyperferritinemic sepsis different from sepsis without hyperferritinemia? *Pediatr Crit Care Med*, 21(5):509–512, 2020.

[9] G. Cavalli, G. De Luca, C. Campochiaro, E. Della-Torre, M. Ripa, D. Canetti, C. Oltolini, B. Castiglioni, C. Tassan Din, N. Boffini, A. Tomelleri, N. Farina, A. Ruggeri, P. Rovere-Querini, G. Di Lucca, S. Martinenghi, R. Scotti, M. Tresoldi, F. Ciceri, G. Landoni,

A. Zangrillo, P. Scarpellini, and L. Dagna. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol*, 2(6):e325–e331, 2020.

[10] G. Cavalli, A. Larcher, A. Tomelleri, C. Campochiaro, E. Della-Torre, G. De Luca, N. Farina, N. Boffini, A. Ruggeri, A. Poli, P. Scarpellini, P. Rovere-Querini, M. Tresoldi, A. Salonia, F. Montorsi, G. Landoni, A. Castagna, F. Ciceri, A. Zangrillo, and L. Dagna. Interleukin-1 and interleukin-6 inhibition compared with standard management in patients with COVID-19 and hyperinflammation: a cohort study. *Lancet Rheumatol*, 2021.

[11] G. Ceneviva, J. A. Paschall, F. Maffei, and J. A. Carcillo. Hemodynamic support in fluid-refractory pediatric septic shock. *Pediatrics*, 102(2):e19, 1998.

[12] J. E. G. Charlesworth, S. Wilson, A. Qureshi, E. Blanco, A. Mitchell, S. Segal, D. Kelly, J. Weitz, D. O'Shea, K. Bailey, and A. Kavirayani. Continuous intravenous anakinra for treating severe secondary haemophagocytic lymphohistiocytosis/macrophage activation syndrome in critically ill children. *Pediatr Blood Cancer*, page e29102, 2021.

[13] J. Cohen and J. Carlet. INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. *Crit Care Med*, 24(9):1431–40, 1996.

[14] J. S. Cornish, E. Wirthgen, and J. Dabritz. Biomarkers predictive of response to thiopurine therapy in inflammatory bowel disease. *Front Med (Lausanne)*, 7:8, 2020.

[15] E. Della-Torre, M. Lanzillotta, C. Campochiaro, G. Cavalli, G. De Luca, A. Tomelleri, N. Boffini, R. De Lorenzo, A. Ruggeri, P. Rovere-Querini, A. Castagna, G. Landoni, M. Tresoldi, F. Ciceri, A. Zangrillo, and L. Dagna. Respiratory impairment predicts response to IL-1 and IL-6 blockade in COVID-19 patients with severe pneumonia and hyper-inflammation. *Front Immunol*, 12:675678, 2021.

[16] K. J. Downes, J. C. Fitzgerald, E. Schriver, C. L. K. Boge, M. E. Russo, S. L. Weiss, F. Balamuth, S. E. Kubis, P. Tolomeo, W. B. Bilker, J. H. Han, E. Lautenbach, S. E. Coffin, and J. S. Gerber. Implementation of a pragmatic biomarker-driven algorithm to guide antibiotic use in the pediatric intensive care unit: the Optimizing Antibiotic Strategies in Sepsis (OASIS) II Study. *J Pediatric Infect Dis Soc*, 9(1):36–43, 2020.

[17] H. C. Emsley, C. J. Smith, R. F. Georgiou, A. Vail, S. J. Hopkins, N. J. Rothwell, P. J. Tyrrell, and I. Acute Stroke. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. *J Neurol Neurosurg Psychiatry*, 76(10):1366–72, 2005.

[18] A. M. Fein, G. R. Bernard, G. J. Criner, E. C. Fletcher, J. Good, J. T., W. A. Knaus, H. Levy, G. M. Matuschak, H. M. Shanies, R. W. Taylor, and T. C. Rodell. Treatment of severe systemic inflammatory response syndrome and sepsis with a novel bradykinin antagonist, deltibant (CP-0127). results of a randomized, double-blind, placebo-controlled trial. CP-0127 SIRS and Sepsis Study Group. *JAMA*, 277(6):482–7, 1997.

[19] J. Fernandez-Sarmiento, D. De Souza, R. Jabornisky, G. A. Gonzalez, M. D. P. Arias Lopez, and G. Palacio. Paediatric inflammatory multisystem syndrome temporally associated with COVID-19 (PIMS-TS): a narrative review and the viewpoint of the Latin American Society of Pediatric Intensive Care (SLACIP) Sepsis Committee. *BMJ Paediatr Open*, 5(1):e000894, 2021.

[20] A. Fiore-Gartland, A. Panoskaltsis-Mortari, A. A. Agan, A. J. Mistry, P. G. Thomas, M. A. Matthay, P. P. Investigators, T. Hertz, and A. G. Randolph. Cytokine profiles of severe influenza virus-related complications in children. *Front Immunol*, 8:1423, 2017.

[21] J. Fisher, C. J., J. F. Dhainaut, S. M. Opal, J. P. Pribble, R. A. Balk, G. J. Slotman, T. J. Iberti, E. C. Rackow, M. J. Shapiro, R. L. Greenman, and et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA*, 271(23):1836–43, 1994.

[22] J. Fisher, C. J., G. J. Slotman, S. M. Opal, J. P. Pribble, R. C. Bone, G. Emmanuel, D. Ng, D. C. Bloedow, and M. A. Catalano. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. *Crit Care Med*, 22(1):12–21, 1994.

[23] M. Franzetti, A. Forastieri, N. Borsa, A. Pandolfo, C. Molteni, L. Borghesi, S. Pontiggia, G. Evasi, L. Guiotto, M. Erba, U. Pozzetti, A. Ronchetti, L. Valsecchi, G. Castaldo, E. Longoni, D. Colombo, M. Soncini, S. Crespi, S. Maggiolini, D. Guzzon, and S. Piconi. IL-1 receptor antagonist anakinra in the treatment of COVID-19 acute respiratory distress syndrome: A retrospective, observational study. *J Immunol*, 2021.

[24] J. Galea, K. Ogungbenro, S. Hulme, H. Patel, S. Scarth, M. Hoadley, K. Illingworth, C. J. McMahon, N. Tzerakis, A. T. King, A. Vail, S. J. Hopkins, N. Rothwell, and P. Tyrrell. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. *J Neurosurg*, 128(2):515–523, 2018.

[25] C. A. Gogos, E. Drosou, H. P. Bassaris, and A. Skoutelis. Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. *J Infect Dis*, 181(1):176–80, 2000.

[26] R. Goldbach-Mansky, N. J. Dailey, S. W. Canna, A. Gelabert, J. Jones, B. I. Rubin, H. J. Kim, C. Brewer, C. Zalewski, E. Wiggs, S. Hill, M. L. Turner, B. I. Karp, I. Aksentijevich, F. Pucino, S. R. Penzak, M. H. Haverkamp, L. Stein, B. S. Adams, T. L. Moore, R. C. Fuhlbrigge, B. Shaham, J. N. Jarvis, K. O'Neil, R. K. Vehe, L. O. Beitz, G. Gardner, W. P. Hannan, R. W. Warren, W. Horn, J. L. Cole, S. M. Paul, P. N. Hawkins, T. H. Pham, C. Snyder, R. A. Wesley, S. C. Hoffmann, S. M. Holland, J. A. Butman, and D. L. Kastner. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N Engl J Med*, 355(6):581–92, 2006.

[27] C. Group. Effect of anakinra versus usual care in adults in hospital with COVID-19 and mild-to-moderate pneumonia (CORIMUNO-ANA-1): a randomised controlled trial. *Lancet Respir Med*, 9(3):295–304, 2021.

[28] M. W. Hall, S. M. Geyer, C. Y. Guo, A. Panoskaltsis-Mortari, P. Jouvet, J. Ferdinands, D. K. Shay, J. Nateri, K. Greathouse, R. Sullivan, T. Tran, S. Keisling, and A. G. Randolph. Innate immune function and mortality in critically ill children with influenza: a multicenter study. *Crit Care Med*, 41(1):224–36, 2013.

[29] M. W. Hall, N. L. Knatz, C. Vetterly, S. Tomarello, M. D. Wewers, H. D. Volk, and J. A. Carcillo. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. *Intensive Care Med*, 37(3):525–532, 2011.

[30] R. Harwood, B. Allin, C. E. Jones, E. Whittaker, P. Ramnarayan, A. V. Ramanan, M. Kaleem, R. Tulloh, M. J. Peters, S. Almond, P. J. Davis, M. Levin, A. Tometzki, S. N. Faust, M. Knight, S. Kenny, and P.-T. N. C. M. S. Group. A national consensus management pathway for paediatric inflammatory multisystem syndrome temporally associated with covid-19 (pims-ts): results of a national delphi process. *Lancet Child Adolesc Health*, 5(2):133–141, 2021.

[31] A. Helmy, M. R. Guilfoyle, K. L. Carpenter, J. D. Pickard, D. K. Menon, and P. J. Hutchinson. Recombinant human interleukin-1 receptor antagonist in severe traumatic brain injury: a phase II randomized control trial. *J Cereb Blood Flow Metab*, 34(5):845–51, 2014.

[32] L. A. Henderson, S. W. Canna, K. G. Friedman, M. Gorelik, S. K. Lapidus, H. Bassiri, E. M. Behrens, A. Ferris, K. F. Kernan, G. S. Schulert, P. Seo, M. B. F. Son, A. H. Tremoulet, R. S. M. Yeung, A. S. Mudano, A. S. Turner, D. R. Karp, and J. J. Mehta.

American College of Rheumatology Clinical Guidance for Multisystem Inflammatory Syndrome in Children Associated With SARS-CoV-2 and Hyperinflammation in Pediatric COVID-19: Version 2. *Arthritis Rheumatol*, 73(4):e13–e29, 2021.

[33] A. M. Hung, C. D. Ellis, A. Shintani, C. Booker, and T. A. Ikizler. Il-1beta receptor antagonist reduces inflammation in hemodialysis patients. *J Am Soc Nephrol*, 22(3):437–42, 2011.

[34] A. Kavirayani, J. E. G. Charlesworth, S. Segal, D. Kelly, S. Wilson, A. Qureshi, E. Blanco, J. Weitz, D. O'Shea, and K. Bailey. The Lazarus effect of very high-dose intravenous anakinra in severe non-familial CNS-HLH. *Lancet Rheumatol*, 2(12):e736–e738, 2020.

[35] J. A. Kellum, L. Kong, M. P. Fink, L. A. Weissfeld, D. M. Yealy, M. R. Pinsky, J. Fine, A. Krichevsky, R. L. Delude, and D. C. Angus. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med*, 167(15):1655–63, 2007.

[36] K. F. Kernan, L. Ghaloul-Gonzalez, B. Shakoory, J. A. Kellum, D. C. Angus, and J. A. Carcillo. Adults with septic shock and extreme hyperferritinemia exhibit pathogenic immune variation. *Genes Immun*, 20(6):520–526, 2019.

[37] S. R. Kim, T. Kissoon-Larkin, B. Horn, and M. Elder. Anakinra as an agent to control hemophagocytic lymphohistiocytosis in Griscelli type 2. *Pediatr Blood Cancer*, 66(12):e27997, 2019.

[38] W. A. Knaus, J. Harrell, F. E., J. F. LaBrecque, D. P. Wagner, J. P. Pribble, E. A. Draper, J. Fisher, C. J., and L. Soll. Use of predicted risk of mortality to evaluate the efficacy of anticytokine therapy in sepsis. The rhIL-1ra Phase III Sepsis Syndrome Study Group. *Crit Care Med*, 24(1):46–56, 1996.

[39] I. Kone-Paut, R. Cimaz, J. Herberg, O. Bates, A. Carbasse, J. P. Saulnier, M. C. Maggio, J. Anton, and M. Piram. The use of interleukin 1 receptor antagonist (anakinra) in Kawasaki disease: A retrospective cases series. *Autoimmun Rev*, 17(8):768–774, 2018.

[40] E. J. Kooistra, N. J. B. Waalders, I. Grondman, N. A. F. Janssen, A. H. de Nooijer, M. G. Netea, F. L. van de Veerdonk, E. Ewalds, J. G. van der Hoeven, M. Kox, P. Pickkers, and R.-C.-S. Group. Anakinra treatment in critically ill COVID-19 patients: a prospective cohort study. *Crit Care*, 24(1):688, 2020.

[41] E. J. Kooistra, N. J. B. Waalders, M. Kox, and P. Pickkers. Effect of anakinra in COVID-19. *Lancet Rheumatol*, 2(9):e523–e524, 2020.

[42] S. Leteurtre, A. Duhamel, J. Salleron, B. Grandbastien, J. Lacroix, and F. Leclerc. PELOD-2: an update of the PEdiatric Logistic Organ Dysfunction score. *Crit Care Med*, 41(7):1761–73, 2013.

[43] L. McCulloch, S. M. Allan, H. C. Emsley, C. J. Smith, and B. W. McColl. Interleukin-1 receptor antagonist treatment in acute ischaemic stroke does not alter systemic markers of anti-microbial defence. *F1000Res*, 8:1039, 2019.

[44] C. Meisel, J. C. Schefold, R. Pschowski, T. Baumann, K. Hetzger, J. Gregor, S. Weber-Carstens, D. Hasper, D. Keh, H. Zuckermann, P. Reinke, and H. D. Volk. GM-CSF to reverse sepsis-associated immunosuppression: A double-blind randomized placebo-controlled multicenter trial. *Am J Respir Crit Care Med*, 2009.

[45] N. J. Meyer, J. P. Reilly, B. J. Anderson, J. A. Palakshappa, T. K. Jones, T. G. Dunn, M. G. S. Shashaty, R. Feng, J. D. Christie, and S. M. Opal. Mortality benefit of recombinant human interleukin-1 receptor antagonist for sepsis varies by initial interleukin-1 receptor antagonist plasma concentration. *Crit Care Med*, 46(1):21–28, 2018.

[46] L. A. Monteagudo, A. Boothby, and E. Gertner. Continuous intravenous anakinra infusion to calm the cytokine storm in macrophage activation syndrome. *ACR Open Rheumatol*, 2(5):276–282, 2020.

[47] G. Monti, C. Campochiaro, A. Zangrillo, A. M. Scandroglio, E. Fominskiy, G. Cavalli, G. Landoni, L. Beretta, M. Mucci, M. G. Calabro, M. Pieri, P. Nardelli, M. Sartorelli, M. Baiardo Redaelli, F. Morselli, A. Serpa Neto, R. Bellomo, L. Dagna, and C. O.-B. S. Group. Immunosuppressive strategies in invasively ventilated ARDS COVID-19 patients. *Minerva Anestesiol*, 2021.

[48] A. C. Morton, A. M. Rothman, J. P. Greenwood, J. Gunn, A. Chase, B. Clarke, A. S. Hall, K. Fox, C. Foley, W. Banya, D. Wang, M. D. Flather, and D. C. Crossman. The effect of interleukin-1 receptor antagonist therapy on markers of inflammation in non-ST elevation acute coronary syndromes: the MRC-ILA Heart Study. *Eur Heart J*, 36(6):377–84, 2015.

[49] J. A. Muszynski, R. Nofziger, M. Moore-Clingenpeel, K. Greathouse, L. Anglim, L. Steele, J. Hensley, L. Hanson-Huber, J. Nateri, O. Ramilo, and M. W. Hall. Early immune function and duration of organ dysfunction in critically ill children with sepsis. *Am J Respir Crit Care Med*, 198(3):361–369, 2018.

[50] B. Neven, I. Marvillet, C. Terrada, A. Ferster, N. Boddaert, V. Couloignier, G. Pinto, A. Pagnier, C. Bodemer, B. Bodaghi, M. Tardieu, A. M. Prieur, and P. Quartier. Long-term

efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome. *Arthritis Rheum*, 62(1):258–67, 2010.

[51] G. Noguchi, N. Nakaigawa, S. Umemoto, K. Kobayashi, Y. Shibata, S. Tsutsumi, M. Yasui, S. Ohtake, T. Suzuki, K. Osaka, K. Muraoka, H. Hasumi, K. Kondo, Y. Igarashi, T. Sasada, T. Kishida, and M. Yao. C-reactive protein at 1 month after treatment of nivolumab as a predictive marker of efficacy in advanced renal cell carcinoma. *Cancer Chemother Pharmacol*, 86(1):75–85, 2020.

[52] T. Novak, M. W. Hall, D. R. McDonald, M. M. Newhams, A. J. Mistry, A. Panoskaltsis-Mortari, P. M. Mourani, L. L. Loftis, S. L. Weiss, K. M. Tarquinio, B. Markovitz, M. E. Hartman, A. Schwarz, W. G. Junger, A. G. Randolph, and P. P. I. C. I. N. Investigators. RIG-I and TLR4 responses and adverse outcomes in pediatric influenza-related critical illness. *J Allergy Clin Immunol*, 145(6):1673–1680 e11, 2020.

[53] S. M. Opal, J. Fisher, C. J., J. F. Dhainaut, J. L. Vincent, R. Brase, S. F. Lowry, J. C. Sadoff, G. J. Slotman, H. Levy, R. A. Balk, M. P. Shelly, J. P. Pribble, J. F. LaBrecque, J. Lookabaugh, H. Donovan, H. Dubin, R. Baughman, J. Norman, E. DeMaria, K. Matzel, E. Abraham, and M. Seneff. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. *Crit Care Med*, 25(7):1115–24, 1997.

[54] L. Pasin, G. Cavalli, P. Navalesi, N. Sella, G. Landoni, A. G. Yavorovskiy, V. V. Likhvantsev, A. Zangrillo, L. Dagna, and G. Monti. Anakinra for patients with COVID-19: a meta-analysis of non-randomized cohort studies. *Eur J Intern Med*, 2021.

[55] O. Phadke, K. Rouster-Stevens, H. Giannopoulos, S. Chandrakasan, and S. Prahalad. Intravenous administration of anakinra in children with macrophage activation syndrome. *Pediatr Rheumatol Online J*, 19(1):98, 2021.

[56] E. Pontali, S. Volpi, A. Signori, G. Antonucci, M. Castellaneta, D. Buzzi, A. Montale, M. Bustaffa, A. Angelelli, R. Caorsi, E. Giambruno, N. Bobbio, M. Feasi, I. Gueli, F. Tricerri, F. Calautti, E. Castagnola, A. Moscatelli, G. A. Rollandi, A. Ravelli, G. Cassola, M. P. Sormani, and M. Gattorno. Efficacy of early anti-inflammatory treatment with high doses of intravenous anakinra with or without glucocorticoids in patients with severe COVID-19 pneumonia. *J Allergy Clin Immunol*, 2021.

[57] S. Rajasekaran, K. Kruse, K. Kovey, A. T. Davis, N. E. Hassan, A. N. Ndika, S. Zuiderveen, and J. Birmingham. Therapeutic role of anakinra, an interleukin-1 receptor antagonist, in

the management of secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction/macrophage activating syndrome in critically ill children*. *Pediatr Crit Care Med*, 15(5):401–8, 2014.

[58] A. J. Rosenbloom, P. K. Linden, A. Dorrance, N. Penkosky, M. H. Cohen-Melamed, and M. R. Pinsky. Effect of granulocyte-monocyte colony-stimulating factor therapy on leukocyte function and clearance of serious infection in non-neutropenic patients. *Chest*, 127(6):2139–50, 2005.

[59] G. S. Schulert, F. Minoia, J. Bohnsack, R. Q. Cron, S. Hashad, E. P. I. Kon, M. Kostik, D. Lovell, D. Maritsi, P. A. Nigrovic, P. Pal, A. Ravelli, M. Shimizu, V. Stanevicha, S. Vastert, A. Woerner, F. de Benedetti, and A. A. Grom. Effect of biologic therapy on clinical and laboratory features of macrophage activation syndrome associated with systemic juvenile idiopathic arthritis. *Arthritis Care Res (Hoboken)*, 70(3):409–419, 2018.

[60] B. Shakoory, J. A. Carcillo, W. W. Chatham, R. L. Amdur, H. Zhao, C. A. Dinarello, R. Q. Cron, and S. M. Opal. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: Reanalysis of a prior phase III trial. *Crit Care Med*, 44(2):275–81, 2016.

[61] T. P. Shanley, N. Cvijanovich, R. Lin, G. L. Allen, N. J. Thomas, A. Doctor, M. Kalyaraman, N. M. Tofil, S. Penfil, M. Monaco, K. Odoms, M. Barnes, B. Sakthivel, B. J. Aronow, and H. R. Wong. Genome-level longitudinal expression of signaling pathways and gene networks in pediatric septic shock. *Mol Med*, 13(9-10):495–508, 2007.

[62] C. H. Sibley, N. Plass, J. Snow, E. A. Wiggs, C. C. Brewer, K. A. King, C. Zalewski, H. J. Kim, R. Bishop, S. Hill, S. M. Paul, P. Kicker, Z. Phillips, J. G. Dolan, B. Widemann, N. Jayaprakash, F. Pucino, D. L. Stone, D. Chapelle, C. Snyder, J. A. Butman, R. Wesley, and R. Goldbach-Mansky. Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease treated with anakinra: a cohort study to determine three- and five-year outcomes. *Arthritis Rheum*, 64(7):2375–86, 2012.

[63] C. J. Smith, H. C. Emsley, C. T. Udeh, A. Vail, M. E. Hoadley, N. J. Rothwell, P. J. Tyrrell, and S. J. Hopkins. Interleukin-1 receptor antagonist reverses stroke-associated peripheral immune suppression. *Cytokine*, 58(3):384–9, 2012.

[64] C. J. Smith, S. Hulme, A. Vail, C. Heal, A. R. Parry-Jones, S. Scarth, K. Hopkins, M. Hoadley, S. M. Allan, N. J. Rothwell, S. J. Hopkins, and P. J. Tyrrell. SCIL-STROKE (Subcutaneous Interleukin-1 Receptor Antagonist in Ischemic Stroke): A Randomized Controlled Phase 2 Trial. *Stroke*, 49(5):1210–1216, 2018.

[65] M. K. R. Somagutta, M. K. Lourdes Pormento, P. Hamid, A. Hamdan, M. A. Khan, R. Desir, R. Vijayan, S. Shirke, R. Jeyakumar, Z. Dogar, S. S. Makkar, P. Guntipalli, N. N. Ngardig, M. S. Nagineni, T. Paul, E. Luvsannyam, C. Riddick, and M. A. Sanchez-Gonzalez. The safety and efficacy of anakinra, an interleukin-1 antagonist in severe cases of COVID-19: A systematic review and meta-analysis. *Infect Chemother*, 53(2):221–237, 2021.

[66] N. M. Ter Haar, E. H. P. van Dijkhuizen, J. F. Swart, A. van Royen-Kerkhof, A. El Idrissi, A. P. Leek, W. de Jager, M. C. H. de Groot, S. Haitjema, D. Holzinger, D. Foell, J. van Loosdregt, N. M. Wulffraat, S. de Roock, and S. J. Vastert. Treatment to target using recombinant interleukin-1 receptor antagonist as first-line monotherapy in new-onset systemic juvenile idiopathic arthritis: Results from a five-year follow-up study. *Arthritis Rheumatol*, 71(7):1163–1173, 2019.

[67] B. W. Van Tassell, C. R. Trankle, J. M. Canada, S. Carbone, L. Buckley, D. Kadariya, M. G. Del Buono, H. Billingsley, G. Wohlford, M. Viscusi, C. Oddi-Erdle, N. A. Abouzaki, D. Dixon, G. Biondi-Zoccai, R. Arena, and A. Abbate. Il-1 blockade in patients with heart failure with preserved ejection fraction. *Circ Heart Fail*, 11(8):e005036, 2018.

[68] K. Viele, B. R. Saville, A. McGlothlin, and K. Broglio. Comparison of response adaptive randomization features in multiarm clinical trials with control. *Pharm Stat*, 2020.

[69] S. L. Weiss, F. Balamuth, J. Hensley, J. C. Fitzgerald, J. Bush, V. M. Nadkarni, N. J. Thomas, M. Hall, and J. Muszynski. The epidemiology of hospital death following pediatric severe sepsis: When, why, and how children with sepsis die. *Pediatr Crit Care Med*, 18(9):823–830, 2017.

[70] S. L. Weiss, M. J. Peters, W. Alhazzani, M. S. D. Agus, H. R. Flori, D. P. Inwald, S. Nadel, L. J. Schlapbach, R. C. Tasker, A. C. Argent, J. Brierley, J. Carcillo, E. D. Carrol, C. L. Carroll, I. M. Cheifetz, K. Choong, J. J. Cies, A. T. Cruz, D. De Luca, A. Deep, S. N. Faust, C. F. De Oliveira, M. W. Hall, P. Ishimine, E. Javouhey, K. F. M. Joosten, P. Joshi, O. Karam, M. C. J. Kneyber, J. Lemson, G. MacLaren, N. M. Mehta, M. H. Moller, C. J. L. Newth, T. C. Nguyen, A. Nishisaki, M. E. Nunnally, M. M. Parker, R. M. Paul, A. G. Randolph, S. Ranjit, L. H. Romer, H. F. Scott, L. N. Tume, J. T. Verger, E. A. Williams, J. Wolf, H. R. Wong, J. J. Zimmerman, N. Kissoon, and P. Tissieres. Surviving Sepsis Campaign International Guidelines for the management of septic shock and sepsis-associated organ dysfunction in children. *Pediatr Crit Care Med*, 21(2):e52–e106, 2020.

[71] H. R. Wong, N. Z. Cvijanovich, N. Anas, G. L. Allen, N. J. Thomas, M. T. Bigham, S. L. Weiss, J. Fitzgerald, P. A. Checchia, K. Meyer, M. Quasney, M. Hall, R. Gedeit,

R. J. Freishtat, J. Nowak, S. S. Raj, S. Gertz, K. Howard, K. Harmon, P. Lahni, E. Frank, K. W. Hart, T. C. Nguyen, and C. J. Lindsell. Pediatric Sepsis Biomarker Risk Model-II: Redefining the pediatric sepsis biomarker risk model with septic shock phenotype. *Crit Care Med*, 44(11):2010–2017, 2016.

[72] B. B. Yang, S. Baughman, and J. T. Sullivan. Pharmacokinetics of anakinra in subjects with different levels of renal function. *Clin Pharmacol Ther*, 74(1):85–94, 2003.

[73] E. Zittan, I. M. Gralnek, and M. S. Berns. The new proactive approach and precision medicine in Crohn's Disease. *Biomedicines*, 8(7), 2020.