Pilot Randomized Clinical Trial of Therapeutic Hypothermia Plus Neuromuscular Blockade vs. Standard of Care in Patients with Moderate to Severe ARDS – the Cooling to Help Injured lungs (CHILL) Pilot Study

NCT03376854

Study Protocol

Date: July 29, 2019
A. Background:

1. **Clinical Definition and Epidemiology of Acute Respiratory Distress Syndrome (ARDS)**: ARDS is a life-threatening condition often of rapid and unpredictable onset and characterized by hypoxemia and reduced lung compliance. The current clinical definition of ARDS is based on the 2012 Berlin criteria and include: (1) onset of respiratory failure within one week of a known precipitating illness or event; (2) bilateral pulmonary opacities on chest imaging not explained by effusions, atelectasis, or nodules; (3) respiratory failure not fully explained by cardiac failure or fluid overload; and (4) oxygenation defect. The severity of ARDS is based on the degree of oxygen transfer impairment, specifically the PaO$_2$/FiO$_2$ (P/F) ratio with at least 5 cm H$_2$O positive end-expiratory pressure (PEEP) provided. The P/F ratio is 200–300, 100–200, and less than 100 in mild, moderate, and severe ARDS, respectively. The risk factors for ARDS are shared by military and civilian populations and include hypertransfusion, severe sepsis, inhalational injuries, severe trauma, burns, and respiratory infections including influenza and Streptococcal pneumonia. Recent epidemiologic data show an incidence of 7-26 cases of ARDS per 100,000 person-years in Europe. Older studies suggest a much higher incidence in the USA with ~190,000 cases and ~74,000 deaths per year. ARDS occurs in 19-30% of combat injuries, including both direct thoracic trauma and peripheral injuries and burns. The ~40% mortality associated with moderate to severe ARDS is similar in military and civilian, and veteran populations. Those ARDS patients who survive have high morbidity rates related to impaired gas transfer, reduced exercise tolerance, depression, impaired cognition, and inability to work. Of additional relevance to the veteran population, older age and co-morbidities are associated with poorer outcome in patients with ARDS.

2. **Current Pathophysiology-directed Management**: ARDS is characterized by acute onset of non-hydrostatic pulmonary edema and refractory hypoxemia due to direct or indirect, predominantly neutrophil-mediated, injury to the alveolar epithelial and capillary endothelial barrier. Although the pathophysiology of ARDS has been extensively studied and a complex network of immunologic mediators has been implicated in its pathogenesis, no therapeutic agents to date have been shown to benefit patients with ARDS. Only two interventions have been shown to improve survival in Phase III randomized controlled trials (RCTs), low tidal volume mechanical ventilation and prone positioning. A third intervention, early neuromuscular blockade (NMB), improved adjusted 90-day survival in the ARDS et Curarisation Systematique (ACURASYS) trial, but the recently completed NHLBI Reevaluation of Systemic Early Neuromuscular Blockade (ROSE) trial failed to confirm benefit of NMB in ARDS. Of specific relevance to this grant application, both ROSE and ACURASYS trials demonstrated that early treatment with NMB in patients with ARDS caused no significant adverse effects, including ICU-acquired weakness. The role of extracorporeal gas exchange (ECMO) in ARDS, an expensive, labor-intensive therapy limited to tertiary care centers, has not yet been defined.

The rationale for low tidal volume ventilation is based on the recognition that cyclical recruitment, overdistention, and de-recruitment of alveoli by mechanical ventilation can itself cause neutrophil-dependent inflammation and injury even to previously normal lungs. The conceptual framework of ARDS pathogenesis and the contribution of mechanical ventilation has been further refined by Gattinoni et al. based on heterogeneous distribution of aeration and atelectasis in chest CT scans of patients with ARDS. Gattinoni proposed that regions of the ARDS lung, collectively referred to as the “baby lung,” retain near normal compliance and are thus disproportionately over-ventilated and predominantly responsible for gas exchange in the injured lung. The size of the “baby lung” fraction depends on the severity of ARDS and the position of the patient. Importantly, cyclic overdistension of these compliant lung units leads to progressive injury and reduction in size of the “baby lung,” so that the shrinking “baby lung” is subjected to progressively greater overdistension. Prone positioning is thought to increase the size of the “baby lung” by exploiting the geometry of the lung, thoracic cavity and mediastinum thereby distributing ventilation over more lung units. Thus, low tidal volume ventilation and prone positioning both reduce progression of lung injury by reducing cyclic overdistension of the most vulnerable lung units. ECMO may also reduce cyclic overdistension of vulnerable lung in ARDS by reducing the requirement for pulmonary gas exchange and mechanical ventilation.

3. **Theoretical Basis for Therapeutic Hypothermia (TH) in Management of ARDS**: If shivering is blocked by co-treatment with NMB, overall metabolism and requirement for pulmonary gas exchange is reduced by ~10% for...
each 1°C decrease in core temperature. Thus, TH can confer a benefit similar to ECMO by reducing the requirement for mechanical ventilation, but with much less cost and risk than ECMO. However, in addition to its potential to limit overdistension of vulnerable lung, TH also exerts effects on at least two signaling pathways that are directly involved in the pathogenesis of lung injury, Transient Receptor Potential Cation Channel Subfamily V Member 4 (TRPV4) and p38 Mitogen-activated kinase (MAPK).

TRPV4 is a stretch-activated nonselective anion channel, which is expressed on pulmonary endothelium and increases calcium entry in response to increased endothelial stretch and increasing temperature between 24°C and 40°C. Elevated intracellular calcium levels increase endothelial permeability by modifying adherens junction components and activating myosin light chain kinase and increase recruitment of inflammatory cells by increasing endothelial expression of P-selectin. In an isolated perfused mouse lung model of acute lung injury (ALI), hyperthermia (39°C) and high-pressure ventilation each caused lung injury that was additive, and lungs from TRPV4-deficient mice were protected from both high-pressure- and hyperthermia-induced injury.

The p38 MAPK signaling pathway is also activated by stretch and inflammatory mediators and it contributes to the pathogenesis of ARDS/ALI. The p38 family comprises four serine/threonine protein kinases of which p38α is the proinflammatory isoform and p38β and p38γ are cytoprotective. Among the many biological processes regulated by p38α, endothelial and epithelial barrier function, leukocyte trafficking, and cytokine expression, are central to the pathogenesis of ARDS/ALI. Although at least 62 p38 substrates have been identified, p38 achieves specificity through the selective binding of substrates to various substrate docking domains, which are distinct from the catalytic site. We previously identified p38 phosphorylation of MAPK-activated protein kinase-2 (MK2) as a potentially temperature-sensitive pathway that increases endothelial permeability, neutrophil migration, and cytokine expression at febrile temperatures. Other laboratories have shown that hypothermia reduces TNFα-induced activation of p38-dependent endothelial permeability. We recently discovered that MK2 phosphorylation by p38α increased a remarkable 14.5-fold as temperature increased from 33°C to 39.5°C compared with only ~2-fold change for other substrates. The temperature-dependent change in MK2 phosphorylation was associated with changes in p38α:MK2 binding affinity and a conformational change in p38α MK2 binding site. Since TH not only facilitates lung-protective ventilator strategies by reducing metabolism and has direct effects on at least two cell signaling pathways that contribute to the pathogenesis of the exudative phase of ARDS, we believe it can confer lung protection in ARDS that is additive with current therapies.

4. Preclinical animal and cell model data demonstrating the potential of febrile-range hyperthermia (FRH) to increase and hypothermia to mitigate lung injury:

a. In vitro studies showing FRH augments cellular processes that contribute to ALI: We have shown that exposure to FRH enhances permeability and neutrophil transendothelial migration in human lung endothelial cells, increases apoptosis in lung epithelium, and modifies expression of cytokines, chemokines, GM-CSF, and G-CSF.

b. Animal studies showing FRH worsens ALI: We have shown that co-exposing mice to FRH (core temperature 39.5°C) augments ALI induced by hyperoxia, lipopolysaccharide (LPS), Klebsiella pneumonia, and mechanical ventilation by modifying cytokine expression, increasing epithelial necrosis, and promoting transalveolar neutrophil migration. Other labs have confirmed the ALI-promoting effects of FRH in vivo. These studies model fever occurring in the setting of ALI (which is common in ARDS) in contrast with other studies showing that pre-conditioning animals by exposure to much higher temperatures (42-45°C) can be lung protective.

c. In vitro studies showing hypothermia mitigates cellular processes that contribute to ARDS pathogenesis: Following publication of the first two Phase III RCTs of TH (32°-34°C) after cardiac arrest, in vitro studies have sought to elucidate the biological effects of hypothermia. A partial list of effects relevant to ARDS includes modified monocyte gene expression, suppression of inflammatory cytokine expression, prolongation of LPS-induced NFκB activation in human monocytes, stabilization of mitochondria, resistance to apoptosis, augmentation of autophagy, stabilization of endothelial barrier, reduction in oxidant generation, inhibition of AMPK, PKCδ. We showed that exposing human small airway epithelial cells to 32°C induced expression of miRNAs that target PKCα and relieve cell cycle progression block.
d. Animal studies showing hypothermia mitigates ALI: We and others have shown the potential of clinically relevant hypothermia to reduce lung injury in animal models of ALI induced by i.t. LPS \(^{104-106}\), paraquat \(^{107}\), hemorrhage \(^{108}\), mechanical ventilation \(^{85,109,110}\), pneumonia \(^{111}\), sepsis \(^{112,113}\), air embolism \(^{114}\), and smoke \(^{115}\).

5. Clinical studies showing potential of fever to increase and hypothermia to mitigate ARDS:
   a. Retrospective clinical studies suggesting fever may impact outcome in ARDS: The reported incidence of fever in ARDS varies from 23% \(^{116}\) to 65% \(^{117}\) and depends on temperature measurement technique and underlying cause of ARDS. Our reanalysis of ICAP data showed that 65% of patients have fever (>38°C) within 3 days of ARDS diagnosis and that each additional day with fever reduces the likelihood of successful ventilator liberation by 33% \(^{117}\). In contrast, a reanalysis of ARDSNet FACCT data found that fever at time of diagnosis was associated with improved 90-day survival. However, temperature measurements in that study included axillary and tympanic measurements \(^{116}\), which are unreliable in the critically ill \(^{118}\).

   b. Prospective clinical studies suggesting fever suppression may be lung protective: There have been no RCTs of fever prevention in ARDS. However, in two studies of fever prevention in septic shock, pneumonia was the predominant cause of sepsis. In the Ibuprofen in Sepsis study, ibuprofen did not affect mortality, but it reduced fever and tended to increase days free of pulmonary dysfunction and ARDS \(^{119}\). Schortgen et al. showed that aggressive fever suppression in patients with septic shock reduced 14-day mortality to 19% vs. 34% in the standard care group, but measures of lung function and injury were not mentioned \(^{120}\). These studies not only suggest that fever suppression may be lung-protective during sepsis, but also provide reassurance about the safety of fever suppression during sepsis. We note the study by Schulman et al. \(^{121}\) which found increased mortality in trauma patients who were treated with aggressive fever suppression (7 vs. 1 death in controls), but this small study was flawed as there was no difference in temperature between the two groups and no mention of management of shivering and its metabolic and hemodynamic impact during cooling, which may have increased mortality in the fever suppression group.

   c. Retrospective and anecdotal studies suggesting hypothermia is lung-protective in ARDS: Compared to fever/hyperthermia, there are less data about the effects of cooling on lung injury in humans. Several anecdotal reports describe favorable outcomes in individual patients treated with TH for severe ARDS \(^{122-125}\) and acute post-transplant lung graft injury \(^{126}\). A small retrospective study from one of our clinical site directors (Mayo) showed that cardiac arrest survivors treated with TH tended to have better respiratory compliance and improved P/F ratio than similar patients who were not cooled \(^{127}\).

   d. Prospective trials of TH in ARDS: A small non-randomized controlled trial of TH (33.7±0.6°C for 70±15h) in moribund patients with septic shock and ARDS showed improved survival (3/9 vs. 0/10) and improved PaO\(_2\)/PAO\(_2\) ratio (0.19±0.04 vs. 0.15±0.04) in the TH group \(^{128}\). Despite preceding modern definitions and treatments for ARDS, this study supports our hypothesis that early TH will reduce lung injury in ARDS. A trial of TH (35°C) vs. normothermia in 31 patients with renal transplant and respiratory failure due to infection showed improved patient and renal graft survival in the TH group \(^{129}\), but no measures of lung function were included. Although flawed, these studies increase confidence that TH will be beneficial in ARDS. The more recent Cooling and Surviving Septic Shock (CASS) trial of TH in patients with septic shock demonstrated no benefit of hypothermia in patients with septic shock and respiratory failure and a trend to increased death attributed to refractory shock in the hypothermia arm. The lack of relevance of the CASS trial to the proposed CHILL trial is discussed later in this application, but we note here that the number of deaths attributable to respiratory dysfunction was lower in the hypothermia arm compared with controls.

6. Summary of the CHILL pilot study of TH in patients with ARDS treated with NMB \(^{130}\):
   a. Historical control patients receiving NMB for moderate to severe ARDS: To avoid the negative impact of shivering, we tested the feasibility of performing TH in patients already receiving NMB as part of their clinical management and compared them with historical controls who had ARDS (P/F<150), received continuous infusion of cisatracurium, and were supported with ARDSNet protocols for mechanical ventilation and fluid management in the UMMC MICU. We retrospectively identified 58 control patients who had ARDS and received early treatment with NMB. At onset of NMB, APACHE II scores were 23.5 (19.75-30) (median, range), P/F was 91 (75-110), and oxygenation index (OI) was 24 (15.8-34.6) (Table 1). Underlying causes of ARDS were extrapulmonary
sepsis (52%), pneumonia (38%), blood transfusion (7%) and other (3%). Duration of NMB was 57.5 (29.75-82) hrs. These patients had 0 (0-12) 28-day ventilator-free days (VFDs), 0 (0-6) 28-day ICU-FDs, and 53.5% hospital mortality. Temperature was measured via esophageal probe (34), urinary catheter (1), rectally (11), orally (3), axillary (7), and not specified (2). Core temperature was 36.9 (36.2-37.8)°C at initiation of NMB and averaged 36.6 (36-37.3)°C during NMB. Of the 53 (91%) controls who survived ≥3 days after initiation of NMB, median P/F improved from 91 (75.5-109.5) on day 0 to 168 (121-214) (p<0.0001) on day 3. Median OI improved from 23.3 (15.6-34.2) to 11.1 (8.43-19) (p<0.0001). Ventilator-associated pneumonia (VAP) occurred in 7 patients, significant bleeding in 8 (requiring ≥2 units blood products), and symptomatic bradycardia in 1. Since the retrospective analysis period preceded publication of the Prone Positioning in Severe Acute Respiratory Distress Syndrome (PROSEVA) trial 37, only 12 of 58 patients underwent prone positioning.

b. Pilot open-label trial of TH in patients receiving NMB for ARDS: We enrolled 8 consecutive patients with ARDS who were receiving continuous infusion of cisatracurium in an open-label pilot trial of cooling in ARDS (Table 1, 2). Inclusion criteria were: (1) age 18-80 yrs; (2) endotracheal tube or tracheostomy in place and mechanically ventilated; (3) P/F ratio<150 with PEEP ≥5 cm H₂O; and (4) radiologic evidence of bilateral pulmonary infiltrates (criteria 3 and 4 met within previous 72 hrs, not fully explained by hydrostatic pulmonary edema and occurring with an ARDS-associated condition); (5) current treatment with NMB; and (6) access to an authorized proxy for consent. The only exclusion criteria were: (1) contraindication to cooling (intracranial hemorrhage, severe hemorrhage, refractory hypotension), (2) not likely to remain intubated and receive NMB for ≥48 hrs; (3) moribund and not likely to survive ≥48 hrs, (4) skin lesions precluding placement of cooling devices, and (5) ECMO during the ICU course. Disease severity in the cooled patients was at least as high compared with the historical controls with median baseline APACHE II score 30 (24-32.8) and median P/F ratio 85.6 (63-104). ARDS was caused by pneumonia in all 8 cooled subjects. We used the UMMC institutional TH protocol with modified target temperature range (34-36°C), duration (48 hrs), 0.3°C/hr rewarming, without subsequent fever suppression. Cooling using the Arctic Sun™ system in 2 subjects and Blanketrol™ cooling blankets in 6 subjects was similarly effective. Target temperature was reached within 4 (2.25-9) hrs of cooling initiation and maintained for 43 (32-56) hrs (92% of the cooling period) without fever or SAEs.

Because the cooled patients tended to have higher baseline APACHE II scores (30 vs. 23.5; p= 0.15) and lower baseline P/F ratios (86.5 vs. 91; p=0.4) than the historical controls, we performed parallel analyses comparing cooled patients with all 58 controls and with a subset of 16 controls with baseline APACHE II scores ≤25. The median change in APACHE II was -4.5 (1.32-10.1) in the cooled patients and 1.3 (1.32-3) in the historical controls (p<0.0001). Median changes in bias-adjusted P/F ratio were 0 (0-25.3) in the cooled patients and 25.3 (5.01-25.3) in the historical controls (p=0.0576). Day 3 P/F and Day 3 OI were also significantly decreased in the cooled patients compared with the controls (p=0.0237 and p=0.0137, respectively).

### Table 1. Demographics for Cooled Subjects and Controls

<table>
<thead>
<tr>
<th>Number</th>
<th>All controls</th>
<th>Matched Controls</th>
<th>Cooled patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>56</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Age</td>
<td>58 (36-57)</td>
<td>56 (29-58)</td>
<td>55 (49-59)</td>
</tr>
<tr>
<td>% male</td>
<td>55</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>% White</td>
<td>62</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>% AA</td>
<td>34</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>% Asian</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>BMI</td>
<td>32 (28-8.25)</td>
<td>35 (29.75-42.25)</td>
<td>31.5 (29.44)</td>
</tr>
<tr>
<td>APACHE II</td>
<td>23.5 (19.75-30)</td>
<td>20 (23.75-32)</td>
<td>20 (24-32.75)</td>
</tr>
<tr>
<td>P/F ratio</td>
<td>91 (75-110)</td>
<td>80 (72-129)</td>
<td>85.6 (63-104)</td>
</tr>
</tbody>
</table>

### Table 2. Clinical Information for Cooled Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>BMI</th>
<th>Baseline APACHE II</th>
<th>Baseline P/F ratio/OI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>M</td>
<td>White</td>
<td>29</td>
<td>29</td>
<td>100/13</td>
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<tr>
<td>2</td>
<td>63</td>
<td>F</td>
<td>AA</td>
<td>22</td>
<td>29</td>
<td>63/23</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>F</td>
<td>White</td>
<td>34</td>
<td>31</td>
<td>81/24</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>M</td>
<td>AA</td>
<td>50</td>
<td>33</td>
<td>142/152</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>M</td>
<td>Asian</td>
<td>32</td>
<td>19</td>
<td>49/53</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>F</td>
<td>Hispanic</td>
<td>33</td>
<td>19</td>
<td>92/20</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>F</td>
<td>Hispanic</td>
<td>35</td>
<td>21</td>
<td>105/17</td>
</tr>
</tbody>
</table>

### Table 3. Outcomes in Cooled Subjects, Controls

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Cooled patients</th>
<th>Matched Controls</th>
<th>All controls (p vs. cooled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>16</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Temperature¹</td>
<td>34.35 (34-34.75)</td>
<td>36.65 (35.83-37.48)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>25%</td>
<td>68.75%</td>
<td>86% (p=0.04)</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>25%</td>
<td>75.0%</td>
<td>83.4% (p=0.02)</td>
</tr>
<tr>
<td>Number febrile D0</td>
<td>2</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Number febrile D1</td>
<td>5</td>
<td>5</td>
<td>5 (p=0.0578)</td>
</tr>
<tr>
<td>Number febrile D2</td>
<td>1</td>
<td>2</td>
<td>2 (p=0.04)</td>
</tr>
<tr>
<td>Number febrile D3</td>
<td>2</td>
<td>2</td>
<td>2 (p=0.04)</td>
</tr>
<tr>
<td>VFDs</td>
<td>9 (0-21)</td>
<td>10 (0-21)</td>
<td>10 (p=0.04)</td>
</tr>
<tr>
<td>ICU-FD</td>
<td>5.50 (17.75)</td>
<td>4.00 (6.00)</td>
<td>6.23 (p=0.0576)</td>
</tr>
<tr>
<td>Day 3 P/F</td>
<td>265 (160-270)</td>
<td>175 (175-195)</td>
<td>171 (120-214)</td>
</tr>
<tr>
<td>Day 3 OI</td>
<td>9.84 (11.75)</td>
<td>12.91 (5.21-13.3)</td>
<td>11.6 (12-15)</td>
</tr>
</tbody>
</table>

¹Average temperature (°C) during cooling or during cisatracurium infusion in controls. (Medians [interquartile ranges] shown.)
controls matched for APACHE II scores, P/F ratio, underlying cause of ARDS, age, BMI, and gender. Cooled patients had more VFDs (9 (0-21.5)) and ICU-FDs (5.5 (0-17.5)) than the matched controls (0 (0-0) VFDs, p=0.009) and 0 (0-0) ICU-FDs, p=0.014) and tended to have more VFDs and ICU-FDs than all 58 controls (0 (0-12) VFDs, p=0.16) (0 (0-6) ICU-FDs, p=0.06) (Table 3 and Fig. 1). Day-3 P/F ratios were higher in the cooled patients than the 58 controls (255 (160-270) vs. 171(120-214), p=0.024) and tended to be higher than the matched controls (175 (75-231), p=0.16). Day-3 OI values were similar in the cooled patients and the 58 controls (9.8 (4.7-11.5) vs. 11.1 (8.4-19), p=0.14) and the matched controls (12.1 (8.5-31.3), p=0.16).

The incidence of significant bleeding (1 of 8) and symptomatic bradycardia (0 of 8) during the cooling period was similar to the control group (Table 4). The incidence of VAP (1 of 8) was similar in the cooled patients and controls. There were no significant differences in proportion of controls and cooled patients with abnormal values of serum glucose, potassium, or magnesium. Hypokalemia occurred in 5 patients during cooling, but only one of these patients had hypokalemia post-cooling on day-3. One patient in the cooling group, 13 of 58 controls, and 5 of 16 matched controls received continuous renal replacement therapy (CRRT) prior to starting NMB. Of the patients not already on CRRT at the start of cisatracurium infusion, 4 of 7 prospectively cooled patients, 14 of 58 controls and 5 of 11 matched controls required initiation of CRRT. Five of 8 cooled patients, 12 of 58 controls, and 3 of 16 matched controls received proning. Since the PROSEVA trial showed proning improves survival, we compared controls who were proned with those who were not proned and found no significant differences between the two groups in mortality, VFDs, ICU-FDs, or day-3 P:F ratio or OI. Despite the limitations of historical controls, this pilot demonstrates the feasibility and safety of treating ARDS patients with TH and suggests the treatment may be effective.

Potential controversies about TH: We acknowledge a recent meta-analysis showing that endogenous fever was associated with improved survival and spontaneous hypothermia with reduced survival in patients with sepsis. However, two critical features of this study invalidate its relevance to the CHILL trial. First, it analyzes trials of patients with sepsis rather than ARDS. Even more importantly, this study analyzed the impact of spontaneous hypothermia rather than TH. Spontaneous hypothermia is known to be a marker of increased inflammation and greater disease acuity in sepsis. We also acknowledge the recently published Cooling and Surviving Septic Shock (CASS) trial that demonstrated no benefit of hypothermia in patients with septic shock and respiratory failure. Several aspects of the CASS trial invalidate its relevance to the proposed CHILL trial. The CASS study population had a primary diagnosis of septic shock but no mention of ARDS, was substantially older (age for inclusion was >50 years and median age was 71 yrs) than planned for CHILL (age for inclusion is 18-65 yrs), and CASS included patients with preexisting lung disease and heart failure or receiving high levels of vasopressor support. These conditions are exclusion criteria in the CHILL trial because they independently increase mortality and time on ventilator and are not relevant to the patient population of critically ill or injured military personnel. The target temperature in the CASS trial (32-34°C) was lower and the rewarming rate
(0.5°C/h) faster than in CHILL, which increases the potential for adverse cardiovascular events, especially in the older, hemodynamically unstable patients studied. Since shivering in the CASS trial was treated with intermittent dosing of rocuronium when shivering was observed rather than continuous co-treatment with NMB to prevent shivering as planned for CHILL, several of the patients in the CASS trial likely experienced periods of increased cardiopulmonary demand that might have negatively impacted this older, hemodynamically unstable patient population. Interestingly, in the CASS trial there were fewer deaths attributed to respiratory dysfunction in the TH than the control arm (17 vs. 22). The results of the CASS trial and the CHILL pilot guided the design of the proposed CHILL RCT to improve safety of TH in ARDS patients and avoid confounding factors that might obscure benefit.

7. **Summary of Background and Preliminary Data:** In summary, we provide a strong mechanistic basis to support the hypothesis that TH will mitigate lung injury in patients with ARDS. Our review of the existing *in vitro* and clinical data, including our own historical-control pilot study, suggest that mild TH is feasible, well tolerated, and likely to be beneficial in patients with moderate to severe ARDS who are receiving NMB to block shivering. Collectively, these data underscore the importance of conducting the proposed CHILL randomized control trial of TH in patients with moderate to severe ARDS.

**B. Specific Aims:**

Our **long-term goal** is to develop new therapies for ARDS based on an improved understanding of disease pathophysiology, specifically, modifying body temperature. To advance this program, we have just applied to the DoD to support a multicenter RCT of TH+NMB vs. usual temperature management in moderate to severe ARDS. This is a change in the original design of the protocol, which initially randomized patients with moderate to severe ARDS and already receiving NMB to TH vs. usual temperature management (patients in both arms receive NMB). This original design avoided an imbalance between NMB between the two arms. However, the recent NHLBI Reevaluation of Systemic Early Neuromuscular Blockade (ROSE) trial showed that NMB treatment for 48 hrs neither benefited nor harmed patients with moderate to severe ARDS 39. The **overall objective** of this protocol is to begin data collection in a single-site RTC of TH+NMB vs. usual temperature management in moderate to severe ARDS patients at our UMMC using the same protocol as proposed in our DoD grant application. **Our central hypothesis** is that mild TH (core temperature 34º-35ºC) for 48 hrs, co-administered with NMB to block shivering, will be lung protective in moderate to severe ARDS (P/F<200). This hypothesis is supported by: (1) mechanistic studies showing that clinically relevant shifts in temperature modify the activity of two signaling pathways that are central to ARDS pathogenesis 49,78; (2) cell culture and animal models that show fever worsens and hypothermia mitigates cellular responses that contribute to ARDS pathogenesis 56,57,79,81-85,104,105,107-109,111,114,135; (3) Villar and Slutsky’s small prospective non-randomized, controlled trial showing benefit of TH in moribund patients with ARDS and septic shock 128; (4) a retrospective study of cardiac arrest survivors that showed a trend toward improved lung compliance and oxygen exchange in those receiving TH 127; and (5) the results of our CHILL pilot 130.

We will objectively test our central hypothesis by conducting a single-site randomized, controlled Cooling to Help Injured Lungs (CHILL) Phase IIb RCT of mild TH+NMB in patients with ARDS and P/F < 200 to address the following **specific aims:**

**1. Analyze the potential lung protective effect of mild TH (core temperature 34º-35ºC for 48 hrs)+NMB in patients with ARDS and P/F < 200 compared with controls receiving standard temperature management.** We expect the TH-treated group to have at least four more 28-day VFDs (primary), more 28-day ICU-FDs, improved day-3 driving pressure and oxygen saturation index (OSI) compared with the controls.

**2. Evaluate the effects of TH and NMB on systemic inflammation and extrapulmonary organ dysfunction.** We expect the treated group to have reduced levels of proinflammatory mediators, including those regulated by the inflammasome, and lower Sequential Organ Failure (SOFA) scores than controls.

**3. Analyze safety of TH and NMB in patients with ARDS.** We expect ARDS patients cooled to only 34º-35ºC to exhibit few of the known complications of more extreme post-cardiac arrest TH (infections, insulin resistance, electrolyte abnormalities, bradycardia, and hypotension).
We anticipate that the proposed Phase IIb trial will demonstrate a large enough effect of TH/NMB on VFDs and ICU-FDs to support the introduction of TH/NMB into the DoD Joint Trauma System Clinical Practice Guidelines for ARDS. We also expect the results to inform a decision about whether to proceed with a subsequent civilian population Phase III clinical trial of TH to reduce mortality in ARDS and to direct its study design.

C. Study Design

1. Overview and Justification of the CHILL RCT study design (see individual sections for more details and justification): The proposed CHILL multicenter Phase IIb unblinded RCT will compare combined treatment with TH to maintain core temperature at 34°-35°C for 48 hrs and NMB to block shivering vs. usual temperature management in patients with moderate to severe ARDS. The primary outcome will be 28-day VFDs. Multiple physiologic, clinical, and safety secondary outcomes will also be measured. The proposed study design is based on our experience in conducting the CHILL open label pilot and our more recent single-center CHILL pilot RCT. The CHILL Phase IIb trial uses a controlled, randomized study design with randomization stratified by clinical site and prone-positioning status. Men and women 18-65 years of age with moderate to severe ARDS (P/F ratio <200) for ≤48 hrs will be randomized to TH+NMB or usual temperature management. Temperature management in the control group will be protocolized to maintain core temperature between 37° and 38.5°C during the ~54 hr treatment period (including cooling and rewarminng time). All CHILL trial subjects will be supported using ARDSNet protocols for lung-protective mechanical ventilation and fluid management and protocol compliance will be monitored in real-time. Clinical, physiologic and clinical laboratory parameters to assess lung injury and extrapulmonary organ dysfunction will be recorded on case report forms (CRFs) and research blood samples will be collected for mediator analysis at baseline and on study days 1, 2, 3, 4, and 7. The primary outcome, 28-day VFD and secondary efficacy and safety outcomes will be determined prospectively and recorded on appropriate CRFs. Subject participation will last up to 90 days at which time their clinical status will be assessed by patient visit (if still hospitalized), checking the medical record, and telephone call.

Because the nature of the therapeutic intervention, TH and NMB, precludes blinding and because the primary outcome (28-day VFDs), major secondary outcomes (e.g. 28-day ICU-FDs), and potential confounding treatments (e.g. proning) are dependent on subjective decisions by the clinical ICU team, protocols have been developed to direct these decisions. The proposed study design includes both pragmatic and explanatory aspects. The former includes the broad inclusion criteria regarding underlying cause of ARDS and comorbidities, intention to treat analysis, and unspecified cooling devices to reach the target temperature range and choice of NMB agents. The explanatory aspects include randomization, strict protocolization of supportive care and clinical decision making, and use of mortality surrogates as outcomes.

2. Study Population: Men and women 18-65 yrs of age of any race and ethnicity who meet Berlin criteria for moderate to severe ARDS the following inclusion and exclusion criteria will be enrolled and randomized.

a. Inclusion Criteria: (1) 18-65 yrs of age; (2) endotracheally intubated for or have a tracheostomy in place, and mechanically ventilated ≤7 days; (3) admitted to a participating ICU; (4) have a P/F ratio <200 with PEEP ≥8 cm H₂O; (5) have radiologic evidence of bilateral pulmonary infiltrates; (6) able to give consent or have a legally authorized representative (LAR) to provide consent. Criteria 4 and 5 must be met within 48 hrs, not be fully explained by pleural effusions, atelectasis, or hydrostatic pulmonary edema, and must have occurred within 7 days of an underlying condition associated with ARDS. If arterial blood gas (ABG) data are not available, the P/F ratio can be inferred based on SpO₂ values using the nonlinear imputation strategy described by Brown et al. 136 and used in the ROSE trial 39. The values for P/F ratio will be inferred using Table e2 from the Brown et al. paper as long as the following conditions are met: (1) SpO₂ values are 80-96%; (2) SpO₂ is measured ≥10 min after any change in F₂O₂; (3) PEEP is ≥ 8 cm H₂O; (4) the pulse oximeter waveform tracing is adequate; and (5) the qualifying inferred P/F ratio is confirmed 1-6 hrs after the initial determination.

b. Exclusion criteria: (1) missed ARDS window (≥48 hrs); (2) currently on NMB for ≥12 hrs; (3) missed mechanical ventilation window (>7 days); (4) refractory hypotension (requiring > 0.2 µg/kg/min of norepinephrine or equivalent dose for minimum of 6 hrs); (5) core temperature <35.5°C while not receiving CRRT; (6) patient is unable to give consent and no legally authorized representative is available; (7) significant, active bleeding (≥3u blood products and/or surgical/IR intervention); (8) platelets <10K/mm³ (uncorrected); (9) active hematologic malignancy; (10) skin process precludes cooling device; (11) moribund, not likely to survive 72 hrs; (12)
pre-morbid condition makes it unlikely that patient will survive 28 days; (13) Do Not Resuscitate status; (14) not likely to remain intubated for ≥48 hrs; (15) physician of record unwilling to participate; (16) severe underlying lung disease (on home O2 or non-invasive positive pressure ventilation (except for OSA) or prior lung transplantation); (17) BMI >50 kg/m²; (18) known New York Heart Association class IV heart disease; (19) acute coronary syndrome past 30 days (MI, unstable angina); (20) cardiac arrest within 30 days of enrollment; (21) burns over >20% of the body surface; (22) severe chronic liver disease (Child-Pugh of 12-15); (23) previously randomized in CHILL.

c. Justification of Study Population: The inclusion of patients with moderate to severe ARDS (based on the modified Berlin criteria used in the ROSE trial of P/F ratio <200 with PEEP ≥ 8 cm H2O) provides a study population with an expected mortality >40% 10,39,137 We have elected to not include a 12-24 hr stabilization period for meeting ARDS criteria as was used in PROSEVA 131 since Villar et al. 137 showed that 95% of patients initially presenting with ARDS and P/F ratio <200 still met ARDS criteria 24 hrs later, 72.4% with P/F ratio remaining <200, and with an overall 42.8% mortality. Moreover, based on the proposed mechanisms of TH in ARDS, the earlier TH treatment can be initiated, the more likely it will confer benefit. The upper age limit for inclusion was lowered to 65 yrs to skew the age of study population toward a younger age that is more representative of active military personnel. The exclusion of patients with high vasopressor requirements is based on results of the recent CASS trial 134, which found no benefit of TH in patients with septic shock and respiratory failure and a trend toward increased deaths due to refractory shock in an older, hemodynamically unstable patient population. Based on experience from the CHILL pilot, we have excluded patients with comorbidities that are likely to reduce VFDs and ICU-FDs independent of the effects of hypothermia on ARDS, including active hematologic malignancies, severe immune suppression, and pre-existing cardiomyopathy with LV ejection fraction <30%. Exclusion of patients with spontaneous hypothermia (<35.5°C) in the absence CRRT is based on our finding that hypothermia is independently associated with decreased VFDs and increased mortality in the patients in the ICAP database 117.

3. Interventions:

a. Therapeutic Hypothermia Arm: Subjects in the TH arm will receive sedation to Richmond Agitation-Sedation Scale (RASS) -4 or -5, then a continuous infusion of cisatracurium. The NMB infusion rate will be adjusted to achieve two twitches on train-of-four testing and further adjusted to eliminate visible shivering. Once sedation and NMB are confirmed, hypothermia to 34°-35°C will be achieved as quickly as possible, within 6 hrs of randomization, using either Blanketrol II cooling blankets, or Arctic Sun™ cooling system, whichever is most readily available. Temperature will be measured continuously from a central probe (esophageal, urinary, or intravascular). Once the target temperature is reached, it will be maintained for 48 hrs. Subjects will then be rewarmed to 36°C by 0.3°C per hr and the cooling devices will then be removed. NMB will then be discontinued unless the ICU team feels there is clinical indication for continued NMB (e.g. ventilator dyssynchrony). Although not expected to occur with modest TH to 34°-35°C, we have developed the following criteria for early termination of TH and NMB: (1) persistent severe bradycardia (heart rate <30 associated with mean arterial pressure <65 without vasopressors); (2) uncontrolled bleeding, and (3) intractable ventricular arrhythmias. An exemption for an IDE for the cooling devices has been granted by FDA. A formal Q-Sub Study Risk Determination request for an IND exemption regarding the NMB agents has been submitted to FDA.

b. Usual Temperature Management Arm: We will use the same light sedation protocol in the control group as was used in the ROSE trial 39 with RASS ~1 or 0 and will protocolize treatment of fever and CRRT-related hypothermia. During the 54 hr post-randomization period (corresponding with cooling and rewarming in the TH arm), acetaminophen will be given for core temperature ≥38°C and surface cooling initiated for core temperature ≥38.5°C and adjusted to maintain temperature ≤38°C. Administration of anti-shivering therapy will be at the discretion of the ICU providers. If subjects are hypothermic (core temperature ≤36°C) during CRRT, surface warming will be initiated to restore core temperature to 37°C. Following the 54 hr treatment period (including cooling and rewarming time), temperature management in both arms will be directed by the ICU team.

c. Justification of TH Protocol: Target temperature (34°-35°C) is based on (1) results of the Villar and Slutsky nonrandomized trial of hypothermia in severe ARDS in which mean core temperature was 33.7°C 128; (2) positive outcome trends in our CHILL open-label pilot in which median core temperature during cooling was 34.4°C 130; (3) a study in cardiac arrest survivors showing that less extreme hypothermia (36°C) exerted the
same benefit as cooling to 33°C and tended to cause less adverse events \(^{138}\); (4) findings of similar lung protection in various animal models of lung disease by hypothermia to 34°C compared with hypothermia between 27° and 32°C \(^{104-115}\); (5) CASS trial results showing excess cardiovascular deaths in older patients with septic shock who were treated with more extreme hypothermia (32°-34°C) \(^{134}\), and (6) an inverse correlation between temperature and significant adverse events in humans \(^{139}\). The duration of cooling (48 hrs) is based on animal lung injury models showing peak neutrophil accumulation and protein leak within the first 48 hrs \(^{82,86,140}\), suggestion of improved outcomes associated with 48 hrs cooling in our historically controlled open-label pilot, and concerns about prolonging NMB administration much beyond what was shown to be safe in the ROSE and ACURASYS trials \(^{39,134}\). Our recent survey of NMB use in ARDS patients \(^{141}\) suggests that NMB administration for ~48 hrs would be acceptable to most academic intensivists. Choice of cooling device: Since our CHILL pilot showed that cooling blankets and a gel pad cooling system were equally effective in ARDS patients who are receiving NMB \(^{130}\), we have not designated a specific surface cooling device.

d. Temperature management in the control arm: Based on our previous retrospective analysis of ARDS patients demonstrating that fever is common in ARDS patients \(^{117,130}\), we have also developed a protocol for fever management during the 54 hr treatment period in the control arm. Mandated post-cooling fever suppression was not used in our open-label pilot study \(^{130}\) and is not part of the TH protocol; fever management will be directed by the ICU providers.

e. Justification of combined NMB and cooling in the TH arm is based on (1) our previous study showing that cooling critically ill patients without blocking shivering increases oxygen utilization by ~60% \(^{142}\); (2) results of the CHILL open-label study \(^{128}\) showing that TH in ARDS patients receiving NMB is feasible and well tolerated; (3) established clinical guidelines recommending use of NMB to prevent shivering during TH \(^{143}\); and (4) results of the ACURASYS and ROSE trials showing early NMB in patients with moderate to severe ARDS did not cause serious adverse effects including ICU-acquired myopathy \(^{38,134}\). Although multiple alternative pharmacologic and nonpharmacologic anti-shivering therapies are used during TH, NMB is the most consistently effective and is used when all other modalities fail. We included NMB in the TH treatment protocol to avoid periods of shivering and its untoward metabolic effects and the potential confounding effects from variable use of various other anti-shivering medications. Although this study design will result in an imbalance between the two groups in use of NMB, the results of the ROSE trial \(^{39}\) showing neither benefit nor harm of early NMB use in ARDS, reduces concern about the significance of this imbalance. Most medical centers, including all of our clinical sites, have an established institutional protocol for TH and experience in applying TH, which will facilitate rapid initiation of TH following randomization.

f. Additional Study Procedures: Patients will be supported using ARDSNet protocols for mechanical ventilation and fluid management similar to those used in the ROSE trial \(^{134}\) for at least the first 7 days. Decisions about mechanical ventilation weaning, extubation, proning, referral for ECMO, and transfer from ICU will be based on pre-specified criteria. Compliance with all study protocols will be encouraged by a checklist included in the day 1, 2, 3, 4, and 7 CRFs with pertinent queries regarding appropriate temperature management, timely blood draws and physiological measurement, tidal volume deviations from low tidal volume targets, whether maintenance fluids were administered and total intake and output volumes. Details about transitioning to Unassisted Breathing within the first 28 days will be recorded on the Unassisted Breathing Checklist.

g. Treatment stopping rules: Although not expected to occur with modest TH to 34°-35°C, we have developed the following criteria for early termination of TH and NMB: (1) persistent severe bradycardia (heart rate <30 associated with mean arterial pressure <65 without vasopressors); (2) uncontrolled bleeding, and (3) intractable ventricular arrhythmias.

4. Participating ICUs: CHILL will be conducted in three ICUs at the University of Maryland Medical Center (UMMC), the Medical Intensive Care Unit (MICU), Critical Care Receiving Unit (CCRU), and the Shock-Trauma Multitrauma Critical Care Unit (MTCC).

5. Description and Justification of Study Endpoints:
   a. Primary endpoint: There is no perfect surrogate for mortality in RCTs for ARDS \(^{144}\). We elected to use 28-day VFDs \(^{145}\) as the primary endpoint in the proposed CHILL trial because: (1) it has been used as a secondary
outcome in most Phase III ARDS trials, including ARMA, ACURASYS, and PROSEVA; (2) it is a measure of both survival and lung function; (3) we found a significant effect size of TH on 28-day VFD compared with historical controls in our CHILL-pilot study; and; (4) it is relevant to clinical practice guidelines for the immediate care and transport of critically ill or injured military. We realize that VFDs can be biased by physician decision-making about transitioning to unassisted breathing and by confounding factors that prolong respiratory failure. We have addressed these potential pitfalls by protocolizing and monitoring decision-making regarding liberation from mechanical ventilation and by excluding conditions that may independently reduce VFDs (e.g. active hematologic malignancy, severe underlying lung disease, cardiomyopathy). Details will be documented in the Transition to Unassisted Breathing CRF. The 28-day VFDs will be calculated at day 28 and recorded on the day 28 CRF. For subjects still alive after 28 days, the 28-day VFD is calculated by adding the number of days in the 28 days post-enrollment that the subject was ventilator-independent. Transient ventilator-independence time will be included as long as the subject was continuously ventilator-independent for >48 hrs. VFDs for subjects who die before day 28 will be assigned a value of zero whether or not they successfully weaned from mechanical ventilation as initially described by Schoenfeld et al. There has been ongoing debate about the most appropriate outcomes in trials involving mechanical ventilation. We will consider supplementing the analysis of VFDs as defined by Schoenfeld as the primary outcome with secondary analyses using a mixture model that jointly estimates treatment effect on two competing event time outcomes: (1) time to unassisted breathing followed by discharge home alive; and (2) time to in-hospital death.

b. Intermediate endpoint: The low and high core temperature measurements in each 2-hr period will be recorded for the first three study days and used to evaluate the effectiveness of our intervention in maintaining the target core temperature. In our open-label pilot, median core temperature was 37 (36.1-38)°C at randomization and 34.4 (34-34.8)°C during cooling. We will analyze the duration of cooling and percent of treatment period within the target temperature range in the TH arm, need for temperature management in the control arm, and separation of core temperature between the two arms.

c. Secondary endpoints:

i. Clinical: The following outcomes were selected to provide measures of overall recovery: (a) 28-day ICU-FDs; (b) SOFA scores; (c) 60- and 90-day survival; and (d) 90-day functional status; (e) neurocognitive screening performed at ICU and hospital discharge using the Montreal Cognitive Assessment (MoCA); and hospital, 60-day, and 90-day mortality. SOFA scores for days 0-3 will exclude the neurologic component when subjects in the TH arm will be receiving NMB. Decisions about transfer out of the ICU will be protocolized. These data will demonstrate potential impact of TH on pulmonary and extra-pulmonary organ injury.

ii. Physiologic: Changes in driving pressure and OSI between baseline (at randomization) and study days 3 and 7. The driving pressure (Plateau pressure – PEEP during non-patient initiated breath) reflects interactions between the ventilator and injured lungs that correlate with mortality in ARDS. The OSI (Mean airway pressure x 100 x FIO2/SpO2) takes into account Mean Airway Pressure and FIO2 as does the Oxygenation Index, but avoids the need for repeated arterial blood sampling. Although early measures of oxygenation are poor predictors of survival, the trend may provide a measure of alveolar recovery.

![Figure 1. Schedule of study procedures](image-url)
iii. **Biomarker**: The plasma biomarkers measured on study days 0, 1, 2, 3, 4, and 7 were selected to provide insight into mechanism of TH effect on ARDS pathogenesis and includes: (a) IL-6 and 8 (marker of inflammation; predictive of survival in reanalysis of ARMA and ALVEOLI data 150), IL-18 and 18 (products of inflammation, an indicator of p38 activation), soluble-RAGE 151 and surfactant protein (SP)-D 152 (indicator of Type I and II alveolar epithelial injury), soluble ICAM-1 (indicator of endothelial activation/injury 153), MMP8 (marker of neutrophil activation; elevated in ARDS 154), and sTNFRI. Unused samples will be stored for at least 2 yrs after study completion for additional analyses by CHILL investigators and other qualified investigators.

iii. **Safety**: Safety measures to be monitored for the first 54 hrs (during cooling and rewarming), based on the post-cardiac arrest experience 138, include: (a) continuous cardiac monitoring for bradycardia and arrhythmias; (b) every 6 hr point of care (POC) blood glucose monitoring; (c) every 12 hr serum potassium, magnesium and phosphate measurement and (d) monitoring for significant bleeding event (requiring ≥3u packed red blood cells or surgical/interventional radiologic intervention). Based on the theoretical concern for immunosuppression, we will monitor for VAP and other secondary infections during the first study week. Oxygenation will be measured as a secondary outcome of efficacy.

6. **Subject Recruitment**: To maximize randomization within the inclusion window, we will employ a 2-step strategy. We will consent and enroll subjects who meet all Berlin criteria regardless of meeting the P/F <200 criterion. If the P/F ratio (or the inferred P/F ratio based on S\textsubscript{O}2) is < 200 at time of enrollment, the subject will also undergo randomization. Those subjects who have not yet met the P/F < 200 criterion will be followed until P/F ratio (or the inferred P/F ratio based on S\textsubscript{O}2) drops below 200 or they exit the 48 hrs ARDS window. Just prior to randomization, a research blood sample will be drawn, an esophageal temperature probe will be placed if no other probe is present, and subjects will be randomized to TH+NMB or usual temperature management. Consideration was given to requiring that subjects meet ARDS criteria for 12-24 hrs to be enrolled as was done in PROSEVA 131, but not ACURASYS 38 or ROSE 39. An analysis of stability of ARDS phenotype in 478 patients with moderate or severe ARDS receiving usual care demonstrated that 95% still met ARDS criteria 24 hrs later 137, 72.4% were still characterized as moderate or severe ARDS, and the patients who did transition to mild ARDS had the same mortality (39.8%) as those who were still classified as moderate ARDS after 24 hrs. Based on these data and in the interest of facilitating enrollment and early initiation of treatment, we have not required demonstrated stability of ARDS phenotype prior to enrollment.

a. **Patient Screening**: All patients in participating ICUs who are 18-65 yrs old and receiving mechanical ventilation for <7 days will be screened daily for potential inclusion in CHILL 7 days per week. The physicians and nurses in the participating ICUs will be educated about the CHILL trial and signs with trial description, inclusion/exclusion criteria, and contact information will be placed in all participating ICUs. During our single-site CHILL RCT pilot, we have developed Epic electronic medical record (EMR)-based tools to facilitate screening, which will be shared with all clinical sites.

b. **Informed Consent procedure**: Patients found on screening to qualify for CHILL and whose primary ICU care provider agrees will be offered participation in CHILL. Because of the nature of the disease we anticipate that most patients will be unable to give consent. In this case, informed consent will be obtained from the patient’s LAR by the CHILL PI, CHILL sub-investigators, or the CHILL coordinator. If the CHILL study personnel is also the clinical ICU provider for a potential subject, he/she will not participate in the consent discussion with the patient/LAR to avoid undue pressure to agree to the study. The information provided in the consent covers the elements listed in the 21 CFR Part 50.25. This includes the investigational nature and objectives of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and potential alternative therapies, alternative to not participate and right to withdraw without penalty will be explained. The discussion about consent will take place in a private location in or nearby the participating ICU. Study staff personnel will offer to answer any questions. Consent will be documented by LAR signature on the IRB approved consent form or witnessed verbal consent or nonverbal agreement by the patient. A copy of the informed consent document will be given to the LAR. No study procedure will be done prior to obtaining signed informed consent. Once subjects regain decision making capacity (using a capacity assessment tool that has been approved by the IRB), informed consent for continuing participation in CHILL will be obtained by the PI, coordinator, or designee.
c. Retention strategies: We anticipate that most participating subjects will remain hospitalized, likely in the ICU, through study day-7 and accessible to study personnel. Subjects will be seen daily by study personnel through the first 7 days for data collection, protocol adherence, and collection of research samples. All data will be recorded on paper CRFs by the CHILL PI, sub-investigators, or coordinator. Beyond study day-7, clinical status will be monitored by examining the medical record and, as needed, bedside visit. Study personnel will complete CRFs for ICU discharge, study day-28 status, and hospital discharge. The hospital discharge CRF will contain subject disposition and all contact numbers, including the subject’s LAR to facilitate 60- and 90-day follow-up.

d. Safeguards for vulnerable population: We anticipate that most potential CHILL subjects will be cognitively impaired, at least temporarily, as a result of the inclusion criteria for this study for study entry. In these cases, informed consent will be obtained from an LAR and subjects will be re-consented once they regain decision-making capacity. Such subjects will be considered vulnerable patients. Based on our underlying hypothesis, the subjects randomized to the TH + NMB arm may derive benefit from the experimental therapy. Subjects in both arms will derive benefit from the additional monitoring by the research team and the use of protocolized evidence-based care as a consequence of participating in the CHILL trial.

e. Outreach to minorities and women: The potential CHILL patient population in our participating ICUs is well-represented in minorities and women (44% African American, 9% Hispanic). There are no exclusions based on race, ethnicity, or gender. Pregnancy will be an exclusion because there are no data to insure safety of mild hypothermia for the fetus. Pregnancy testing will be performed in all women of child-bearing age prior to randomization.

f. Engagement of clinical community to encourage recruitment: Presentations about CHILL will be given as Grand Rounds at each clinical site and their referring hospitals as well as to institutional critical care committees. The site PI and coordinator will educate staff and physicians for all participating ICUs about the CHILL trial. Signs with information about the CHILL trial including inclusion and exclusion criteria and easy contact information will be prominently displayed in all participating ICUs.

7. Research Sample Handling and Shipping: Venous blood will be collected through indwelling catheters into two 6 ml lavender-topped Becton-Dickinson tubes just prior to randomization and on study days 1, 2, 3, 4, and 7. The day-1 research blood draw will be performed as close to 0800 on the day after the enrollment day (e.g. if enrolled on Monday at 0005, the day-1 research blood draw would be performed on Tuesday morning at 0800; if enrolled on Sunday at 2355, the day-1 research blood draw would be performed on Monday at 0800). The blood will be placed on ice and centrifuged at 1000 g for 5 min at 4°C within 60 min of blood draw, dispensed in 0.5 ml aliquots in labelled cryotubes, and stored in a remotely-monitored -80°C freezer with liquid CO2 back-up in the Hasday laboratory in UMB Health Science Facility-II, until batches of samples are transferred to the University of Maryland Cytokine Core Laboratory (CCL) in the adjacent Bressler Research Building. The CCL has been performing ELISA and Luminex™-based mediator analysis using Good Laboratory Practice for >25 years.

8. Randomization Plan: Patients with moderate to severe ARDS for ≤48 hrs based on the Berlin criteria ¹ will be enrolled and randomized. Because prone positioning improves survival in ARDS 27, patients will be stratified at enrollment by both proning status and clinical site and randomized within each stratum using a 1:1 assignment ratio in small blocks of randomly varying size. Patients without ABG data may be enrolled using a P/F ratio inferred from SpO2 data as described by Brown et al. 136 and used in the ROSE trial 39. To facilitate successful consent and randomization within the inclusion window, patients who meet all Berlin criteria but have not yet met the P/F <200 criterion will be consented, enrolled, and followed until the P/F ratio <200 criterion (measured or inferred from SpO2 readings) is documented or they exit the 48 hr ARDS window. Just prior to randomization, a research blood sample will be drawn, an esophageal temperature probe will be placed if no other central temperature probe is present, and patients will be randomized to TH+NMB or usual temperature management using the protected access Excel-based randomization tool on the University One-drive.

9. Statistical Design and Power:
   a. Sample Size and Stratification Design: We performed a power analysis to detect a 4-day increase in 28-day VFDs between the TH arm and the control arm. A 4-day increase was found in the PROSEVA trial of proning.
We assumed a standard deviation (SD) of 10, the average SD among the ACURASYS, PROSEVA, and ROSE trials, resulting in the effect size to be detected equal to $4/10 = 0.40$. Setting power $= 0.90$ and alpha level $= 0.05$ (two-tailed), we calculate $n = 2\times[(1.96+1.28)\times(10)^2/(4)^2 = 131.2$. The sample size was then corrected to allow 10% one-way crossover assuming that subjects randomized to TH+NMB may not receive study treatment because of technical issues but that subjects randomized to standard treatment would not likely receive hypothermia treatment. The 10% is conservative (resulting in a larger total N) as the cross-over is typically ~3-4% in similar trials. This adjustment resulted in a sample size equal to $131.2/(1-0.1)^2 = 162.0$ per group. Hence, the final total N = 324. Since proning has been shown to improve survival in ARDS patients when added to NMB, randomization will be stratified for baseline proning status (i.e. for both center and proning status) and decisions to start or stop proning will be based on protocolized study-wide criteria.

10. Data Analysis Plan: This is a pilot study to demonstrate feasibility and to rest and refine study protocols and data collection to facilitate a planned subsequent multicenter Phase IIb RCT. Since we anticipate hearing about possible DoD funding by November, 2019, we anticipate enrolling 12 patients and randomizing 8 patients in the next twelve months. Since the protocol for this pilot and the follow-up multicenter Phase IIb trial will be similar, we plan on merging the patients from the pilot with the larger study database and analyzing as follows.

Primary and secondary analyses will be performed according to the principle of intention-to-treat. Per protocol analyses will also be performed as part of assessing the feasibility of a civilian clinical trial with a mortality outcome. The treatment group difference on the primary outcome, 28-Day VFD’s following randomization will be tested with a mixed effects model with 28-Day VFD’s as the dependent variable, treatment group as the primary independent variable, proning status (binary) as covariate, and treatment center as a random effect. The alpha level of the test will be 0.05. The random treatment center effect will account for site variability and intra-site correlation. As prior studies and our own pilot data indicate that 28-Day VFD’s is a skewed variable with more zeros than would be expected in a normal distribution we will select a mixed effects model that is of adequate fit for this distribution and use robust Huber-White standard errors.

We will examine two interactions in secondary analyses. First, we will examine variability of treatment effect by treatment center by adding an interaction term between the treatment group indicator and site. Non-zero site variability will be tested by likelihood ratio test of improved fit comparing the model to the model without this random interaction term. If there is significant site variability we will estimate and report treatment effects by site (coded) as well. Second, we will test whether the treatment effect differs by proning status by adding the interaction term between treatment group indicator and proning status. If this interaction is significant we will estimate and report treatment effects separately by proning status.

Three interim analyses will be performed after ~25% (n=81), ~50% (n=162) and ~75% (n=243) of planned enrollment has either been followed-up for 28 days or has died prior to the 28th day. To inform the decision whether to stop the trial early due to superiority of the TH+NMB treatment arm (greater 28-Day VFD’s), we will use the Lan-DeMets $^{156}$ alpha spending function with O’Brien-Fleming $^{157}$ boundaries, i.e. $\alpha(t^*) = 2 - 2\Phi(Z_{\alpha/2}/\sqrt{t^*})$ where $t^* = n/N$ and $n$ equals the interim sample size (i.e., 81, 162, 243). Whether to stop the study early due to futility at an interim analysis will be informed by a conditional power calculation $^{158,159}$. We approximated conditional power (alpha-level=0.05) for detecting a group difference in mean VFD’s using the method of Lan and Wittes $^{159}$ for a range of differences. For example, at the 2nd interim analysis, conditional power was 0.85, 0.41, 0.07, and 0.00 when the difference in mean VFDs = 3, 2, 1, and 0, respectively. Based on these calculations, if the difference was 1 or less, we would recommend that the trial be paused due to futility as the chances of finding a VFD difference that would be compelling would be small.

Several secondary analyses will be performed. First, group comparisons will be performed on baseline clinical and physiologic measurements with potential prognostic value. If there are one or more significant group differences on prognostic baseline variables, we will perform secondary analyses using the above mixed effects model to adjust for these variables. Second, group comparisons will be performed on secondary clinical outcomes and physiologic variables using mixed effects models, as above, including adjustment for proning status and including site as a random effect. Variable transformations such as ln(y) for right skew and y^2 for left skew will be used if needed to satisfy model assumptions. An interaction term will be added between treatment group
indicator and baseline proning status and, if significant, separate results of these secondary analyses will be reported by baseline proning status. A mixed logistic regression model will be used to compare the proportion who die within 60 and 90 days. For outcomes with missing data that are not obviously informative, regression multiple imputation\textsuperscript{160,161} will be used. Third, a repeated measures mixed model (RMMM) ANOVA will be used to test group differences on physiologic and biomarker outcomes over days 0, 1, 2, 3, 4, and 7 (i.e., 0, 24, 48, 72, 96, and 168 hrs). To address the issue of truncation due to death and minimize resulting bias we will use a “pattern-mixture” model\textsuperscript{160} approach to estimate group mean trajectories separately for different days of death. This approach will be implemented using the above RMMM stratified by day to death. We will test linear contrasts from the above fitted model to test the hypothesis that the level of cytokines (IL-6 and IL-8) are significantly different in the two study arms at the 48-hr time point and remain different out to the 168-hr time point. If there are significant group differences, we will follow this with analysis of group trajectories. For other types of missing data not due to death, if they occur more than infrequently, we will generally use multiple imputation.

11. Data Management Plan: The Data Management Core (DC) will be jointly run by Clayton Brown, PhD, and Michael Terrin, MD, CM, MPH. The DC is responsible for oversight of randomization and data management, data quality, and providing data summaries to external groups. Data management will be performed according to standard operating procedures using paper case report forms (CRFs) that will be migrated to an electronic data capture (eDC) system, probably the RAVE eDC (Medidata, Inc.) by the Cooperative Studies Program Coordinating Center (CSPCC) in Perry Point, MD should DoD funding be awarded. Screening logs, medical records and other notes containing direct identifiers will be stored securely in a locked file cabinet in a locked office in the Pulmonary and Critical Care Office suite in Paca Pratt 2nd floor. Each screened patient will be assigned a unique study ID (SID) number and letter code unrelated to initials or any other personally identifying information for the purposes of individual specific information linkage during the clinical trial. We will document that clinical site staff who handle study data are all trained in human subjects, good clinical practice (GCP) and CHILL-specific data and specimen collection.

12. Data Safety Monitoring: Dr. Avelino Verceles, MD will serve as the Data Safety Monitor for this pilot RCT. He is a pulmonary/critical care physician scientist with experience in clinical research involving critically ill and mechanically ventilated patients who is not otherwise involved in CHILL-pilot. All deaths or life-threatening events temporally related with study procedures, including cooling and rewarming, will be reported to Drs. Hasday or Shanholtz, who will submit to the IRB with their assessment of relatedness to study procedures within 24 hours. All other possible adverse events will be evaluated for severity and study-relatedness by Dr. Verceles biannually.
References:
