Efficacy of Coenzyme Q10 Supplementation on Multi-Organ Dysfunction in Severely Burned Patients

NCT03968640

PROTOCOL

Updated 04-26-2019

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I. PRINCIPAL INVESTIGATOR:

Herb A. Phelan, MD, MSCS
Department of Surgery
Division of Burns/Trauma/Critical Care
UT-Southwestern Medical Center/Parkland Memorial Hospital
5323 Harry Hines Blvd., E5.508A
Dallas, TX 75390-9158
214-648-6841
herb.phelan@utsouthwestern.edu

II. CO-INVESTIGATORS AND KEY STUDY PERSONNEL:

James H. Holmes IV, MD
Wake Forest University School of Medicine
jholmes@wakehealth.edu

Steve E. Wolf, MD
University of Texas Medical Branch- Galveston
swolf@utmb.edu

Amalia Cochran, MD
The Ohio State University Wexner Medical Center
Amalia.Cochran@osumc.edu

James Hwang, MD
University of Alabama-Birmingham Medical Center
jameshwang@uabmc.edu

Dhaval Bhavsar, MD
University of Kansas Medical Center
dbhavsar@kumc.edu

Gary Alec Vercruysse, MD
University of Michigan
vercruys@med.umich.edu

Anjay Khandelwal, MD
Case Western Reserve University School of Medicine
akhandelwal@metrohealth.org

Jeremy Goverman, MD
Massachusetts General Hospital
JGOVERMAN@mgh.harvard.edu

Tony Baldea, MD
Loyola University Medical Center
abaldea@lumc.edu

Janelle Wagner, MD
Temple University
Janelle.Wagner@tuhs.temple.edu

Nicholas Meyer, MD
Columbia St.Mary's-Wisconsin
Nicholas.meyer@ascension.org

Tina Palmieri, MD
University of California- Davis
Tina.Palmieri@ucdmc.ucdavis.edu

Rachel Karlnoski, PhD
University of South Florida
rkarlnos@health.usf.edu

David Smith Jr., MD
University of South Florida
dsmith3@health.usf.edu

Fred Endorf, MD
Hennepin County Medical Center
frederick.endorf@hcmed.org

Lori Palfalvi, CRA
American Burn Association
palfalvi@ameriburn.org

Mary Beth Lawless, RN, MSN
UC Davis, American Burn Association
mblawless@ucdavis.edu

III. BACKGROUND

Based on estimates reported in 2010, burn injuries account for 603,000 visits to US emergency departments and 50,000 hospital admissions[1] with inpatient hospital costs totaling $1 billion[1]. Multiple organ dysfunction syndrome (MODS) is a major factor in the morbidity and mortality of severely burned patients. The elements of MODS after burn injury include acute kidney injury (AKI), acute respiratory distress syndrome (ARDS), liver and cardiovascular dysfunction, and coagulopathy. Although the molecular mechanisms underlying MODS in burns are not fully understood, inflammation and mitochondrial dysfunction play important roles in the development of multi-organ dysfunction[2-5].

The inflammatory cascade behind MODS complicates attempts to treat another leading cause of mortality in burn patients, that of sepsis[6]. Effective pharmacologic strategies to prevent and/or treat MODS and sepsis in burns or major trauma remain elusive due in part to the belief that any anti-inflammatory strategies used to treat MODS patients will of their nature suppress immune function, thereby increasing susceptibility to infection and sepsis. Hence, new strategies with the dual capability of inhibiting inflammation/dysfunction in organs (e.g., lung, heart, kidney, liver), while preserving or enhancing the bactericidal capability of immune cells, are eagerlyawaited.

Mitochondrial dysfunction and multi-organ dysfunction in burns: An increasing body of evidence implicates the role of the mitochondria in clinical outcome after burn injury as mitochondrial dysfunction has been implicated in the pathogenesis of MODS after critical illness and severe burn injury[7-10] . Additionally, mitochondrial dysfunction appears to play a part in the metabolic changes that accompany thermal injury such as hyperglycemia, insulin resistance[11], hypermetabolism[12], hyperlactatemia, increased glutamine consumption[13], and muscle wasting. Although the underlying molecular pathogenesis is incompletely understood, mitochondrial dysfunction appears to be a major commonality of these burn- induced metabolic aberrations. In light of this, improving mitochondrial function would appear to be a reasonable approach to ameliorating burn-induced MODS. Finding a suitable strategy to accomplish this goal, however, has been a major challenge and the safety and efficacy of therapies that target the mitochondria have not yet been evaluated in a clinical trial in burns.

Coenzyme Q10 (CoQ10) deficiency and mitochondrial dysfunction: Coenzyme Q (CoQ) is an essential co- factor in the mitochondrial electron transport chain (ETC). CoQ10 is the major species of CoQ found in humans and contains 10 isoprenyl side chains (**Fig. 1**). It exists in oxidized (ubiquinone) and reduced (ubiquinol) forms. In its reduced form, it acts as a lipophilic anti-oxidant[14] and while both forms are used as dietary supplements, ubiquinol has greater bioavailability compared to ubiquinone[15]. Primary (congenital) and acquired CoQ10 deficiencies cause mitochondrial dysfunction, which, in turn, leads to metabolic aberration and dysfunction of multiple organs and systems, including heart, skeletal muscle, vasculature (endothelium), immune, and central nervous system[16]. Conversely, mitochondrial dysfunction and damage can lead to CoQ10

deficiency[17-20], since CoQ10 is biosynthesized in the mitochondria. Thus, mitochondrial damage and CoQ10 deficiency form a vicious cycle.

Dr. Masao Kaneki showed that plasma CoQ10 concentrations are lower in surgical intensive care unit (ICU) patients[21] and septic patients[22] than in healthy controls. Further, his preliminary data show that plasma CoQ10 levels are significantly lower in burn patients compared to healthy controls. In addition, CoQ10 content in peripheral blood mononuclear cells (PBMCs) tends to decline after burn injury and therefore is lower in burn patients compared to healthy controls. These results suggest that burn-induced CoQ10 deficiency may contribute to mitochondrial dysfunction, leading to metabolic alterations and multiple organ dysfunction. While CoQ10 supplementation may reverse or lessen the severity of these complications in burn patients, this therapy has not yet been fully explored.

Decreased complex I activity in burn patients: Complex I (NADH:ubiquinone oxidoreductase) is an essential component of the mitochondrial electron transport chain (ETC), participating not only in cell respiration, but also in cellular/organismal reactive oxygen species (ROS) homeostasis, apoptosis initiation or modulation, and O2 sensing[23]. CoQ10 receives electrons from nicotinamide adenine dinucleotide hydrate (NADH) dehydrogenase at complex I or from succinate dehydrogenase at complex II and transfers electrons to cytochrome C at complex III in the mitochondrial ETC (**Fig. 2**). Complex I is made up of at least 46 subunits and is the largest complex in mitochondrial ETC. A previous study has shown that a decrease in complex I, but not complex II, activity in skeletal muscle predicts the prognosis of critically ill patients[24, 25]. Moreover, decreased complex I activity in peripheral blood mononuclear cells (PBMCs) is associated with immune dysregulation[24]. These findings indicate the clinical importance of decreased complex I in burns and critical illness. In fact, Dr. Kaneki's preliminary experiments showed that complex I activity in PBMCs was significantly

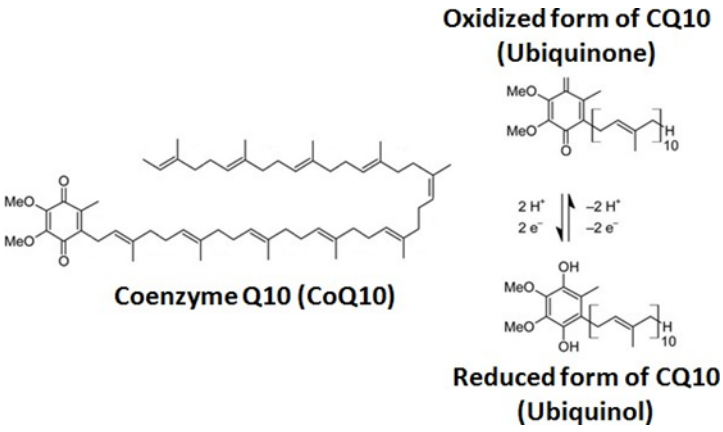

Figure 1. *CoQ10 exists in exists in oxidized (ubiquinone) and reduced (ubiquinol) forms*

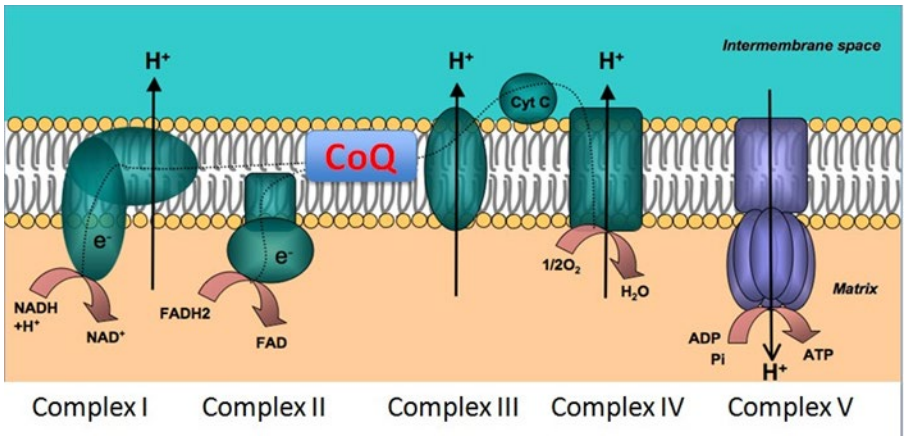

Figure 2. *CoQ10 receives electrons from NADH dehydrogenase at complex I or from succinate dehydrogenase at complex II and transfers electrons to cytochrome C at complex III in the mitochondrial ETC*

lower in burn patients than healthy controls.

Disruption of mitochondrial integrity and inflammatory response: Mitochondria are not just the power plants of cells. They lie at the crossroads of energy metabolism, apoptosis and inflammatory response, and in fact, regulate their function. When mitochondrial integrity is disrupted, mitochondrial DNA (mtDNA) is released into the cytosol where it activates DNA sensors (e.g., toll-like receptor-9 [TLR9], AIM2), leading to an inflammatory response[26], particularly activation of NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3) inflammasome[27]. Once NLRP3 inflammasome is activated, it causes activation and cleavage of caspase-1, which, in turn, cleaves pro-interleukin (IL)-1β, converting it into mature, active IL-1β. This is reminiscent of the induction of apoptosis by cytochrome C release from the mitochondria. Of note, CoQ10 content in PBMCs is lower in fibromyalgia patients than healthy controls, which parallels inflammation in PBMCs. CoQ10 supplementation reduced inflammation in PBMCs of fibromyalgia patients[28]. The latter study indicates that CoQ10 deficiency can cause inflammation, which is reversed or ameliorated by CoQ10 supplementation. Similarly, CoQ10 supplementation prevented burn-induced NLRP3 activation, as indicated by cleavage of pro-caspase-1 and pro-IL-1β, in mouse skeletal muscle.

Circulating mitochondrial damage-associated molecular patterns (DAMPs): mtDNA and mitochondrial proteins (e.g., heat shock protein 60, formyl peptides) are increased in the circulation after major trauma, including burn injury, owing in a significant part to mitochondrial disintegrity and apoptotic cell death[29-31]. These mitochondrial damage-associated molecular patterns (DAMPs, aka alarmins) play a crucial role in sterile systemic inflammatory response in major trauma. Dr. Kaneki's preliminary data showed that plasma mtDNA concentrations were significantly greater in burn patients than healthy controls, consistent with previous studies in non-burn major trauma patients[29-33]. In his preclinical study in mice, CoQ10 administration prevented burn-induced increases in circulating mtDNA and high-mobility group protein B1 (HMGB1) concentrations. Together, these findings suggest that CoQ10 supplementation may reduce elevated DAMPs and control systemic inflammatory response in burn patients. Of note, in his pilot study in burn patients, CoQ10 supplementation tended to inhibit increased plasma mtDNA levels compared with placebo. These findings suggest that CoQ10 supplementation may ameliorate the disruption of mitochondrial intergrity and thereby inhibit an elevation in mitochondrial DAMPs in the circulation in burn patients.

CoQ10 as a protector of mitochondria from oxidative stress: Burn injury induces oxidative stress[34, 35], which, in turn contributes to organ damage and dysfunction. Oxidative stress causes damage and disintegrity of the mitochondria. Conversely, the mitochondria serve as the major site of reactive oxygen species (ROS) generation in cells, and the mitochondria generate increased amounts of ROS when they are damaged. Hence, inherent defense mechanisms that protect against oxidative stress are necessary to the mitochondria. The reduced form of CoQ10 (ubiquinol) is a membrane-localized antioxidant that resides in the mitochondria and is an integral component of the mitochondrial anti-oxidative stress defense mechanism[36]. Reduced form CoQ10 (ubiquinol) supplementation mitigates oxidative stress in many human diseases[37-39] and increases resistance to oxidative stress in mice[40]. CoQ10 deficiency, therefore, increases susceptibility to oxidative stress, leading to aggravated oxidative damage and mitochondrial dysfunction, and CoQ10 administration prevented burn-induced oxidative stress in the mitochondria of mouse skeletal muscle in Dr. Kaneki's work. These

several lines of evidence support our expectation that CoQ10 supplementation will exert protective effects against oxidative stress in burns.

Protective effects of CoQ10 in burned mice: Recently, Dr. Kaneki's preliminary data showed that supplementation with reduced form CoQ10 (ubiquinol) (40 mg/kg, SC, b.i.d.) prevents burn-induced mitochondrial dysfunction/disintegrity, insulin resistance, hyperlactatemia, oxidative stress in the mitochondria, and NLRP3 inflammasome activation in mice. Full thickness third degree burn injury (30% TBSA) induced the following effects: (1) decreased activity in mitochondrial ETC (e.g., complex I) and decreased mtDNA-to-nDNA ratio in muscle; (2) morphological abnormalities in the mitochondria (i.e., elongation of mitochondria, loss of cristae structure); (3) insulin resistance in skeletal muscle; (4) hyperlactatemia; (5) mtDNA translocation to the cytosolic fraction; (6) NLRP3 inflammasome activation; and (7) increased circulating levels of mtDNA and HMGB1. These changes were reversed or lessened by CoQ10 supplementation. These findings indicate that CoQ10 supplementation reduces the severity of burn-induced mitochondrial dysfunction/disintegrity and metabolic derangements, thereby mitigating multi-organ dysfunction. Moreover, his preliminary study showed that CoQ10 supplementation improved survival, bacterial clearance, and systemic inflammatory response in septic mice.

Safety of CoQ10 supplementation: CoQ10 is an essential nutrient and has an excellent safety profile[41]. CoQ10 is commercially available to the general public in doses up to 600 mg/day. Previous clinical studies have reported the safety of high-dose, long-term CoQ10 supplementation in patients with amyotrophic lateral sclerosis (3,000 mg/day)[42] and Huntington's disease (3,600 mg/day)[43]. CoQ10 is a nutrient, but since it is biosynthesized in the mitochondria of every cell type of our body, it is not considered a vitamin. To date, CoQ10 status has not been studied in burns or major trauma. Although the majority of CoQ10 in the human body is produced endogenously in healthy adults, it has been estimated that approximately 20% to 25% of circulating CoQ10 is derived from dietary sources[44]. Nonetheless, current standard enteral and parenteral nutritional support preparations do not include CoQ10.

IV. PRELIMINARY STUDIES

Pilot clinical study: Dr. Kaneki conducted a pilot double-blind randomized, placebo-controlled clinical study of CoQ10 (ubiquinol) supplementation (1,800 mg/day) after burn injury at the Massachusetts General Hospital (MGH) (Clinical Trials.gov. Identifier: NCT02251626; PI: Masao Kaneki). The objectives of this study were to evaluate the safety and bioavailability of CoQ10 supplementation in burn patients. Eligible adult patients with ≥5% TBSA burn were enrolled within 72 hours after burn injury. The intervention consisted of enteral CoQ10 supplementation or placebo for 4 weeks or until hospital discharge, whichever came first. Prior to CoQ10/placebo supplementation, blood samples were collected (designated as Day 0). The effects of CoQ10 supplementation on CoQ10 levels in plasma and peripheral blood mononuclear cells (PBMCs) were studied. Thirty patients were enrolled and studied (each arm: n=15). Block randomization was used and enrollment was not stratified by burn size in the pilot study. An investigational new drug (IND) exemption was obtained from the U.S. Food and Drug Administration (FDA) for CoQ10 (ubiquinol) (1,800 mg/day) in adult burn patients.

Overall demographics: There was no difference in age, gender, burn size (%TBSA) or Revised Baux score between the CoQ10 and the placebo groups. The characteristics of the cohort can be seen in **Table 1**. No adverse events or protocol deviations were noted in the

conduct of the overall study.

CoQ10 deficiency in burn patients in comparison with healthy controls. The pilot study showed that plasma total and reduced CoQ10 concentrations, reduced-to-total CoQ10 ratio and CoQ10 content in PBMCs were significantly lower in burn patients on Day 0 (baseline) prior to the CoQ10 or placebo supplementation (n=30) compared with healthy controls (n=12) (age [y.o.]: 40 ± 4 [mean ± SEM]). In the placebo group, CoQ10 levels in plasma and PBMCs remained significantly lower on Day 3 and Day 6 as well as Day 0 compared with healthy controls.

Table 1. *Pilot study population*	**Age (year)**	**Female, n (%)**	**%TBSA**	**LOS (days)**	**Revised Baux Score**
CoQ10 Grp (n=15)	48.9 ± 3.9	3 (20.0)	19.4 ± 4.9	22.0 ± 3.4	69.5 ± 6.3
Placebo Grp (n=15)	48.2 ± 4.7	5 (33.3)	13.9 ± 2.8	21.9 ± 5.3	65.0 ± 7.0
P value	0.90	-	0.33	0.99	0.63

CoQ10 supplementation effectively increased CoQ10 levels in burn patients. CoQ10 supplementation significantly increased plasma total and reduced CoQ10 concentrations, % reduced CoQ10, and CoQ10 content in PBMCs in burn patients compared with the placebo group and with the baseline levels prior to the supplementation (Day 0). These data indicate that burn injury decreases CoQ10 levels, which are reversed by CoQ10 supplementation.

CoQ10 supplementation tended to inhibit increased circulating mtDNA levels in burn patients. The pilot data showed that plasma mtDNA levels were significantly greater in burn patients than healthy controls. These results are consistent with previous studies in non-burn major trauma patients[29-33]. In the placebo group, plasma mtDNA levels tended to increase on Day 3 relative to Day 0 although there was no statistical significance ($p<0.10$). On the other hand, in the CoQ10 group there was a trend toward decreased plasma mtDNA levels on Day 3 relative to Day 0 ($p<0.10$). The Day 3-to-Day 0 ratio of plasma mtDNA levels tended to be lower in the CoQ10 group than the placebo group ($p=0.0765$), but there was no statistical significance possibly owing to the small sample size. Our data suggest that CoQ10 supplementation may protect against burn-induced mitochondrial disintegrity and thereby inhibit an increase in circulating mtDNA levels.

Taken together with previous studies and Dr. Kaneki's preliminary data in mice, these findings provide sufficient scientific evidence to test the hypothesis that CoQ10 supplementation prevents MODS by ameliorating mitochondrial dysfunction/disintegrity in burn patients. Collectively, our data warrant a large-scale clinical trial to evaluate the safety and efficacy of CoQ10 supplementation in severely burned patients.

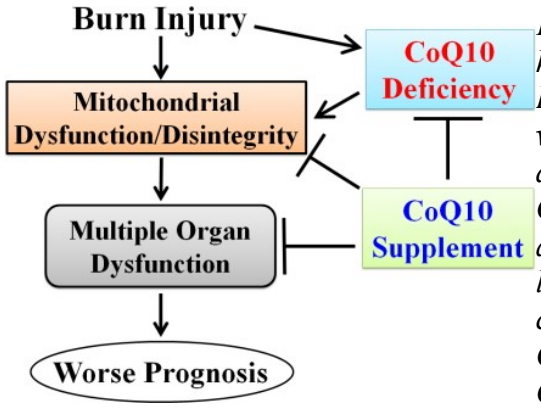

Fig. 3. *Schematic presentation of the overall hypothesis. Based on previous studies and Dr. Kaneki's preclinical and clinical pilot results, we posit that burn injury causes mitochondrial dysfunction/ disintegrity in parallel with CoQ10 deficiency, which in turn induces and/or exacerbates multi-organ dysfunction, leading to worse prognosis. Importantly, pilot clinical data showed that burn injury causes CoQ10 deficiency, which was reversed by CoQ10 supplementation. Moreover, this preliminary data showed that CoQ10 supplementation prevents burn-induced mitochondrial dysfunction/disintegrity, metabolic aberration, oxidative stress and inflammation. These results warrant a clinical trial of CoQ10 supplementation in burn patients.*

STUDY OBJECTIVES AND HYPOTHESES

Primary objective: To evaluate the efficacy of reduced form CoQ10 (ubiquinol) supplementation in severely burned adult patients in the mitigation of MODS between 72 hours after burn injury and 12 weeks, death, or discharge (whichever comes first).

Primary objective hypothesis: Reduced form CoQ10 supplementation will result in a statistically significant reduction in the incidence of MODS between 72 hours after burn injury and 12 weeks, death, or discharge (whichever comes first) as compared to placebo.

Secondary objectives: To evaluate the effect of reduced form CoQ10 (ubiquinol) supplementation in severely burned adult patients on the following clinical outcome events and biochemical measurements in plasma and urine between 72 hours after burn injury and 12 weeks, death, or discharge (whichever occurs first):

1. The six individual organ systems comprising the MODS score and their times of occurrences,
2. The weighted composite score of the six constituents
3. The maximum total Sequential Organ Failure Assessment (SOFA) score
4. The maximum SOFA score for each organ
5. AUC of total SOFA score (score x days)
6. AUC of SOFA score for each organ (score x days)
7. Delirium (CAM-ICU) (number of days)
8. Length of hospital stay (number of days)
9. Sepsis (Sepsis-3) (number of incidence)
10. Septic Shock (Sepsis-3) (number of incidence)
11. Plasma mitochondrial DNA (4 blood collections)
12. 3-Methylhistidine (3-MH) and creatinine in urine (a biomarker of muscle wasting)

Secondary objective hypothesis: Reduced form CoQ10 supplementation will result in a statistically significant reduction in each of the secondary endpoints between 72 hours after burn injury and 12 weeks, death, or discharge (whichever comes first) as compared to placebo.

RESEARCH DESIGN

Brief study overview: We will conduct a two-arm, prospective, double-blind, randomized, placebo-controlled clinical trial to evaluate the efficacy and safety of reduced form CoQ10 (ubiquinol) supplementation in severely burned adult patients (total n=290) at 15 mature burn centers across North America. Stratified block randomization will take place by site and Revised Baux score. The formula for the Revised Baux score is: Age + Percent Burn (%TBSA) + 17 x (Inhalation Injury, 1 = yes, 0 = no). This formula has been widely adopted for mortality prediction in burn patients[45,46]. In previous studies, a trend has been shown for a sharp increase in mortality when the Revised Baux score exceeds approximately 110[45,47,48]. Therefore, patients enrolled in this study will be stratified for randomization into two groups based on the Revised Baux score: (1) less than 110; and (2) equal to or greater than 110. The intervention will consist of a loading dose of reduced form CoQ10 of 1,800 mg/day tid for 4 weeks to be followed by a maintenance dose of 600 mg/day once daily from weeks 5 to 12. The intervention or allocation-controlled placebo will be administered by 72 hours after injury and will continue until 12 weeks after injury or until death or discharge, whichever comes first. Oral tablets (600 mg/tablet) will be administered to CoQ10 subjects who can swallow while a liquid form (100 mg/mL) will be administered to CoQ10 subjects requiring an enteral tube for nutrition. There will be no post-discharge study procedures.

Study investigators, personnel, and centers:

Herb A. Phelan MD, MSCS; UT Southwestern Medical Center/Parkland Memorial Hospital
James H. Holmes IV, MD; Wake Forest University School of Medicine
Steve E. Wolf, MD; University of Texas Medical Branch- Galveston
Amalia Cochran, MD; The Ohio State University Wexner Medical Center
James Hwang, MD; University of Alabama-Birmingham Medical Center
Dhaval Bhavsar, MD; University of Kansas Medical Center
Gary Alec Vercruysse, MD; University of Michigan
Anjay Khandelwal, MD; Case Western Reserve University School of Medicine
Jeremy Goverman, MD; Massachusetts General Hospital
Tony Baldea, MD; Loyola University Medical Center
Janelle Wagner, MD; Temple University
Nicholas Meyer, MD; Columbia St. Mary's-Wisconsin
Tina Palmieri, MD; University of California- Davis
Rachel Karlnoski, PhD; University of South Florida
David Smith Jr., MD; University of South Florida
Fred Endorf, MD; Hennepin County Medical center

Lori Palfalvi, CRA; American Burn Association

Mary Beth Lawless, RN, MSN; UC Davis, American Burn Association

***Description of the Recruitment Process*:** Study coordinators will screen the medical records of newly admitted patients using the inclusion/exclusion criteria. Only English and/or Spanish-speaking adult subjects or surrogates (≥18 years) will be approached to consider study participation since this represents the overwhelming majority of patients who receive care in the study sites. No remuneration will be provided.

***Description of the Informed Consent Process*:** The physician, who is known to the potential subject and has firsthand knowledge of the patient's medical history, will approach the patient/family at the bedside to briefly introduce the study and emphasize that the patient/family is free to participate or not, and ask if the patient/family would like to hear more information about the study from a physician-associate. If answered in the affirmative, a co- investigator or their designated research coordinator will be notified. This study collaborator will subsequently come to the bedside and provide more detail about what the study would involve for the patient and again emphasize the voluntary nature of participation. If the patient/family expresses reluctance, then the topic of participation would end at that point. If the patient/family expresses an interest in participation, the MD co-investigator or study coordinator would discuss the study in more detail, answer questions, and obtain informed consent in a private room. Human subjects' questions will be addressed by physician investigators listed on the IRB protocol during the consent process and throughout the trial.

In compliance with the guidelines, the following categories of surrogates (listed in preferred order) may provide consent in writing on behalf of potential subjects incapable of providing informed consent: i) court appointed guardian with specific authority to consent to participation in research or authority to make health care decisions for a class of diagnostic and therapeutic decisions inclusive of the proposed research; ii) health care proxy/person with durable power of attorney with specific authority for making health care decisions inclusive of the proposed research; or iii) spouse, adult child, or other close family member who knows the subject well and has been involved in their care. Assent of subjects will be a requirement for participation in the research unless the subject is incapable of giving assent due to his/her medical condition. A licensed physician colleague or a well-trained study investigator will obtain informed consent. Participants will be given as much time as necessary to consider participation or to discuss the study with anyone before making a decision. Routine care will be provided to the patient regardless of enrollment to the study. This fact will be explained to the patients and/or legal guardian.

***Randomization procedure*:** Permuted-block randomization stratified by study site and Revised Baux score will be performed. This study procedure will utilize the RS2 system that was developed by the ABA's Data Coordinating Center (DCC) based out of UC-Davis and which has been used in previous burn trials successfully.

Study population:

Table 2.	

Inclusion Criteria	Rationale
Age 18 years and older	Military age subjects. There are no limitations on gender, race, or ethnicity.
Burn patients with 20% or greater of total body surface area (TBSA) burn and equal to or less than 70% TBSA burn	Burns greater than 20% are considered to be severe and potentially life threatening. Burns larger than 70% carry an excessive mortality and confound the primary endpoint of MODS
Capable of receiving routine oral, enteral nutrition, or a combination of routine oral and enteral nutrition	CoQ10 is not available in injectable form for humans. The enrolled patients will receive tablets (softgels) or liquid form of CoQ10 or placebo.
Enrolled within 72 hours after burn injury	This timepoint is early enough that a preventative effect of CoQ10 replacement can manifest
Patient or legally authorized representative (LAR) who is capable of giving full informed consent	Principle of autonomy
Anticipated hospital stay: 2 weeks or more	This duration is the common timeframe for MODS development should it occur.
Exclusion Criteria	**Rationale**
Patients with liver disease (bilirubin greater than 3 or diagnosis of liver cirrhosis) at the time of admission, hyperthyroidism that currently requires treatment, diagnosis of chronic heart failure, chronic renal failure requiring hemodialysis, malignancy currently undergoing treatment, or history of cancer or hematological malignancy treatment within 5 years	The primary endpoint of MODS scoring is intended for acute organ dysfunction. Subjects with chronic, pre-existing organ dysfunction are confounders
History of HIV or AIDS	MODS is driven by the immune system so pre-existing immune dysfunction is a confounder
Presence of concurrent injuries apart from burn injury that may produce long-term disabilities (e.g., spinal cord injury, anoxic brain injury)	Nonthermal injury is a MODS risk by itself and therefore methodologically problematic

Participation in another research study that may confound the results of this study in the opinion of the site principal investigator	Concurrent enrollments in prospective studies with non-competing endpoints are common events in academic burn centers
Pregnant women	Vulnerable population
Prisoners	Vulnerable population

***Rationale for the dosing regimen*:** The dosing regimen for CoQ10 supplementation consists of a loading dose of 600 mg po tid (ie, 1800 mg/day total)for 4 weeks followed by a maintenance dose of 600 mg/day (once daily) for an additional 8 weeks (12 weeks total) or until death or hospital discharge, whichever comes first. This regimen is based on Dr. Kaneki's pilot study in adult burn patients. This dose effectively increased CoQ10 levels both in plasma and in PBMCs in the randomized burn patients including those with 20% - 70% TBSA burn injury. In contrast, in the placebo group, CoQ10 levels declined after burn injury. A relatively high dose (i.e., 1,800 mg/day) is necessary to effectively increase intracellular CoQ10 levels in the initial period of CoQ10 supplementation. As shown in previous studies and pilot data, plasma CoQ10 concentration increased within an hour after oral intake of CoQ10. However, intracellular CoQ10 is delayed with oral CoQ10 intake. Presumably, this occurs because cells lack the specific molecular machinery required to uptake extracellular CoQ10, since CoQ10 is endogenously synthesized in every cell type. On the other hand, a relatively low-dose of CoQ10 (200-300 mg/day) supplementation achieves the plateau level of intracellular CoQ10 within 4 weeks in healthy individuals[49]. Once the CoQ10 has reached the desired plateau within 4 weeks, a lower dose (600 mg/day) is sufficient to maintain the plateau.

Because of the lipophilic nature of CoQ10, bile acids secreted in the intestine at the time of meals or enteral feeding facilitate absorption of CoQ10 as well as other lipids by the intestine. We propose, therefore, to give CoQ10 supplementation within 30 minutes after meals or enteral feeding. CoQ10 tablets (softgels) and liquid form (100 mg/mL) and respective placebo will be provided by Kaneka Nutrient Corp. (Pasadena, TX).

Measurement of Biomarkers

Plasma samples for mitchondrial DNA (mtDNA) assays: Plasma samples will be collected on the first 100 subjects enrolled across all centers. These four plasma samples will be collected at four separate time points: 1) at the time of enrollment (and therefore within 72 hours of injury), 2) on post-burn day 7, 3) on post-burn day 14, and 4) on post-burn day 21). Each specimen will be handled in an identical fashion. To remove residual cells, platelets, microparticles, and debris, EDTA-plasma samples will be obtained from whole blood (10 mL) and then the plasma samples will be spun twice at 5,000g[50]. mtDNA will be extracted using the mtDNA extraction kit (Wako Chemicals), and mtDNA levels will be evaluated by real-time PCR using specific primers for mtDNA (forward: 5'-CACTTTCCACACAGACATCA-3'; reverse: 5'-TGGTTAGGCTGGTGTTAGGG-3')[51]. Plasma samples will be stored at -80ºC freezer until the measurement. As a standard for the measurement of mtDNA levels, we will use the plasmid vector, in which we subcloned the PCR product from the human PBMC-derived DNA using these primers.

Urine samples for 3-methylhistidine and creatinine assays*:* 24-hour urine samples will be collected on the first 100 subjects enrolled across all centers. 24-h urine samples will be

collected for three consecutive days at four time points, namely on Days 1-3 (first), 7-9 (second), 14-16 (third), and 21-23 (fourth). Each specimen will be handled in an identical fashion. First, 20 mL of 6N HCl will be added to each container prior to each 24 hour urine collection. 24 hours later, the total 24 hour urine volume will be recorded and that given 24-hour urine specimen will be placed on ice. Once all three 24 hour urine specimens for a given time point are completed, a 20 mL urine sample from each individual 24-hour urine specimen will be collected (2 tubes of 10 mL each) and frozen at -80 degrees C until the measurements. This process will be repeated on each of the three days' collection at the 4 time points. Therefore, each of the first 100 subjects enrolled will generate twelve urine specimens over the four time points.

3-Methylhistidine concentrations will be quantified by negative chemical ionization gas chromatography mass spectrometry as described in our previous studies[52,53]. Urinary creatinine concentrations will be measured by a commercial kit. The average of three measurements on the three consecutive days at each time point will be used for statistical analysis to minimize possible day-to-day variations.

STATISTICAL ENDPOINTS AND APPROACH TO DATA ANALYSIS

This two-arm balanced double blind parallel Randomized Clinical Trial (RCT) study will be performed using an intention to treat (ITT) analysis population that includes all randomized patients.

Primary endpoint: The primary endpoint will be the count of the following events. This count will be used to create a composite score ranging from 0 to 6 by assigning 1 to each event regardless of stage, scores and severity that individual subjects encounter from 72 hours after burn injury through 12 weeks or until death or discharge, whichever occurs first:

1. Acute Kidney Injury (AKI) (KDIGO Stages 1 – 3)
2. ARDS (The Berlin ARDS Definition: Mild, Moderate, Severe)
3. Cardiovascular Dysfunction (SOFA Scores 1 – 4)
4. Coagulopathy (SOFA Scores 1 – 4)
5. Liver dysfunction (SOFA Scores 1 – 4)
6. Death (all causes)

We will calculate mean values of the composite sum scores among the study patients in the CoQ10 and placebo arms, and then test their difference by using an independent samples *t*-test if the observed data are evident to follow a normal distribution by Shapiro-Wilk's test. Otherwise, Mann-Whitney U test will be applied and the group specific medians and quartiles will be used as the summary statistics.

AKI: AKI represents the acute loss of kidney function and is associated with a profound and severe increase in morbidity and mortality in burn patients[54]. Early AKI, which occurs less than 3 days post-burn, is usually related to inadequate fluid replacement during the initial resuscitation. AKI can occur later in the hospitalization and late AKI is multifactorial in origin and often associated with sepsis development[55]. For the purposes of this study, we will follow the definitions and classification guidelines set forth in "AKI of Kidney Disease: Improving Global Outcomes (KDIGO)"[56,57] (**Table 3**). Published in 2012, this guideline has been used in critically ill patients and in combat-injured populations[58].

Stage	Table 3. AKI (KDIGO)
1	Increase in serum creatinine (SCr) by ≧ 0.3mg/dL within 48 hours or increase in SCr 1.5 to 1.9 times baseline which is known or presumed to have occurred within the prior 7 days
2	Increase in SCr to 2.0 to 2.9 times baseline
3	Increase in SCr to 3.0 times baseline or increase in SCr to ≧ 4.0 mg/dL or initiation of renal replacement therapy

ARDS: ARDS is a common complication in critically ill burn patients leading to increased mortality. The American European Consensus Conference (AECC) ARDS definition was revised by the Berlin definition in 2012 (**Table 4**). Since then, the ARDS Berlin definition has been used in critically ill patients[59], including wartime military burns[60]. We will use the ARDS Berlin definition in this study.

Table 4. The ARDS Berlin Definition	
	Oxygenation
Mild	200mmHg < PaO_2/FiO_2 ≦ 300mmHg
Moderate	100mmHg < PaO_2/FiO_2 ≦ 200mmHg
Severe	PaO_2/FiO_2 ≦ 100mmHg

Cardiovascular dysfunction: To assess cardiovascular dysfunction, we will use the sequential organ failure assessment (SOFA) scores (**Table 5**). The SOFA score is a simple and objective score that allows for calculation of both the number and the severity of organ dysfunction in six organ systems (e.g., respiratory, coagulation, liver, cardiovascular, renal), and the score can measure individual or aggregate organ dysfunction[61] and has been used in many previous studies in critically ill patients, including burn victims. Cardiovascular dysfunction is a common complication of severe burn injury. CoQ10 deficiency causes and/or exacerbates heart failure and clinical trials have shown the protective effects of CoQ10 supplementation in chronic heart failure.

Coagulopathy**:** Severe burn injury is associated with systemic coagulopathy, which is characterized by procoagulant changes, as well as impaired fibrinolytic and endogenous anticoagulation systems[62-65]. The exact pathophysiology of burn-induced coagulopathy is incompletely understood, but recent studies indicate that circulating mtDNA and mitochondrial damage can induce hypercoagulopathy and thrombocytopenia[66-68]. It is conceivable, therefore, that CoQ10 supplementation may ameliorate coagulopathy by mitigating mitochondrial dysfunction/disintegrity in burn patients.

Liver dysfunction: Hepatic function plays pivotal roles in immune function, acute-phase response, systemic inflammatory response, detoxication, and metabolism. Burn causes dysfunction and morphological changes (e.g., hepatomegaly) of the liver[69]. Although liver failure is not a frequent complication of burn injury, increased serum bilirubin level is associated with the mortality of severely burned patients[70].

	Cardiovascular System	**Coagulation**	**Liver**
SOFA Score	Hypotension	Platelets ($10^3/\mu l$)	Bilirubin (mg/dl)
1	MAP＜70 mm Hg	＜150	1.2 - 1.9
2	dop≦5 or dob (any dose)	＜100	2.0 - 5.9
3	dop＞5 OR epi≦0.1 OR nor≦0.1	＜50	6.0 - 11.9

4	dop＞15 OR epi＞0.1 OR nor＞0.1	＜20	＞12.0

Table 5. Cardiovascular, coagulation and liver SOFA score. *MAP: mean arterial pressure; dop: dopamine; dod: dobutamine; epi: epinephrine; nor: norepinephrine. Adrenergic agents administered for at least 1 hour (doses given are in μg/kg/min).*

Note that "coma" is not included within the primary endpoint calculations, even though mitochondrial dysfunction impairs the central nervous system. The rationale for this exclusion is that precise evaluation of coma and consciousness (e.g., Glasgow coma scale) is sometimes difficult to carry out due to intubation and sedation in severely burned patients. However, delirium will be evaluated by the Confusion Assessment Method for Intensive Care Unit (CAM-ICU) as one of our secondary endpoints.

Scientific rationale for the primary endpoint: To evaluate the efficacy of CoQ10 supplementation in severely burned patients, we have chosen dysfunction of multiple organs and systems as the primary endpoint based on the following scientific rationale. *First,* multi-organ dysfunction is a major determinant of the mortality and long-term clinical and functional outcomes of severely burned patients. *Second,* mitochondrial dysfunction/ dysintegrity is involved in dysfunction of multiple organs and various cell types, including heart, kidney, lung, liver, and platelets. *Third,* CoQ10 is biosynthesized in the mitochondria and required for function and integrity of the mitochondria in every cell type and CoQ10 deficiency causes mitochondrial dysfunction in various cell types and a broad range of organs and systems. *Fourth,* previous clinical trials have shown that CoQ10 supplementation improves heart, kidney, and liver function in various human diseases, although the effects of CoQ10 have not been studied in patients with burn injury or major trauma. *Fifth,* in burn patients CoQ10 supplementation tended to lessen the burn-induced increase of circulating mtDNA levels in our pilot study. Increased circulating mtDNA is both an indicator of systemic mitochondrial disintegrity and an inducer or enhanced systemic inflammation. In burned mice, CoQ10 supplementation prevented burn-induced mitochondrial dysfunction/disintegrity and metabolic aberration, while simultaneously inhibiting increased circulating mtDNA levels. Collectively, these data support the hypothesis that CoQ10 supplementation mitigates dysfunction of multiple organs and systems by ameliorating burn-induced mitochondrial dysfunction and disintegrity.

Scientific rationale for the observation period: Studies in trauma patients have suggested the organ dysfunction that develops soon after trauma (i.e., during the first 48 -72 h) rapidly resolves in a high proportion of patients and may reflect the reversible derangement of organ function induced by the inciting event or incomplete resuscitation. Thus, it has been proposed that later assessment of organ function (i.e., 3 days after injury) will yield a more accurate definition of multiple organ failure in trauma patients, which is needed to evaluate the relationship between trauma-induced organ dysfunction and prognosis[71,72]. In the acute phase, particularly the first 72 hours after severe burn injury, organ dysfunction frequently occurs, causing AKI (e.g., oliguria), cardiovascular dysfunction (e.g., hypotension), and coagulopathy (e.g., thrombocytopenia)[73]. In some cases, the organ dysfunction is transient and the patient may recover during the late-acute or sub-acute phase. In such cases, the recovery from organ dysfunction is associated with relatively good prognosis. In contrast, if the organ dysfunction continues beyond the acute phase or if it occurs or recurs at a later time, the clinical outcome is typically worse. Therefore, we will evaluate the effects of

CoQ10 supplementation on the aforementioned multi-organ dysfunction that patients encounter beginning 72 hours post-burn injury and thereafter.

Secondary endpoints: Secondary endpoints will be the following outcome events observed from 72 hours after burn injury through 12 weeks or until death or discharge (whichever occurs first) and the biochemical measurements in plasma and urine.

1. The six individual binary outcome MODS events and their times of occurrences, (i.e., the constituents of the primary composite score)
2. Weighted composite score of the six constituents
3. Maximum total SOFA score
4. Maximum SOFA score for each organ
5. AUC of total SOFA score (score x days)
6. AUC of SOFA score for each organ (score x days)
7. Delirium (CAM-ICU) (number of days)
8. Length of hospital stay (number of days)
9. Sepsis (Sepsis-3) (number of incidence)
10. Septic Shock (Sepsis-3) (number of incidence)
11. Plasma mitochondrial DNA (4 blood collections)
12. 3-Methylhistidine (3-MH) and creatinine in urine (a biomarker of muscle wasting)

Scientific rationale for the clinical secondary endpoints: To comprehensively evaluate the effects of CoQ10 supplementation on dysfunction of organs and systems, we will examine total and individual scores of multiple organs/systems. Severity of multiple organ dysfunction is associated with the morbidity and mortality of burn victims. We will, therefore, evaluate weighted composite score of the six events that constitute the primary endpoint, where stages 1, 2 and 3 of AKI, and mild, moderate and severe ARDS are counted as score 1, 2 and 3, respectively. Regarding cardiovascular dysfunction, coagulopathy and liver dysfunction, we will use the SOFA scores (**Table 5**) for the weighted composite score. Moreover, over and above the maximum severity of organ dysfunction, its duration also affects the clinical trajectory of severely burned patients. Therefore, to take both severity and duration of organ dysfunction into account, we will analyze the area under the curve (AUC) for the SOFA score as previously described[74].

Our preclinical and pilot clinical studies suggest that CoQ10 supplementation may prevent the development and/or exacerbation of mitochondrial dysfunction/disintegrity and multi-organ dysfunction in burn victims. However, it is possible that CoQ10 supplementation may exert beneficial effects in the recovery phase as well as acute and subacute phases. We will, therefore, investigate the time-dependent effects of CoQ10 supplementation longitudinally.

Delirium, a common complication and significant clinical issue in burn patients, is associated with long-term outcome[75]. Dr. Kaneki's preliminary data showed in mice that mitochondrial dysfunction in the central nervous system plays a critical role in surgery/anesthesia-induced delirium[76]. However, the effect of CoQ10 on delirium has not yet been studied in any disease. CAM-ICU will be used to assess delirium[77].

Sepsis and septic shock are major causes of mortality in burn patients. Dr. Kaneki's preliminary study showed in mice that CoQ10 supplementation improved bacterial clearance and survival of septic mice and prevented inflammation organ inflammation (e.g., heart, liver, kidney). For the diagnosis of sepsis and septic shock, we will use the Third

International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) that was issued in 2016[78,79].

Scientific rationale for the biochemical secondary endpoints: To evaluate the effect of CoQ10 supplementation on systemic mitochondrial disintegrity, we will measure mtDNA abundance in the circulation. Mitochondrial disintegrity and apoptotic cell death have been implicated in the rise of circulating mtDNA. Moreover, circulating mtDNA functions as a damage-associated molecular pattern (DAMP), which induces and/or exacerbates systemic inflammatory response in major trauma[29, 33]. In our pilot study, plasma mtDNA levels were significantly higher in burn patients compared with healthy controls. These results are consistent with previous studies in non- burn trauma patients. In our preclinical study in mice, burn caused an increase of mtDNA levels in plasma, which was mitigated by CoQ10 administration. Similarly, there was a trend toward decreased circulating mtDNA levels in the CoQ10 group compared with the placebo group ($P<0.10$), although statistical significance was not achieved possibly due to the small sample size (n=15 per group).

Muscle wasting is a major metabolic complication of burn injury and worsens the clinical outcome of burn patients. Muscle wasting interferes with the ability to wean off mechanical ventilation, recovery, and rehabilitation after burn injury. Moreover, muscle wasting is an essential component of burn cachexia and hypermetabolism, both of which are major contributors to worse prognosis after severe burn injury. Increased proteolysis is a major component of burn-induced muscle wasting. After burn injury, both breakdown and synthesis of proteins increase in skeletal muscle; however, the increase in protein breakdown exceeds that of protein synthesis, leading to muscle atrophy[80,81]. To evaluate muscle wasting, therefore, we will measure 3-methylhistdine in 24-h urine samples, an indicator of protein breakdown in muscle, and normalize it to creatinine concentrations. Because 3-methylhistdine is present only in actin and myosin and is not reutilized for protein synthesis after it is released during proteolysis[82], increased 3-methylhistdine-to-creatinine ratio in urine indicates accelerated myofibrillar protein breakdown[83]. Burn injury increases 3- methylhistdine-to-creatinine ratio in human and rat urine[52,53].

Data Analysis: For between-arm comparisons of single valued (either measured at once or as an aggregated variable such as AUC over a period) outcomes, we will apply chi-square test if the outcome is binary, and *t*-test or Mann-Whitney *U*-test to continuous outcomes based on the best fit of Shapiro-Wilk's test. The between-arm difference in the distributions of times to the first event and multiple events (of the six constituents) will be examined by using single event time Cox's model and the times to correlated multiple events using Cox's model allowing multivariate frailty, respectively. The incidence rates of each individual event, with- and without differentiating its severity, as a binary outcome will be compared between the two arms by means of longitudinal logistic modeling with generalized estimating equation (GEE)[84] to allow more than one events per patient. For the repeated measures analysis of the longitudinal plasma mitochondrial DNA and urine 3- methylhistidine (3-MH), we will examine if the mean levels over time are different between the two arms by using longitudinal general linear mixed effects model[85] which will provide the within group time dependency patterns as a linear effect or more complex form of non-linear patterns which would be able to characterize the time dependent treatment effects over time along with testing for time × group interaction(s) of those model parameter(s). Total and organ-specific SOFA scores over time will also be examined by this mixed effects models of which the

particular interest is to test if the improvement in the longitudinal mean SOFA scores is greater during certain time window. All analyses will also be extended to adjust for the influencing effects of any baseline demographic and/or clinical indications. Missing data may be unavoidable. Because the informed consent from many study enrollees will be obtained through the family members (i.e., surrogates), a small portion (up to 2% attrition rate) of the enrolled patients may voluntarily withdraw from the study. Before final analysis, we will check the missing data pattern of the outcomes first. If there are systematic differences in missing data patterns between the study groups and/or patient characteristics (e.g., burn type), we will take into account such problems in the analysis. The longitudinal mixed effects modeling will provide unbiased and consistent estimates of the effects provided that the missing data mechanism is 'missing at random' (MAR). If necessary, multiple-imputation will be applied to all endpoints analyses. All statistical tests will be two-sided and will adopt a 5% significance level.

Sample size calculation: The sample size of 290 study patients for this study is determined to achieve an adequate statistical power at a 5% Type-1 error rate for the primary endpoint analysis by the following statistical calculations. We first estimated the expected means and variances of the composite scores (assuming the six individual events are independent) for the Control and CoQ10 treatment arms under the assumption depicted in **Table 6** via 1000 repeated simulated data sets (n = 5000 each), instead of calculating them algebraically.

Each simulation run calculated the means and variances of the two arms, and the resulting average means (and variances) over 1000 simulations were 2.43 (1.04) in the placebo arm and 1.94 (1.05) in CoQ10 arm respectively. Based on the assumption about the event incidence rates and the estimated means and variances of the composite scores from the simulated data sets, the calculated required sample size per treatment arm (using PASS[110]) to detect such a difference (i.e., 20% reduction in the overall events) with 80% power at a 5% Type-1 error rate using 2-sided independent samples t-test was 142 per arm.

We have estimated the incidence rates of dysfunction of each organ or system and mortality under the null hypothesis (H0), which are listed below in **Table 6** (Control Arm), based on previous studies[6,46,48,59,60,64,74,86-95] and Dr. Kaneki's pilot study.

Table 6. *Elements of the composite endpoint and their assumed incidence rates*	**Control Incidence Rate**	**CoQ10 Incidence Rate**
Acute Kidney Injury (KDIGO) (Stage 1 -3)	40%	32%
ARDS (Berlin Definition) (Mild – Severe)	40%	32%
Cardiovascular Dysfunction (Score 1 - 4)	70%	56%
Coagulopathy (Score 1 - 4)	70%	56%
Liver dysfunction (Score 1 - 4)	10%	8%
Death (All Causes)	8%	6.4%

Quarterly Enrollment Target: 290 burn patients will be enrolled at the 15 sites and each site is anticipated to enroll 19 patients during the study period. The quarterly enrollment targets are shown below in **Table 7**.

Table 7	Year 1				Year 2				Year 3				Year 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4

Quarterly	-	-	24	24	25	24	24	25	24	24	25	24	24	25	-	-
Cumulative	-	-	24	48	73	97	121	146	170	194	219	241	265	290	290	290

DATA MANAGEMENT AND DATE SAFETY MONITORING PLAN

Data management: Data will be housed in a secure database at the University of California Davis Data Coordinating Center (UCD-DCC). The UCD-DCC will utilize the RedCap data management tool to manage this clinical study. Records containing PHI will be managed according to the requirements of the HIPAA Privacy Rule. All subject case study forms and records will be de-identified and assigned a unique study number to protect patient privacy and maintain confidentiality. PHI will be maintained at the participating sites for the purpose of data verification. Any hard copy source documents will be stored in a locked file at each site for a minimum of three years after study closure and completion of analysis. If any information from this study is presented at scientific meetings or published, subject names and PHI will not be used.

***Data safety monitoring plan and interim analysis*:** We will follow the guidelines of the DoD to form the Data Safety Monitoring Board (DSMB). The DSMB will monitor the overall status of the trial: number of patients enrolled overall and per each center, adherence to protocol overall and per center and results of the interim analysis.

Risk management and emergency response: We do not anticipate any severe adverse event (SAE) related to the supplement or placebo. No adverse events (AEs) occurred in Dr. Kaneki's pilot study of CoQ10 that enrolled 30 burn patients. We will, however, establish risk management and emergency response plans. Site PIs and site Co-investigators will monitor the safety environment of the participants. If an adverse event (AE) or unanticipated problem occurs during the study period, it will be reported to the overall PI, the site PI, the overall study IRB, and the UCD-DCC. In that case the overall PI will report the AE to other site PIs. The site PI and/or overall PI will submit an expedited SAE report to the American Burn Association and the USAMRMC within 24 hours of the event being reported to the PI. The expedited report will be followed by a detailed, written SAE report. The DSMB will convene and decide necessary responses to unpredictable risks or changes in the protocols such as dose reduction and inclusion/exclusion criteria.

RISKS OF STUDY PARTICIPATION

Possible complications of non-surgical procedures: The blood samples will be withdrawn from an existing central IV line or venipuncture if a central IV line is not available. No new indwelling catheters will be placed in order to obtain blood samples. Blood draw from central venous lines may be associated to a small risk of infection; however the procedure will be only performed by authorized and experienced healthcare personnel. The risk of venipuncture is minimal. Hematoma formation and phlebitis are possible complications; however the occurrence is very rare with rapid resolution and venipuncture will be performed only when the patient needs other labs drawn in her/his clinical care.

Supplementation side effects and toxicities: Risks and discomfort associated with this

study will be small. CoQ10 is a nutrient and not a drug. Dr. Kaneki obtained an IND exemption from the FDA for his pilot study of CoQ10 supplementation (1,800 mg/day) in burn patients. The safety of CoQ10 and placebo supplementation in both tablet and liquid formulas has been shown in previous clinical studies in healthy subjects and patients with other disease conditions. High doses of CoQ10 (e.g., 2400 - 3,600 mg/day) have been used in clinical studies in the United States and the long-term safety of high-dose CoQ10 (3,000 mg/day or 3,600 mg/day) has been shown in patients with Huntington's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease, and in healthy subjects. To date, no serious adverse side effect of CoQ10 has been reported regardless of the dosage. Side effects are typically mild and brief, stopping without any treatment needed. Reactions are rare but may include nausea, vomiting, stomach upset, heartburn, diarrhea, loss of appetite, skin itching, rash, insomnia, headache, dizziness, increased light sensitivity of the eyes, fatigue, or flu-like symptoms. There may be other risks that are currently unknown about supplementation of CoQ10 or placebo.

Psychosocial (non-medical) risks: Other potential major risks involve privacy concerns. As such, all data will be coded and only available to the immediate research team and the UCD-DCC. Data will be stored in the HIPPA- compliant REDCap (Research Data Capture) system. Whole blood samples will be labeled according to an established code to be able to identify patients when/if needed. All samples will be stored in a secure -80°C freezer at UTMB. Subject identifying information and the informed consent will be maintained in a locked cabinet in a locked room in the Clinical Research Department of each study site. Results obtained from individual subjects will be analyzed, reported, and discussed in reference to Subject ID only. All investigators and study staff are required to receive training in the ethical use of humans in research. We will not obtain any genetic information, such as DNA sequence, single-nucleotide polymorphism, haplotype and gene expression, although we will measure total amounts of mitochondrial DNA in plasma.

IND EXEMPTION

Given that CoQ10 is classified as a nutrient, Dr. Kaneki received an IND exemption for the original pilot trial. As we will be using the same preparation in the same route of administration and in the same dosage ranges, we do not have plans to repeat obtaining an IND exemption for the present study.

PROJECT MILESTONES

(1) Regulatory documents and research protocols (1-6 months): It will take at least 2- 3 months for regulatory review and approval of this clinical trial by the USAMRMC Human Research Protection Office (HRPO). In the meantime, the protocol will be reviewed and approved by the UT Southwestern (UTSW) Institutional Review Board (IRB). As an institution, UTSW participates in SmartIRB, a platform designed to facilitate multicenter clinical research among participating institutions through single IRB reliance. We anticipate that the overall PI will have the protocol approved by the UTSW IRB prior to the funding start date and will have secured IRB reliance from the other 14 participating centers. A Data Safety Monitoring Board (DSMB) will be formed.

(2) Inception of patient enrollment: After the IRB protocols are approved and data and sample collection methods are confirmed by site visits of the overall PI and the data management staff of the UCD-DCC within the first 6 months of Year 1, we will start enrollment of eligible patients by month 6. Human subjects protection, compliance, quality of data collection, and pace of enrollment will be monitored by the DSMB, the UCD-DCC, and the overall and site PIs.

(3) Data analysis and reports: In the latter half of Year 4, we will perform all the statistical analyses of clinical data and biomarkers and write reports and a manuscript.

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