VIOLET: Vitamin D to **I**mprove **O**utcomes by **L**everaging **E**arly **T**reatment

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1 ABBREVIATIONS AND DEFINITIONS

1,25OH$_2$D = 1,25-dihydroxyvitamin D
25OHD = 25-hydroxvitamin D
ABG = arterial blood gases
AE = Adverse Event
APACHE = Acute Physiology and Chronic Health Evaluation
ARDS = Acute Respiratory Distress Syndrome
BiPAP = Bilevel Positive Airway Pressure
BMI = Body Mass Index
BUN = Blood Urea Nitrogen
CCC = Clinical Coordinating Center
CHF = congestive heart failure
CPAP = Continuous Positive Airway Pressure
CT = computerized tomography scan
CXR = chest x-ray
DNA = deoxyribonucleic acid
DSMB = Data Safety Monitoring Board
ED = Emergency Department
FACTT = Fluid and Catheter Treatment Trial
FDA = Food and Drug Administration
FiO2 = Fraction of Inspired Oxygen
GCS = Glasgow Coma Scale
IBW = Ideal Body Weight
ICU = Intensive Care Unit
ID = Identification
IFN-γ = interferon-γ
IL-1β = Interleukin 1β
IL-2 = interleukin-2
IL-6 = Interleukin 6
IL-8 = Interleukin 8
IL-17 = interleukin-17
IND = Investigational New Drug
IRB = Institutional Review Board
IU = International Unit
INR = International Normalized Ratio
ITT = Intent to Treat
LC/MS/MS = Liquid Chromatography-Tandem Mass Spectrometry (gold standard Vitamin D measurement)
LAR=Legally Authorized Representative
LIPS = Lung Injury Prediction Score
LTAC = Long Term Acute Care Facility
LTFU = Lost to Followup
MAP = Mean arterial pressure
mBW = Measured Body Weight
mRNA = messenger ribonucleic acid

NBAC = National Bioethics Advisory Committee
NDI = National Death Index
NHLBI = National Heart Lung and Blood Institute
NIH = National Institutes of Health
NNT = Number Needed to Treat
OHRP = Office of Human Research Protections
OR = Operating Room
PBW = predicted body weight
PETAL = Prevention and Early Treatment of Acute Lung Injury
P/F = PaO_2/FiO_2 ratio
$PaCO_2$ = Partial pressure of arterial carbon dioxide
PaO_2 = Partial pressure of arterial oxygen
PB = Barometric Pressure
PBW = Predicted Body Weight
PEEP = Positive End-Expiratory Pressure
PI = Principal Investigator
PSV = Pressure Support Ventilation
SACE = Survival Average Causal Effect
S/F = SpO2/FiO2 ratio
SOFA = Sequential Organ Failure Assessment
SBP = Systolic Blood Pressure
SBT = Spontaneous Breathing Trial
SNF = skilled nursing facility
SpO_2 = Oxygen Saturation via pulse oximetry
SSN = Social Security Number
SUSAR = Serious and Unexpected Suspected Adverse Reactions
SAEs = Adverse events that are serious and unexpected and have a reasonable possibility that the event was due to a study procedure
TNF-α = tumor necrosis factor-α
VDR = vitamin D receptor
VFD = Ventilator-free days
WBC = White Blood Cell

Adverse Event: Any untoward medical occurrence associated with the use of a drug or a study procedure, whether or not considered drug related.

Adverse reaction: An adverse reaction means any adverse event caused by a drug. An adverse reaction is a subset of all suspected adverse reactions where there is a reason to conclude that the drug caused the event.

All-cause, all-location mortality: Primary outcome to be assessed by phone call at 90 days for patients discharged alive from the hospital

Assisted breathing: Any level of ventilatory support at pressures higher than the unassisted breathing thresholds (defined below). Completing 48 hours of UAB is defined as the date (calendar day) that the subject reaches exactly 48 hours of UAB. Example: if subject meets

UAB at 1900 on 6/1/15 and does not return to assisted breathing, then the date of completing 48 hours of UAB would be 6/3/15.

Controlled Ventilation: Any mode with a backup rate that allows clinicians to either set tidal volume to a target or adjust pressures to target a tidal volume. Examples include volume assist control, pressure assist control, pressure regulated volume control.

Eligible patient: All patients ≥ 18 years old with an intention to admit to ICU from emergency department, hospital ward, operating room (except uncomplicated, elective post-operative admissions to ICU for routine monitoring), or outside facility **with one or more acute risk factors** for ARDS or mortality and no exclusion criteria. The time of eligibility will be based on documented time of intention to admit to ICU (see below).

Enrolled participants: All eligible patients who have completed the consenting process **and are eligible to receive the vitamin D screening test**

Extubation: Removal of an orotracheal, nasotracheal tube, or unassisted breathing with a tracheostomy

Home: Level of residence or health care facility where the patient was residing prior to hospital admission.

Intention to Admit to Intensive Care Unit (ICU): Documentation of plan to admit to ICU from emergency department (ED), hospital ward, operating room, or referring hospital that sets time zero for the 12-hour enrollment window.

Intention to Treat (ITT): All eligible and consented patients who undergo randomization will be included in the ITT cohort for the purposes of analyzing the primary and secondary study outcomes.

Invasive Mechanical Ventilation: Assisted ventilation delivered by a nasotracheal, orotracheal, or tracheostomy tube

Legal Representative: An individual, judicial, or other body authorized under applicable law to consent on behalf of a prospective patient to the patient's participation in the clinical study.

Funding: National Institutes of Health (National Heart Lung and Blood Institute)

Nonrandomized participants: The subset of eligible and enrolled patients with screening 25OHD levels 20 ng/mL or greater.

Screen/Failure: Patients who meeting eligibility criteria 1-3 **AND** one or more exclusions.

Sponsor: The Clinical Coordinating Center at Massachusetts General Hospital

Study Day: The day of randomization is study day zero. The next day is study day one etc.

Study Drug: Randomly assigned vitamin D_3 (cholecalciferol) or placebo

Study hospital: Defined as the hospital where the patient was randomized and enrolled.

Study withdrawal: Defined as permanent withdrawal from study before completion of study activities. This does not include those participants who have completed the protocol procedures or stopped procedures because they have reached unassisted breathing. If a patient or surrogate requests withdrawal from the study the clinician should seek explicit permission to continue data collection.

Suspected adverse reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality that adverse reaction (21 CFR 312.32(a))

Randomized participants: The subset of eligible and enrolled patients with screening 25OHD levels less than 20 ng/mL that are randomized to receive either study drug or placebo.

UAB (Unassisted Breathing): Spontaneously breathing with face mask, nasal prong oxygen, room air, T-tube breathing, tracheostomy mask breathing, CPAP ≤ 5 without PS or IMV assistance, the use of noninvasive ventilation solely for sleep-disordered breathing, or use of a nasal high flow system.

Valid SpO2: Defined as SpO2 <97%, FiO2 ≥40%, PEEP ≥5 cm H_2O, and at least 10 minutes after any documented change to FiO2 that is not considered spurious by the study staff and treating clinician.

2 TRIAL SUMMARY

2.1 Title

Vitamin D to Improve Outcomes by Leveraging Early Treatment (VIOLET)

2.2 Objective

To assess the efficacy and safety of early administration of vitamin D_3 (cholecalciferol) in reducing mortality and morbidity for vitamin D deficient patients at high risk for ARDS and mortality.

2.3 Hypothesis

Early administration of vitamin D_3 (cholecalciferol) will improve all-cause, all-location mortality to day 90 in vitamin D deficient patients at high risk for ARDS and mortality.

2.4 Study Design

VIOLET is a randomized, double-blinded, placebo-controlled, phase III trial (up to maximum n=3000) of early vitamin D_3 in vitamin D deficient patients at high risk for ARDS and mortality.

We will screen all patients for whom there is an intention to admit to ICU for study eligibility and will approach patients meeting inclusion/exclusion criteria for study enrollment. Screening will require screening for vitamin D deficiency using an FDA approved testing method for 25OHD, either by the hospital's clinical laboratory or using the FastPack IP device (Qualigen Inc., Carlsbad, CA). We will obtain written informed consent for the protocol prior to the vitamin D screening test.

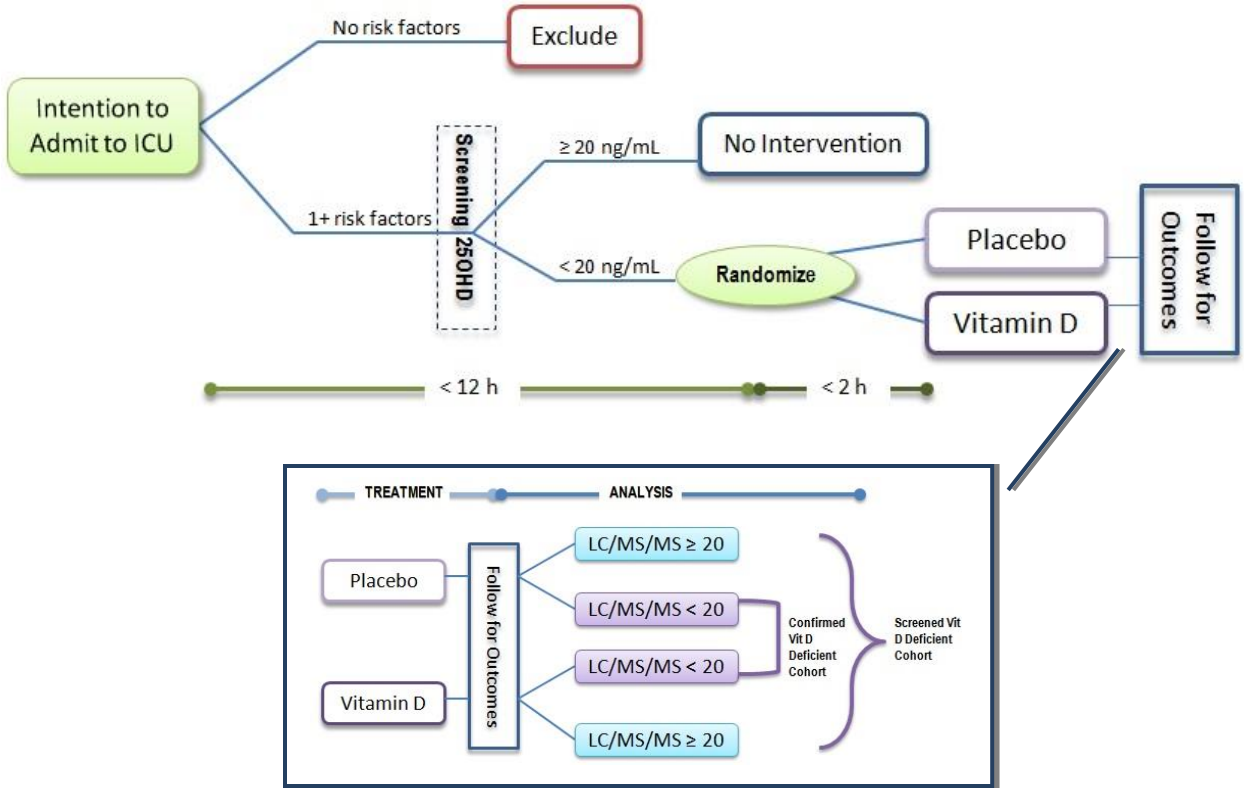
We will randomize enrolled participants who are vitamin D deficient (initial screening 25OHD levels <20 ng/mL) to receive either 540,000 IU vitamin D_3 (cholecalciferol) or placebo as a single, liquid enteral dose, administered either orally or via naso/orogastric tube. Randomization must occur within 12 hours of ICU admission decision and study drug administered within 2 hours of randomization. We will assess trial endpoints for randomized participants by chart review and will contact participants or proxies at day 90.

At baseline for all randomized participants, we will measure serum calcium (in real time, if not clinically available) and plasma 25OHD levels (batched and measured initially in monthly increments using the gold-standard liquid chromatography-tandem mass spectrometry [LC/MS/MS] method). We will collect additional plasma and whole blood for DNA banking. For the first 300 randomized participants, we will measure day 3 serum calcium (in real time, if not clinically available) and baseline plasma IL-6. We will also collect plasma at day 3 in the first 300 randomized participants to measure 25OHD by LC/MS/MS and IL-6 (both batched) and for cryopreservation in the biorepository.

The primary efficacy analysis cohort (confirmed vitamin D deficient cohort) will be defined as randomized participants having LC/MS/MS levels less than 20 ng/mL. The primary efficacy analysis will only involve these participants. The FastPack device had some discordance with LC/MS/MS at a 20 ng/mL cutoff, and the previous study only showed a treatment effect in patients with low vitamin D levels. [1]Since LC/MS/MS values will take at least 24 hours to process, we need to treat participants on the basis of the screening test result (FastPack or

clinical laboratory test) available within three hours of patient consent. A secondary analysis cohort (screened vitamin D deficient cohort), which will also be used for the primary safety analysis, will include all randomized participants who receive vitamin D or placebo.

Figure 1 Study Flow



2.5 Inclusion Criteria

1. Age ≥ 18 years
2. Intention to admit to ICU from emergency department, hospital ward, operating room, or outside facility
3. One or more of the following acute risk factors for ARDS and mortality contributing directly to the need for ICU admission:
   Pulmonary
   a) Pneumonia
   b) Aspiration
   c) Smoke Inhalation
   d) Lung contusion
   e) Mechanical ventilation for acute hypoxemic or hypercarbic respiratory failure
   Extra-Pulmonary
   f) Shock
   g) Sepsis
   h) Pancreatitis
4. Vitamin D deficiency (screening 25OHD level <20 ng/mL)

2.6 Exclusion Criteria

1. Inability to obtain informed consent
2. Unable to randomize within 12 hours of ICU admission decision
3. Unable to take study medication by mouth or enteral tube
4. Baseline serum calcium >10.2 mg/dL (2.54 mmol/L) or ionized calcium >5.2 mg/dL (1.30 mmol/L)
5. Known kidney stone in past year or history of multiple (>1) prior kidney stone episodes
6. Decision to withhold or withdraw life-sustaining treatment (patients are still eligible if they are committed to full support except cardiopulmonary resuscitation if a cardiac arrest occurs)
7. Expect <48 hour survival
8. If no other risk factors present, a) mechanical ventilation primarily for airway protection, pain/agitation control, or procedure; or b) elective surgical patients with routine postoperative mechanical ventilation; or c) anticipated mechanical ventilation duration <24 hours; or d) chronic/home mechanical ventilation for chronic lung or neuromuscular disease (non-invasive ventilation used solely for sleep-disordered breathing is not an exclusion).
9. Prisoner
10. Pregnancy
11. Greater than 72 hours since hospital presentation (or > 3 calendar days since hospital presentation if transferred with no time stamp).

2.7 Randomization and Study Initiation Time Window

All participants must be enrolled and randomized within 12 hours of meeting inclusion criteria. Study medication must be administered within 2 hours of randomization.

2.8 Primary endpoint

The primary endpoint is all-cause, all-location mortality to day 90. The primary analysis will be based on randomized participants with baseline 25OHD levels <20 ng/mL, as measured by LC/MS/MS (***confirmed vitamin D deficient cohort***). As below, additional secondary analyses will be based on all randomized participants (***screened vitamin D deficient cohort***).

2.9 Secondary endpoints

Clinical Endpoints

- Hospital length of stay among survivors to day 90
- Healthcare facility length of stay among survivors to day 90
- Alive and home (prior level of care) at day 90
- Ventilator-free days to day 28
- Time to mortality to day 90

Physiological Endpoints

- Development and severity of ARDS to day 7
- Change in organ failure severity to day 7
- 25OHD levels to day 3
- IL-6 levels to day 3

Safety Endpoints

- Calcium levels to day 14

- Kidney stones to day 90
- Fall-related fractures to day 90

The primary analyses will be in the confirmed vitamin D deficient cohort. These analyses will include an assessment of the secondary endpoints defined above. In addition, we will estimate the treatment effects on the primary and secondary endpoints in the screened vitamin D deficient cohort. If the analysis in the confirmed vitamin D deficient cohort is significant then estimates in the screened vitamin D deficient cohort will measure the treatment effect in patients who would be given the treatment if early LC/MS/MS is not available (or until next generation screening tests more closely approximate LC/MS/MS results). We will also estimate the treatment effects on the endpoints above as a continuous function of baseline 25OHD level by LC/MS/MS. This function will provide an estimate of the extent to which efficacy (and safety) depends on vitamin D level.

2.10 Sample Size/ Interim Monitoring

1. We base our assumptions on finding a 5% or greater absolute difference in mortality. With a 20% mortality rate in the control arm and a 15% mortality rate in the intervention arm, the maximum required total sample size is 3000 randomized participants with 87.4% power based on the assumption that 80% of the randomized participants will have baseline 25OHD levels < 20 ng/mL, as measured by LC/MS/MS, and one sided $\alpha = 0.025$.
2. The principal analysis will be based on randomized participants with baseline 25OHD levels <20 ng/mL, as measured by LC/MS/MS (confirmed vitamin D deficient cohort), following an intention-to-treat principle (analysis according to randomization assignment). We anticipate that approximately 80% of randomized participants will have baseline 25OHD levels <20 ng/mL by LC/MS/MS. The maximum size of the screened vitamin D deficient cohort will be 3000 patients and the futility stopping boundaries will consider the possibility that the sample size will not reach 2400 (80% of 3,000) in the confirmed vitamin D deficient cohort if too small a fraction of randomized patients would have baseline 25OHD levels <20 ng/mL by LC/MS/MS.
3. An independent Data and Safety Monitoring Board (DSMB) will determine if the study should stop for superiority or futility of the intervention, or safety concerns. There will be three interim analyses throughout the trial (at subject enrollments 750, 1500, and 2250 randomized participants).
4. For the first 300 randomized participants, we will analyze calcium levels at baseline and day 3 and 25OHD levels at day 3 using LC/MS/MS. This analysis will evaluate the effectiveness and safety of the vitamin D intervention in correcting vitamin D deficiency (clinical outcomes will not be evaluated at this time). Based on pre-specified rules and in consultation with the DSMB (who will have access to additional adverse event data), we may require changes to the vitamin D dose or extend the duration of 25OHD/calcium monitoring.

3 TRIAL DESCRIPTION

3.1 Background

Acute respiratory distress syndrome (ARDS) is a common and devastating complication of critical illness or injury. Since effective therapies to reduce morbidity and mortality from ARDS are limited, prevention of ARDS in high-risk patients has become a high priority for the acute and critical care communities and a major goal of the PETAL Network.[2] Currently, there are no safe or effective therapies for ARDS prevention. Vitamin D has emerged as a promising intervention to prevent ARDS and improve associated morbidity in critically ill patients. Specifically, strong preclinical data support the protective role of vitamin D in regulating pulmonary inflammation and disruption of the alveolar-capillary membrane that are fundamental to ARDS pathogenesis. Observational data indicate that vitamin D deficiency is common in critically ill patients and a key ARDS risk factor. Phase II trial data demonstrate high potential for early vitamin D supplementation to safely and effectively correct vitamin D deficiency and improve clinical outcomes in critically ill patients. Accordingly, this phase III trial is warranted to definitively test the efficacy and safety of early vitamin D repletion to reduce mortality and morbidity for vitamin D deficient patients at high risk for ARDS and mortality.

3.2 Vitamin D-Potential Benefits and Mechanisms

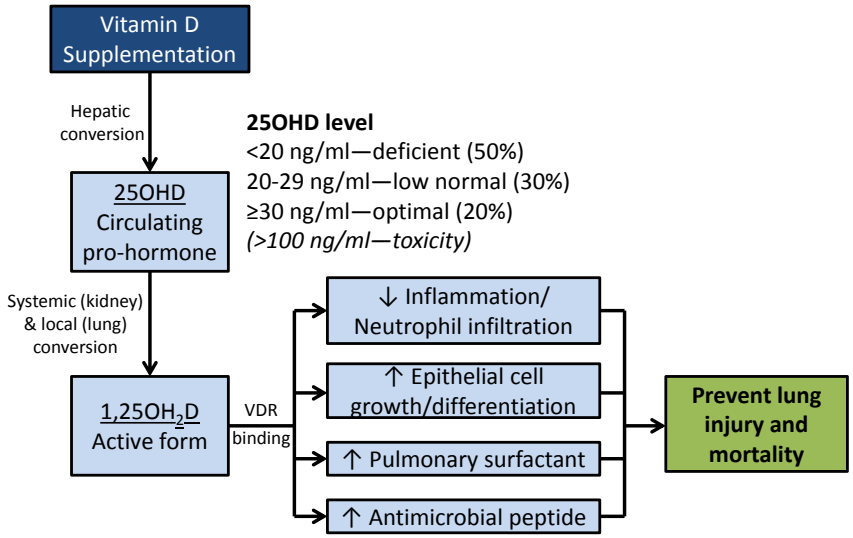
In addition to its role in calcium and phosphorus homeostasis, vitamin D has pleiotropic roles regulating immune function and maintaining epithelial surface integrity. The major circulating form of vitamin D, 25-hydroxvitamin D (25OHD) is converted systemically and locally in the lung to the active hormone 1,25-dihydroxyvitamin D ($1,25OH_2D$), which binds to the vitamin D receptor (VDR) found on most immune and epithelial cells. Pre-clinical data indicate that vitamin D is a potent anti-inflammatory agent[3,4] that reduces neutrophil infiltration,[5] and is essential for: a) epithelial cell growth/differentiation,[6,7] b) pulmonary surfactant synthesis,[8,9] and c) expression of endogenous antimicrobial peptides such as cathelicidin (LL-37) by epithelial cells and leukocytes.[10,11]

- Inflammation: The role of vitamin D in regulation of persistent inflammation is also critical in mitigating secondary organ injury (including ARDS), often seen in lung injury. Specifically, $1,25OH_2D$ inhibits synthesis of pro-inflammatory cytokines IL-1β, IL-2, IL-6, IL-17, TNF-α, and IFN-γ *in vitro* and in animal models of sepsis. However, unlike other more non-specific anti-inflammatory interventions (e.g., glucocorticoids), vitamin D dampens pro-inflammatory responses without negatively impacting pathogen clearance,[12,13] which is critical to recovery from infection-related ARDS.
- Neutrophil Infiltration: $1,25OH_2D$ also suppresses IL-8 production,[14-22] an endogenous chemotactic factor for neutrophils key in the pathogenesis of ARDS.[23-29] In a hamster model of lipopolysaccharide-induced ARDS, $1,25OH_2D$ inhibited neutrophil recruitment in the lung by 40%.[5] In this study, vitamin D supplementation inhibited neutrophils *when administered early*, but not later, in the course of ARDS development. This supports initiating vitamin D therapy early in the hospital course (i.e., in the ED) to optimize prevention of ARDS.
- Epithelial Cell Growth/Differentiation: 25OHD or $1,25OH_2D$ increases airway epithelial cell growth and differentiation in a dose-dependent manner.[6,7] These trophic roles for

vitamin D are important in the preserving pulmonary epithelial integrity that is compromised during ARDS pathogenesis.

- Pulmonary Surfactant: 1,25OH_2D stimulates surfactant protein-B mRNA and protein synthesis in alveolar type II cells.[8,9] Pulmonary surfactant is a complex of phospholipids and proteins that maintains alveolar integrity by reducing surface tension at the alveolar air-liquid interface. Since disruption of pulmonary surfactant is a contributor to the pathophysiology of ARDS, the ability of vitamin D to enhance pulmonary surfactant hold promise as a mechanism to prevent ARDS.
- Antimicrobial: Vitamin D upregulates the production of the antimicrobial peptide cathelicidin (LL-37), aiding host defenses by killing a wide variety of pathogens.[4,10,30-33] Antimicrobial activity of vitamin D leads to control of the source infection and limits the host responses that lead to secondary tissue damage, including ARDS.

Figure 2: Proposed mechanisms of vitamin D and prevent ARDS and improve clinical outcomes



3.3 Recent Clinical Trials

Vitamin D deficiency is common in critically ill patients, with approximately 40-50% meeting the Institute of Medicine definition for vitamin D deficiency (25OHD levels <20 ng/mL [50 nmol/L]).[34] Consistent with preclinical data, observational clinical studies also support that vitamin D deficiency is a common, potentially reversible risk factor for ARDS.[34-45] Specifically, early vitamin D deficiency in the ED or at ICU admission is associated with higher hospital and ICU lengths of stay, lung and other organ injury, prolonged mechanical ventilation, and mortality.

Based on the rationale outlined above, several recent phase II trials have evaluated the potential of acute vitamin D repletion to improve outcomes in medical and surgical ICU. In ICU patients with sepsis, Leaf et al found that active vitamin D (calcitriol)-treated patients had increased expression of the potent antimicrobial peptide LL-37 (p=0.04) and observed a lower mortality rate vs. placebo (17% vs. 23%; p=ns).[46] Dancer et al demonstrated that a 200,000 IU pre-operative vitamin D_3 dose decreased post-operative extravascular lung water and pulmonary vascular permeability indices in esophagectomy patients at high risk for ARDS.[7] Quraishi et al found that a 400,000 IU vitamin D_3 dose in ICU patients with sepsis increased 25OHD levels by an average 12 ng/mL and led to increased expression of LL-37, as well as decreased pro-inflammatory cytokines (IL-1 and IL-6).[47] Han et al found that 500,000 IU

(administered over 5 days) safely increased 25OHD levels in ventilated ICU patients and was associated a lower hospital length of stay[48]

Amrein et al published two randomized controlled trials in a mixed medical-surgical population of ICU patients in Austria that directly support the dose and rationale for the VIOLET trial. An initial pilot trial (n=25) demonstrated that a single 540,000 IU vitamin D_3 dose administered enterally to vitamin D deficient (25OHD <20 ng/mL) ICU patients increased 25OHD levels by day 1 and above the target concentration (25OHD 30 ng/mL) by day 2.[49] In a larger follow-up trial using the same 540,000 IU dose in 475 ICU patients, 25OHD levels increased above the target concentration (30 ng/mL) prior to day 3 and was sustained through at least day 7. Like prior trials, there were no vitamin D-related adverse events, particularly no clinically significant episodes of hypercalcemia nor incident kidney stones. Compared to placebo, vitamin D-treated patients had a lower observed 28-day mortality (21.9% vs. 28.6%; p=0.14) and 6-month mortality (35.0% vs. 42.9%; p=0.09).[1] While the trial was underpowered for mortality, these results justify the need for a larger phase III trial to evaluate the impact of acute vitamin D supplementation on clinical outcomes in at-risk patients.

The VIOLET trial incorporates three important differences from previous efforts. First, while preclinical and observational data support early supplementation to improve physiology and outcomes, the recent Amrein trial randomized patients later into their illness, a median of 3 days into the ICU course.[1] VIOLET will be the first study to test *early* vitamin D supplementation, prior to ICU admission, which we believe will optimize the potential for clinical benefit. Utilizing PETAL infrastructure that includes acute care specialists (emergency medicine and acute care surgery), early administration (within 12 hours) will likely increase the probability of modifying disease progression and enhance the potential clinical benefit of the vitamin D intervention. Second, prior data strongly support plausible mechanism and potential benefit in patients at higher risk for ARDS and mortality. Compared to the Amrein trial, which enrolled all ICU patients, we focus enrollment on patients at higher risk for ARDS and mortality to increase the potential to improve clinical outcomes. Third, due to known variability in vitamin D measurement techniques, VIOLET will base the primary analysis on the gold standard LC/MS/MS measurement (i.e., confirmed vitamin D deficient cohort with LC/MS/MS <20 ng/mL) to decrease the heterogeneity of treatment effect according to baseline risk and maximize trial power to detect an efficacy signal. Further, there is scientific interest in the efficacy and safety of the intervention on the screened vitamin D deficient cohort including patients with LC/MS/MS ≥20 ng/mL, which will also be addressed in VIOLET.

3.4 Summary of Rationale

Based on these data, there are several reasons why the PETAL Network should conduct a trial of early vitamin D repletion to improve clinical outcomes in vitamin D deficient patients at high risk for ARDS and mortality:

1. Vitamin D deficiency is common in critically ill patients, and high dose supplementation can rapidly correct this deficiency.
2. Promising pre-clinical and epidemiological data strongly suggest potential clinical benefit and plausible mechanism for high-dose vitamin D supplementation to prevent ARDS and improve clinical outcomes.

3. Robust phase II trial data support the dosing regimen, safety, and potential clinical efficacy in ICU patients.
4. The trial leverages the PETAL infrastructure for early intervention prior to ICU admission, which is both feasible and desirable to optimize potential for clinical benefit.
5. The data collection requirements are modest to monitor safety and measure clinically relevant outcomes, which facilitate this large, simple, generalizable and cost-efficient trial that is consistent with NHLBI and PETAL Network goals.
6. Vitamin D is inexpensive, easy to administer, and safe, supporting the high potential to rapidly disseminate and change clinical practice if the trial results are positive.

3.5 Objectives

3.5.1 Primary Objective

To assess the efficacy and safety of early administration of vitamin D_3 (cholecalciferol) in reducing mortality and morbidity for vitamin D deficient patients at high risk for ARDS and mortality.

3.5.2 Primary Hypothesis

Early administration of vitamin D_3 (cholecalciferol) will improve all-cause, all-location mortality to day 90 in vitamin D deficient patients at high risk for ARDS and mortality.

3.6 Endpoints

3.6.1 Primary Endpoint

The primary endpoint is all-cause, all-location mortality to day 90. We will ascertain vital status at day 90 by chart review (for participants that remain in the hospital at day 90 or died in hospital prior to day 90). For participants that were discharged alive from the hospital prior to day 90, we will call the patient or proxy (e.g., research contact list and next-of-kin) on/after day 90 to determine vital status. For those that we are unable to verify vital status at day 90, we will use evidence from the medical record (e.g., healthcare visits after day 90), review of obituaries, or phone calls to healthcare facilities to determine that participants are alive. Finally, for participants that have missing 90-day mortality, we will use the Centers for Disease Control and Prevention's National Death Index (NDI) to determine vital status, using each patient's social security number (SSN) for an exact NDI match. At the time of final analysis, we will use best available mortality data and if vital status remains unknown (anticipated for <5%), will impute 90-day mortality based on last known location.

3.6.2 Secondary Endpoints

3.6.2.1 Clinical Endpoints

1. **Study hospital length of stay among survivors to day 90:** Study hospital length of stay is defined as the number of days from enrollment (typically the day of hospital admission) to the day of study hospital discharge up to day 90. This endpoint will be analyzed only in survivors because hospital length of stay in those who die in the hospital is non-informative for this endpoint. In addition, we will use survivor average causal effect (SACE) methods to estimate hospital length of stay among participants that would have survived in both

treatment and control groups.[50] Accordingly, these methods adjust for the impact of differential survival between treatment groups.

2. **Healthcare facility length of stay among survivors to day 90:** Healthcare facility length of stay is the time spent in another hospital or healthcare facility (e.g. long-term acute care [LTAC] hospitals or acute rehabilitation/skilled nursing facility), for the subgroup of participants that were discharged to another healthcare facility after the initial hospitalization. This measure is defined as the number of days from initial hospital discharge to the first facility discharge to home (pre-hospitalization level of care) up to day 90. Healthcare facility LOS is zero for patients discharged to home (pre-hospitalization level of care) from the study hospital. This endpoint will be analyzed only in survivors using SACE methods because healthcare facility length of stay in those who die during the follow-up period is non-informative for this endpoint.

3. **Alive and home at day 90:** This endpoint is the proportion of participants who have survived and are present at home, defined as pre-hospitalization level of care, at day 90.

4. **Ventilator free days (VFD) to day 28:** VFD depends on both duration of ventilation and mortality through study day 28. In participants who survive 28 days, VFD is defined as 28 minus duration of ventilation. Duration of ventilation is counted from the first study day of assisted breathing through the last day of assisted breathing provided the last day is prior to day 28. Otherwise, it is counted from the first study day of assisted breathing through day 28. For participants discharged with assisted ventilation (e.g., to LTAC facility) prior to day 28, a phone call will be required to assess ventilator status at day 28. Participants discharged prior to day 28 (but not to home) on unassisted breathing will be assumed to remain on unassisted breathing through day 28. Isolated periods of ventilation briefer than 24 hours for surgical procedures and ventilation solely for sleep disordered breathing do not count towards duration of ventilation. In participants who never require assisted breathing, duration of ventilation is zero. Participants who do not survive 28 days will be assigned zero VFD. VFD is undefined in participants with chronic/home mechanical ventilation (except solely for sleep disordered breathing) and they will be excluded from this analysis.

5. **Time to mortality to day 90:** Time to mortality will use best available data on vital status and date of death and censor based on last known alive date for participants with missing vital status.

3.6.2.2 Physiological Endpoints

6. **Development and severity of ARDS to day 7:** We will determine the presence and severity of ARDS for each day of mechanical ventilation to day 7 using the following approach:
   a. For each ventilator day: if ABG available between 2:00 AM and 8:00 AM, measure P/F (PaO2, FiO2 and PEEP) for all ABGs during this time window daily to day 7. Or, for ventilator days that no ABG available between 2:00 AM and 8:00 AM, determine lowest imputed P/F from measured S/F (SpO2, FiO2, and PEEP). See Appendix B for P/F imputation table.
   b. For participants with P/F <300 or imputed P/F <300, FiO2 ≥40%, and PEEP ≥5 cm H_2O, determine if hypoxemia is valid, acute, and not fully explained by CHF or fluid overload
   c. If yes to item (b), local investigators will:

i. Review the first CXR (or CT) performed on each ventilated day with valid P/F or imputed P/F <300 (to day 7). If no chest imaging studies are present that day, site investigators may review imaging one day before or after to determine if ARDS imaging criteria met.
ii. Assess if the images are consistent with ARDS (bilateral opacities not fully explained by effusions, lobar/lung collapse, or nodules). If equivocal, the reviewing investigator will adjudicate with additional investigators.

7. **Change in organ failure severity to day 7:** We will measure acute organ failure severity daily to day 7 for three key organ systems: acute hypoxemic respiratory failure, acute kidney injury, and cardiovascular failure (i.e., shock). We will measure acute hypoxemic respiratory failure using data from the ARDS assessment, except this outcome does not require chest imaging consistent with ARDS. We will measure acute kidney injury by highest daily creatinine values or new use of dialysis/ renal replacement therapy (chronic dialysis participants are excluded). We will measure cardiovascular failure by highest daily dose of vasopressors (epinephrine, norepinephrine, dopamine, phenylephrine, or vasopressin).

8. **Plasma 25OHD levels:** In addition to the screening vitamin D test (for trial entry), we will measure gold-standard plasma 25OHD levels at baseline for all randomized participants and at day 3 in the first 300 randomized participants to confirm biomarker response to the intervention. The gold-standard test will be done in batch using LC/MS/MS methods. Individual results will not be available to investigators and coordinators to avoid unblinding. Based on pre-specified rules and in consultation with the DSMB (who will have access to additional adverse event data), we may require changes to the vitamin D dose or extend the duration of 25OHD/calcium monitoring.

9. **Plasma IL-6 levels:** We will measure plasma IL-6 levels at baseline and day 3 in the first 300 randomized participants in batch concurrent with 25OHD testing. IL-6 is a pro-inflammatory cytokine associated with increased incidence and severity of ARDS severity and worse clinical outcomes. Vitamin D may improve outcomes in critically ill patients by reducing levels of IL-6 (either causal association or as a result of improved recovery).

3.6.2.3 Safety Endpoints

10. **Calcium levels to day 14:** Because the half-life of 25OHD is approximately 2 weeks, we will record clinically available serum or ionized calcium levels through day 14 for all randomized participants. In addition, we will measure baseline and day 3 in the first 300 randomized participants in real time, either as part of routine clinical care or as a research procedure if not available. Day 3 is the when 25OHD levels peak in ICU patients given 540,000 IU of vitamin D.[1,49] While no prior study of acute vitamin D repletion, using similar high doses as our trial, has observed clinically important hypercalcemia, we will confirm these findings in the VIOLET trial. Because little change in calcium levels is anticipated, access to individual-level calcium results will not unblind study staff. During the trial, the DSMB may seek additional subject measurements for safety assessment, though this is not anticipated.

11. **Kidney stones to day 90:** While an association between hypervitaminosis D and kidney stones has been suggested, the relationship with high dose vitamin D supplementation and kidney stones remains controversial. We will assess for incident kidney stones by chart

review at the end of the hospitalization and by self-report at the 90 day phone call for those discharged from the hospital prior to day 90.

12. **Fall-related fractures to day 90:** The association between acute vitamin D supplementation and falls/fractures remains unclear. Most data suggest that high dose vitamin D in healthy outpatients may improve muscle function, balance, and bone mineral density, and thus decrease fall-related fractures, but other data suggest that high dose vitamin D supplementation may actually increase the incidence of falls/fractures. Because of this uncertainty and limited data in hospitalized patients, we will assess for incident fall-related fractures by chart review at the end of the hospitalization and by self-report at the 90 day phone call for those discharged from the hospital prior to day 90.

3.7 Subgroups

The primary analysis will be based on randomized participants with baseline 25OHD levels <20 ng/mL by LC/MS/MS (confirmed vitamin D deficient cohort). Secondary analysis will be based on the screened vitamin D deficient cohort (all randomized patients). *A priori* subgroups for analysis will include age, sex, race/ethnicity, residence (independent vs. long-term care facility), BMI, pre-hospitalization vitamin D supplementation, baseline 25OHD level (by the LC/MS/MS method), baseline renal function, ARDS risk factor (e.g., infectious vs non-infectious), LIPS score, pre-randomization mechanical ventilation status, pre-randomization presence of ARDS, and source of ICU admission.

4 STUDY POPULATION AND ENROLLMENT

4.1 Number/Source/Screening

The trial will randomize a maximum of 3000 participants from the EDs, hospital wards, operating rooms, ICUs and other acute care areas of the PETAL Network Clinical Centers. The 12-hour enrollment window requires engagement of the Network's infrastructure for early recruitment. We will screen and enroll all medical, trauma, or post-operative patients at each site prior to ICU admission largely using available clinical data to determine potential eligibility.

Tactics will include:

i. **Emergency Department (ED) screening:** We anticipate that the majority of participants for this trial will be screened and enrolled in the ED. Eligible patients must have ICU admission intended (defined as an ICU consult or bed request/order) and meet inclusion and exclusion criteria.

   This assessment will be in person, by telephone, or through electronic screening. For capable sites, we encourage electronic triggers for hypotension, mechanical ventilation, hyperlactemia, trauma team activation, and vasopressor order that will identify patients likely to meet trial inclusion criteria even before the ICU admission is triggered. We also will engage key leaders and the ED staff to enhance knowledge of and effort to enroll in the trial as early as possible.

ii. **Hospital ward screening:** Ward patients who are destined for the ICU will be eligible for study inclusion. The primary trigger for screening will be hospital rapid response team, ICU consult or bed order, and clinician referral. Because typically these patients arrive rapidly to the ICU, we anticipate that enrollment and randomization will occur primarily in the ICU.

iii. **Operating room screening:** Emergent/unscheduled operating room patients destined for the ICU will be eligible for study inclusion. In addition, patients with elective operations will be eligible if unanticipated complications occur that meet inclusion criteria (e.g., hemorrhagic shock, unplanned prolonged mechanical ventilation).

iv. **ICU screening:** We will screen patients admitted to ICUs upon arrival if still within the enrollment time window, including admissions from the ED, wards, operating room, and outside facilities. Because the 12-hour enrollment window begins when the ICU admission decision is made (defined in Section 4, Inclusion Criteria), we anticipate that most trial participants will have screening and enrollment procedures begun prior to ICU arrival.

v. **Communication and hand-off:** We will facilitate hand-offs with use of a unified screening log (to be used for ED, inpatient, and ICU screening); inservice training by site PIs to ED, OR, and ICU staff; and coordination by research staff for enrolled patients. When patients are enrolled prior to ICU admission (as we commonly anticipate in this trial), ED and other pre-ICU research staff will communicate with the ICU research staff to report patient information and current status (screening, consent, randomization, administration of study medication).

   As a Network, we will develop and update best practices for communication hand-offs to encourage interdisciplinary communication among research staff and between research staff and clinical providers. These will include enrollment notes and scanned consent forms in the electronic medical record; study acronym with coordinator name/contact on the white board in the patient's room; and communication about VIOLET enrollment upon transfer to new units. Implemented strategies will be tailored to each hospital's unique processes.

vi. **Study staff available for consent:** Each site will have dedicated study physicians and coordinators who are certified and trained in human subjects protection and understand the study protocol.

4.2 Inclusion Criteria

1. Age ≥ 18 years
2. Intention to admit to ICU from emergency department, hospital ward, operating room, or outside facility
3. One or more of the following acute risk factors for ARDS and mortality contributing directly to the need for ICU admission:
   <u>Pulmonary</u>
   a) Pneumonia
   b) Aspiration
   c) Smoke Inhalation
   d) Lung contusion
   e) Mechanical ventilation for acute hypoxemic or hypercarbic respiratory failure

Extra-Pulmonary

f) Shock
g) Sepsis
h) Pancreatitis

4. Vitamin D deficiency (screening 25OHD level <20 ng/mL)

4.2.1 Intention to Admit to ICU

Randomization must occur within 12 hours of ICU admission decision. The occurrence of one or more risk factor conditions should contribute directly to the need for ICU admission per treating physician. The criteria recognize that recruiting sites have different processes for ICU admission, and therefore provides some flexibility, while ensuring that participants across sites would be recruited at similar/consistent times. The first occurrence of any of the following criteria will serve as time zero to start the 12-hour enrollment window:

1. ED, hospital ward, or operating room patients at study hospital
   Whichever comes first:
   a. Initiation of ICU consult, OR
   b. Written order for ICU bed, OR
   c. Physician documentation of plan for ICU admission, OR
   d. Discussion with treating physician of intention to admit to ICU, documented by study coordinator or investigator
2. Patients transferred from outside facility to study hospital ICU
   a. Acceptance of ICU transfer
3. Patients transferred from outside facility to study hospital ED, hospital ward, or operating room at study hospital.
   a. Use same criteria as in item 1 above.

4.2.2 Pulmonary risk factors

Pneumonia

Known or suspected based on physician charting or discussion with treating physician
OR, both 1 and 2 below:

1. Chest radiographs showing infiltrates, consolidation, or cavitation that is not known to be pre-existing (on prior chest imaging)
2. Clinical signs of pneumonia (new cough, sputum, fever, or WBC>12,000)

Aspiration

Witnessed or suggestive history of inhalation of food, liquid, or regurgitated gastric contents

Smoke inhalation

Witness or suggestive history of smoke inhalation

Lung contusion

Blunt or penetrating trauma to thorax that results in related infiltrates in chest x-ray or CT.

Mechanical ventilation

Patients receiving invasive or non-invasive mechanical ventilation for acute hypoxemic or hypercarbic respiratory failure per treating physician. Mechanical ventilation exposes patients to ventilator-associated lung injury and is a marker for higher illness severity in ICU patients.

Therefore, these patients are at high risk for mortality,[51] and prior data support the potential of vitamin D to improve outcomes in this population.[52]

4.2.3 Extra-pulmonary risk factors

Shock

Infusion of vasopressors (epinephrine, norepinephrine, dopamine (>5 µg/kg/min), phenylephrine, or vasopressin)
OR MAP <65 mmHg or SBP <90 mmHg after at least 1 liter intravenous fluids or 1 unit of blood product

Sepsis

Both 1 and 2 below:

1. Suspected or proven infection
    - Treating physician suspects bacterial or fungal infection (document suspected source(s))
    - Or, cultures: ordered in past 48 hours or positive cultures from referring ED
    - Or orders for antimicrobial medication
2. Organ dysfunction defined as an acute change in total SOFA score ≥2 points
    - The baseline SOFA score can be assumed to be zero in patients not known to have preexisting organ dysfunction

Pancreatitis

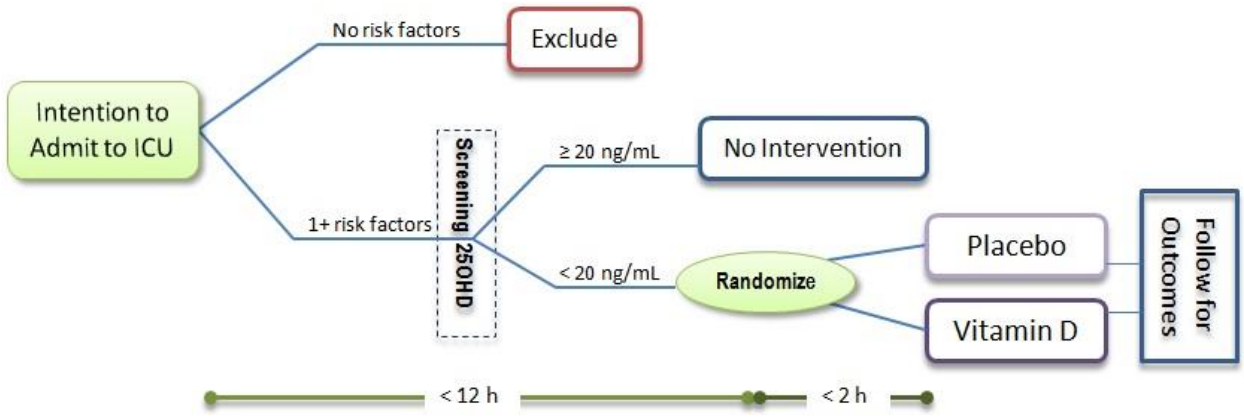
Defined as two of the three following features, not explained by another obvious cause (e.g., diabetic ketoacidosis):

a. Symptoms consistent with acute pancreatitis (e.g., abdominal pain or vomiting)
b. Serum Amylase and/or Lipase ≥ 3 times the upper limit of normal
c. Characteristic findings of acute pancreatitis on CT scan

4.2.4 Screening Vitamin D Test

After initial study screening, eligible patients will be approached by research staff for written informed consent for the protocol, prior to secondary screening for vitamin D deficiency. Vitamin D screening will use an FDA-approved test, by either the hospital's clinical laboratory or the FastPack IP device (Qualigen Inc., Carlsbad, CA). Results of the screening test will be interpreted as vitamin D deficient (25OHD <20 ng/mL) or not vitamin D deficient (25OHD ≥20 ng/mL). We will randomize participants with a screening 25OHD level <20 ng/mL within 12 hours of ICU admission decision and administer study drug within 2 hours of randomization.

Figure 3 Study Flow: screening & enrollment



4.3 Exclusion Criteria

1. Unable to obtain informed consent
2. Unable to randomize within 12 hours of ICU admission decision
3. Unable to take study medication by mouth or enteral tube
4. Baseline serum calcium >10.2 mg/dL (2.54 mmol/L) or ionized calcium >5.2 mg/dL (1.30 mmol/L)
5. Known kidney stone in past year or history of multiple (>1) prior kidney stone episodes
6. Decision to withhold or withdraw life-sustaining treatment (patients are still eligible if they are committed to full support except cardiopulmonary resuscitation if a cardiac arrest occurs)
7. Expect <48 hour survival
8. If no other risk factors present, a) mechanical ventilation primarily for airway protection, pain/agitation control, or procedure; or b) elective surgical patients with routine postoperative mechanical ventilation; or c) anticipated mechanical ventilation duration <24 hours; or d) chronic/home mechanical ventilation for chronic lung or neuromuscular disease (non-invasive ventilation used solely for sleep-disordered breathing is not an exclusion).
9. Prisoner
10. Pregnancy
11. Greater than 72 hours since hospital presentation (or > 3 calendar days since hospital presentation if transferred with no time stamp).

4.3.1 Reasons for Exclusions

We exclude patients <18 years old because of limited data on the safety of high dose vitamin D during critical illness in children. The 12-hour enrollment window underscores the trial rationale and PETAL Network goals for early intervention to optimize potential for benefit (criterion 2). The study drug is administered enterally and thus we must exclude patients who are unable to take the single vitamin D dose by mouth or enteral tube (criterion 3). Because vitamin D increases gastrointestinal calcium absorption, we will exclude patients with hypercalcemia to avoid further increases to calcium levels (criterion 4). Calcium levels should be obtained in the majority of ICU-bound patients as part of the routine clinical care. Results will be typically available within 1 hour of hospital arrival. If not available clinically in the pre-consent screening period (up to 72 hours prior to consent) and the clinical team does not plan to order a calcium level, the study team will order a serum calcium level as a research test in consented patients. Whether vitamin D may cause hypercalciuria and related kidney stones is controversial, it is prudent and consistent with prior vitamin D trials to exclude patients with a recent history or multiple prior episodes of kidney stones (criterion 5). Criteria 6 and 7 exclude patients who may not survive to important study endpoints and have limited chance to see benefit from the intervention. Criterion 8 limits enrollment of patients that have lower relative rates of mortality related to mechanical ventilation or confounds the interpretation of important trial endpoints (eg, VFD). Prisoners will not be enrolled due to their protected status as a vulnerable population, and preservation of autonomy for these patients will be difficult in the setting of critical illness (criterion 9). Exclusion of pregnant women is required because the safety of the single high dose of vitamin D is unknown for pregnant women and their fetuses, as this dosing has never

been studied even in healthy pregnant women (criterion 10). We would anticipate very few pregnant women would meet the eligibility criteria for this trial and thus, this trial would have limited potential to gain knowledge about the safety and efficacy of the intervention in pregnant women. We will ensure that non-pregnant women of childbearing age are not biased against inclusion in the trial. Criterion 11 limits enrollment to acute conditions and presentations.

Patients who meet inclusion criteria 1-3 and no exclusion criteria will be eligible for participation in the trial. After completing the informed consent process, eligible participants will then be assessed for randomized study treatment with a screening test for vitamin D deficiency. Participants meeting inclusion criteria 4 (screening 25OHD level <20 ng/mL) will be randomized 1:1 to study drug or placebo.

4.4 Randomization and Study Initiation Time Window

All participants with screening 25OHD <20 ng/mL must be randomized within 12 hours of ICU admission decision, regardless of patient location. Study medication must be administered within 2 hours of randomization**.**

4.5 Informed Consent

All patients meeting inclusion criteria 1-3 will be entered on a screening log. If the patient meets an exclusion criterion we will document the reason and collect a minimum data set to the extent allowed. We will not seek informed consent. We will seek informed consent from each patient not excluded (or from their legally authorized representative (LAR)) prior to Vitamin D screening. We will assess the patient's decisional capacity prior to consent. In order to facilitate possible long term follow-up ancillary studies separate from this primary trial, we will request consent to obtain social security number and Medicare number for all participants who consent to participation (both vitamin D deficient and not vitamin D deficient by the screening vitamin D test).

4.6 Randomization

After informed consent and completion of vitamin D screening, we will randomize each participant that has screened in as vitamin D deficient (screening 25OHD level <20 ng/mL). Randomization procedures will be specified by the CCC. Designated research team members will have permission to randomize participants. At randomization, each subject will be assigned a unique randomization ID number that the site pharmacy will use to determine study arm assignment to vitamin D or placebo. The site research staff, clinical staff, patient and LAR will remain blinded to assignment. The CCC will send a confirming email to the randomizer containing the randomization ID number. We will stratify randomization by enrolling hospital.

4.7 Minorities and Women

No patients will be excluded on the basis of race, ethnicity, or sex. NHLBI considered the composition of minorities and women in the target study population in selecting the PETAL Network Clinical Centers so that studies reflect the population of the US census. The PETAL Network CCC will monitor recruitment of minorities and women. If necessary, additional recruitment efforts will be made at specific centers to ensure that the aggregate patient sample contains representative race/ethnicity and sex subsets.

5 STUDY PROCEDURES

Trials should be conducted in a setting reflective of good clinical practice that can be clearly described and reproduced in a clinical (non-trial) setting. NHLBI selected Clinical Centers already providing high quality standardized ED and ICU care. We will monitor the provision and results of key processes of care and implement strict protocols (with training, monitoring, and feedback) for the administration of vitamin D. Almost all PETAL centers have existing protocols and order sets for the routine management of ED and ICU patients

5.1 Intervention Group (Vitamin D)

Participants randomized to the intervention group will receive a single enteral (oral or per naso/orogastric tube) dose of 540,000 IU vitamin D_3 (cholecalciferol). Study medication will be provided in liquid form by BioTech Pharmacal (Fayetteville, AR), which has successfully provided study medication for prior NIH-sponsored vitamin D clinical trials. Because of the high dose of vitamin D and intention to prevent and treat disease, we filed a FDA Investigational New Drug (IND) application for this protocol.

The two primary forms of vitamin D supplementation are enteral pre-hormone vitamin D (e.g., cholecalciferol, ergocalciferol) and enteral or parenteral active hormone (e.g., calcitriol or VDR agonists [e.g., paricalcitol]). The primary advantages of nutritional vitamin D supplementation include greater experience with its use, dosing, and safety; ability to measure dose-response using clinically relevant 25OHD levels; and a longer (2 week) half-life. Though VDR agonists can be administered parenterally, there are limited safety data with its use and dosing, particularly in critically ill patients. Therefore, we have selected nutritional vitamin D supplementation for this trial. Single bolus doses of 50,000-750,000 IU vitamin D are safe in the general population, and doses up to 540,000 IU in studies of ICU patients have not triggered clinically significant hypercalcemia, hypercalciuria, or other known vitamin D-related adverse events. Specifically, in a previous clinical trial, a single dose of 540,000 IU to ICU patients was safe, rapid, and effective in repleting vitamin D deficiency (25OHD <20 ng/mL).[1] We chose this relatively high dose because of its known excellent safety profile in ICU patients and to maximize the chance to improve clinical outcomes through effective vitamin D repletion.

5.1.1 Stopping Rules for Vitamin D Administration

Because the intervention is a single dose of vitamin D, stopping treatment is not relevant to individual trial participants. However, trial participants will be followed for endpoints through day 90, and the overall ongoing safety of the trial and the vitamin D intervention will be closely monitored during the conduct of the trial.

5.2 Randomized Control Group

Participants randomized to the control group will receive a single liquid enteral dose of placebo that is matched to the vitamin D intervention to ensure blinding.

5.3 Common Strategies for Both Groups

This trial will not mandate aspects of routine clinical care because 1) the intention is to evaluate the efficacy and safety of high dose vitamin D in the context of routine clinical care; 2) there is a broad spectrum of conditions that we will enroll in this trial; and 3) randomization in this large trial will balance routine care and we anticipate minimal impact on observed differences.

We will encourage sites to inform providers of this trial broadly and to ensure communication between research and clinical teams of specific participants that are enrolled. This will allow clinical decisions to be made in the context of the trial, such as any additional vitamin D supplementation or testing, to ensure the safety of participants and limit crossover and unblinding. Information on these items will be collected passively from the medical record for descriptive summary and analysis.

6 DATA VARIABLES AND SPECIMENS

6.1 Background Assessments

1. Inclusion/exclusion criteria
2. Demographic and admission data (including age, sex, race/ethnicity, pre-hospitalization level of care)
3. Pertinent medical history (including Charlson co-morbidity score, renal function, chronic ventilation)
4. Height; measured body weight (MBW); calculated predicted body weight (PBW); body mass index (BMI)
5. Ventilator status prior to randomization
6. Location when inclusion criteria met
    - ED, ward, operation room, referring hospital
7. ICU Admission Service
    - Medical
    - Surgical scheduled
    - Surgical unscheduled
    - Trauma
    - Other

6.2 Baseline Assessments

1. Home vitamin D and calcium supplementation (as medication or supplement including multivitamin)
2. SOFA Score: Cardiovascular, renal, respiratory, hepatic, and hematology organ function, as clinically available
3. Lung Injury Prediction Score (LIPS)
4. Calcium level, if not performed in routine clinical care
5. Pregnancy test for women of reproductive age, if not performed in routine clinical care
6. Health-related quality of life by EuroQol (EQ-5D-5L)

6.3 Assessment during Study

We will collect the following in-hospital data by medical record review to assess trial efficacy and safety endpoints. Most data may be assessed by medical record review.

1. Duration of ventilation to day 28 (may require call to LTAC facility if hospital discharge with assisted breathing prior to day 28).
2. Organ failure assessments daily to day 7 (ARDS, creatinine, and vasopressor use), using clinically available data.

3. In-hospital vitamin D supplementation daily to day 14 (medication only, not related to dietary intake or nutritional supplementation).
4. In-hospital calcium levels daily to day 14, (required on day 3 for first 300 randomized participants, otherwise if clinically available).
5. Adverse events and serious adverse events to day 14
6. Safety endpoints (i.e., kidney stones and fall related fractures)
7. Vital status and ventilator status (among survivors) at hospital discharge
8. Date of hospital discharge or date of death, as applicable
9. Hospital discharge disposition. "Home" will be defined as pre-hospitalization level of care.

6.3.1 Specimen Collection

We will collect 15 mL of blood at baseline (within 2 hours of randomization) for all randomized participants and 15 mL of blood at study day 3 for approximately the first 300 randomized participants (300 participants with match pairs of baseline and day 3 samples). We will measure plasma 25OHD on baseline samples for all randomized participants and on study day 3 samples for approximately the first 300 randomized participants. Additionally, we will measure plasma IL-6 at baseline and on study day 3 for approximately the first 300 randomized participants. Some of the blood collected on randomized participants at baseline will also be used for banking of plasma and DNA for future research.

Table 1 Specimen Collection Schedule

Subjects	Collection Type	Draw Time Points
All randomized subjects	Plasma for 25OHD	Baseline
	Plasma for banking	
	Whole blood for DNA banking	
Approximately the first 300 randomized subjects	Plasma for IL-6	Baseline & Day 3
	Plasma for banking	Day 3
	Plasma for 25OHD	Day 3

The 25OHD and IL-6 samples will be shipped to a central lab for batched measurements. The baseline 25OHD samples will be measured by the LC/MS/MS method and compared to the screening test results, initially in monthly increments for quality control purposes. The samples for future research (plasma and whole blood for DNA) will be shipped to the network central repository. All samples will be identified by a coded number during shipment and storage in both the central repository and central lab.

The primary purpose of this trial is to evaluate efficacy and safety of the proposed vitamin D intervention, and we have attempted to limit additional data and specimen collection that extend beyond this aim. Accordingly, we designed the limited biospecimen collection plan to confirm that the vitamin D dose successfully corrects vitamin D deficiency and changes an important biomarker in critical illness (IL-6); this requires a small subsample (n=300) to evaluate. In addition, we will be able to stratify results based on clinical variables and vitamin D levels to see if there are subgroups that may have more or less potential to benefit. Although additional explanatory and mechanistic data collection would be beyond the scope (and budget) of the present trial, we will store baseline plasma/DNA to facilitate additional (ancillary) studies that would be potentially useful in explaining negative or positive results.

6.4 Assessments after Hospitalization

For participants discharged to home or post-acute care facilities (e.g., LTAC facilities, SNF, or acute rehabilitation facilities) prior to day 90, we will obtain study endpoints by a telephone call with the patient, LAR, or facility staff to assess:

1. Vital status at day 90 and date of death (if applicable)
2. Location at day 90 (home, LTAC/SNF/acute rehab, hospital)
3. Date of discharge home (prior level of care) for participants discharged from the hospital to post-acute care facility
4. Safety endpoints (kidney stones and fall-related fractures)
5. Health-related quality of life by EuroQol (EQ-5D-5L)

The EQ-5D-5L[53] is a brief, validated, reliable, and widely used[54] QOL instrument that determines whether respondents have problems in each of five domains: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Responses can be used to calculate a health utility score[55] associated with the given health state that ranges from -0.11 to 1.00 (higher scores are better; 1.00 is perfect health).

At the 90-day follow-up, patients may still be impaired. While proxies differ from primary respondents on almost all instruments, including the EQ-5D-5L,[56,57] we are hopeful that proxy respondents will not be differentially distributed among intervention and control, allowing us to minimize confounding related to proxy vs. individual responses.

We will collect contact information for the patient and alternative contact information for up to 3 individuals. For participants who we are unable to contact by phone, we will use evidence from the medical record (e.g., healthcare visits after day 90) to identify participants that are still alive. We will also verify duration of survival for participants lost to follow-up or noted to have died using the Centers for Disease Control and Prevention's National Death Index (NDI) and other federal databases. We will use each patient's social security number (SSN) for an exact NDI match. Although we do not plan to collect long-term outcomes as part of primary trial endpoints, we will record Medicare number to facilitate future ancillary studies that wish to link with Medicare data on utilization and functional status.

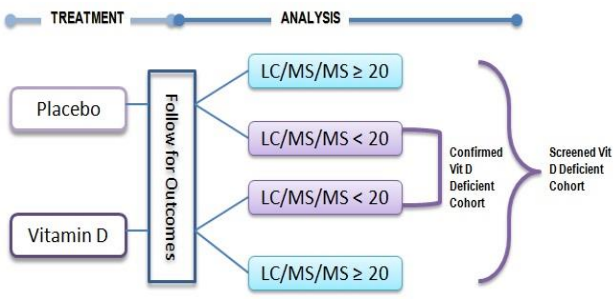
7 STATISTICAL CONSIDERATIONS

7.1 Statistical Methods Primary Analyses

The primary endpoint is intention to treat all-cause, all-location 90 day mortality in the confirmed vitamin D deficient cohort (LC/MS/MS <20 ng/mL). We will conduct analyses on a modified intention to treat basis (all randomized participants who received either vitamin D formulation or placebo in the confirmed vitamin D deficient cohort).

Sample size is based on a comparison of binomial proportions with an overall one-sided alpha level of 0.025. With 20% mortality rate in the control group and 15% rate in the vitamin D group, three interim looks at the data, and 80% of randomized patients with

Figure 4 Study Flow: analysis plan



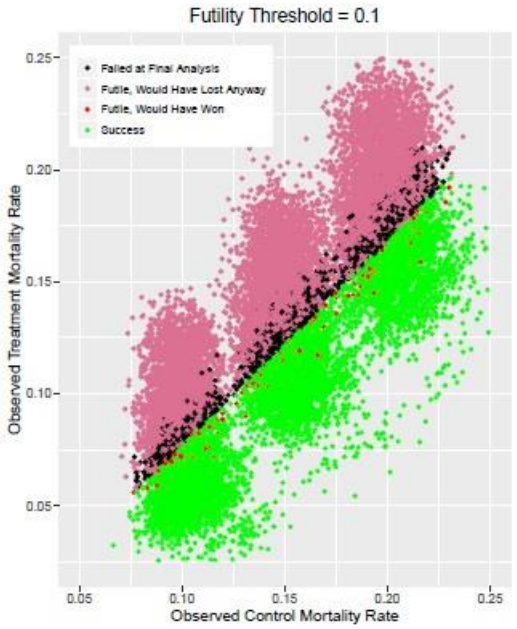
confirmed vitamin D deficiency by the LC/MS/MS method, the study will have a power of 87.4%. The trial will stop when the screened vitamin D deficient cohort reaches a maximum 3000 randomized participants.

The presumed 20% mortality rate in the control group is based on several recently published clinical trials. In the original Amrein study, mortality prior to discharge home before day 90 was 40% in the control group.[1] Because these participants were enrolled a median 3 days after ICU admission (some lower risk participants were already discharged from the ICU) and European ICUs tend to admit sicker patients that U.S. ICUs (some lower risk ICU patients in the U.S. would be managed in a non-ICU setting in Austria), we anticipate a lower mortality in our trial. In the ProCESS trial, which recruited ED patients early in the course of septic shock, the 90 day mortality was 32%.[58] While similar septic shock patients will be recruited in VIOLET, other eligible patients (in addition to septic shock) will likely have a modestly lower mortality. For example, in the LIPS-A trial, which enrolled ED patients across broader ARDS criteria, the subgroup of patients admitted to the ICU had 11% *in-hospital* mortality (vs. 19% in-hospital mortality in ProCESS). While 90 day mortality was not collected in the LIPS-A trial, we would anticipate a 90 day mortality rate of approximately 18%. However, LIPS-A excluded many potentially eligible patients because of aspirin use, which selected against an older, more chronically ill population. These patients would be included in VIOLET (no aspirin exclusion criteria), which would likely increase the event rate.

We plan to have three interim analyses at 750, 1500, and 2250 randomized (screened vitamin D deficient) participants. In addition, there will be an initial evaluation specifically of 25OHD and calcium levels after the first 300 randomized participants to confirm that the intervention repletes vitamin D deficiency without hypervitaminosis D or clinically significant hypercalcemia, consistent with prior trials using the same dose in ICU patients. DSMB meetings will occur as close to these planned analyses as scheduling allows. The study will be stopped for efficacy based on an analysis of the confirmed vitamin D deficient (LC/MS<20 ng/mL) participants. There will be an efficacy stopping rule at each interim analysis based on a the Lan-DeMets alpha spending function of 0.025t^4, where t is the proportion of the sample size that has accrued.

In addition, Berry Consultants developed the futility stopping rules for this study according to the following specification. The study will continue until there are 3000 patients randomized, unless it stops early for efficacy or futility. The futility stopping rules will be based on the death rates in each treatment and the proportion of randomized participants who have LC/MS/MS level <20 ng/mL (ie, the inclusion rate). The rule will calculate the predictive probability that the trial will reach statistical significance, when 3000 total randomized participants are enrolled. The futility rules will not be binding on the DSMB, as they will have other information on the secondary analyses of the study as well as information from outside the study. The following table gives the power of the study under different scenarios under the assumption that 80% of the randomized participants will have a LC/MS/MS level <20 ng/mL. The plot below shows individual simulated trials with colors indicating whether they were positive and whether they were stopped early for futility.

Futility Stopping Boundaries



Control Rate	Effect	Power	Success (N=750)	Success (N<=1500)	Success (N<=2250)	Futility (N=750)	Futility (N<=1500)	Futility (N<=2250)	E[N]	E[n]
0.1	0.02	0	0	0	0	0.742	0.963	0.998	973	778
	0	0.02	0	0	0.005	0.467	0.728	0.892	1431	1145
	−0.01	0.106	0.001	0.009	0.044	0.328	0.545	0.72	1765	1412
	−0.02	0.356	0.002	0.043	0.16	0.177	0.295	0.421	2176	1741
	−0.03	0.685	0.006	0.13	0.422	0.076	0.135	0.192	2279	1824
	−0.04	0.918	0.017	0.333	0.743	0.033	0.043	0.054	2083	1666
	−0.05	0.985	0.068	0.649	0.951	0.007	0.009	0.01	1730	1384
0.15	0.02	0	0	0	0	0.703	0.933	0.986	1034	827
	0	0.033	0	0.005	0.018	0.442	0.724	0.877	1450	1160
	−0.01	0.089	0.001	0.005	0.031	0.317	0.539	0.727	1785	1428
	−0.02	0.289	0.003	0.027	0.121	0.18	0.329	0.484	2142	1714
	−0.03	0.524	0.003	0.069	0.291	0.13	0.207	0.292	2256	1805
	−0.04	0.758	0.007	0.167	0.524	0.069	0.116	0.149	2226	1781
	−0.05	0.934	0.031	0.36	0.777	0.029	0.041	0.051	2033	1626
0.2	0.02	0.001	0	0.001	0.001	0.704	0.935	0.991	1026	821
	0	0.023	0	0	0.007	0.473	0.74	0.89	1418	1134
	−0.01	0.095	0.002	0.006	0.034	0.341	0.567	0.738	1734	1387
	−0.02	0.228	0.002	0.025	0.099	0.236	0.395	0.545	2024	1619
	−0.03	0.44	0	0.049	0.216	0.144	0.271	0.377	2207	1766
	−0.04	0.661	0.004	0.123	0.41	0.095	0.138	0.201	2272	1816
	−0.05	0.874	0.016	0.244	0.637	0.035	0.06	0.08	2196	1757

7.2 Statistical Methods for the Screened Vitamin D Deficient Cohort

The effect of treatment will be estimated in the screened vitamin D deficient cohort. Furthermore we will use a quadratic smoothing spline to estimate the relationship between the treatment effect and the baseline 25OHD level.

8 DATA COLLECTION AND SITE MONITORING

8.1 Data Collection

The research staff will collect and record data on paper collection sheets or in a custom-designed computer database. Data will be transferred to the Clinical Coordinating Center on a prescribed basis through a web-based data collection program. There will be recommendations but no protocol-mandated aspects of routine clinical care.

8.2 Site Monitoring

Data quality will be reviewed remotely using front-end range and logic checks at the time of data entry and back-end monitoring of data using SAS reports. Additionally, Clinical Center on site visits will be performed on a regular basis by the Clinical Coordinating Center to ensure that all regulatory requirements are being met and to monitor data quality. Patient records and case report forms will be examined on a spot check basis to evaluate the accuracy of the data entered into the database and monitor for protocol compliance.

8.3 Vitamin D Screening Quality Assurance

We will utilize a quality assurance plan to monitor accuracy of Vitamin D screening using Qualigen FastPack.

9 RISK ASSESSMENT

9.1 Potential Risks to Subjects

As noted in Section 5, high dose enteral vitamin D appears to be safe in critically ill patients with no related adverse events reported in a recent large trial using the dose proposed for this study.[1] As such, the potential risks below are theoretical and have not been observed in previous clinical trials.

Hypercalcemia: The primary risk associated with use of vitamin D supplementation is hypercalcemia, which may cause gastrointestinal symptoms, dizziness, fatigue, and less commonly cardiac arrhythmia. This risk is largely theoretical, as trials using similar vitamin D doses as in this protocol, including in ICU patients, have not observed clinically significant hypercalcemia. For example, in the largest trial to date using 540,000 IU vitamin D_3 as the intervention (Amrein et al, n=475),[1] the maximum total serum calcium level observed in the vitamin D group during the first 28 days was 11.2 mg/dL with no clinically or statistically significant difference in mean total serum or ionized calcium levels. To enhance trial safety, we will exclude patients with baseline hypercalcemia or 25OHD levels ≥20 ng/mL.

We will confirm the minimal effect of acute vitamin D repletion on calcium levels by collecting and analyzing clinically available calcium levels to day 14 (after which 25OHD levels decline). We will also measure day 3 calcium levels (after plateau of 25OHD levels) as protocol specified testing in the first 300 randomized participants, and in additional participants or timepoints if required by the DSMB or cIRB to monitor ongoing safety.

Kidney stones: While an association between hypervitaminosis D and kidney stones has been suggested, the relationship with high dose vitamin D supplementation and kidney stones remains controversial. However, we will exclude patients with a personal history of recent (past year) or multiple prior kidney stones and will closely monitor participants for incident kidney stones during the hospitalization and follow-up period.

Fall-related fractures: The association between acute vitamin D supplementation and falls/fractures remains unclear. Some data suggest that higher doses of daily vitamin D supplementation (1,000-4,000 IU per day) may improve muscle function and balance, and thus decrease falls, which is also highlighted by a recent American Geriatrics Society clinical guideline recommending higher doses of daily vitamin D supplementation for fall *prevention*.[60] In addition, the National Institute on Aging has recently sponsored a large phase III clinical trial with this hypothesis for evaluating vitamin D and fall prevention (NCT02166333). However, one report published in JAMA suggested that a single dose of 500,000 IU vitamin D supplementation annually increased the incidence of falls and possibly fractures in healthy older women.[61] In addition, two recent trials in older adults found that monthly vitamin D supplementation (60,000-100,000 IU) increased the incidence of falls but not fractures during the 1 year follow-up[62,63]. The recent VITdAL-ICU trial showed no difference in falls with 540,000 IU vitamin D_3 in critically ill patients (6 month fall rate: 17.7% vitamin D and 24.3% placebo; p=0.17) with no difference in the rate of fractures (two fractures per group at 6 months follow-up).[1] Because there remains some uncertainty around this issue, we will track fall-related fractures during the hospitalization and at the 90 day phone call as a secondary endpoint that is reviewed by the DSMB and reported in the final manuscript.

Placebo for those with vitamin D deficiency: Although vitamin D levels are not routinely measured as part of clinical inpatient care, during the course of this protocol clinicians may detect previously unrecognized vitamin D deficiency. However, the time span for vitamin D repletion to receive known benefit (e.g., osteoporosis prevention) is on the time scale of years. Acute vitamin D repletion is not the current standard of care in hospitalized patients for any clinical outcome. Thus, it is proposed that there is clinical equipoise to randomize vitamin D deficient participants to the placebo group for the duration of the study.

Phlebotomy: All participants will have blood drawn for research purposes. As almost all participants will have invasive lines placed for clinical purposes, risk of blood draws are essentially nil, as blood can be easily obtained from these lines. In the rare case an invasive line is not present, the risks of drawing blood are uncommon and include bleeding and bruising. Commonly, drawing blood is painful, and rarely, drawing blood can lead to infections at the site of the blood draw.

9.2 Minimization of risk

Federal regulations at 45 CFR 46.111(a)(1) require that risks to participants are minimized by using procedures which are consistent with sound research design. There are several elements of study design inherent in the present protocol that meets this human subject protection requirement. High dose vitamin D has been used safely in a number of populations, including ICU patients. We selected the 540,000 IU dose as the highest dose studied in ICU patients with reassuring safety profile. In addition, we will exclude patients with baseline screening 25OHD levels ≥20 ng/mL, hypercalcemia, or significant kidney stones to further mitigate potential for risk. We anticipate that approximately 20% of randomized participants will have baseline 25OHD levels ≥20 ng/mL by LC/MS/MS measurement. Based on our pilot data, nearly all of these participants will have a baseline LC/MS/MS level <30 ng/mL, and in this range, we believe there is still potential for benefit and low risk for vitamin D-related adverse events. Based on pilot data, we anticipate that the screening 25OHD testing will prevent randomization of participants with baseline LC/MS/MS levels >40 ng/mL, which further minimizes risk for participants. The DSMB will review data as outlined above and will examine not only efficacy but safety (inclusive of mortality) and may recommend halting the study at any time.

9.3 Potential Benefits

Study participants may or may not receive any direct benefits from their participation in this study. Early vitamin D repletion may result in reduced incidence and severity of ARDS and a lower associated morbidity and mortality.

9.4 Risks in Relation to anticipated benefit

Federal regulations at 45 CFR 46.111 (a)(2) require that “the risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.” Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits.

9.4.1 Procedures

Blood draws: The risks associated with these common clinical practices are small, however the knowledge gained in furthering our understanding of the pathophysiology and potentially leading to better and targeted therapy may be substantial.

9.4.2 Intervention

High dose vitamin D has been previously studied in ICU patients and there is substantial data to support safety and potential efficacy.

9.5 Safety Monitoring

An independent Data and Safety Monitoring Board will monitor trial progress to determine if the study should stop for safety, futility, or efficacy. The DSMB will also monitor trial quality and feasibility. The first analysis will occur after randomization of 300 patients and will include a review of plasma 25OHD levels and safety data for the first 150 vitamin D-treated patients. Based on dose-response data in ICU patients[1,49], we anticipate that the peak level will occur by day 3. We anticipate that the mean 25OHD level among vitamin D-treated participants will be ~35-40 ng/mL, with the nearly all being in the normal range (20-80 ng/mL). Acute vitamin D toxicity has been reported only with 25OHD levels >150 ng/mL, with hypervitaminosis D being defined as >120 ng/mL. If mean 25OHD levels are lower than 30 ng/mL and there is no evidence of hypercalcemia, hypervitaminosis D, or other safety concerns, then the Steering Committee may recommend increasing the vitamin D dose. If mean 25OHD levels are higher than 50 ng/mL, then the Steering Committee may recommend decreasing the vitamin D dose. The DSMB will then review recommendations to modify the current dose. The DSMB will have access to unblinded rates of adverse events, clinical and laboratory findings, and study endpoints. The DSMB may also recommend prolonged (beyond 300 randomized patients) or additional monitoring of 25OHD or calcium levels if more data are deemed necessary to monitor trial safety. Additional DSMB reviews will occur at interim analyses after randomization of 750 and 1500 and 2250 randomized participants and include safety, efficacy, and feasibility data (see Section 7, Statistical Considerations).

10 HUMAN SUBJECTS

Each study participant or a LAR must sign and date an informed consent form. Institutional review board approval will be required before any subject is entered into the study. PETAL will use a central IRB.

10.1 Selection of subjects

Federal regulations at 45 CFR 46(a)(3) require the equitable selection of subjects. The EDs, operating rooms, hospital wards, and ICUs of PETAL sites will be screened to determine if any patient meets inclusion and exclusion criteria. Data that have been collected as part of the routine management of the subject will be reviewed to determine eligibility. If any patient meets criteria for study enrollment, then the attending physician will be asked for permission to approach the patient or his/her LAR for informed consent. The screening vitamin D test will be performed as part of the eligibility process after written consent obtained. Study exclusion criteria neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals from participation in the research. Hence, the recruitment of subjects conforms to the principle of distributive justice.

10.2 Justification of including vulnerable subjects

The present research aims to investigate the safety and efficacy of vitamin D repletion to improve clinical outcomes in vitamin D deficient patients at high risk for ARDS and mortality. Due to the nature of this patient population, the majority of these patients will have impaired

decision-making capabilities. Moreover, those with intact decision-making capacities probably have milder disease than those with impaired capacity. Therefore, this study could not be conducted if limited to enrolling only those subjects with retained decision-making capacity. Hence, subjects recruited for this trial are not being unfairly burdened with involvement in this research simply because they are easily available.

10.3 Informed consent

Federal regulations 45 CFR 46.111(a)(5) require that informed consent will be sought from each prospective subject or the subject's LAR. We anticipate many consents will be from the subject's LAR, and thus the remainder of this section will focus on LARs. Study personnel obtaining informed consent are responsible for ensuring that the LAR understands the risks and benefits of participating in the study, answering any questions the LAR may have throughout the study and sharing any new information in a timely manner that may be relevant to the LAR's willingness to permit the subject's continued participation in the trial. The consenter will make every effort to minimize coercion. All study participants or their LARs will be informed of the objectives of the study and the potential risks. The informed consent document will be used to explain the risks and benefits of study participation to the LAR in simple terms before the patient is entered into the study, and to document that the LAR is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each LAR. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures including administration of study agent.

10.4 Continuing consent

Subjects for whom consent was initially obtained from a LAR, but who subsequently regain decision-making capacity while in hospital will be approached for consent for continuing participation, including continuance of data acquisition. The consent form signed by the LAR should reflect that such consent should be obtained.

10.5 Withdrawal of consent

Participants may withdraw or be withdrawn (by the LAR) from the trial at any time without prejudice. Data recorded up to the point of withdrawal will be included in the trial analysis, unless consent to use their data has also been withdrawn. If a patient or LAR wishes to withdraw from the trial after completion of trial treatment, permission to access medical records for trial data will be sought. Due to the single dose of study drug, it will not be possible for subjects or their LARs to withdraw consent to terminate the study drug. Withdrawal of consent prior to randomization will constitute a screen-failure. Withdrawal of consent after randomization will lead to subject discontinuation but site staff will request access to medical records for data related to the trial.

10.6 Identification of legally authorized representatives

Many of the patients approached for participation in this research protocol will have limitations of decision-making abilities due to their critical illness. Hence, most patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's legally authorized representative (LAR).

Regarding proxy consent, the existing federal research regulations ('the Common Rule') states at 45 CFR 46.116 that "no investigator may involve a human being as a subject in research…unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative"; and defines at 45 CFR 46 102 (c) a legally authorized representative (LAR) as "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedures(s) involved in the research." The Office of Human Research Protections (OHRP) defined examples of "applicable law" as being state statutes, regulations, case law, or formal opinion of a State Attorney General that addresses the issue of surrogate consent to medical procedures. Such "applicable law" could then be considered as empowering the LAR to provide consent for subject participation in the research. Interpretation of "applicable law" may be state specific and will be addressed by the PETAL central IRB.

According to a previous President's Bioethics Committee (National Bioethics Advisory Committee (NBAC)), an investigator should accept a relative or friend of the potential subject who is recognized as an LAR for purposes of clinical decision making under the law of the state where the research takes place [64]. Finally, OHRP has stated in their determination letters that a surrogate could serve as a LAR for research decision making if such an individual is authorized under applicable state law to provide consent for the "procedures" involved in the research study. [65]

10.7 Justification of surrogate consent

According to the Belmont Report, respect for persons incorporates at least two ethical convictions; first, that individuals should be treated as autonomous agents, and second, that person with diminished autonomy are entitled to protection. One method that serves to protect subjects is restrictions on the participation of subjects in research that presents greater than minimal risks. Commentators and research ethics commissions have held the view that it is permissible to include incapable subjects in greater than minimal risk research as long as there is the potential for beneficial effects and that the research presents a balance of risks and expected direct benefits ***similar*** to that available in the clinical setting [66]. Several U.S. task forces have deemed it is permissible to include incapable subjects in research. For example, the American College of Physicians' document allows surrogates to consent to research involving incapable subjects only "if the net additional risks of participation are not substantially greater than the risks of standard treatment." [67]. Finally, NBAC stated that an IRB may approve a protocol that presents greater than minimal risk but offers the prospect of direct medical benefits to the subject, provided that "the potential subject's LAR gives permission…" [64]

Consistent with the above ethical sensibilities regarding the participation of decisionally incapable subjects in research and the previous assessment of risks and benefits in the previous section, the present trial presents a balance of risks and potential direct benefits that is ***similar*** to that available in the clinical setting, with the exception of the additional blood draws.

10.8 Additional safeguards for vulnerable subjects

The present research will involve subjects who might be vulnerable to coercion or undue influence. As required in 45CFR46.111(b), we recommend that sites utilize additional safeguards to protect the rights and welfare of these subjects. Such safeguards might include, but are not limited to: a) assessment of the potential subject's capacity to provide informed

consent, b) the availability of the LAR to monitor the subject's subsequent participation and withdrawal from the study. The specific nature of the additional safeguards will be left to the discretion of the central IRB, in conjunction with the sites.

10.9 Confidentiality

Federal regulations at 45 CFR 46 111 (a) (7) requires that when appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data. To maintain confidentiality, all laboratory specimens, evaluation forms, and reports will be identified only by a coded number. The coded number will be generated by a computer, and only the study team will have access to the codes. All records will be kept in a locked, password protected computer. All computer entry and networking programs will be done with coded numbers only. All paper case report forms will be maintained inside a locked office. Study information will not be released without the written permission of the patient, except as necessary for monitoring by the National Heart, Lung, and Blood Institute, and the PETAL Clinical Coordinating Center or their designees.

11 ADVERSE EVENTS

11.1 Safety Monitoring

Assuring patient safety is an essential component of this protocol. Each participating investigator has primary responsibility for the safety of the individual participants under his or her care. A clinical trial adverse event is defined as any untoward medical event associated with the use of a drug or study procedure in humans, whether or not it is considered related to a drug or study procedure.

The Investigators will determine daily if any adverse events occur during the period from enrollment (signing of the informed consent) through study day 14 or hospital discharge, whichever occurs first. Investigators will determine if the event is serious or related to the study drug. The rationale for this time window is the 2 week half-life of 25OHD, which helps to define the period at risk from vitamin D.

The following adverse events will be collected in the adverse event case report forms:

- Serious adverse events
- Non-serious adverse events that are considered by the investigator to be related to study drug or study procedures

Investigators will assess if there is a reasonable possibility that the study drug or procedure caused the event. Investigators will also consider if the event is explained by the patient's underlying medical conditions, anticipated clinical course, previous medical conditions, and concomitant medications.

11.2 Serious Adverse Events

A serious adverse event is any adverse event that results in one of the following outcomes:

- Death
- A life-threatening experience (that is, immediate risk of dying)
- Prolonged inpatient hospitalization

  As per http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm: Report if admission to the hospital or prolongation of hospitalization was a result of the adverse event. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).

- Persistent or significant disability/incapacity

  As per http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm: Report if the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Serious adverse events will be collected during the first **14 study days** or until hospital discharge, whichever occurs first, regardless of the investigator's opinion of causation. Thereafter, serious adverse events are not required to be reported unless the investigator feels the events were related to either study drug or a protocol procedure.

Study site personnel must alert the CCC of any **serious and study drug or study procedure related** adverse event within 24 hours of investigator awareness of the event. Alerts issued via telephone are to be immediately followed with official notification on the adverse event case report form. See Appendix for reporting timelines for serious, unexpected, study related events (SAEs) and serious, unexpected suspected adverse reactions (SUSARs).

APPENDICES

A. Time-Events Schedule

Measurement/Event	0	1	2	3	4	5	6	7	8-14	HOSP D/C	90
Demographics, history and physical	X										
HCG (females)	X										
Screening 25OHD test	X										
Risk factors for ARDS and mortality	X										
Home meds (calcium and Vitamin D)	X										
Charlson Co-morbidity score	X										
SOFA	X										
LIPS	X										
Study drug administration	X										
ARDS Assessment	X	X	X	X	X	X	X	X			
Vasopressors (yes/no)	X	X	X	X	X	X	X	X			
Highest creatinine		A	A	A	A	A	A	A			
Serum albumin level	A	A	A	A	A	A	A	A	A		
On-study Vitamin D medication		X	X	X	X	X	X	X	X		
Calcium level (safety labs)†	X	A	A	X	A	A	A	A	A		
Ventilator status	X									X‡	
Plasma for IL-6 (first 300 randomized participants)	X			X							
Plasma for banking†	X			X							
Whole blood for DNA banking	X										
Plasma for 250HD level†	X			X							
EQ-5D-5L	X										X
Length of stay (hospital and healthcare facility)										X	X
Assessment of kidney stones and fall related fractures										X	X
Vital status										X	X

X: required

A: if available

†: Baseline required for all randomized participants; Day 3 required for first 300 randomized participants only

‡: If patient discharged from hospital prior to day 28 with ventilator-assisted breathing, day 28 ventilator status should be obtained (e.g., phone call to LTAC)

B. Imputed P/F using SpO2 and FiO2

The equivalence table below determines the estimated P/F ratio from the FiO2 and SpO2. This data was generated by investigators at the University of Utah, on a cohort of critically ill patients with pneumonia [68-74].

SPO2	FiO2														
	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1
80%	148	127	111	98	89	81	74	68	63	59	55	52	49	47	44
81%	151	129	113	101	91	82	76	70	65	60	57	53	50	48	45
82%	155	132	116	103	93	84	77	71	66	62	58	55	52	49	46
83%	158	136	119	106	95	86	79	73	68	63	59	56	53	50	47
84%	162	139	122	108	97	89	81	75	70	65	61	57	54	51	49
85%	167	143	125	111	100	91	83	77	71	67	63	59	56	53	50
86%	171	147	129	114	103	94	86	79	73	69	64	61	57	54	51
87%	177	151	132	118	106	96	88	81	76	71	66	62	59	56	53
88%	182	156	137	121	109	99	91	84	78	73	68	64	61	58	55
89%	189	162	141	126	113	103	94	87	81	75	71	67	63	60	57
90%	196	168	147	130	117	107	98	90	84	78	73	69	65	62	59
91%	203	174	153	136	122	111	102	94	87	81	76	72	68	64	61
92%	213	182	159	142	128	116	106	98	91	85	80	75	71	67	64
93%	223	191	168	149	134	122	112	103	96	89	84	79	74	71	67
94%	236	202	177	157	142	129	118	109	101	94	89	83	79	75	71
95%	252	216	189	168	151	138	126	116	108	101	95	89	84	80	76
96%	273	234	205	182	164	149	136	126	117	109	102	96	91	86	82

For altitude adjustment, we will incorporate the practice from prior ARDS Network studies of using average ambient to sea level barometric pressure (for Utah, 0.86; for Denver, 0.84).

Additional requirements for the use of the S/F ratio include:

1. SpO_2 between 80-96%
2. SpO_2 should be measured at least 10 minutes after any change in FiO_2.
3. PEEP ≥ 5 cm H_2O
4. An adequate pulse oximeter waveform tracing

C. Adverse Event Reporting and Unanticipated Problems

As noted in section 11, investigators will report all adverse events that are serious and study drug or study procedure related to the CCC within 24 hours. The CCC will then notify the NHLBI and cIRB.

The Medical Monitor at the CCC will work collaboratively with the reporting investigator to determine if a serious adverse event has a reasonable possibility of having been caused by the study drug or procedure (see Appendix C2). The Medical Monitor will also determine if the event is unexpected. An adverse is considered "unexpected" if it is not listed in the investigator brochure or the study protocol (21 CFR 312.32(a)). If a determination is made that a serious adverse event has a reasonable possibility of having been caused by the drug, it will be classified as a suspected adverse reaction. If the suspected adverse reaction is unexpected, it will be classified as a serious unexpected suspected adverse reaction (SUSAR).

The CCC will report all unexpected and study related deaths, SAEs, and SUSARs to the DSMB, NHLBI, and cIRB within 7 days after receipt of the report from a clinical site. A written report will be sent to the NHLBI, DSMB, FDA and the cIRB within 15 calendar days. All unexpected and study related deaths and life threatening SUSARS will be reported to the Food and Drug Administration (FDA) within 7 days; all other SUSARS will be reported to the FDA within 15 days. The DSMB will also review all adverse events and clinical outcomes during scheduled interim analyses. If the DSMB determines that the overall rate of adverse events is higher in the vitamin D group than the control group the cIRB and the FDA will be notified within 15 days of this determination (the latter via an IND safety report (21 CFR 312.32(c)(1)(i)(A)). The CCC will distribute the written summary of the DSMB's periodic review of adverse events to the cIRB in accordance with NIH guidelines (http://grants.nih.gov/grants/guide/notice-files/not99-107.html).

Unanticipated Problems (UP)

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug or study procedures within 24 hours. An unanticipated problem is defined as follows: any incident, experience, or outcome that meets all of the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
- Related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Determining Relationship of adverse events to study drug or procedures

Investigators will be asked to grade the strength of the relationship of an adverse event to vitamin D or study procedures as follows:

- Definitely Related: The event follows: a) A reasonable, temporal sequence from a study procedure; and b) Cannot be explained by the known characteristics of the patient's clinical state or other therapies; and c) Evaluation of the patient's clinical state indicates to the investigator that the experience is definitely related to study procedures.

- Probably or Possibly Related: The event should be assessed following the same criteria for "Definitely Associated". If in the investigator's opinion at least one or more of the criteria are not present, then "probably" or "possibly" associated should be selected.

- Probably Not Related: The event occurred while the patient was on the study but can reasonably be explained by the known characteristics of the patient's clinical state or other therapies.

- Definitely Not Related: The event is definitely produced by the patient's clinical state or by other modes of therapy administered to the patient.
  .
- Uncertain Relationship: The event does not meet any of the criteria previously outlined.

Events that are definitely, probably, or possibly related or of unknown relationship will be considered "study related" for the purposes of expedited reporting to the CCC. As noted above, the Medical Monitor at the CCC will work collaboratively with the reporting investigator to make a final determination if an adverse event or reaction has a reasonable possibility of having been caused by the study drug or procedure as outlined in 21 CFR 312.32**(a)**(1).

Clinical Outcomes that may be exempt from adverse event reporting

Study-specific clinical outcomes are exempt from adverse event reporting unless the investigator deems the event to be related to the study drug or the conduct of study procedures (or of uncertain relationship). The following are examples of events that will be considered study specific clinical outcomes:

- Death not related to the study drug or procedures.
- Multiple organ failures
- Hypercalcemia
- Kidney stone
- Falls resulting in fractures

D Data and Safety Monitoring Board

The principal role of the DSMB is to assure the safety of participants in the VIOLET trial. They will regularly monitor data from this trial, review and assess the performance of its operations, and make recommendations to the NHLBI with respect to:

- Review of adverse events
- Interim results of the study for evidence of efficacy or adverse events
- Possible early termination of the trial because of early attainment of study objectives, safety concerns, or inadequate performance
- Possible modifications in the clinical trial protocol
- Performance of individual centers

Three interim analyses will be conducted at approximately 25%, 50%, and 75% of target enrollment accrual.

The NHLBI PETAL Network DSMB is appointed by the Director, NHLBI and makes recommendations to the NHLBI Director. The DSMB reviews all protocols for safety following review by an independent NHLBI Protocol Review Committee. The DSMB will consist of members with expertise in acute lung injury, emergency medicine, biostatistics, ethics, and clinical trials. An NHLBI staff member not associated with PETAL will serve as Executive Secretary. Appointment of all members is contingent upon the absence of any conflicts of interest. All the members of the DSMB are voting members. The Principal Investigator and the Medical Monitor of the CCC will be responsible for the preparation of all DSMB and adverse event reports and may review unblinded data. The DSMB will develop a charter and review the protocol and sample consent form during its first meeting. Subsequent DSMB meetings will be scheduled in accordance with the DSMB Charter with the assistance of the CCC. When appropriate, conference calls may be held in place of face-to-face meetings. Recommendations to end, modify, or continue the trial will be prepared by the DSMB executive secretary for review by Director, NHLBI. Recommendations for major changes, such as stopping, will be reviewed by the NHLBI Director and communicated immediately. Other recommendations will be reviewed by the NHLBI director and distributed in writing to the CCC, which will distribute to the PETAL steering committee with instructions for reporting to local IRBs when appropriate.

Details of the NHLBI policies regarding DSMBs can be found at the following URL:
http://www.nhlbi.nih.gov/funding/policies/dsmb_inst.htm

E PETAL Network Steering Committee

The PETAL Network Steering Committee is comprised of the Principal Investigators and Co-investigators of all the Clinical sites, the CCC, and the NHLBI Project Officer who represents the NHLBI. All sites have two votes and the CCC has one.

F SOFA Scoring System

SOFA Score	0	1	2	3	4
Respiration[A] PaO_2/F_IO_2 (mm Hg) or imputed P/F using SaO_2/F_IO_2	>400	<400	<300	<200	<100
Coagulation Platelets $10^3/mm^3$	>150	<150	<100	<50	<20
Liver Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular[B] Hypotension	No hypotension	MAP <70	Dopamine </= 5 or dobutamine (any)	Dopamine >5 or norepinephrine </= 0.1	Dopamine >15 or norepinephrine >0.1
CNS Glasgow Coma Score	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dL) or urine output (mL/d)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

A: Values for scores 3 and 4 are with respiratory support

B: Adrenergic agents administered for at least one hour (doses given in µg/kg/min)

G De-identified data elements for screened, non-enrolled

The following data elements will be collected on screened subjects who met the inclusion criteria but were not enrolled.

- Month of the year that patient met screening criteria (1-12).
- Gender
- Ethnicity
- Age (if age >89, 89 will be entered for age)
- Screening vitamin D level (25OHD level)
- Method used for vitamin D level (clinical lab or Qualigen FastPack IP)
- Patient location (e.g. MICU, SICU, etc.)
- Reason(s) patient excluded from study
- If not excluded, not enrolled, why?
- ARDS risk factors
    - If shock, etiology
    - If sepsis, site of infection

H References

1. Amrein K, Schnedl C, Holl A, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. *JAMA.* 2014;312(15):1520-1530.
2. Levitt JE, Matthay MA. Clinical review: Early treatment of acute lung injury--paradigm shift toward prevention and treatment prior to respiratory failure. *Crit Care.* 2012;16(3):223.
3. Zhang Y, Leung DY, Richers BN, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol.* 2012;188(5):2127-2135.
4. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. *Immunology.* 2011;134(2):123-139.
5. Takano Y, Mitsuhashi H, Ueno K. 1alpha,25-Dihydroxyvitamin D(3) inhibits neutrophil recruitment in hamster model of acute lung injury. *Steroids.* 2011;76(12):1305-1309.
6. Brockman-Schneider RA, Pickles RJ, Gern JE. Effects of vitamin D on airway epithelial cell morphology and rhinovirus replication. *PLoS One.* 2014;9(1):e86755.
7. Dancer RC, Parekh D, Lax S, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). *Thorax.* 2015;70(7):617-624.
8. Phokela SS, Peleg S, Moya FR, Alcorn JL. Regulation of human pulmonary surfactant protein gene expression by 1alpha,25-dihydroxyvitamin D3. *Am J Physiol Lung Cell Mol Physiol.* 2005;289(4):L617-626.
9. Rehan VK, Torday JS, Peleg S, et al. 1Alpha,25-dihydroxy-3-epi-vitamin D3, a natural metabolite of 1alpha,25-dihydroxy vitamin D3: production and biological activity studies in pulmonary alveolar type II cells. *Mol Genet Metab.* 2002;76(1):46-56.
10. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* 2006;311(5768):1770-1773.
11. Hewison M. Antibacterial effects of vitamin D. *Nat Rev Endocrinol.* 2011;7(6):337-345.
12. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol.* 2010;184(2):965-974.
13. Coussens AK, Wilkinson RJ, Hanifa Y, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc Natl Acad Sci U S A.* 2012;109(38):15449-15454.
14. Fukuoka M, Ogino Y, Sato H, Ohta T, Komoriya K. Regulation of RANTES and IL-8 production in normal human dermal fibroblasts by active vitamin D3 (tacalcitol). *Br J Pharmacol.* 1998;124(7):1433-1438.
15. Takahashi K, Horiuchi H, Ohta T, Komoriya K, Ohmori H, Kamimura T. 1 alpha,25-dihydroxyvitamin D3 suppresses interleukin-1beta-induced interleukin-8 production in human whole blood: an involvement of erythrocytes in the inhibition. *Immunopharmacol Immunotoxicol.* 2002;24(1):1-15.
16. Fukuoka M, Ogino Y, Sato H, et al. RANTES expression in psoriatic skin, and regulation of RANTES and IL-8 production in cultured epidermal keratinocytes by active vitamin D3 (tacalcitol). *Br J Dermatol.* 1998;138(1):63-70.
17. Krüger S, Kreft B. 1,25-dihydroxyvitamin D3 differentially regulates IL-1alpha-stimulated IL-8 and MCP-1 mRNA expression and chemokine secretion by human primary proximal tubular epithelial cells. *Exp Nephrol.* 2001;9(3):223-228.
18. Rostkowska-Nadolska B, Sliupkas-Dyrda E, Potyka J, et al. Vitamin D derivatives: calcitriol and tacalcitol inhibits interleukin-6 and interleukin-8 expression in human nasal polyp fibroblast cultures. *Adv Med Sci.* 2010;55(1):86-92.
19. Penna G, Fibbi B, Amuchastegui S, et al. The vitamin D receptor agonist elocalcitol inhibits IL-8-dependent benign prostatic hyperplasia stromal cell proliferation and inflammatory response by targeting the RhoA/Rho kinase and NF-kappaB pathways. *Prostate.* 2009;69(5):480-493.

20. Harant H, Andrew PJ, Reddy GS, Foglar E, Lindley IJ. 1alpha,25-dihydroxyvitamin D3 and a variety of its natural metabolites transcriptionally repress nuclear-factor-kappaB-mediated interleukin-8 gene expression. *Eur J Biochem.* 1997;250(1):63-71.
21. Harant H, Wolff B, Lindley IJ. 1Alpha,25-dihydroxyvitamin D3 decreases DNA binding of nuclear factor-kappaB in human fibroblasts. *FEBS Lett.* 1998;436(3):329-334.
22. Sato H, Nakayama Y, Yamashita C, Uno H. Anti-inflammatory effects of tacalcitol (1,24(R)(OH)2D3, TV-02) in the skin of TPA-treated hairless mice. *J Dermatol.* 2004;31(3):200-217.
23. Kunkel SL, Standiford T, Kasahara K, Strieter RM. Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. *Exp Lung Res.* 1991;17(1):17-23.
24. Mitsuhashi H, Hata J, Asano S, Kishimoto T. Appearance of cytokine-induced neutrophil chemoattractant isoforms and immunolocalization of them in lipopolysaccharide-induced acute lung inflammation in rats. *Inflamm Res.* 1999;48(11):588-593.
25. Ge ZJ, Jiang GJ, Zhao YP, Wang GX, Tan YF. Systemic perfluorohexane attenuates lung injury induced by lipopolysaccharide in rats: the role of heme oxygenase-1. *Pharmacol Rep.* 2010;62(1):170-177.
26. Slofstra SH, Groot AP, Maris NA, Reitsma PH, Cate HT, Spek CA. Inhalation of activated protein C inhibits endotoxin-induced pulmonary inflammation in mice independent of neutrophil recruitment. *Br J Pharmacol.* 2006;149(6):740-746.
27. Zarbock A, Allegretti M, Ley K. Therapeutic inhibition of CXCR2 by Reparixin attenuates acute lung injury in mice. *Br J Pharmacol.* 2008;155(3):357-364.
28. Gonçalves de Moraes VL, Singer M, Vargaftig BB, Chignard M. Effects of rolipram on cyclic AMP levels in alveolar macrophages and lipopolysaccharide-induced inflammation in mouse lung. *Br J Pharmacol.* 1998;123(4):631-636.
29. Bao Z, Ye Q, Gong W, Xiang Y, Wan H. Humanized monoclonal antibody against the chemokine CXCL-8 (IL-8) effectively prevents acute lung injury. *Int Immunopharmacol.* 2010;10(2):259-263.
30. Hiemstra PS. The role of epithelial beta-defensins and cathelicidins in host defense of the lung. *Exp Lung Res.* 2007;33(10):537-542.
31. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J.* 2005;19(9):1067-1077.
32. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol.* 2007;179(4):2060-2063.
33. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol.* 2004;173(5):2909-2912.
34. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med.* 1998;338(12):777-783.
35. Ginde AA, Camargo CA, Shapiro NI. Vitamin D insufficiency and sepsis severity in emergency department patients with suspected infection. *Acad Emerg Med.* 2011;18(5):551-554.
36. Kiebzak GM, Moore NL, Margolis S, Hollis B, Kevorkian CG. Vitamin D status of patients admitted to a hospital rehabilitation unit: relationship to function and progress. *Am J Phys Med Rehabil.* 2007;86(6):435-445.
37. Giusti A, Barone A, Razzano M, et al. High prevalence of secondary hyperparathyroidism due to hypovitaminosis D in hospitalized elderly with and without hip fracture. *J Endocrinol Invest.* 2006;29(9):809-813.
38. Jeng L, Yamshchikov AV, Judd SE, et al. Alterations in vitamin D status and anti-microbial peptide levels in patients in the intensive care unit with sepsis. *J Transl Med.* 2009;7:28.
39. Nierman DM, Mechanick JI. Bone hyperresorption is prevalent in chronically critically ill patients. *Chest.* 1998;114(4):1122-1128.

40. Lucidarme O, Messai E, Mazzoni T, Arcade M, du Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. *Intensive Care Med.* 2010;36(9):1609-1611.
41. Braun A, Chang D, Mahadevappa K, et al. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. *Crit Care Med.* 2011;39(4):671-677.
42. Braun AB, Gibbons FK, Litonjua AA, Giovannucci E, Christopher KB. Low serum 25-hydroxyvitamin D at critical care initiation is associated with increased mortality. *Crit Care Med.* 2012;40(1):63-72.
43. Kale SS, Yende S. Effects of Aging on Inflammation and Hemostasis through the Continuum of Critical Illness. *Aging Dis.* 2011;2(6):501-511.
44. Galloway P, McMillan DC, Sattar N. Effect of the inflammatory response on trace element and vitamin status. *Ann Clin Biochem.* 2000;37 (Pt 3):289-297.
45. Krishnan A, Ochola J, Mundy J, et al. Acute fluid shifts influence the assessment of serum vitamin D status in critically ill patients. *Crit Care.* 2010;14(6):R216.
46. Leaf DE, Raed A, Donnino MW, Ginde AA, Waikar SS. Randomized controlled trial of calcitriol in severe sepsis. *Am J Respir Crit Care Med.* 2014;190(5):533-541.
47. Quraishi SA, De Pascale G, Needleman JS, et al. Effect of Cholecalciferol Supplementation on Vitamin D Status and Cathelicidin Levels in Sepsis: A Randomized, Placebo-Controlled Trial. *Crit Care Med.* 2015;43(9):1928-1937.
48. Han JE, Jones JL, Tangpricha V, et al. High Dose Vitamin D Administration in Ventilated Intensive Care Unit Patients: A Pilot Double Blind Randomized Controlled Trial. *J Clin Transl Endocrinol.* 2016;4:59-65.
49. Amrein K, Sourij H, Wagner G, et al. Short-term effects of high-dose oral vitamin D3 in critically ill vitamin D deficient patients: a randomized, double-blind, placebo-controlled pilot study. *Crit Care.* 2011;15(2):R104.
50. Hayden D, Pauler DK, Schoenfeld D. An estimator for treatment comparisons among survivors in randomized trials. *Biometrics.* 2005;61(1):305-310.
51. Mehta AB, Syeda SN, Wiener RS, Walkey AJ. Epidemiological trends in invasive mechanical ventilation in the United States: A population-based study. *Journal of Critical Care.*30(6):1217-1221.
52. Quraishi SA, McCarthy C, Blum L, Cobb JP, Camargo CA. Plasma 25-Hydroxyvitamin D Levels at Initiation of Care and Duration of Mechanical Ventilation in Critically Ill Surgical Patients. *JPEN J Parenter Enteral Nutr.* 2015.
53. The EuroQol Group. EuroQol--a new facility for the measurement of health-related quality of life. *Health policy.* 1990;16(3):199-208.
54. Angus DC, Carlet J, Brussels Roundtable P. Surviving intensive care: a report from the 2002 Brussels Roundtable. *Intensive Care Med.* 2003;29(3):368-377.
55. Shaw JW, Johnson JA, Coons SJ. US valuation of the EQ-5D health states: development and testing of the D1 valuation model. *Medical care.* 2005;43(3):203-220.
56. Dinglas VD, Gifford JM, Husain N, Colantuoni E, Needham DM. Quality of life before intensive care using EQ-5D: patient versus proxy responses. *Crit Care Med.* 2013;41(1):9-14.
57. Gifford JM, Husain N, Dinglas VD, Colantuoni E, Needham DM. Baseline quality of life before intensive care: a comparison of patient versus proxy responses. *Crit Care Med.* 2010;38(3):855-860.
58. Lilly CM. The ProCESS trial--a new era of sepsis management. *N Engl J Med.* 2014;370(18):1750-1751.
59. Jennison C, Turnbull BW. *Group sequential methods with applications to clinical trials.* CRC Press; 1999.

60. Adults AGSWoVDSfO. Recommendations abstracted from the American Geriatrics Society Consensus Statement on vitamin D for Prevention of Falls and Their Consequences. *J Am Geriatr Soc.* 2014;62(1):147-152.
61. Sanders KM, Stuart AL, Williamson EJ, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA.* 2010;303(18):1815-1822.
62. Ginde AA, Blatchford P, Breese K, et al. High-Dose Monthly Vitamin D for Prevention of Acute Respiratory Infection in Older Long-Term Care Residents: A Randomized Clinical Trial. *J Am Geriatr Soc.* 2016.
63. Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ, et al. Monthly High-Dose Vitamin D Treatment for the Prevention of Functional Decline: A Randomized Clinical Trial. *JAMA Intern Med.* 2016;176(2):175-183.
64. National Bioethics Advisory Commitee (NBAC). *Research Involving Persons with Mental Disorders That May Affect Decisionmaking Capacity.* Rockville: U.S. Government Printing Office; 1998.
65. Office of Human Research Protections (OHRP). Compliance Determination Letters. 2002; http://ohrp.osophs.dhhs.gov/detrm_letrs/.
66. Dresser R. Research Involving Persons with Mental Disabilities: A Review of Policy Issues and Proposals. *National Bioethics Advisory Commission*. Rockville: U.S. Government Printing Office; 1999:5-28.
67. American College of Physicians. Cognitively Impaired Subjects. *Ann Int Med.* 1989;111:843-848.
68. Ellis RK. Determination of PO2 from saturation. *Journal of applied physiology.* 1989;67(2):902.
69. Severinghaus JW. Simple, accurate equations for human blood O2 dissociation computations. *Journal of applied physiology: respiratory, environmental and exercise physiology.* 1979;46(3):599-602.
70. Rice TW, Wheeler AP, Bernard GR, et al. Comparison of the SpO2/FIO2 ratio and the PaO2/FIO2 ratio in patients with acute lung injury or ARDS. *Chest.* 2007;132(2):410-417.
71. Lanspa MJ, Jones BE, Brown SM, Dean NC. Mortality, morbidity, and disease severity of patients with aspiration pneumonia. *Journal of hospital medicine : an official publication of the Society of Hospital Medicine.* 2013;8(2):83-90.
72. Dean NC, Jones JP, Aronsky D, et al. Hospital admission decision for patients with community-acquired pneumonia: variability among physicians in an emergency department. *Annals of emergency medicine.* 2012;59(1):35-41.
73. Brown SM, Jones BE, Jephson AR, Dean NC, Infectious Disease Society of America/American Thoracic S. Validation of the Infectious Disease Society of America/American Thoracic Society 2007 guidelines for severe community-acquired pneumonia. *Crit Care Med.* 2009;37(12):3010-3016.
74. Van de Louw A, Cracco C, Cerf C, et al. Accuracy of pulse oximetry in the intensive care unit. *Intensive Care Med.* 2001;27(10):1606-1613.