1. Administrative Information

Title	Vitamin D to Improve Outcomes by Leveraging Early Treatment: Long-term Brain Outcomes in Vitamin D Deficient Patients (VIOLET-BUD)
Trial Registration	https://clinicaltrials.gov/ct2/show/NCT03733418
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Date	Draft Date: March 3, 2020
SAP Version	Version 1.0

2. Introduction

This is the Statistical Analysis Plan (SAP) for the study titled, "Vitamin D to Improve Outcomes by Leveraging Early Treatment: Long-term Brain Outcomes in Vitamin D Deficient Patientof Acute Lung Injury (PETAL) randomized control trial titled, "Vitamin D to Improve Outcomes by Leveraging Early Treatment" (VIOLET, NCT03096314) which was published in the *New England Journal of Medicine*.[1] VIOLET-BUD was sponsored by the NHLBI (R56HL141567).

2.1. Background and rationale

One out of four intensive care unit (ICU) survivors will develop long-term cognitive impairment (LTCI) similar in severity to mild Alzheimer's disease.[2] A substantial proportion will also develop impairments in executive function,[2] which leads to increased disability, worsening quality of life, and reduced employment.[3,4] Interventions that preserve long-term cognition and executive function after critical illness are lacking.

Early, high-dose oral Vitamin D repletion could potentially help preserve long-term cognition in critically ill patients. Vitamin D is a pleiotropic secosteroid hormone that modulates systemic and central nervous system (CNS) inflammatory responses.[5-8] Inflammation in response to an acute illness plays a prominent role in dementia pathogenesis.[9-14] An abundance of recent observational data suggest Vitamin D deficiency is associated with poorer long-term cognition[15,16] and increased risk of Alzheimer's dementia in community-dwelling adults.[16] Vitamin D deficiency is also associated with accelerated cognitive decline in hospitalized, acutely ill patients.[17] To our knowledge, no studies have evaluated if Vitamin D repletion improves these cognitive outcomes in the setting of an acute or critical illness.

2.2. Objectives

VIOLET-BUD is an ancillary study to the parent VIOLET study. Its primary objective and hypothesis are:

> To determine if early administration of a single high-dose (540,000 IU) oral vitamin D3 (cholecalciferol) treatment improves long-term global cognition and executive function as determined by comprehensive neuropsychological testing in critically ill patients with Vitamin D deficiency at enrollment. *We hypothesize that critically ill patients with Vitamin D deficiency who are treated with a single high-dose of Vitamin D3 will have significantly better long-term global cognition and executive function than those treated with placebo.*

3. Study Population

VIOLET-BUD enrolled subjects from November 2018 to October 2019 in 7 (out of 42) PETAL sites that participated in VIOLET. The local site contacted all patients who were enrolled in VIOLET to recruit patients for VIOLET-BUD. Originally, the objective was to conduct the follow-up assessments 12 ± 4 months after randomization. Due to slower than anticipated enrollment, VIOLET-BUD expanded the follow-up window from 12 months to include all survivors. The median (interquartile range) time from randomization to follow-up was 443 (386.5, 481.5) days.

Patients were excluded from VIOLET-BUD if they were deaf, blind, or non-English speaking. Patients who were deaf or blind were excluded, because the neuropsychological testing has auditory and visual components. Non-English speaking patients were excluded because the neuropsychological raters can only perform the assessments in English.

4. Variables Collected for VIOLET-BUD

4.1 Primary Outcome Variables

The primary outcomes for VIOLET-BUD are long-term global cognition and executive function, as determined by a comprehensive neuropsychological assessment. Global cognition was assessed for using the ***Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)***. The RBANS is a comprehensive neuropsychological battery for the evaluation of global cognition and has been validated in subjects with mild cognitive impairment, moderate to severe traumatic brain injuries, vascular dementias, and Alzheimer's disease.[18-22] In addition to providing a score for global cognition, it also provides individual scores for immediate and delayed memory, attention, visuospatial construction, and language.

To quantify executive function, we used tests from the ***Delis–Kaplan Executive Function System (D-KEFS)***. An executive function composite score was calculated using scores from the ***DKEFS Proverbs, Trail Making-Number/Letter Switching, and Verbal Fluency Category Switching subscales.***

4.2. Secondary Outcomes are listed in Table 1.

Study Assessment	Description
RBANS cognitive domains	Immediate memory, delayed memory, attention, visuospatial construction, and language
Katz Activities of Daily Living (ADL)[23]	Quantifies basic ADLs such as bathing, dressing, toileting, transferring, continence, and feeding.
Lawton Instrumental Activities of Daily Living Scale (IADL)[24]	Quantifies the patient's IADLs, such as his/her ability to use the telephone, shopping, food preparation, housekeeping, laundry, transportation, medication management, and finances.

Table 1. Secondary outcomes for VIOLET-BUD.

4.3. Additional Variables

The patient was considered to have ***pre-existing cognitive impairment*** if he/she had a past history of dementia or was on an acetylcholinesterase inhibitor medication (galantamine [Reminyl], Donepezil [Aricept] and rivastigmine [Exelon]) prior to randomization. ***Pre-illness intelligence*** was estimated using the Barona Index, which is based upon age, region of residence, education, and highest occupation.[27,28] ***Severity of illness*** at enrollment was characterized using the Sequential Organ Failure Assessment.[29] ***Infection*** at enrollment was defined by the presence of sepsis or pneumonia.

5. Statistical Methods

All long-term outcomes will be analyzed using both univariate methods and multivariable regression, adjusting for covariates noted below. Though baseline patient characteristics should theoretically be balanced between treatment groups due to randomization, adjustment increases our power and precision. Adjusted analyses will be considered the primary analyses.

5.1. Unadjusted analyses

We will analyze normally distributed outcomes using the t-test and non-normally distributed outcomes using the Mann-Whitney test.

5.2. Adjusted analyses

Long-term RBANS = **Vitamin D versus Placebo** **+ Covariates**

Long-term executive function composite score = **Vitamin D versus Placebo** **+ Covariates**

Figure 1. Multivariable linear regression models

Covariates	Variable Type	df	Comment
Vitamin D vs placebo	Dichotomous	1	Primary independent variable
Age at enrollment	Continuous	2	
Pre-existing cognitive impairment	Dichotomous	1	Dementia or use of cholinesterase inhibitor prior to randomization
Severity of illness at enrollment	Continuous	2	Sequential Organ Failure Assessment (SOFA) score prior to randomization
Infection (Sepsis + Pneumonia) at enrollment	Dichotomous	1	Obtained by medical record review
Pre-illness intelligence at enrollment	Continuous	2	The Barona Index which is based on level of education, highest occupation, and region of residence[27,28]

Table 2. Covariates for the multivariable linear regression models in order of importance. The models will be able to accommodate 9 degrees of freedom.

To determine if single, high-dose enteral Vitamin D treatment improves long-term global cognition and executive function compared with placebo, we will use multivariable linear regression or proportional odds logistic regression models based on the distribution of the continuous outcomes (**Figure 1**). Separate models will be constructed for long-term RBANS and executive function and adjusted for the baseline covariates listed in **Table 2.** Post-randomization covariates (e.g., interventions provided during hospitalization) will not be adjusted for because they may potentially be affected by the randomized intervention and be on the causal pathway. Prior to modeling, we will perform redundancy analyses to ensure that no covariates completely explain any of the others (resulting in multicollinearity) using an adjusted R^2 cutoff of 0.7. If any covariates are highly correlated, only one of them based on clinical relevance will be kept in the model. If the multivariable models do not converge due to limited variability or low number of patients in specific levels of certain covariates, the number of covariates will be reduced based on the least rank in the order specified above. Restricted cubic splines for continuous variables will be incorporated into the models based on the distribution. To account for correlation among patients within a given site, we will adjust standard errors using Huber-White sandwich estimate.[30] Partially missing long-term outcomes will be imputed using single imputation.

As exploratory heterogeneity of treatment effect analyses for primary outcomes, we will evaluate for effect modification by age, pre-existing cognitive impairment (e.g., dementia), baseline severity of illness (Sequential Organ Failure Assessment), infection (pneumonia or sepsis diagnosis), and pre-morbid intelligence (Barona). Because this is an exploratory analysis, we will consider an interaction term p-value less than 0.20 to suggest the potential presence of effect modification.

To determine how time to neuropsychological assessment impacted our findings, we will also conduct a sensitivity analysis; we will re-run the multivariable regression models adjusting for time to neuropsychological assessment. Patients were included in VIOLET (and VIOLET-BUD) if their plasma Vitamin D concentrations were < 20 mg/dL as determined by local immunoassay (clinical laboratory or point-of-care) testing. The VIOLET parent trial also confirmed baseline (pre-randomization) Vitamin D deficiency using liquid chromatography-mass spectrometry (LC-MS), the gold standard for Vitamin D measurement, conducted at the University of Washington on batched samples after 90-day follow-up was completed. VIOLET-BUD's primary analyses will use immunoassay Vitamin D measurements. We will re-run the multivariable regression models and adjust for baseline Vitamin D levels as measured by LC-MS as a sensitivity analysis. Because the VIOLET parent study did not observe a significant difference in 90-day mortality between the Vitamin D and placebo groups,[1] analyses accounting for differential mortality will not be performed.

Other secondary analyses will include associations between Vitamin D treatment and individual RBANS cognitive domains (e.g., immediate and delayed memory, attention, visuospatial construction, and language) and executive function subscales (DKEFS Proverbs, Trail Making-Number/Letter Switching, and Verbal Fluency Category Switching). We will also determine if treatment is associated with functional status (Katz and Lawton).

Level of statistical significance will be set at 5%. All tests will be two-sided and 95% confidence intervals will be reported along with all effect estimates. Presentation of results will emphasize clinical significance, effect sizes, and confidence intervals, over statistical significance. Regarding the analyses of all *a priori*-defined secondary outcomes described herein, no adjustments will be made for multiple comparisons in keeping with recommendations on this topic and standard practice when analyzing multiple, prospectively defined outcomes in a clinical trial.[31,32]

The Outcomes After Critical Illness and Surgery (OACIS) Employment Status Questionnaire[25,26] and place of residence were originally intended to be secondary outcomes. Due to the categorical nature of and low number of patients with these outcomes (loss of employment or nursing home), their statistical analyses will be descriptive in nature. Death was also a secondary outcome but will be analyzed as a separate manuscript.

D5.2. Sample Size Calculations

In our original sample size calculations, we used half a standard deviation (SD) of that particular test to define the minimum clinically important difference (MCID) threshold.[33-36] The SD for the RBANS in our population from previous studies was 12.4.[2] Thus, we considered a 6-point difference to be the MCID threshold for the RBANS. Based upon a two-sided alpha of 0.05 and 80% power, we computed that we would need outcome data in a total of 140 patients with completed follow-up data (~70 patients in each group) to detect a 6-point difference in RBANS between treatment groups. However, VIOLET-BUD enrolled 95 patients; because the VIOLET parent trial stopped enrollment early, no additional patients could be recruited for this ancillary study. However, this sample size still provides us with more than 80% power to detect at least a

7.2-point difference. Some have considered an RBANS difference of 8 points to be the MCID threshold.[37] For our executive function composite score, the SD in our previous population was 2.34. With 95 patients assessed, we will have greater than 80% power to detect a 1.4-point difference in the executive function composite score assuming a two-sided alpha of 0.05.

For our multiple linear regression models, we will have one degree of freedom available for every 10 to 15 patients with completed outcome measurements.[38] Consequently, we will be able to accommodate 9 degrees of freedom for our multivariable models without overfitting.

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