
# Vitamin D Oral Replacement in Asthma

**NCT03686150**

**Protocol Document**

**December 14, 2020**

# Vitamin D Oral Replacement in Asthma (VDORA1)

**UAMS IRB Protocol Number: 228446**

**Version Number: 07**

**14 December 2020**

**Sponsor: ISPCTN DCOC**

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**Funded by: The National Institutes of Health (NIH)**

### Summary of Changes from Version 01 to 02:

| Affected Section(s) | Summary of Revisions Made (01 to 02) | Rationale |
|---|---|---|
| 1.1<br>Schedule of Activities | Corrected footnote "h" in Schedule of Activities so that the footnote matched the table. Specifically changed "screening" to "baseline." | Inadvertent oversight during proofreading. |
| 6.5<br>Concomitant Therapy | Added information about allowable Vitamin D dosage in over-the-counter vitamins that the participants may take. | Requested in UAMS IRB contingency letter of 09/13/2018 |
| 8.1.2<br>Laboratory Evaluations | Corrected "complete blood count" to "platelet" where the change was not made prior to initial IRB submission. | Prior to initial IRB submission, a decision was made to change one of the lab tests from "complete blood count" to "platelet count." This change was inadvertently not made consistently throughout the protocol. |
| 10.1.9.1<br>Data Collection & Management Responsibilities | Added paragraph noting that data will not be de-identified by the sites, but will be de-identified before it is shared outside of the network. | Requested in UAMS IRB contingency letter of 09/13/2018 |

### Summary of Changes from Version 02 to 03:

| Affected Section(s) | Summary of Revisions Made (02 to 03) | Rationale |
|---|---|---|
| Title page and section 10.1.5 | Added Jason Lang, MD, PhD to title page and corrected affiliation | Simple correction. |
| Title page and section 10.1.5 | Removed Dr. Greg Kearns name from protocol. | Dr. Kearns is no longer working on protocol. |
| Sections 1 and 4.1, | Increase number of participants that can be randomized in part 1 of the study. Increased from 32 to 50. | This is to ensure adequate numbers in each group for pharmacokinetic analyses. |
| Sections 1; 1.1; 3; 8.1.2; and 9.4.10 | Made specimen collection optional for platelets. | Specimens for platelet analysis cannot be frozen or held for extended periods. This will allow sites to schedule participants for Friday and Saturday visits |
| Sections 1.1; 4.1; 4.4; 6.1.2; 8.1.1.1; and 8.2 | Corrected/clarified timeframes for scheduling visits to make it clear that all visits are in relation to baseline visit (Visit 2). | There were inconsistencies within the protocol regarding scheduling of visits. |

| Affected Section(s) | Summary of Revisions Made (02 to 03) | Rationale |
|---|---|---|
| Section 1.1; 8.1.1.1 (screening and baseline visits) | Removed requirement to do a focused physical examination as a routine part of the study. | Not essential to protocol. |
| Sections 2.3.3; 4.1; and 5.2, | Corrected use of terminology for randomization and enrollment. | Technical correction/clarification. |
| Section 5.1 | Annotated “abnormal liver function” to indicate values need to also be clinically significant (per site investigator). | Clarification. |
| Sections 5.2 | Clarified/revised used of “restricted” and “excluded” with regard to medication. Noted that excluded medication cannot be used, not only within 30 days prior to enrollment, but also at any time during the run-in period. | Clarification. |
| Section 5.2 and 8.1.1.1 (Visit 2) | Changed “return greater than or equal to 75% of diary cards” to “return diary cards with a completion rate greater than or equal to 75%.” | Clarification/correction. |
| Section 5.4 | Inserted “Participants who were terminated due to a screen fail must sign new consent/assent documents prior to rescreening.” | Clarification. |
| Section 5.5, (Recruit-ment) | Added paragraph to allow for collection of information about why potential participants decline to be in the study. | New. |
| Sections 6.1.2; 6.4; and 8.1.1.1 | Added that diary cards will be used for follow-up visits in addition to Visits 1-6. These diary cards will not have a line for recording if vitamin D pills were taken. Concomitantly, diary cards for visits in which Vitamin D is dispensed will be “diary cards for dosing visits.” | New. |
| Section 6.5 | Clarified what is a study deviation with regard to use of excluded medication. | Clarification. |
| Section 6.5 | In excluded medication list, the “calcifediol (vitamin D2)” was changed to “Vitamin D2.” | Technical correction. |
| Sections 7.1; and 8.2 | Deleted “calcium and creatinine” each measured in mg/ml” | Phrase was causing confusion at sites. |
| Section 7.2 | Add “at the discretion of the site investigator” to discontinuation due to non-compliance. | Clarification. |
| Section 8.1.1.1 (Visit 1) | Revised daily diary cards for visit 1. Cards for visit 1 will no longer have a line to collect information about taking Vitamin D pills. | Correction made to diary cards. |

| Affected Section(s) | Summary of Revisions Made (02 to 03) | Rationale |
|---|---|---|
| Section 8.1.1.1 (Visit 1) | Added that participants whose safety laboratory values exceed thresholds will be referred to primary care physician. | Clarification for safety. |
| Section 8.1.1.1 (Visit 2). | Clarified that sites must verify participants continue to meet inclusion/exclusion criteria at baseline (visit 2). | Clarification/reinforcement of requirement. |
| Section 8.1.2. | Referenced lab manual for specimen collection methods for central lab; deleted specifications for collection methods that were in this protocol. | Some specification in protocol were not accurate. |
| Section 8.1.2 | Revised inclusion criteria in later part of protocol to include the specification that Vitamin D level must be greater than 10 ng/ml, as well as less than 30 ng/ml, to meet inclusion criteria. | Corrected within protocol inconsistency. |
| Section 8.2. | Deleted "…or they may proceed to a total serum calcium measurement (if preferred in order to obviate a return visit)." A second urine calcium/creatinine is required prior to getting a specimen for blood calcium. | Corrected within protocol inconsistency. A second urine calcium/creatinine is required prior to collecting blood for a blood calcium level. |
| Section 8.3.3.3 | Added list of expected AEs | Clarification. |
| Section 8.3.9 | "Reporting of Pregnancy" section re-written | Clarification and to eliminate the requirement to follow infants for "up to 2 months." |
| Section 9.4.3 | Corrected "…25(OH)D greater than or equal to 0 ng/ml during a desired time period.." to "…25(OH)D greater than or equal to 40 ng/ml during a desired time period.." | Typographical error. |
| Section 10.2 | Deleted unused abbreviations (GLP and IDE) from table. | Correction of inconsistency. |

## Summary of Changes from Version 03 to 04:

*(primarily addressing contingencies from version 03 but also removing requirement to collect specimens for platelets and allowing for more than weekly contact between visits.)*

| Affected Section(s) | Summary of Revisions Made (03 to 04) | Rationale |
|---|---|---|
| Sections 1; 1.1; 2.2; 3; 8.1.1.1; 8.1.2; and 9.4.10 | Deleted requirement/option to collect platelets from entire protocol. Specimens for platelets shall no longer be collected. | Study team (chairs/PIs) determined this was not an essential part of the protocol. Requirement to collect platelets had potential to cause undue noncompliance issues related to scheduling problems, which were secondary to the limited stability of platelet samples. |

| Affected Section(s) | Summary of Revisions Made (03 to 04) | Rationale |
|---|---|---|
| Section 1 and 5.5 | Changed total randomized for entire study to 107 and total enrolled for entire study to 321. | Change to total enrollment because enrollment in part 1 of the study was increased. |
| Sections 1.1; 4.1; 4.4; 8.1.1.1; 8.1.2; 8.2; 9.4.7; | Noted that weeks 20, 24, and 28 are visits 7, 8, and 9, respectively. | Contingency response: clarified to match informed consent. |
| Sections 1.1; 6.4; 8.1.1; 8.1.1.1; | Changed weekly calls to "at least weekly." | This will allow sites to be able to have additional contact and send additional reminders to participants. |
| Sections 1.1; 8.1.1; 8.1.1.1; and 8.1.2 | Clarified what was optional with regard to venipuncture and collection of blood for inflammatory markers. Collection of blood for inflammatory markers is not optional if blood obtained by venipuncture. Method of blood collection (venipuncture vs fingerstick) is, however, optional for most visits. | Contingency response: clarification. |
| Section 4.1 | Changed number of participants to be enrolled in part I to 150. | This is to match the 3:1 ratio used to estimate enrollment:randomization for part II of the study |
| Section 8.1.1.1 | Added requirement for source documentation of participants' choice of venipuncture or fingerstick for each visit. | Ensure adequate documentation of study conduct. |
| Section 8.1.2. | Changed statement FROM "25(OH) vitamin D level by liquid chromatography/tandem mass spectrometry (specimen collection per laboratory manual) to be done at Baseline and study Visits 3, 4, 5, and 6 and optionally at Follow-up and between Week 1 and 3 if the participant volunteers for this.." TO 25(OH) vitamin D level by liquid chromatography/tandem mass spectrometry (specimen collection per laboratory manual) to be done at Baseline (visit 2) and study Visits 3, 4, 5, and 6 as well as Follow-up (visits 7, 8, and 9). There is an optional visit between Week 1 and 3, if the participant volunteers for this. | Corrected internal inconsistency. Vitamin D central laboratory assays are required for all visits 2 through 9, inclusive. |

## Summary of Key Changes from Version 04 to 05:

| Affected Section(s) | Summary of Key Revisions Made (04 to 05) | Rationale |
|---|---|---|
| Title Page, Header, protocol summary and section 10.2 | Changed Protocol Name to "Vitamin D Oral Replacement in Asthma" | Study team decided the name change would be useful in reducing the stigma associated with obesity language for potential participants |

| Affected Section(s) | Summary of Key Revisions Made (04 to 05) | Rationale |
|---|---|---|
| Sections 1.1; 6.4; 8.1.1; 8.1.1.1; | Changed weekly calls to “at least biweekly” (meaning every 2 weeks). | Reduce the required burden on coordinators and participants |
| Protocol Summary | Changed study duration to 34 months and Part 1 duration to 17 months | Correction |
| Protocol Summary and section 4.3 | Changed the names of Part 2 Cohorts 1 and 2 to Cohorts A and B, respectively | Changed to keep Part 1 and Part 2 Cohorts separate |
| Protocol Summary and section 4.3 | Changed “recommended dose based on Part 1 PK analysis” to Single 50,000 IU loading dose + 8,000 IU daily dose | Specified recommended dose for Part 2 |
| Section 4.1 | Added actual enrollment start date | Update |
| Section 4.3 | Added Justification for Part 2 dosing | New |
| Schedule of Assessments and section 8.1.1.1 | Increased the number of days between clinic visits from 28 ± 3 days to 28 ± 7 days | Extended visit window per study team recommendation |
| Schedule of Assessments | Updated the footnote concerning requirement of focused physical examination. | Updated per protocol revision |
| Schedule of Assessments | Instruction added to safety lab footnote:<br>“If urine Ca++/Cr sample is not obtained at the scheduled visit, the sample must be obtained within 2 business days since it is a safety assessment.” | Clarification |
| Section 5.2 | Added clarifying language for spot urine calcium creatinine ratio exclusion criteria | Clarification |
| Section 5.5 | Rewording in recruitment section | Clarification |
| Section 6.1.2 | Added the recommended dose for Part 2 | New |
| Section 6.2.3 | Added information from drug vendor concerning drug stability at certain temperatures | Clarification |
| Section 6.4 | Added a requirement for pharmacists/designees to perform a pill count prior to dispensing study drug to participants. | New |
| Section 6.4 | Added the instruction that study staff will be trained to review the diary cards with the participants | New |
| Section 6.5/6.5.1 | Added clarifying language concerning steroid use | Clarification |

| Affected Section(s) | Summary of Key Revisions Made (04 to 05) | Rationale |
|---|---|---|
| Section 7.1 | Added verbiage for an intended termination visit for unplanned study termination and outlined what the visit will include. | New |
| Section 7.1 | Clarified the "Rules for Halting of Study Drug" to indicate that serum calcium and 25(OH) D are collected locally to determine if a participant develops elevated serum calcium greater than or equal to 1.5×ULN or 25(OH)D level greater than 100 ng/ml [following elevated urine calcium/creatinine ratio > 0.37 on repeat test] | Clarification |
| Section 10.1.1 | Added that unemancipated minors who are 7 years old or older will sign the separate assent form | |

## Summary of Key Changes from Version 05 to 06:

| Affected Section(s) | Summary of Revisions Made (05 to 06) | Rationale |
|---|---|---|
| Section 1.1. Schedule of Activities | In footnote "b" changed "an unplanned study termination visit occurs" to "the participant is asked to stop taking vitamin D and terminates from the study." | Clarification. |
| Section 4.4 | Changed "day 196 ±3" to "day 196 ±7." | Simple correction for internal consistency. |
| Section 6.2.2. | Specified that the loading doses would not be sent home with participants; added that loading doses will be given to participants during visit 2. | Simple correction for internal consistency and clarification regarding dispensing of loading doses. |
| Section 6.2.3. | Deleted "According to the study drug vendor, vitamin D3 should be stable for months at 45 $^{o}$C." | Data not provided by company. |
| Section 6.2.4 | Added second sentence regarding counting of pills for confirmation of number prior to dispensing. | To be consistent with pharmacy manual. |
| Section 7.1 | Deleted reference to an unplanned study termination. Replaced with "If a participant who has been asked to stop taking study drug terminates (for any reason) from the study…" | Terminology (i.e., "unplanned study termination") was not defined. Clarification of when a focused physical exam would be done. |
| Section 8.1.1.1, Visit 3 and throughout | Corrected "weekly" to "biweekly" in section "weeks 5-7" of Study Visit 3 section and indicated that "biweekly" means every 2 weeks | Simply correction and clarification because biweekly has more than one definition. |
| Section 10.1.5 | Corrected address and phone number for Jessica Snowden, MD | Change of office location. |
| Section 10.1.7 | Added that monitoring visits can be conducted remotely as well as at the site | Personnel safety accommodation due to COVID-19 pandemic. |

**Summary of Key Changes from Version 06 to 07:**

| Affected Section(s) | Summary of Revisions Made (06 to 07) | Rationale |
|---|---|---|
| Protocol Summary Table (1);<br>4.1, Overall design;<br>4.3, Justification for Dose;<br>4.4 End of Study Definition;<br>9.2, Sample size determination | Randomization maximum changed:<br>FROM 57 TO 63. | DSMB-approved increase due to potential increase in dropout rate secondary to COVID-19 pandemic. |

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## STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, and 21 CFR Part 56).

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from current participants who have provided consent using a previously approved consent form.

## 1 PROTOCOL SUMMARY

| | |
|---|---|
| **Title:** | Vitamin D Oral Replacement in Asthma (VDORA1) |
| **Study Description:** | Multi-center, randomized, open-label, pharmacokinetic (PK) and safety study of oral vitamin D3 supplementation. This will be performed in two parts. The data from both parts will be combined for PK calculations. |
| **Objectives:** | *Study objective*: The overall objective of the study is to determine the pharmacokinetics of Vitamin D supplementation in children who have asthma and are overweight or obese. |
| | *Part 1 objective: Identify dosing to use in Part 2 using PK analysis on a subset of participants.*<br>*Part 2 objective*: Determine the effectiveness of a recommended 16-week dosing regimen to achieve a serum level of 25(OH)D greater than or equal to 40 ng/ml in vitamin D insufficient or deficient children who have asthma and are overweight or obese. |
| | *Safety objective*: Assess the safety and tolerability of oral vitamin D3 |

| | |
|---|---|
| | *Exploratory objectives:*<br>1. Characterize selected inflammatory markers in children receiving vitamin D3 supplementation.<br>2. Characterize asthma symptoms in children receiving vitamin D3 supplementation.<br>3. Compare the proportion of participants randomized to receive an optimal dose of vitamin D based on PK data versus those who are exposed to standard of care doses that achieve serum 25(OH)D greater than or equal to 40 ng/ml. |
| **Endpoints:** | *Study endpoint*: Pharmacokinetic analysis of vitamin D supplementation in children who have asthma and are overweight/obese. |
| | *Part 1 endpoint: Serum 25(OH)D will be evaluated at baseline and various times including post-dose elimination to determine preliminary PK analysis for the best dose for increasing vitamin D levels above 40 ng/ml in children with asthma who are overweight or obese.*<br>*Part 2 endpoint:* Did participants in the Part 2 active dosing cohort achieve a serum 25(OH)D of greater than or equal to 40 ng/ml at 16 weeks? |
| | Safety endpoints: Adverse events (AEs), serious adverse events (SAEs), adverse reactions (ARs), and serious adverse reactions (SARs) |
| | Exploratory endpoints:<br>1. Inflammatory biomarker panel to include serum leptin as well as tumor necrosis factor alpha (TNFα), interleukin-2 (IL-2), IL-6, IL-10 and IL-17 (cytokine panel)<br>2. Asthma exacerbations<br>3. Asthma Symptom Days (ASDs)<br>4. Asthma Control Test (ACT) (or Childhood Asthma Control Test [c-ACT] for participants less than12 years of age)<br>*5.* Differences in the proportion of participants that achieve serum 25(OH)D levels greater than or equal to 40 ng/ml |

| | |
|---|---|
| **Study Population:** | Approximately 107 participants randomized (321 enrolled), ages 6 to less than18 years of age at enrollment, body mass index (BMI) greater than or equal to 85% for age and sex, with physician-diagnosed asthma and baseline serum 25(OH)D level less than 30 ng/ml and greater than 10 ng/ml |
| **Phase:** | PK / dose finding study |
| **Description of Sites/Facilities Enrolling Participants:** | Open to sites of the IDeA States Pediatric Clinical Trials Network (ISPCTN) |
| **Description of Study Intervention:** | **Part 1: (Dose Finding)**<br>A total of 50 participants were randomized (1:1:1:1) into one of four 16-week vitamin D3 oral regimens:<br>Cohort 1: Single 50,000 IU loading dose + 6000 IU daily dose<br>Cohort 2: Single 50,000 IU loading dose + 10,000 IU daily dose<br>Cohort 3: 6000 IU daily dose<br>Cohort 4: 600 IU daily dose (standard of care comparator [SoC] group)<br>After a minimum of 16 participants (4 in each group) completed PK sampling at Week 20, we performed an interim analysis to determine the recommended dose for use in Cohort A for Part 2 of the study. |
| | **Part 2: (Dose Confirming)**<br>Up to 63 additional participants will be randomized into one of two 16-week vitamin D3 oral regimens in a 2:1 ratio:<br>Cohort A: Single 50,000 IU loading dose + 8,000 IU daily dose<br>Cohort B: 600 IU daily dose (SoC) |
| **Study Duration:** | 42 months<br>Part 1: 17 months (assumes 6 randomizations/month)<br>Interim Analysis period: 2 months<br>Part 2: 17 months (assumes 6 randomizations/month)<br>Final Analysis period: 6 months |

| | |
|---|---|
| **Participant Duration:** | 30 weeks (2-week screening/run-in, 16-week intervention, 12-week follow-up period) |

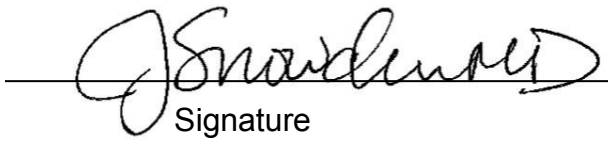
## SIGNATURE PAGE

The signature below documents my review and approval of this protocol, including all appendices and product information. I agree to personally oversee the conduct of this study as described herein and to follow this protocol version as approved by the Institutional Review Board. I agree that this clinical study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements and to the principles outlined in applicable US federal regulations and ICH guidelines.

**Operational Principal Investigator**

Jessica Snowden, MD

Printed name



Signature

12/14/2020

Date

## 1.1 SCHEDULE OF ACTIVITIES (SOA)

A summary of the study procedures is provided in Table 1. An illustration of the study design is provided in Figure 1.

**Table 1. Schedule of Assessments and Procedures**

| Schedule of Assessments and Procedures | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Activities** | **Screening** | **Dosing Phase** | | | | | | **Follow-Up** |
| | Screening (Visit 1) | Baseline (Visit 2) | Optional Blood Draw | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visits 7, 8, and 9 |
| | Day -14 [a] | Day 0 | Day 7- 21 | Day 28 [a] | Day 56 [a] | Day 84 [a] | Day 112 [a] | |
| | | Week 1 | Week 1 to Week 3 | Week 4 | Week 8 | Week 12 | Week 16 | Weeks 20, 24, and 28[a] |
| Informed consent/assent | X | | | | | | | |
| Demographics | X | | | | | | | |
| Inclusion/exclusion criteria | X | X | | | | | | |
| Medical history | X | | | | | | | |
| Focused physical examination[b] | | | | | | | | |
| Collection of height and weight | X | X | | X | X | X | X | |
| Urine pregnancy test[c] | X | | | | X | | X | |
| Begin run-in period[d] | X | | | | | | | |
| Dispense and explain/review diary cards | X | X | | X | X | X | X | X |
| Randomize to dose group | | X | | | | | | |
| Dispense study drug | | X | | X | X | X | | |
| Collect unused study drug | | | | X | X | X | X | |
| Assess adherence[e] | | X | | X | X | X | X | |
| Adherence communication[f] | | X | | X | X | X | X | X |
| Urine calcium/creatinine ratio[g] | X | | | X | X | X | X | |
| Blood inflammatory markers[h] | | X | | X[i] | X[i] | X[i] | X | X[i] |
| Serum 25(OH) vitamin D (local laboratory) | X | | | | | | | X[j] |

| Schedule of Assessments and Procedures | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Activities** | **Screening** | **Dosing Phase** | | | | | | **Follow-Up** |
| | Screening (Visit 1) | Baseline (Visit 2) | Optional Blood Draw | Visit 3 | Visit 4 | Visit 5 | Visit 6 | Visits 7, 8, and 9 |
| | Day -14 [a] | Day 0 | Day 7- 21 | Day 28 [a] | Day 56 [a] | Day 84 [a] | Day 112 [a] | |
| | | Week 1 | Week 1 to Week 3 | Week 4 | Week 8 | Week 12 | Week 16 | Weeks 20, 24, and 28[a] |
| Serum 25(OH) vitamin D (central laboratory)[k] | | X | X[l] | X | X | X | X | X |
| Asthma assessments (ACT, c-ACT) | X | X | | X | X | X | X | |
| Concomitant medications review | X | X | | X | X | X | X | X |
| Adverse event assessment | X | X | | X | X | X | X | X |

a Screening is 14 (±7) days PRIOR to baseline. Except for Optional Blood Draw and Baseline, days are ±7.

b a focused physical examination will only be done if the participant is asked to stop taking vitamin D and terminates from the study..

c for females of child-bearing potential only; a pregnancy test will also be done if a female participant does not remain in the study through Visit 6.

d start of daily diary cards

e weekly adherence will be assessed by diary cards and pill count at visits.

f periodic communication may be via phone, text, or email as participant prefers. Communication will occur at least every other week in weeks without a study visit between Visits 2 and 6, and then contact will be made, minimally, during the week prior to each Follow-up Visit (visits 7, 8 and 9)

g performed locally; if urine Ca++/Cr ratio is greater than 0.37 on repeat testing [after adequate hydration and within 2 business days of initial test], the patient will return for a local serum calcium and 25(OH)D. If urine Ca++/Cr sample is not obtained at the scheduled visit, the sample must be obtained within 2 business days. This is necessary because Ca++/Cr is a safety assessment.

h leptin plus TNFα, IL-2, IL-6, IL-10, and IL-17 (cytokine panel). These will be drawn by venipuncture at baseline and Visit 6. If blood for vitamin D level is drawn by venipuncture (vs fingerstick) at other times, sufficient blood will be collected for inflammatory marker determination.

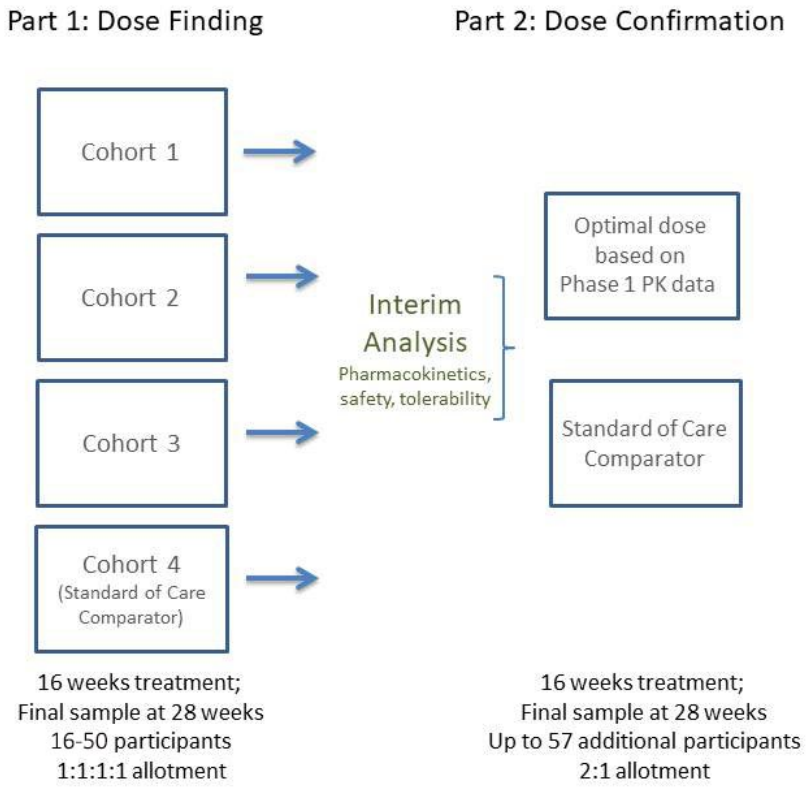
i for participants having venipuncture (vs fingerstick) blood draw

j local laboratory determination of serum 25(OH)D will be performed at week 28 (visit 9).

k blood for central laboratory vitamin D determination can be collected by venipuncture or by fingerstick using a microtainer collection tube

l an optional blood draw (fingerstick or venipuncture) can occur any time between week 1 and 3 (from day 7 to day 21, inclusive).

**Figure 1.Study Design**




# 2 INTRODUCTION

## 2.1 STUDY RATIONALE

Obesity and asthma are epidemic in the United States. Low vitamin D levels have been recognized as a common denominator in both inflammatory diseases, making vitamin D supplementation a potential low cost, readily available way to ameliorate the shared inflammatory aberration in these 2 conditions. A premise of the current study is that raising low 25(OH)D levels to greater than or equal to 40 ng/ml will achieve immunomodulatory effects beneficial to asthma control among children who are obese. Optimal dosing to achieve levels greater than or equal to 40 ng/ml is not known, and there is no recommended dose for vitamin D supplementation in this population that will reliably achieve a serum 25(OH)D level sufficient to affect inflammation.

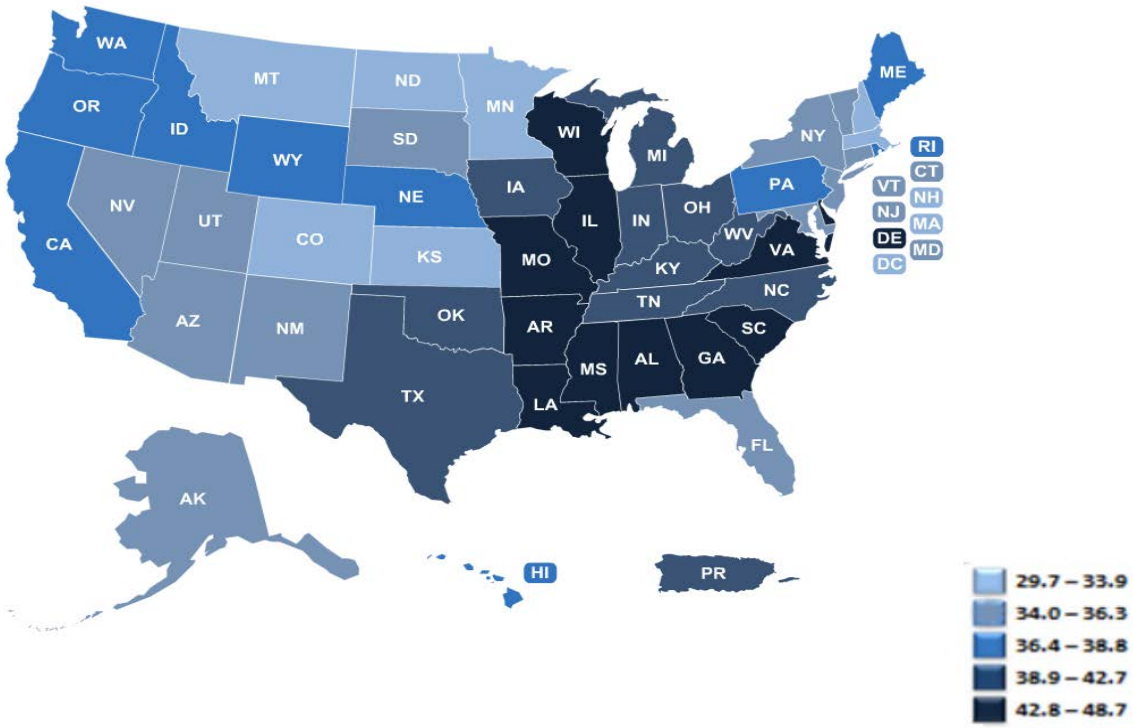
This PK study is being conducted in a population of children with obesity and asthma to obtain dosing and safety data to inform the design of a future interventional trial. Since the intervention trial will be performed in children with obesity-related asthma, it is

important that this PK study be performed in an identical population. The PK of vitamin D are considerably more complex than a standard pharmacological agent because of the required stepwise hydroxylation into the active form, $1,25(OH)_2$ D, and the presence of vitamin D and its metabolites in the circulation and tissues. Vitamin D's binding to the vitamin D-binding protein influences the properties of vitamin D metabolites.(1) In fact, data from large community-based health maintenance programs predict that supplemental vitamin D intake for individuals who are obese should be 2 to 3 times higher than for those of normal weight.(2) Therefore, it is imperative that the PK of vitamin D supplementation in the target population of children with asthma and obesity be well-studied prior to the design of any intervention study in this population.

There are confounding variables between asthma, obesity, and vitamin D deficiency. We believe studying a group with all 3 variables is important to achieve our goal of determining the most appropriate dose for a randomized, controlled intervention trial at a later date. Children who are overweight/obese represent an understudied population in asthma treatment and the data obtained through this trial will contribute to the network's future studies and to other pediatric studies involving lipophilic compounds.

The IDeA States Pediatric Clinical Trials Network (ISPCTN) is part of the Environmental Influences on Child Health Outcomes (ECHO) program with a focus on the ECHO priority areas: upper and lower airway disease; pediatric obesity; neurodevelopment; and pre-, peri-, and post-natal outcomes. Its purpose is to provide an opportunity for children in IDeA states to participate in clinical trials and to build clinical trial research capacity in the IDeA states. The ISPCTN is perfectly suited for this study given the prevalence of both asthma and obesity in rural areas (Figure 2) and the inclusion of both of these issues in the ECHO portfolio. ISPTCN sites have developed a close working relationship with each other and with the central coordinating center. This PK study is ideal as a "first in network" trial to build capacity, experience, and to help the coordinating center gain experience running a multi-center trial. It will inform us as to the ability of this network to recruit participants into future PK and intervention trials.

**Figure 2. Percent of Asthma Patients in Each State Who Are Also Obese**




Source: Centers for Disease Control and Prevention Asthma States, CDC.gov

## 2.2 BACKGROUND

Asthma is one of the most common chronic illnesses in childhood and a major cause of hospitalization. The incidence and prevalence of asthma have increased since 2000.(3) The disease burden of asthma in children includes missed school days, parental work absence, limitation of activities of daily living, Emergency Department (ED) visits, and hospitalizations.

Similarly, obesity is a burgeoning problem throughout the United States, particularly in rural areas.(4) There is a growing body of literature regarding the effect that obesity has on asthma control. The Centers for Disease Control and Prevention report 39% of adults with asthma in the United States are obese.(5) There are several aspects of obesity that contribute to more severe asthma symptoms including mass loading on the chest wall, airway stiffness, and inflammation. Childhood obesity-related asthma likely differs from adult-onset obesity-related asthma in that the pro-inflammatory nature of adipose tissue may be more critical for children.(6, 7, 8) Supporting this, a study in young children with asthma and obesity suggested that obese children are more responsive to inhaled corticosteroid therapy than their adult counterparts.(9)

Vitamin D plays a role in modulating inflammation, and deficiency of this pro-hormone may exacerbate poor asthma control. Vitamin D insufficiency/deficiency is common in children who are overweight or obese (10) and studies have suggested a relationship

between low serum 25(OH)D and asthma symptoms.(11, 12) It is possible that vitamin D supplementation could improve asthma control through its anti-inflammatory mechanisms. A Cochrane review published in 2016 supported this assertion, although noted that better studies, particularly in subpopulations, were needed.(13) A meta-analysis of studies in children showed a trend toward fewer asthma exacerbations in children given relatively small amounts of supplemental vitamin D.(14) Greater supplementation, which is considered to be very safe, may have even more salubrious effects.

The conversion of vitamin D3 to 25(OH)D has been well studied. (15) It is noted that the vitamin D-25-hydroxylase operates well below its $V_{max}$ in most individuals due to a lack of substrate. The $V_{max}$ of the conversion of D3 to 25(OH)D occurs when the 2 are in equimolar concentrations, which occurs with a 25(OH)D level of approximately 40 ng/ml. Unfortunately, as the authors state, "…it is very difficult and costly to measure circulating vitamin D3 and relate it to circulating 25(OH)D." As D3 will not be measured, the conversion of D3 to 25(OH)D will be considered with absorption of D3 and incorporated in the model as extravascular input for 25(OH)D. This approach has been used in a previously developed model for vitamin D by Foissac et al.(16)

The level of 25(OH)D needed to ameliorate asthma symptoms is not known, but is believed to be greater than that recommended for bone health, 30 ng/ml. It has been suggested that a level of approximately 40 ng/ml is necessary to achieve anti-infective and anti-inflammatory activity in cells critical to asthma control.(17, 18) It is unknown how much vitamin D (as vitamin D3) should be given to children who are overweight/obese to attain a level of greater than or equal to 40 ng/ml. It is likely a much larger dose is needed given the large volume of distribution that excess adipose tissue poses (a potential vitamin D sink). A study that combines PK modeling with exploratory asthma and inflammatory mediator endpoints is of particular interest given the uncertainty regarding an appropriate dose of vitamin D necessary to achieve adequate serum levels of 25(OH)D and the need to verify that reaching a level greater than or equal to 40 ng/ml will in fact lead to decreased inflammation. We propose this study to determine an appropriate regimen for vitamin D supplementation to achieve consistent serum 25(OH)D levels greater than or equal to 40 ng/ml, and we will explore its effect on markers of inflammation and asthma control.

**Obesity Worsens Asthma Severity in Children**

Uncontrolled asthma is a major public health problem. Each year asthma is a leading cause of ED visits, hospitalization, and missed school for children. Obesity in children increases the risk for the development of asthma,(19, 20) through unknown means. Although there are many mechanical reasons why obesity may cause a predisposition to asthma-like symptoms, it is probable that the pro-inflammatory nature of excess adipose tissue is a predisposing factor.(6, 21) A recent Expert Panel proposed that obesity-mediated metabolic and inflammatory dysregulation contributes to the pathogenesis of asthma.(7) Specifically, analysis of data from over 17,000 children demonstrated a link between asthma, body mass index, and dyslipidemia.(22) Obesity is associated with elevated levels of circulating adipokines and cytokines, including leptin, tumor necrosis factor alpha (TNF-α), and numerous interleukins (ILs), all of which

can contribute to airway inflammation and asthma.(21, 23) In one study, serum leptin levels were found to be elevated in asthmatic children who were obese and higher leptin levels were associated with worse airflow through peripheral airways.(24) Several studies have demonstrated that obesity in children is associated with worse asthma symptom control, reduced lung function, and greater health care utilization.(25, 26, 27). Thus, improved asthma control in this high-morbidity group is a leading public health need.

**Vitamin D Levels are Lower in Obese Children and Are Associated With Worse Asthma Symptoms**

Correcting low vitamin D levels may be critically important for improving asthma control in children who are obese. Low vitamin D levels are associated both with obesity in children and with difficult-to-treat asthma.(10,12) Turer, et al. observed that 79% of overweight children (BMI 85th%-95th%) and 86% of obese children (BMI greater than or equal to 95th percentile) met the diagnosis of vitamin D insufficiency (less than 30 ng/ml).(10) In addition, low 25(OH)D levels are associated with increased markers of oxidative stress and inflammation in children who are severely obese.(28) Vitamin D has been demonstrated to down-regulate production of pro-inflammatory cytokines such as TNFα and IL-6 and inhibit NFκB activation,(29) processes important to asthma control. Polymorphisms in the vitamin D receptor that decrease vitamin D action have been associated with the presence of asthma in children and adults.(30) Decreased platelet counts have been noted anecdotally in those increasing their vitamin D consumption (Scott Weiss, personal communication) and platelets play an important role in recruiting effector cells into the lungs of people with asthma .(31)

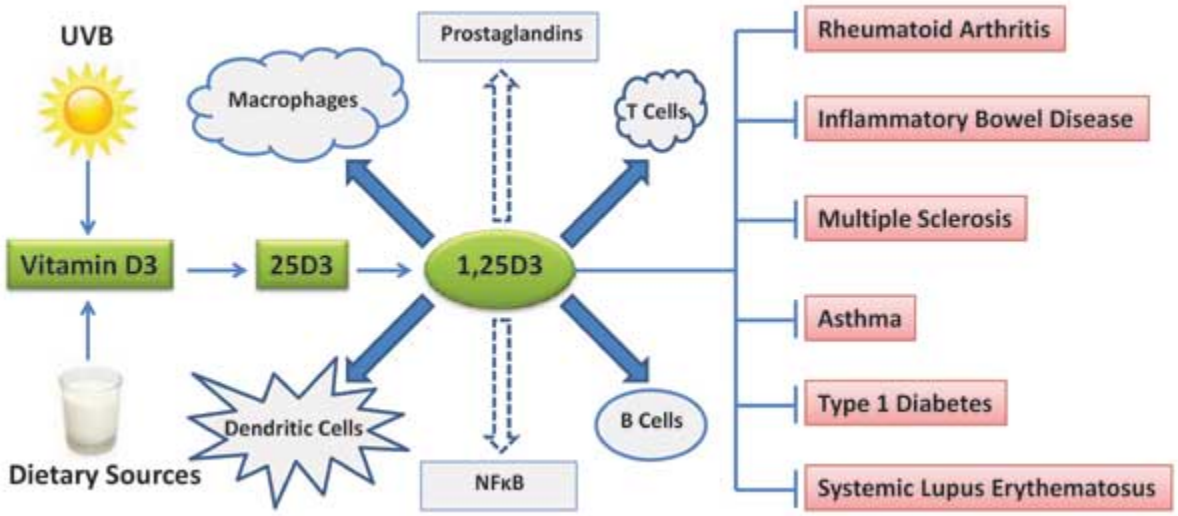
**Children With Asthma Have Lower Vitamin D Levels**

Low vitamin D levels are found in children with asthma irrespective of body weight. There is an association between low vitamin D levels and obesity in childhood. However, even children who are not obese but have asthma are at increased risk of having low serum 25(OH)D levels. In the Childhood Asthma Management Program (CAMP) vitamin D levels were obtained on 1,024 children aged 5 to 12 years who were known to have asthma. Thirty-five percent of these children were vitamin D insufficient (less than 30 ng/ml).(12) A nationwide study using National Health and Nutrition Examination Survey (NHANES) data saw a positive correlation between vitamin D insufficiency defined as serum 25(OH)D level less than 30 ng/ml and current asthma or current wheeze in children.(32) An examination of data from the Childhood Asthma Study from Australia found that 25(OH)D deficiency (less than 20 ng/ml) early in childhood is associated with an increased risk for persistent asthma.(33) These lower levels may be due to increase exposure to oral and inhaled corticosteroids used to treat asthma, decreased sun exposure due to exercise induced symptoms limiting outdoor activities, or a primary effect of asthma on vitamin D metabolism.(34) It will be important, therefore, to evaluate the relationship between asthma, obesity, and vitamin D pharmacokinetics as a whole since the target population for an intervention trial will be children who have both asthma and weight-related issues..

Low vitamin D levels put people at risk for a multitude of inflammatory disorders including asthma.(35) (Figure 3) Several high quality controlled trials have shown that low vitamin D levels place asthmatic children at high risk for severe exacerbations.(12, 36) Gupta and collaborators found lower vitamin D levels in children who had difficult-to-treat asthma or poor asthma control,(37) and vitamin D deficiency has been associated with lower pulmonary function in obese children.(38)

In a longitudinal study, Hollams and colleagues found that the number of times a child was found to be deficient in vitamin D (defined as a level of less than 20 ng/ml) was positively associated with the risk for asthma and wheezing at 10 years of age, suggesting that low vitamin D is instrumental in the inception of asthma.(33) In addition, several studies have concluded that higher 25(OH)D levels appear to protect against respiratory infections, a key trigger for asthma exacerbations.(39, 40)

**Figure 3. Role of Vitamin D in the Immune System**




Source: Lin Z, Li W. Vitamin D and Inflammatory Diseases. Current Top Med Chem. 2016; 16(11), 1242-61

**The Optimal 25(OH)D Level for Treating Asthma**

Obesity among adults with asthma is associated with more severe symptoms and a reduced response to conventional anti-inflammatory asthma treatments. The Endocrine Society states that vitamin D deficiency occurs at levels less than 20 ng/ml and vitamin D insufficiency is present when levels are 21 to 29 ng/ml. (59) Vitamin D insufficiency and deficiency are generally defined in terms of calcium metabolism and bone health. Non-calcemic effects of vitamin D are well known, but the Endocrine Society does not recommend specific levels to achieve effects outside of bone health and fall prevention. The precise level one needs to achieve to have an effect on infection and inflammation

is not known. However, several studies indicate that this is likely greater than or equal to 40 ng/ml.(33, 29)

The anti-infective and anti-inflammatory effects of vitamin D appear to occur when serum levels are high enough to achieve adequate cellular levels of 1,25 hydroxy-vitamin D3 (1,25D3). The expression of many genes in inflammatory cells including macrophages, dendritic cells, T helper cells, and B cells is regulated by 1,25D3 through the vitamin D receptor. Despite levels of 25(OH)D greater than 30 ng/ml being adequate for bone health, higher levels may be necessary to achieve maximal extra-orthopedic vitamin D effects. Hollis, et al. showed that optimal conversion of vitamin D3 to 25(OH)D occurs when serum levels of 25(OH)D are greater than or equal to 40 ng/ml.(15) Further, Ginde et al. reviewed NHANES 2003 to 2006 data and found that optimal vitamin D status, defined by estimated maximum parathyroid hormone (PTH) suppression, does not occur until 25(OH)D levels are greater than or equal to 40 ng/ml. (41) This study included nearly 6000 children between 6 and 19 years old. In a prospective study, Sabetta et al. (40) found that maintaining a serum 25(OH)D level above 38 ng/ml could reduce the risk of acute viral respiratory tract infections in adults. Another study found that pre-eclampsia did not occur in women who had 25(OH)D levels greater than 37 ng/ml early in pregnancy.(42) These studies imply that higher levels of 25(OH)D are necessary to achieve the desired effects beyond bone health.

Indeed, a small number of asthma studies support this target. In the Childhood Asthma Management Program (CAMP) study, the risk of exacerbations was inversely associated with 25(OH)D with the lowest risk seen with levels greater than or equal to 40 ng/ml.(12) Similarly, the large Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma (VIDA) study reported trends toward improved asthma control in a population of adults who achieved a mean 25(OH)D level of 41.9 ng/ml at 12 weeks of treatment.(43) The 95% confidence interval around this mean was quite small, implying that benefit begins to be seen at this level. Some studies of supplementation of vitamin D have not shown a positive effect on asthma control; however, serum 25(OH)D levels greater than or equal to 40 ng/ml were not achieved routinely. Due to these uncertain findings, a 2016 Cochrane review of vitamin D supplementation in asthma noted that the optimum circulating level is as yet undetermined.(13) They note that there is a need for primary trials in under-represented populations to determine this goal. We hope to fill this knowledge gap by aiming to achieve a level that will provide supporting evidence for anti-inflammatory benefits at an appropriate serum level.

**Vitamin D Trials in Children with Asthma Are Few but Encouraging**

Few controlled trials involving vitamin D supplementation have been conducted in children with asthma. Those that have been done have been small but encouraging. A recent meta-analysis of vitamin D supplementation trials in children found that supplementation is likely to reduce the risk of asthma exacerbations.(14) The analysis included 8 randomized trials of greater than 570 children treated for 1 to 12 months with a dose that ranged from 1000 IU per day to 60,000 IU per month. The authors concluded that children receiving vitamin D had reduced odds of exacerbation

compared to placebo treated children, although the evidence was weak. It should be noted that few children achieved a serum 25(OH)D level of greater than or equal to 40 ng/ml, and serum levels were not reported for most studies. None of the trials were designed to achieve a target 25(OH)D level greater than or equal to 40 mg/ml.

A similar meta-analysis was performed in adults taking vitamin D supplementation and showed a significant reduction in asthma attacks requiring ED visits in those supplemented.(44) Again, serum levels greater than or equal to 40 ng/ml were not necessarily reached. Kerley and colleagues evaluated 17 intervention trials of vitamin D supplementation involving 1578 asthmatic adults and children.(45) Study size, vitamin D dose, age of participants, and study lengths differed, making comparisons among these 17 trials difficult. Results were generally supportive of supplementation, including decreased exacerbations in newly diagnosed children (46) and decreased airway eosinophilia.(47) The Cochrane review of 7 trials involving 435 children and 2 trials of 658 adults concluded that improved vitamin D levels reduced the risk of asthma exacerbations; however, vitamin D supplementation had no effect on lung function as assessed by Forced Expiratory Volume in 1 Second.(13) Spirometry does not appear to be an important endpoint for a vitamin D-asthma study.

**Determining Optimal Supplemental Dose Needed to Achieve 25(OH)D Levels Above 40 ng/ml**

Scott Weiss and Augusto Litonjua have studied the asthma-vitamin D link extensively. As they state, “The biology of vitamin D is complex…we feel there is much more to uncover, such as the dose and timing of supplementation and the level that maximizes protection.”(18)

We have examined previous studies of vitamin D supplementation in adults and children in order to try to determine the best way to achieve adequate serum 25(OH)D levels in children who are overweight or obese. Two months of treatment with vitamin D at a dose of 5000 IU daily increased mean 25(OH)D levels from 15 to 40 ng/ml in a small study of children who were obese (n=14).(48)

A vitamin D3 supplementation trial of 4000 IU daily for 6 months was conducted in adolescents with diabetes and obesity.(49) After 3 months, levels increased from an average of 19 ng/ml to roughly 35 ng/ml (mean increase at 3 and 6 months was 15 ng/ml and 19.5 ng/ml, respectively). Treatment was well tolerated. Importantly, this study did not include a loading dose, yet yielded a substantial increase in 25(OH)D in just 3 months in adolescents who were obese. Maximum levels were not achieved until 6 months, consistent with the long half-life of vitamin D.

A loading dose is the amount of drug designed to fill the central volume of distribution for a drug to a concentration that matches the final plateau concentration achieved with the maintenance dose.(50) Loading doses are used in order to achieve this final plateau sooner than the four half-lives required if the drug is simply administered at the maintenance dose rate.(51) The functional half-life for vitamin D3 is extremely long and ranges from 2 to 3 months (52, 53) or longer (54), largely due to the lipophilic nature of the drug and its distribution into adipose tissue. The clinical effect of this long

elimination half-life and high volume of distribution is that dose adjustments require 4 to 5 half-lives to reach steady-state conditions. Thus, achieving target concentrations through a loading dose strategy should allow individuals to achieve target concentrations more quickly (51) and have a more rapid therapeutic effect.

Limited studies have examined the benefit of a loading dose of vitamin D on obtaining the desired 25(OH)D concentration.(50) Luger examined 2 dose regimens in adults with morbid obesity following gastric by-pass surgery; a loading dose (up to 300,000 IU over 4 weeks, divided into 3 doses) achieved the desired vitamin D concentration more rapidly than the standard regimen. Radhakishun examined weekly vitamin D treatment with 25,000 IU in 109 multi-ethnic obese children, 8 to 18 years of age, with vitamin D insufficiency/deficiency.(55) In 84.4% of the children, the vitamin D status improved from deficiency (less than 20 ng/ml) to insufficiency/sufficiency (greater than or equal to 20 ng/ml). No side effects were reported, and the highest level reached was far below the threshold for toxicity.(55) Van Groningen evaluated 3 loading dose strategies in 208 vitamin D deficient adults (ages 18-77 years). This study found that greater responses in vitamin D status were observed as a function of increasing loading dose.(56) A study by Alansari, et al. gave 6 to 14 year-olds 600,000 IU by intramuscular injection as a loading dose followed by just 400 IU daily.(57) Participants who received the loading dose demonstrated a 7 ng/ml greater increase in 25(OH)D than participants who did not receive the loading dose.

In the VIDA trial, a large-scale vitamin D supplementation trial in mainly overweight/obese adults with asthma and vitamin D deficiency (mean BMI = 30, mean 25(OH)D less than 20 ng/ml), participants received a single oral vitamin D3 loading dose of 100,000 IU and 4000 IU daily. This resulted in a mean 25(OH)D level of 41.9 ng/ml at 12 weeks (43), highlighting the benefit of a large loading dose in this population.

Of concern is that most of these studies did not achieve a level over the presumed threshold of 40 ng/ml needed to achieve an anti-inflammatory effect in a majority of their participants. Discussions with endocrinologists, pulmonologists caring for patients with cystic fibrosis, and colleagues in Lipid and Weight Management clinics have raised concerns about achieving our desired goal even with doses similar to those used above. It is likely that even higher doses will be needed to achieve a level greater than or equal to 40 ng/ml in a large proportion (75%) of participants who have large lipid stores. To obviate this concern, we will use a dose approximating that used by Benechia, et al (49) and a higher dose of 10,000 IU/day. The use of a loading dose in 2 groups and the lack of such a dose in another treatment group and the standard of care group will allow PK modeling to determine an optimal dose to get 25(OH)D levels greater than or equal to 40 ng/ml consistently.

### Inclusion of Children With Both Asthma and Obesity

It is the intention of this study to serve as a dose finding study for a large, randomized, controlled intervention trial in overweight/obese children with asthma in the future.

It is self-evident that to determine the best dose of vitamin D to use in a study of children who are obese and have asthma, we should study the PK of vitamin D in children who are obese and have asthma.

There are several reasons that children with both asthma and obesity should be included in this study:

1. Children with asthma and obesity may differ in vitamin D status and response to supplementation compared to children who are only obese.

2. Most children with persistent asthma symptoms are currently treated with inhaled corticosteroids. Both inhaled and oral corticosteroids can affect vitamin D levels.(34)

3. Previous studies of vitamin D levels in non-obese children with asthma have demonstrated the presence of low levels of 25(OH)D in many, which raises the possibility of direct asthma-vitamin D metabolism interactions.

4. Children with asthma may spend more time inside and have decreased exposure to sunlight due to exercise-induced asthma symptoms.

5. In contrast to children with asthma, where there is at least a theoretical benefit to high vitamin D levels, there is no evidence to support the need for 25(OH)D levels greater than equal to 40 ng/ml in children who are obese and do not have asthma. Exposing children with no potential for benefit to higher doses of vitamin D raises ethical concerns.

6. Including children with asthma allows for collection of preliminary data on the effect of vitamin D supplementation on inflammatory markers in a group of children suffering from an inflammatory disease.

7. Including children with asthma in the PK study is important to help develop research capacity in this nascent network. By asking sites to collect diary cards and administer the ACT and record results on case report forms (CRFs) we will build infrastructure and test feasibility for a more sophisticated and intricate asthma study at a later date.

8. Review of the literature reveals that there are few data available on the effects of vitamin D administration in specific patient groups, such as obese children with asthma.

We believe this PK pilot study will be most applicable to our final target population if it includes children with both asthma and obesity.

## Pharmacokinetic Considerations

Ekawaru evaluated 17,614 patients receiving vitamin D and characterized the resulting vitamin D concentrations.(2) The relationship between dose and 25(OH)D appeared to be a non-linear, exponential curve, whereby dose adjustments increased vitamin D concentrations to a point, at which levels began to plateau. BMI, rather than absolute body weight, was found to be the better determinant of the 25(OH)D level. Obese and overweight participants had serum 25(OH)D levels that were 8 ng/ml and 3.2 ng/ml lower, respectively ($p<0.001$), than normal weight participants. Due to the long half-life of 25(OH)D a minimum of 3 months of daily administration is required to attain steady-state concentrations of 25(OH)D.(58)

## Safety of Vitamin D Supplementation

Vitamin D supplementation is extremely safe.(2) Accepted normal serum values for 25(OH)D levels range widely. Some expert groups feel that values less than 30 ng/ml are abnormal, whereas others feel that 20 ng/ml is the lower limit of normal. The upper bound of normal serum 25(OH)D in most laboratories is 100 ng/ml, however toxicity is unlikely to occur at levels less than 150 ng/ml.(59) No published supplementation trials to our knowledge (including those with loading and daily doses far exceeding our proposed dosing) have experienced levels exceeding this safe level. Numerous published trials have demonstrated that the doses we will evaluate are well within a safe dosing range. Recent published dosing regimens including 4000 IU daily for 6 months (49) and repeated 600,000 IU intramuscular loading doses every 3 months over a 6 month period were well tolerated with no untoward effects.(57) Young adults with vitamin D deficiency have been treated with 50,000 IU weekly for 8 weeks and then continued on 50,000 IU every other week for up to 6 years without serious side effects or changes in serum calcium levels.(60) Doses as high as 55,000 IU daily have been administered to obese adults without causing hypercalcemia,(2) and loading doses of 200,000 IU administered to normal weight adults were not associated with hypercalcemia.(51) Celedon and colleagues are currently conducting an NIH-funded trial in 4 to 12-year-olds (61) giving 4000 IU daily for 48 weeks. From personal communication with the study team, we understand there have been no SAEs.

Some drugs interfere with vitamin D absorption or metabolism.(62) These include glucocorticoids, some anti-epileptics, orlistat, and cholestyramine, which have been shown to lower 25(OH)D levels. There is a theoretical concern for hypercalcemia when vitamin D is taken with thiazide diuretics and supplemental calcium. Only 3 such cases of hypercalcemia have been reported. These reports were from 2 studies, both involving elderly patients, and the cases of hypercalcemia were mild and reversible.(63)

## Evaluation of Inflammatory Mediators

Obesity is a pro-inflammatory state characterized by increased circulating levels of adipokines including leptin. It is postulated that the inflammation caused by increased leptin is a contributing factor to the asthma-obesity dyad in children. It will be useful to examine the anti-inflammatory effects of vitamin D on certain biomarkers. Leptin

promotes the release of TNF-α and IL-6 from endotoxin-treated macrophages and lymphocytes.(64) In a study of adults, TNF-α, IL-6, and leptin levels were higher in obese asthma patients compared to non-obese asthma patients.(65) IL-17 is another proinflammatory mediator that regulates tissue inflammation in asthma.(66) Its activity can be inhibited by vitamin D.(67)

Conversely, IL-10 is an anti-inflammatory cytokine. In a mouse model of allergic asthma, 1,25 vitamin D3 has been shown to induce IL-10 release and enhance the effect of immunotherapy.(68)

Examining the effect of vitamin D on leptin plus IL-2, IL-6, IL-10, IL-17, TNF-α (cytokine panel) will help assess its anti-inflammatory role in obesity-related asthma. This will be important to support the benefit of achieving a high level of vitamin D (greater than or equal to 40 ng/ml) in an intervention trial.

## 2.3 RISK/BENEFIT ASSESSMENT

### 2.3.1 KNOWN POTENTIAL RISKS

Compared to most medications, there are few risks to vitamin D supplements. No adverse reactions are listed in the manufacturer's labeling (69). Levels greater than 100 ng/ml have been well tolerated without significant side effects.(70) The Endocrine Society's practice guidelines on vitamin D state that vitamin D intoxication is usually not observed until serum 25(OH)D levels are greater than 150 ng/ml.(59) Vitamin D intoxication associated with hypercalcemia, hyperphosphatemia, and suppressed parathyroid hormone level may occur, but is typically only seen in patients who are receiving massive doses of vitamin D (50,000 to 1 million IU per day for several months to years).(70) Symptoms of vitamin D intoxication may include nausea, vomiting, loss of appetite, constipation, dehydration, fatigue, irritability, confusion, weakness, and/or weight loss(69). Hypersensitivity reactions to vitamin D supplementation are rare but possible. Vitamin D3 is a weak inhibitor of CYP2C9 and may cause drug interactions, although this is considered very rare.

Multiple studies have demonstrated the safety of vitamin D supplementation in pregnancy. Hollis et al. showed that giving pregnant women 4,000 IU/day was safe (71), and another study demonstrated the safety of a single dose of 70,000 IU in pregnant women.(72) In fact, several studies have explicitly looked for the potential benefit of vitamin D supplementation to the mother and fetus and found no harms.(73, 74, 75) However, we will not enroll pregnant women in this study and will ask all females of child-bearing potential to utilize some form of birth control (abstinence qualifies).

Risks of blood draws are minimal and include fainting, bruising, and mild pain. There are small risks to blood sampling, such as pain/discomfort due to the collection process or blood loss. Local pressure will be applied at blood drawing sites to minimize the risk of bruising, and participants who become light-headed will be placed in a supine position. The maximum amount of blood that could be collected per participant over the course of the study is approximately 76.4 ml, and the largest amount of blood drawn in

a single day is 10.6 ml. Both amounts are well below the daily and sustained limits for blood collection in pediatric participants. These amounts will be even lower if microtainer collection of fingerstick samples is used and participants opt out of optional measures – the minimum required amount of blood drawn per participant over the complete course of the study is approximately 23.6 ml.

Protocol elements to mitigate risk include the exclusion of participants with known hypercalcemia, kidney stones, significant renal or parathyroid disease, and/or known diseases complicated by elevated 25(OH)D levels, including sarcoidosis, William Syndrome, and granulomatous diseases. Kidney stones are a known complication of hypercalcemia and hypercalcuria. Spot urine calcium/creatinine ratios will be monitored, and serum calcium levels will be obtained in any participant with elevated urine calcium/creatinine ratios.

### 2.3.2 KNOWN POTENTIAL BENEFITS

**Participant Impact**

Because inclusion criteria include a serum 25(OH)D less than 30 ng/ml —a level considered to be vitamin D insufficient (59) — participating in this study may increase a participant's serum 25(OH)D into the normal range, which potentially could improve bone health. The comparator group will be provided the Institute of Medicine's recommended daily allowance of vitamin D – 600 IU/day.(76) Prior research has shown a trend toward improved asthma control or fewer symptoms in children who have higher levels of vitamin D. It is possible that children in this study may experience fewer and less severe asthma exacerbations with increased serum 25(OH)D levels; however, this cannot be guaranteed. The participants may learn basic information about nutrition and asthma by taking part in this study, potentially resulting in additional health benefits.

**Public Health Impact**

Both asthma and obesity are major public health problems, each contributing significantly to the burden on healthcare systems, patients, and families. Asthma and obesity are particularly prevalent in lower socioeconomic, underserved populations. The Centers for Disease Control and Prevention reports that up to 39% of individuals with asthma are obese, giving a comorbid condition of obesity-related asthma. Notably, obesity is more common in rural areas than in urban areas according to the US Department of Health and Human Services.(77) Vitamin D is an inexpensive nutritional supplement. A low-cost, readily available intervention that could decrease the burden of asthma in the obese asthma pediatric population could have major public health implications. Although speculative at this time, the proposed intervention has the capacity to improve public health in an underserved population with little expense if the correct dose can be determined and shown to be beneficial.

### 2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

All participants randomized will have vitamin D insufficiency and participation in this study will lead to appropriate supplementation. Benefits may be related to improved 25(OH)D level effects on bone health with a potential for a positive effect on asthma control. Risks of blood draws are minimal and not outside those encountered in daily living. We will provide the option for fingerstick collection of small volumes of blood for vitamin D level determination in order to lessen the anxiety of venipuncture in children. Risks will be mitigated by implementation of inclusion and exclusion criteria that decrease the chance of any hypercalcemia-associated problems and by monitoring urine calcium/creatinine ratios.

## 3 OBJECTIVES AND ENDPOINTS

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| **Study** | | |
| The overall objective of the study is to determine the pharmacokinetics of vitamin D supplementation in children who have asthma and are overweight or obese. | Pharmacokinetic analysis of vitamin D supplementation in children who have asthma and are overweight or obese | PK characteristics of 25(OH)D following vitamin D3 supplementation are not known in this population. |
| **Part 1** | | |
| Identify dosing to use in Part 2 using PK analysis on a subset of participants | Serum 25(OH)D will be evaluated at Baseline and various times including post-dose elimination to determine preliminary PK analysis for the best dose for increasing vitamin D levels above 40 ng/ml in children with asthma who are overweight or obese | PK characteristics of 25(OH)D following vitamin D3 supplementation are not known in this population. |
| **Part 2** | | |
| Determine the effectiveness of a recommended 16-week dosing regimen to achieve a serum level of 25(OH)D greater than or equal to 40 ng/ml in vitamin D insufficient or deficient children who have asthma and are overweight or obese. | Did participants in the Part 2 active dosing cohort achieve a serum 25(OH)D of greater than or equal to 40 ng/ml at 16 weeks | Serum 25(OH)D level greater than or equal to 40 ng/ml has been implicated to be anti-inflammatory. |

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| **Safety** | | |
| Assess the safety and tolerability of oral vitamin D3 | Adverse events (AEs), serious adverse events (SAEs), adverse reactions (ARs), and serious adverse reactions (SARs) | Assessing safety is important to determining tolerability of vitamin D in this study population. |
| **Exploratory** | | |
| 1. Characterize selected inflammatory markers in children receiving vitamin D3 supplementation.<br>2. Characterize asthma symptoms in children receiving vitamin D3 supplementation.<br>3. Compare the proportion of participants randomized to receive an optimal dose of vitamin D based on PK data versus those who are exposed to standard of care doses that achieve serum 25(OH)D greater than or equal to 40 ng/ml. | 1. Inflammatory biomarker panel including serum leptin plus TNFα, IL-2, IL-6, IL-10 and IL-17 (cytokine panel)<br>2. Asthma exacerbations<br>3. Asthma symptom days (ASDs)<br>4. Asthma control test (ACT) (or childhood asthma control test [c-ACT] for participants less than 12 years of age)<br>5. Differences in the proportion of participants that achieve serum 25(OH)D levels greater than or equal to 40 ng/ml | Vitamin D levels are negatively associated with asthma symptoms, and supplementation has improved asthma outcomes in children. The anti-inflammatory effect of vitamin D may underlie its utility in asthma. Assure that any increases in vitamin D levels in the experimental group are not solely the impact of unmeasured factors. |

# 4 STUDY DESIGN

## 4.1 OVERALL DESIGN

The trial will comprise a Dose Finding part (Part 1) and a Dose Confirming part (Part 2). A total of 50 children age 6 years to less than 18 years were randomized to 4 cohorts (1:1:1:1) (Table 2) during Part 1. An interim PK analysis was performed when at least 4 participants in each cohort had completed PK sampling at Week 20 (visit 7; days 137-143). Interim PK analysis yielded PK parameters, and a recommended dosing regimen to achieve 25(OH)D greater than or equal to 40 ng/ml in greater than or equal to 75% of participants was determined. In Part 2, up to 63 children will be randomized to

the recommended dose (Single 50,000 IU loading dose + 8,000 IU daily dose) or a standard of care dose of 600 IU/day (2:1).

The age range of 6 to less than 18 years encompasses the major population of children who have both clinical asthma and obesity.

**Table 2. Dosing Schemes for Vitamin D3 in Part 1 (Dose Finding)**

| Cohort | N | Loading Dose (IU) | Maintenance Dose (IU/day) |
|---|---|---|---|
| 1 | 4-8 | 50,000 | 6,000 |
| 2 | 4-8 | 50,000 | 10,000 |
| 3 | 4-8 | 0 | 6,000 |
| 4 | 4-8 | 0 | 600 (SoC) |

### Participant Enrollment and Randomization

There will be enrollment at ISPCTN sites with the goal of enrolling up to 150 participants and randomizing up to 50 for Part 1 and enrolling up to 171 participants and randomizing up to 63 participants for Part 2. During the interim analysis, enrollment continued, which explains the 50 participants randomized in Part 1.

### Study Timeline

We anticipated initiating enrollment in the fourth quarter of 2018. Actual enrollment started in January 2019.

## 4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Low serum 25(OH)D levels are associated with both asthma and obesity in children. Obesity is associated with greater asthma severity. A premise of the current study is that raising low 25(OH)D levels to greater than or equal to 40 ng/ml will achieve immunomodulatory effects beneficial to asthma control among obese children. The optimal dosing strategy of vitamin D for obese and overweight adolescents with asthma and vitamin D insufficiency is unknown. Identifying the appropriate dosing regimen for this population lays the foundation for future efficacy trials, which may be of great public health benefit.

The nonlinear PK of vitamin D3 has been reported in adults, but it is not well studied in children. The multi-level dosing strategy in this study will result in sufficient PK data to better characterize the dose-response relationship of 25(OH)D after vitamin D3 administration. The PK data will be used to model vitamin D dosing regimens that would, based upon the disposition characteristics of the compound, produce a degree of systemic exposure sufficient to produce plasma 25(OH)D concentrations greater than or equal to 40 ng/ml (the putative therapeutic target concentration).

## 4.3 JUSTIFICATION FOR DOSE

Based on published data involving children with obesity, the optimal dosing regimen to achieve serum levels greater than or equal to 40 ng/ml will likely be within the range of doses of vitamin D3 evaluated in Part 1. Based on PK models of vitamin D in adults, the increase in 25(OH)D after vitamin D3 dosing is not linear, but is influenced by the baseline value. A study in obese adults with vitamin D deficiency reported that mean (SD) measured increments in 25(OH)D at Week 21 were 12.4 (9.7) ng/ml, 27.8 (10.2) ng/ml, and 48.1 (19.6) ng/ml following vitamin D3 treatment at 1000 IU/day, 5000 IU/day, and 10,000 IU/day, respectively. Assuming a median baseline of ~20 ng/ml, 5000 IU/day would lead to greater than 50% of participants with 25(OH)D greater than or equal to 40 ng/ml and 10,000 IU/day would lead to greater than 75% participants with 25(OH)D greater than or equal to 40 ng/ml after 21 weeks. To ensure the appropriate exposure of vitamin D in obese children with asthma and in anticipation of a future intervention study, the present study will examine the effect of a loading dose regimen, followed by daily dosing.

Belenchia et al. found that giving obese adolescents with diabetes 4000 IU/day vitamin D3 led to a mean increase in serum 25(OH)D of 19.5 ng/ml at 6 months.(49) That study did not use a loading dose. Assuming our cohort has a similar mean baseline 25(OH)D concentration, we anticipate that we will need more than 4000 IU/day to get 75% of participants above the desired goal of 25(OH)D level of greater than or equal to 40 ng/ml. This is also supported by a study in obese children with vitamin D deficiency, which reported a mean serum 25(OH)D increasing from 15 to 40 ng/ml after 5000 IU/day orally for just 2 months (48). Our group of children, which will include some participants greater than 99th% BMI for age and sex, may require even greater amounts of vitamin D3.

**Vitamin D dosing arms for a PK study**

We explored 3 dosing arms and a SoC arm in Part 1 of this PK trial. Nutrient studies cannot have a true placebo arm since all participants are exposed to some level of vitamin D. An SoC arm has been included to allow for evaluation of changes in 25(OH)D over 16 weeks in a population of participants who are not exposed to high levels of vitamin D. A placebo arm is not appropriate because it is unethical to identify a treatable metabolic deficiency and not intervene.(78) Although the Endocrine Society recommends higher doses for replenishment, the Institute of Medicine recommends 600 IU/day for vitamin D supplementation as SoC.(76) We used this for the comparator group. Given that it is a log-order less than the lowest dose in our treatment arm, it should still act as a reasonable control group. This low dose supplementation also enriched the PK modeling.

This is a randomized study with Part 1 randomization being 1:1:1:1.

Part 1:

- Cohort 1: Single 50,000 IU loading dose + 6,000 IU daily dose
- Cohort 2: Single 50,000 IU loading dose + 10,000 IU daily dose
- Cohort 3: no loading dose + 6,000 IU daily dose
- Cohort 4: no loading dose + 600 IU daily dose (SoC)

We performed interim analysis when a minimum of 4 participants in each group had completed 16 weeks of therapy and had a 20-week serum level drawn. Participants continued to be randomized to one of these 4 groups while the analysis took place. Their data will be collected and utilized in the end-of-study PK modeling.

Following the interim analysis (see statistical methods below), we determined the dose most likely to achieve the Part 2 endpoint of greater than 75% of participants achieving a serum 25(OH)D level greater than or equal to 40 ng/ml. The selection was based on the interim PK analysis as well as safety and tolerability data. This dose is not any of the pre-determined arms used in Part 1.

The DCOC submitted data from Part 1 to the pharmacokinetic analysis team at Duke in November 2019. Based on the PK data, the VDORA1 study team proposed that the dosing regimen that consisted of a single 50,000 IU loading dose + 8,000 IU daily dose be used for part 2. The ECHO ISPCTN Data Safety and Monitoring Board (DSMB) met on February 13, 2020, to review the recommended dose along with a data report prepared by the DCOC. The DSMB approved of the recommended dose.

We will continue to recruit into the SoC arm. These SoC participants will serve as a control/comparator group to help us understand the effect of latitude, season, ethnicity, diet, and skin tone on vitamin D metabolism. This group will also be useful when assessing our ability to achieve serum level greater than or equal to 40 ng/ml in the Part 2 intervention group.

Part 2 will randomize up to 63 additional participants into one of two 16-week regimens in a 2:1 ratio:

Part 2:

- Cohort A: Single 50,000 IU loading dose + 8,000 IU daily dose
- Cohort B: 600 IU daily dose (SoC)

## 4.4 END OF STUDY DEFINITION

The end of the study will be at the completion of the study activities of the 63 randomized participants in Part 2 of the study. For individual participants, the end of the study will be following the Week 28 (visit 9; day 196 ±7) follow-up visit, following participant withdrawal of consent or when the participant has met the protocol-defined criteria for study termination.

# 5 STUDY POPULATION

## 5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following inclusion criteria:

- Greater than or equal to 6 and less than 18 years of age at screening (Visit 1)
- BMI greater than or equal to 85% for age and sex
- Physician-diagnosed asthma
- Ongoing relationship with asthma provider responsible for asthma care (can be specialist or primary care provider)
- Serum 25(OH)D level greater than or equal to 10 ng/ml but less than 30 ng/ml at screening (Visit 1) based on local laboratory test
- Ability to swallow pills similar in size to the vitamin D preparation to be used (Vitamin D supplements will be provided in the form of a size 2 capsule. See Section 6.2.2 for additional information.)
- Signed consent from his or her parent, legal guardian, or caregiver and signed assent from participant (as appropriate)
- Females of child-bearing potential must not be pregnant, must not be lactating, and must agree to practice adequate birth control method (abstinence, single barrier methods, combination barrier and spermicide, or hormonal) for the duration of the study
- Child and parent, legal guardian, or caregiver must speak English or Spanish

## 5.2 EXCLUSION CRITERIA

An individual who meets any of the following exclusion criteria will be excluded from participation in the study:

- Known diseases of calcium metabolism or the parathyroid
- History of renal insufficiency or kidney stones
- Known liver failure or history of abnormal liver function which is deemed by the site investigator to be clinically significant
- History of Williams syndrome, sarcoidosis, or granulomatous disease
- Active tuberculosis

- Spot urine calcium/creatinine ratio greater than 0.37 on 2 consecutive visits. If the ratio is greater than 0.37 on the first visit, this test must be repeated once - following adequate hydration, within 2 business days of the study team being notified of the initial lab results.
- Clinical evidence of rickets
- Taking supplemental vitamin D greater than 1000 IU per day
- Administration of an excluded medication (systemic glucocorticoids, drugs affecting calcium metabolism, drug affecting fat absorption) within 30 days prior to study enrollment – at screening (Visit 1) or at any time during the run-in period.
- Other medical issues that in the opinion of the investigator put the participant at risk or affect results of the study (e.g., fat malabsorption, cystic fibrosis, chronic lung disease of prematurity, congenital heart disease). Type 2 diabetes is not an exclusion criterion.
- Plans to move out of the study area in the next 9 months
- There is another child in the household enrolled in the study
- Plans for significant lifestyle changes in the next 9 months (significant dietary changes while enrolled in the study)
- Participants who are eligible for the run-in period will be ineligible for randomization if they do not demonstrate adherence to run-in procedures (return diary cards with a completion rate greater than or equal to 75%)

## 5.3 LIFESTYLE CONSIDERATIONS

Participants will be advised to not use a tanning bed and to not make significant lifestyle changes (significant dietary changes or changes in their use of sunscreen) while enrolled in the study. Study participants will receive basic health information about the risks of obesity.

## 5.4 SCREEN FAILURES

A screen failure will be defined as a participant who consents to participate in the clinical trial but does not enter the treatment phase of the study. A minimal set of information (demography, informed consent process, reason for screen failure, eligibility criteria, and any AEs) is required for documentation of screen failures to ensure transparent reporting to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Individuals who do not initially meet eligibility criteria may be rescreened 1 time at least 4 weeks later. Participants who were terminated due to a screen fail must sign new consent/assent documents prior to rescreening. Rescreened participants should be assigned the same participant number as for the initial screening.

## 5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Though there are many children with both asthma and obesity, especially in the rural IDeA states, recruitment and retention of eligible participants will be critical to study success. We anticipate that we will need a large number of sites involved in this trial in order to recruit the necessary participants. This study will recruit (enroll) up to 321 participants for a study lasting over 6 months. Although vitamin D supplementation is safe and easy to administer, there are several scheduled blood draws and the time commitment and child concern about blood tests may make it difficult for a small number of sites to recruit enough participants. It is very feasible that a large number of sites can recruit a small number of participants at each site leading to success of this study and success of the Network as a unit working cooperatively.

Within a multi-center network, optimal recruitment approaches may vary from site to site, and network success may require sharing of best-practices between clinical sites. Though each clinical site is responsible for recruiting, enrolling, and retaining study participants, the Data Coordinating and Operations Center (DCOC) will assist each site in creating a site-specific recruitment and retention plan. The DCOC has a Recruitment and Retention Plan summarizing the most common best practices for recruitment and retention of pediatric and adolescent participants and will work with each site to establish their own customized recruitment and retention plan. Site initiation will include review of the individual site's recruitment and retention plans. The trial is registered on *www.ClinicalTrials.gov* (NCT03686150).

**Recruitment**

FDA considers direct advertising (i.e., advertising intended to be seen or heard by prospective participants) to be part of the informed consent process as well as the participant-selection process. Therefore, IRB review and approval is required to ensure that the information is not misleading and that the ad is not coercive. Paper advertising products (flyers, small posters, handbills) and phone recruiting scripts will be created as needed by the DCOC as templates for sites to use. This approach is commonly used with success by pharmaceutical companies and large clinical research organizations.

Following approval by the central (University of Arkansas for Medical Sciences [UAMS]) IRB, the material will be made available in template form to all sites for customization. Sites will be required to use the central (UAMS) IRB for this study. A site may customize recruitment templates and phone scripts – or create its own material - to fit its own community. All advertising and scripts must, however, have written cIRB approval prior to use. A general description of the study, including frequently asked questions and consent and assent forms will be provided to potential participants prior to their initial screening visit. If the potential participants are interested in attending a screening visit, they will meet with the study coordinator and the local physician co-investigators to review the study and answer questions.

Another option for recruitment that local sites may use is a search of medical records for potential participants who may met the inclusion criteria. This will be done in

accordance with their institutional policies and procedures. The cIRB serves as the privacy board as well as the IRB for this study and can grant partial HIPAA waivers to allow sites to use medical records.

If a potential participant appears to meet eligibility requirements, i.e., based on pre-screening assessment, but does not want to be in the study, the potential participant and/or parents/guardians will be asked why they do not want to participate. These data will be recorded in a screening log without any identifiers that could link answers back to an individual or family. The individual/family will be told they do not have to answer any of the questions they do not want to answer.

**Retention**

Similar to recruitment, retention tactics should be tailored to the individual resources and challenges of each site These include the study being too long or burdensome, the participant feeling they are not benefitting from participation, unengaged or unenthusiastic study staff, and lack of physician involvement and attention. The study staff must recognize that the study participants (and caregivers) are providing a service to the study while undergoing risk and participants must feel appreciated.

The target for completion is 80% of randomized participants.

More details about recruitment and retention processes can be found in the Recruitment and Retention Plan in the Manual Operating Procedures (MOP), including detailed descriptions of best practices, strategies to be implemented, and metrics to be monitored.

## 5.6 CLINICAL HELP LINE

Sites can call the VDORA1 Clinical Help Line for specific protocol questions or to speak with one of the protocol PIs. The phone number can be found in the MOP.

# 6 STUDY INTERVENTION

## 6.1 STUDY INTERVENTION(S) ADMINISTRATION

### 6.1.1 STUDY INTERVENTION DESCRIPTION

Participants in VDORA1 will receive oral vitamin D3 (cholecalciferol) at a dose determined by the randomized assignment to 1 of 4 dosing regimens in Part 1 or 1 of 2 dosing regimens in Part 2. This product will be packaged and distributed to study sites for dispensing to the participant's caregiver (parent or guardian).

### 6.1.2 DOSING AND ADMINISTRATION

Once the assigned dose of vitamin D3 is determined, it will be dispensed by the site to the participant's caregiver at Visit 2 (baseline). The caregiver will be instructed to give the participant a loading dose of one 50,000 IU capsule orally on the day of the baseline

visit if the child is randomized to a loading dose cohort. Thereafter, participants will take their assigned vitamin D3 dose (600, 6000, or 10,000 IU (part 1) or 600 or 8,000 IU (part 2)) orally each day, starting on the day after the baseline visit and continuing to Visit 6. Participants with no loading dose will start on their daily dose on the day of the baseline visit. A member of the study team will witness and document the initial dose for each participant during the baseline visit. Participants will be given a dosing diary for recording all doses of vitamin D3 taken through the 16-week dosing phase. Missed doses will also be noted in the daily diary cards for dosing visits.

Following the interim analysis, an optimal dosing regimen was recommended for Part 2.

## 6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

### 6.2.1 ACQUISITION AND ACCOUNTABILITY

We will use a vitamin D3 supplement in this study because vitamin D2 is only approximately 30% as effective as vitamin D3 in maintaining serum 25(OH)D levels.(79) Bio-Tech Pharmacal, Inc. will package and supply all vitamin D3 products for use in this study. The products are licensed, approved drugs. Specific details of this product will be provided to study sites in the MOP. Capsule ingredients include vitamin D3 power (containing cholecalciferol 0.25% and polyethylene glycol 99.5%), microcrystalline cellulose, and gelatin capsule. Ingredients are free from microbiological content and meet USP specification.

### 6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

All vitamin D3 doses will be packed in blister packs or individually labeled bottles. The loading doses will be packaged separately from the daily doses; loading doses will be given directly to participants to take during visit 2. Participants will take home the labeled bottles/packs for their daily (i.e., all but visit 2) doses. Vitamin D supplements will be provided in the form of a size 2 capsule, either gelatin or hypromellose (vegetable). The capsules used for all doses in Part 1 were gelatin. The D3 (4000 IU) capsules for Part 2 are vegetable capsules. Size 2 capsules are approximately 18 mm in length (0.71 inches) with an outside diameter of 6.3 mm (0.24 inches).

### 6.2.3 PRODUCT STORAGE AND STABILITY

This product is to be stored at room temperature approximately 20ºC), away from moisture and light. All temperature excursions at sites must be appropriately recorded and reported to the study sponsor for further evaluation and follow-up.

### 6.2.4 PREPARATION

The product will be kept ready for administration in a sealed bottle or blister packs. No additional preparation is required. However, either the pharmacist or designee will do a pill count prior to giving the bottle to the participant. For details on the pill count, refer to the pharmacy manual.

## 6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

**Randomization**

Randomization of participants will be done online using the randomization module of the DCOC electronic data capture system. For the first part of the study, participants were randomized using a 1:1:1:1 ratio to receive 1 of 4 dose regimens after providing consent and confirmation of eligibility on the study inclusion and exclusion criteria. Upon completion of the interim PK analysis, participants in the second part will be randomized using a 2:1 ratio to receive the treatment regimen or the SoC comparator. The randomization will use a stratified block scheme. Stratification will be by age (6-11 vs 12- less than18 years of age) and BMI age and sex percentile at entrance (overweight = greater than or equal to 85th% to less than 95th% BMI, obese = greater than or equal to 95th% BMI for age and sex, and obese = greater than or equal to 99th% BMI for age and sex).

**Masking**

Masking is not planned for this study.

## 6.4 STUDY INTERVENTION COMPLIANCE

Research assistants will call, email, or text families at least biweekly (every 2 weeks) to provide encouragement regarding adherence to taking the study supplement. The first dose will be witnessed by study staff at Visit 2. Several methods will be employed to optimize intervention compliance and document compliance. Participants will not initiate treatment unless they demonstrate daily diary card adherence during the run-in. This will minimize dropouts due to non-adherence. At Visit 1, participants will be given diary cards (without a line to record Vitamin D dosing) and taught to use them to document asthma medication use and asthma symptoms. At visits 2 -5, diary cards for dosing visits will be given to the participant to use to document adherence to daily Vitamin D dosing as well as to document asthma medication use and asthma symptoms. Pharmacists/designees will perform pill count from sealed bottles prior to dispensing to subjects as outlined in the Drug Accountability memo (March 4, 2020) to pharmacists from the ISPCTN DCOC.

At visits 6-8, participants will again be given daily dairy cards (without the line to record Vitamin D dosing) to document asthma medication use and asthma symptoms. Similar diary cards have been used successfully in numerous large asthma studies to bolster adherence to study drug.(80, 81)

During monthly clinic visits for which Vitamin D was given (at the previous visit), sites are to measure participant dosing compliance by pill counts and by participant diary review, identifying non-compliance (taking less than 75% of vitamin D3). Study staff will be trained to review the diary cards at the visit with the participant to clarify any issues or questions, and to provide positive feedback to participants who demonstrate good adherence and ongoing encouragement when warranted at each visit. Sites are to make every effort to schedule participant clinic visits ±7 days of the scheduled visit, per the schedule of activities. Enrolling ISPCTN study sites will receive a minimum of 1 on-site monitoring visit by a member of the DCOC or their designee, as described in the Clinical Monitoring Plan. During the monitoring visit, drug accountability will be performed. Sites are to maintain all shipment records and non-dispensed product received from Bio-Tech Pharmacal, Inc.

## 6.5 CONCOMITANT THERAPY

All drugs and/or treatments, other than specific excluded medications, are permitted while on study drug. Use of an undisclosed excluded medication will be a protocol deviation. Similarly, if a participant uses an excluded medication and the instructions in this section are not followed, the use of the excluded medication will be a study deviation. Several medications can interfere with the absorption or metabolism of vitamin D and alter 25(OH)D levels. If a participant must start on an excluded medication during the trial period (i.e. after study drug starts), the participant will discontinue study drug but will continue with all other study procedures. New medications prescribed for the control of asthma (including systemic corticosteroids used for brief time periods [less than 14 days] or change in asthma controllers) will not result in withdrawal from study drug but will be recorded. Participants will be advised not to take additional supplements containing more than 1000 international units (IU) of vitamin D per day. All concomitant medications including dates of use, formulation, dose, and frequency will be documented during the study period.

If a participant is taking over-the-counter vitamin supplement(s), study personnel will find out what supplement(s) are being taken. This will be done by showing the participants and caregivers pictures of supplements that are commonly used by the general public. If the supplement is not in the picture set, then the caregiver will be asked to bring in the bottle at the next visit. The caregiver will also be asked how frequently the supplement is taken. Once the supplement is identified, the study team can verify the dose of vitamin D that is being taken by the participant. Children will be allowed to participate if, at the initial visit, the daily dose of vitamin D is less than or equal to 1,000 IU per day.

Concomitant medications will be verified at every visit; the study team will, therefore, be able to provide on-going guidance and counseling regarding participants' daily intake of vitamin D from over-the-counter vitamins. If a study participant changes over-the-counter vitamins and the vitamin D dose changes to greater than 1,000 IU per day, the study team will ask the participant/caregiver to reduce the over-the-counter vitamin D dose to 1,000 IU per day or less. If the participant/caregiver does not follow the request

and the participant's vitamin D dose remains greater than 1,000 IU per day, the participant will be removed from the study.

**Excluded medications**

- Oral or IV steroids (including prednisone and equivalent dexamethasone or other steroid dose) greater than 10 mg/day in the 30 days prior to the screening visit or during the run-in period.
    - Oral or parenteral steroids, including doses greater than 10 mg/day, may be used as an acute asthma control medicine during trial period as stated above.
- Vitamin D2 within the 30 days prior to the screening visit or during the run-in period.
- Vitamin D3 supplementation with a daily dosage of greater than 1000 IUs in an individual supplement form or as part of a multivitamin within the 30 days prior to the screening visit or during the run-in period.

**Concomitant Medications**

Notes: changes in the medications below while on study could alter PK of vitamin D and information regarding such changes must be captured. Participants would not necessarily have to be withdrawn from the study, but changes in dose for any drug in the following list must be documented.

- Anti-epileptic drugs
- Digoxin, calcium channel blockers
- Statins
- Calcium supplements

### 6.5.1 RESCUE MEDICINE

The study team will advise participants to have access to asthma rescue medications (typically albuterol sulfate) for exacerbations of asthma symptoms per standard of care. Management of asthma symptoms will be performed by the participant's routine medical provider. Oral or parenteral steroids are allowed as rescue medicines for asthma exacerbations and should be recorded in the CRF. There is no anticipated need for a rescue medication for the study drug.

# 7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

## 7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from taking vitamin D supplements does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified such as an elevated urine calcium/creatinine ratio (greater than 0.37 on 2 measurements), elevated serum calcium level (greater than 1.5×ULN), or 25(OH)D level greater than100 ng/ml after enrollment, the investigator or qualified designee will determine if any change in participant management is needed including discontinuation of vitamin D supplements. If a participant is discontinued from study drug (vitamin D supplement), he or she will be asked to complete any remaining study visits in order to assure his or her safety and for the sake of completeness of data collection. If a participant who has been asked to stop taking study drug terminates (for any reason) from the study, site coordinators will make reasonable efforts to complete a termination visit. The termination visit will include routine labs (safety labs at minimum) and a focused physical exam.

Rules for halting of study drug:

1. Spot urine calcium/creatinine ratio greater than 0.37 and subsequent serum calcium above 1.5×ULN for the local laboratory. Because dehydration can artificially inflate the calcium/creatinine ratio, participants will be counseled on consuming adequate fluids throughout the study. Participants who have an elevated spot urine calcium/creatinine ratio will be asked to consume 6 to 8 eight-ounce glasses of water per day, and to return within 2 business days of the site team receiving initial lab results for a repeat calcium/creatinine ratio. If the repeat ratio is less than or equal to 0.37, study medication will be continued without interruption. If the ratio remains greater than 0.37, a local serum calcium level and local 25(OH)D will be checked. If the serum calcium is less than 1.5×ULN and 25(OH)D is less than 100 ng/ml, the participant will continue study drug without interruption.

   If a participant develops elevated serum calcium greater than or equal to 1.5×ULN or 25(OH)D level greater than 100 ng/ml by local lab, the participant will remain off study drug through the duration of the study, but will continue with all other study procedures. See Section 8.2 for further safety assessment information.

2. Any documented adverse event, which in the opinion of the site investigator warrants halting of study drug for participant safety.

If a participant develops elevated serum calcium greater than or equal to 1.5×ULN or 25(OH)D level greater than 100 ng/ml, the participant will remain off study drug through the duration of the study, but will continue with all other study procedures.

The data to be collected at the time of study intervention discontinuation will include the following:

- Reason for discontinuation
- Serum 25(OH)D and serum calcium
- Urine calcium/creatinine ratio
- Concomitant medications (including vitamin D3 daily dose at screening in either individual supplement or as a part of a multivitamin)

## 7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Participant request. Participants are free to withdraw from participation in the study at any time for any reason.
- New medical issues that would make continuing in the study dangerous for the participant
- Pregnancy
- Significant study intervention non-compliance at the discretion of the site investigator
- At the discretion of the investigator

The reason for participant discontinuation or withdrawal from the study will be recorded. Participants who sign the informed consent form (i.e., are enrolled) but do not receive the study intervention may be replaced. Participants who sign the informed consent form, receive the study intervention, and subsequently withdraw or are withdrawn or discontinued from the study will not be replaced.

Participants who are withdrawn or who discontinue from the study for any reason are encouraged to return for final study procedures.

## 7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for more than 2 consecutive scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant, reschedule the missed visit within 7 days of the missed visit, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls followed by a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts must be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

# 8 STUDY ASSESSMENTS AND PROCEDURES

## 8.1 STUDY ASSESSMENTS

### 8.1.1 CLINICAL ASSESSMENTS

In the procedure sections below, it should be noted that PK blood samples can be collected by venipuncture or by fingerstick microtainer collection method. If the participant prefers venipuncture, then blood for inflammatory markers will also be drawn. Fingerstick is not an option for visits 2 and 6 since blood inflammatory markers must be assessed for those visits.

Note phone calls will be made at least biweekly (every 2 weeks) from research team to participant/family to promote adherence to medication.

#### 8.1.1.1 PROCEDURES BY STUDY VISIT

**Screening Visit (Visit 1, Days -7 to -21)**

The goal of this visit is to explain the study to potential participants, obtain informed consent/assent, and collect a brief medical history and laboratory studies to assess inclusion/exclusion criteria. After the Screening Visit is complete, eligible participants will begin the run-in period.

1. Inclusion/exclusion criteria will be reviewed. This will include age, BMI, asthma diagnosis, and ability to swallow pills, confounding medical conditions, and concomitant medications per protocol.

2. Informed consent/assent will be obtained for eligible participants (see Section 10.1.1). Once consent and assent have been obtained, the participant is enrolled in the study.

3. Demographic data will be recorded on the data collection form along with measured height and weight.

4. Medical history, including history of asthma exacerbations, will be obtained.

5. Participants (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit 1 blood sample taken from the child's arm or taken from a fingerstick.

6. Participants meeting all other eligibility criteria will have samples collected for local serum 25(OH) vitamin D assessment, urine pregnancy test (for female participants of child-bearing potential), and urine calcium/creatinine ratio.

7. Local safety measures (as described in Section 8.2) will be reviewed by the study coordinator to determine eligibility for continued enrollment. If any safety laboratory value exceeds the thresholds defined by protocol, the participant will be referred to the participant's primary care physician.

8. Diary cards, (without the line to record Vitamin D dosing), will be given to the participant and instructions for completion of the diary will be reviewed. These diary cards will have the questions about asthma control and symptoms and asthma medications, but will not have the questions about vitamin D dosing.

**Baseline (Visit 2, Day 0)**

Study Visit 2 must occur between 10 and 21 days, with a target of 14 days, after Study Visit 1. The goal of this visit is to review eligibility for continued enrollment, provide study procedure training, and randomize participants.

1. Interval history (concomitant medications, asthma symptoms, healthcare utilization) and diary cards (without the line to record Vitamin D dosing) will be reviewed at Visit 2 to assess eligibility for study participation.

    a. Site must confirm that participant continues to meet inclusion/exclusion criteria except for age less than 18 years.

    b. To be randomized, participants must complete and return diary cards with a completion rate greater than or equal to 75%. Participants who do not meet this level of adherence will be removed from the study.

2. An ACT or c-ACT will be completed by the caregiver and/or child, as appropriate.

3. Height and weight measurements for calculation of BMI and BMI-percentile.

4. Blood draw (by venipuncture) for central laboratory 25(OH)D level determination and inflammatory marker assays.

5. Study drug will be dispensed and dosing explained to the participant and his or her parent, legal guardian, or caregiver. (See Section 6.1.2).

6. Diary cards for dosing visits will be given to the participant and instructions for completion of the diary will be reviewed.

7. Study Visit 3 will be scheduled for 28 days (±7) from Study Visit 2.

**Weeks 1-3 (no mandatory visits; optional visit for PK blood draw)**

Adherence communications will occur at least biweekly (every 2 weeks) between study Visits 2 and 3. The study coordinator will interact with the participants and his or her parent, legal guardian, or caregiver through text message, phone, or email to encourage continued participation in the study, monitor adherence to medications, and ask about interval asthma symptoms.

Sites will ask participants to return for serum 25(OH)D level between 1-3 weeks (days 7 to 21, inclusive) after the first dose of vitamin D. Every effort will be made to collect this sample, but this blood draw will be optional. If the participant consents to the optional visit, the participant (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit's blood sample taken by venipuncture or fingerstick.

**Study Visit 3 (Week 4, Day 28 [±7])**

1. Unused medication will be returned and the study coordinator will review the diary cards for dosing visits.
2. ACT (or c-ACT) will be completed by the caregiver and/or child as appropriate.
3. Interval history will be reviewed and recorded including asthma exacerbations, unplanned healthcare utilization, new or changes in concomitant medications (including asthma medications) and adverse events.
4. Height and weight measurements for calculation of BMI and BMI-percentile.
5. Urine will be obtained for calcium/creatinine ratio.
6. Participants (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit's blood sample taken by venipuncture or fingerstick.
7. Blood for 25(OH)D level (required) and inflammatory markers (if blood drawn by venipuncture) will be obtained and sent to central laboratory.
8. Study drug will be dispensed and dosing reviewed with the participant and his or her parent, legal guardian, or caregiver. (See Section 6.1.2)
9. Diary cards for dosing visits will be given to the participant and instructions for completion of the diary will be reviewed.
10. Study Visit 4 will be scheduled for 56 days (±7) from Study Visit 2 (baseline).
11. If a participant is withdrawn from the study drug, he/she will be asked to continue to make any remaining study visits in order to assure his/her safety. (See Section 7.1)

**Weeks 5-7 (No Scheduled Visits)**

Adherence communications will occur at least biweekly (every 2 weeks) between study Visits 3 and 4. The study coordinator will interact with the participants and his or her parent, legal guardian, or caregiver through text message, phone, or email to encourage continued participation in the study, monitor adherence to medications, and ask about interval asthma symptoms.

**Study Visit 4 (Week 8, Day 56 [±7])**

1. Unused medication will be returned and the study coordinator will review the diary cards for dosing visits.

2. ACT (or c-ACT) will be completed by the caregiver and/or child as appropriate.

3. Interval history will be reviewed and recorded including asthma exacerbations and adverse events.

4. Concomitant medications will be reviewed and recorded.

5. Height and weight measurements for calculation of BMI and BMI-percentile.

6. Urine will be obtained for calcium/creatinine ratio (all participants) and for pregnancy tests (for female participants of child-bearing potential).

7. Participants (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit's blood sample taken by venipuncture or fingerstick.

8. Blood for 25(OH)D level (required) and inflammatory markers (if blood drawn by venipuncture) will be obtained and sent to central laboratory.

9. Study drug will be dispensed and dosing reviewed with the participant and his or her parent, legal guardian, or caregiver. (See Section 6.1.2)

10. Diary cards for dosing visits will be given to the participant and instructions for completion of the diary will be reviewed.

11. Study Visit 5 will be scheduled for 84 days (±7) from Study Visit 2 (baseline).

**Weeks 9-11 (No Scheduled Visits)**

Adherence communications will occur at least biweekly (every 2 weeks) between study Visits 4 and 5. The study coordinator will interact with the participants and his or her parent, legal guardian, or caregiver through text message, phone, or email to encourage continued participation in the study, monitor adherence to medications, and ask about interval asthma symptoms.

**Study Visit 5 (Week 12, Day 84 [±7])**

1. Unused medication will be returned and the study coordinator will review the diary cards for dosing visits.
2. ACT (or c-ACT) will be completed by the caregiver and/or child as appropriate.
3. Interval history will be reviewed and recorded including asthma exacerbations and adverse events.
4. Concomitant medications will be reviewed and recorded.
5. Height and weight measurements for calculation of BMI and BMI-percentile.
6. Urine will be obtained for calcium/creatinine ratio.
7. Participants (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit's blood sample taken by venipuncture or fingerstick.
8. Blood for 25(OH)D level (required) and inflammatory markers (if blood drawn by venipuncture) will be obtained and sent to central laboratory.
9. Study drug will be dispensed and dosing reviewed with the participant and his or her parent, legal guardian, or caregiver. (See Section 6.1.2).
10. Diary cards for dosing visits will be given to the participant and instructions for completion of the diary will be reviewed.
11. Study Visit 6 will be scheduled for 112 days (±7) from Study Visit 2 (baseline).

**Weeks 13-15 (No Scheduled Visits)**

Adherence communications will occur at least biweekly (every 2 weeks) between study Visits 5 and 6. The study coordinator will interact with the participants and his or her parent, legal guardian, or caregiver through text message, phone, or email to encourage continued participation in the study, monitor adherence to medications, and ask about interval asthma symptoms.

**Study Visit 6 (Week 16, Day 112 [±7])**

1. Unused medication will be returned and the study coordinator will review the diary cards for dosing visits.

2. ACT (or c-ACT) will be completed by the caregiver and/or child as appropriate.

3. Interval history will be reviewed and recorded including asthma exacerbations and adverse events.

4. Concomitant medications will be reviewed and recorded.

5. Height and weight measurements for calculation of BMI and BMI-percentile.

6. Blood for 25(OH)D level and inflammatory marker assays will be drawn by venipuncture and sent to central laboratory.

7. Urine will be obtained for calcium/creatinine ratio (all participants) and for pregnancy tests (for female participants of child-bearing potential).

8. Diary cards without the line to record Vitamin D dosing will be given to the participant/parent/guardian. These diary cards have the questions about asthma control and symptoms and asthma medications, but do not have the questions about vitamin D dosing. These will be the same cards as those given at Visit 1 (screening visit).

9. Follow-up visit for week 20 (visit 7) will be scheduled for 140 days (±7 days) from Study Visit 2 (baseline).

**Follow-up Visits: Weeks 20 (visit 7), 24 (visit 8), and 28 (visit 9) [Days 140, 168, and 196, respectively, each ±7 days]**

1. Participants (parent/guardian and child) will be asked to initial a form documenting whether they prefer to have this visit's blood sample taken by venipuncture or fingerstick.

2. Post-dosing blood draw for 25(OH)D level (required) and inflammatory markers (if blood drawn by venipuncture) will be obtained and sent to central laboratory.

3. At weeks 20 (visit 7; day 140 ±7) and 24 (visit 8; day168 ±7) only, diary cards without the line to record vitamin D dosing will be given to the participant/parents/guardian. Instructions for completion of the diary will be reviewed. These diary cards will have questions about asthma control and symptoms and asthma medications but will not have any questions about vitamin D. These will be the same cards as those given at Visit 1 (screening visit).

4. Diary cards from the previous visit, as well as additional interval history, will be reviewed. Information, including asthma exacerbations and adverse events, will be recorded.

5. Follow-up visit for week 24 (visit 8) will be scheduled for 168 (±7) days from Visit 2 (baseline) and follow-up visit for week 28 (visit 9) will be schedule for 196 (±7) days from Visit 2 (baseline).

6. At week 28 (visit 9; day 196 ±7), local serum 25(OH)D level will be determined. This is in addition to the central lab.

7. Coordinators will call, text, or email participants at least the week prior to their visit as a reminder.

#### 8.1.1.2 ASTHMA MEASURES

**Asthma Control Test (ACT)**

The ACT is a 4-week recall questionnaire that has been validated and addresses issues of asthma control, symptoms, and nocturnal awakenings for patients with asthma aged 12 and above.(82) The ACT will be administered to 12 to 18-year-olds at all in-person clinic visits.

**Childhood Asthma Control Test (c-ACT)**

The c-ACT is a 4-week recall questionnaire that has been validated and addresses issues of asthma control, symptoms, activity limitation, and nocturnal awakenings for patients aged 4 to 11 years with asthma). The c-ACT will be administered to 6 to 11- year- olds at all in-person visits.

Participants that turn 12 during the course of the study will continue to use the c-ACT test for the duration of their participation.

**Asthma-related Events**

The presence of asthma-related events (ASDs, exacerbations, episodes of problematic asthma, respiratory infections) will be identified by participant documentation on daily diary cards (collected at all clinic visits) and by structured interviews at in-person clinic visits.

1. ASDs:

A diary card will be used to compile information about asthma symptoms. Though diary cards similar to those proposed for VDORA have been used successfully in large highly-controlled pediatric asthma trials, symptom diaries can be criticized for some participants having incomplete data. Symptom diaries however can be used successfully particularly in intervention trials less than 26 weeks (as with VDORA) and when participants are frequently reminded and supported closely by research staff. (76, 77)

An ASD (83) will be defined as a day with any one of the following:

a. Albuterol rescue use (pre-exercise treatment permitted)

b. Use of additional asthma medications including oral steroids. In addition, the 7 days immediately following the end of a course of oral steroids will be considered ASDs.

c. Daytime or nighttime asthma symptoms (wheezing, coughing, phlegm/mucus, chest tightness, or shortness of breath)

d. Unscheduled health care provider visits for asthma, emergency room visit or hospital admission for asthma, or missed work or school due to asthma

Baseline frequency of ASDs will be documented for each participant using the participant's run-in period and Week 1 (if needed) diary cards to explore changes in mean ASD/week longitudinally on treatment.

2. Asthma Exacerbation

In this study an asthma exacerbation will be defined according to the recommendation of the NIH Outcomes Workshop (84) as a worsening of asthma requiring the use of a systemic corticosteroid (at least 2 days of treatment) to prevent a serious outcome. In accordance with the Expert Panel recommendations, data will be captured on the following exacerbation-related outcomes:

a. All worsening asthma events in which systemic corticosteroids were initiated to prevent a serious outcome, including use of systemic corticosteroids in association with any form of healthcare provider encounter

b. All asthma-specific ED or urgent care visits that involved treatment with systemic corticosteroids

c. All asthma-specific hospitalizations that involved treatment with systemic corticosteroids (also reported as an SAE)

d. All asthma-specific intensive care unit admissions or intubations (also reported as an SAE)

e. All deaths (all-cause and asthma-related; also reported as an SAE)

For the purpose of this study, 2 courses of systemic corticosteroids must be separated by at least 1 week to count as 2 exacerbations and to be documented as such.

3. Episode of problematic asthma

   Any asthma-related unscheduled healthcare visit (outpatient, urgent care, ED).

4. Respiratory tract infection

   Patient-reported episodes of upper respiratory tract infection (cold), sore throat, strep throat, bronchitis, pneumonia, ear infection, and acute sinusitis (sinus infection) will be documented from structured interviews occurring at each study visit and planned study phone call. This approach has been used successfully in large pediatric asthma trials. (80)

### 8.1.2 LABORATORY EVALUATIONS

Specimens for evaluation include:

- Safety assessments (volumes to be determined by laboratory facility at local site; methodology to be determined by the laboratory facility at the local site)
  - Urine for pregnancy test for female participants of childbearing potential (done only at Visit 1, Visit 4, and Visit 6/Early Termination).
  - Blood for serum 25(OH)D level at Visit 1 (Screening) and Follow-up visit 9 (week 28).
  - Urine for creatinine/calcium ratio to be done at Screening and study Visits 3, 4, 5, and 6. Blood for serum calcium will be collected if indicated due to a high urine calcium/creatinine ratio.
- Study assessments (to be performed by a College of American Pathologists [CAP] accredited and Clinical Laboratory Improvement Amendments [CLIA] certified central laboratory).
  - 25(OH) vitamin D level by liquid chromatography/tandem mass spectrometry (specimen collection per laboratory manual) to be done at Baseline (visit 2) and study Visits 3, 4, 5, and 6 as well as follow-up (visits 7, 8, and 9). There is an optional visit, with a blood draw for vitamin D analysis, between Week 1 and 3, if the participant volunteers for this.
  - Cytokine panel (IL-2, IL-6, IL-10, IL-17, and TNF-alpha) by immunoassay as cytokine panel (specimen collection per laboratory manual) to be done at Baseline and at Visit 6 and, if the participant agrees to blood collection by venipuncture, at Visits 3, 4, and 5, and Follow-up (visits 7, 8, and 9).

- Leptin by electrochemiluminescence (specimen collection per laboratory manual) to be done at Baseline and at Visit 6 and, if the participant agrees to blood collection by venipuncture, at Visits 3, 4, and 5, and Follow-up (visits 7, 8, and 9).

**Laboratory determinations of vitamin D levels**

25(OH)D levels for study endpoints will be determined at a CAP accredited and CLIA certified central laboratory. Vitamin D levels will be obtained at the local laboratory for determining inclusion criteria (25(OH)D level less than 30 ng/ml but great than 10 ng/mL) and for safety measurements at the local investigator's discretion, ensuring prompt turn around.

## 8.2 SAFETY AND OTHER ASSESSMENTS

Vitamin D is a very safe supplement with a large therapeutic index. Vitamin D sufficiency is considered to be greater than 30 ng/ml. Intoxication does not occur until serum 25(OH)D levels exceed 150 ng/ml (325 nmol/L).(85) However, most clinical laboratories consider 100 ng/ml to be the upper limit in order to maintain a wide safety margin. Reports of vitamin D toxicity are rare and virtually always related to faulty manufacturing or labeling of product such that individuals ingested hundreds to thousands of times the presumed dose.(86, 87) The consequence of vitamin D toxicity is hypercalcemia. Hypercalcemia only results when 25(OH)D concentrations have consistently been above 150-200 ng/ml (375-500 nmol/L).(85)

To ensure safety in this study, spot urine calcium/creatinine levels will be measured at each visit as a screen for hypercalciuria. The study coordinator will instruct the participant and his or her parent, legal guardian, or caregiver to ensure that the child is well-hydrated prior to the study visit. If the urine calcium/creatinine level is greater than 0.37 on a specimen, the participant will be asked again to ensure good hydration and return within 2 business days for a repeat spot urine calcium/creatinine measurement. If the ratio remains greater than 0.37, then a serum calcium level and 25(OH)D for the local laboratory will be obtained.(75) If serum calcium is greater than 1.5×ULN or the 25(OH)D level is greater than 100 ng/ml, the participant will have supplementation stopped but will be asked to return for remaining study visits in order to obtain data on vitamin D clearance and for safety assessments. Vitamin D supplementation ends at 16 weeks (day 112 ±7) for all participants. They will be followed for another twelve weeks for determination of elimination kinetics and for safety assessments. The half-life of vitamin D is variable but is felt to be approximately 2 months.(88) The 12-week follow-up therefore consists of 1.5 half-lives. A serum 25(OH)D level will be drawn for the local laboratory safety measure at week 28 (visit 9; day 196 ±7). If a participant has a serum 25(OH)D level greater than 80 ng/ml at that time, he/she will be asked to return 4 weeks later for a re-check to ensure that the level is not continuing to rise as vitamin D washes out of adipose tissue. If there are any ongoing concerns, further follow up will be determined by the local investigator in collaboration with the child's primary care team and sub-specialists as necessary.

This is not an asthma treatment study. We will monitor asthma symptoms, however. Each participant is required to have an identified asthma-care provider at entry. This can be a specialist or a primary care provider. If it is found that a participant's asthma is poorly controlled, he or she will be referred back to the primary asthma-care provider for reevaluation and further treatment.

If any child is found to be in the midst of an asthma exacerbation at the time of a study visit, the study staff will notify the local PI and take appropriate action to guarantee safety of the child. This may include referral to an Urgent Care or Emergency facility or may involve the local PI treating the participant if he or she is the child's asthma care provider.

## 8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS AND ADVERSE REACTIONS

### 8.3.1 DEFINITION OF ADVERSE EVENTS AND ADVERSE REACTIONS

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related.

AR means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse events where there is reason conclude that the drug caused the event.

### 8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS OR REACTIONS

An AE or suspected AR is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

### 8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

#### 8.3.3.1 SEVERITY OF EVENT

For AEs and ARs not included in the protocol-defined grading system, the following guidelines will be used to describe severity. The site investigator will determine severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

#### 8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

#### 8.3.3.3 EXPECTEDNESS

The expectedness of an AE or suspected AR shall be determined according to the specified reference document containing safety information (e.g., most current product information or product label). Any AE that is not identified in nature, severity, or specificity in the current study drug reference document(s) (e.g., product information) is considered unexpected. Events that are mentioned in the product information as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but not specifically mentioned as occurring with the particular drug in this study are considered unexpected.

Expected AEs that could occur in this study are: bruising related to venipuncture/fingerstick, pain related to venipuncture/fingerstick, increase in calcium in the blood or urine, kidney stones, accidental vitamin D poisoning (associated nausea and increased levels of calcium in the blood), increased urination, decreased appetite, increased fatigue, hypersensitivity to vitamin D, or hypercalcemia (only expected when vitamin D blood level is above 150-200 ng/mL, but the intervention will be stopped if level reaches 100 ng/mL).

If the capsule is ruptured or broken, the powder may cause the following:  irritation or burning of the eyes, skin, or respiratory system (if inhaled).

### 8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs must be followed until 1 of the following criteria is met: resolution, the condition stabilizes, the event is otherwise explained or is judged by the PI to be no longer clinically significant, or the participant is lost to follow-up

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode. At each study visit, the investigator or study coordinator will inquire about the occurrence of AE/SAEs since the last visit. The site investigator will record all reportable events with start dates occurring any time after informed consent is obtained. Events will be followed for outcome information until resolution or stabilization or until 7 days (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation.

### 8.3.5 ADVERSE EVENT AND REACTION REPORTING

All AEs and ARs that occur after informed consent is obtained until 7 days after the last day of study participation should be documented in the patient's source documents in accordance with the Investigator's normal clinical practice and in the AE section of the CRF.

### 8.3.6 SERIOUS ADVERSE EVENT AND REACTION REPORTING

The site investigator will report all SAEs and SARs occurring any time after informed consent is obtained until 30 days after the last day of study participation. The study clinician will immediately report to the sponsor any SAE, whether or not considered study intervention related, including those listed in the protocol or product information and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are SAEs (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

The investigator/qualified designee will enter the required information regarding the SAE into the appropriate module of the electronic case report form (eCRF), which will automatically result in distribution of the information to the appropriate sponsor contact. If the eCRF system is temporarily unavailable, the event, including the investigator-determined causality to study drug, must be reported via a paper back-up SAE form to the appropriate sponsor contact. Upon return of the availability of electronic data capture system, the SAE information must be entered into the eCRF.

All SAEs will be followed until resolution or until the site investigator deems the event to be chronic, or until the participant is stable, or until 30 days after the last day of study participation. Other supporting documentation of the event may be requested by the DCOC/study sponsor and should be provided as soon as possible.

### 8.3.7 REPORTING EVENTS TO PARTICIPANTS

The participants will be notified of those study-related (or potentially study-related) SAEs, SARs and unanticipated problems (UPs) that may affect either the participants' willingness to continue with the study or the future health of the child enrolled in the study. This determination can be made by any of the following: the IRB, the medical monitor, the DSMB, or the sponsor. The person or oversight body that makes the determination will inform the DCOC, which will instruct the site PIs/study coordinators to contact those participants enrolled through their site. Contacts with participants, if necessary, will be recorded on the appropriate CRF and/or study log.

### 8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

### 8.3.9 REPORTING OF PREGNANCY

As noted above in Section 2.3.1, vitamin D supplementation is safe in pregnancy. However, to be extremely cautious, participants of childbearing potential will be counseled to use acceptable forms of birth control as well as condoms during the study period. Abstinence is considered an acceptable form of birth control. There are no known risks of vitamin D intake during pregnancy.

We will follow standard pharmaceutical study practices in regards to pregnancy to make sure we account for the possibility of untoward and unexpected effects on a fetus. If a participant becomes pregnant during the study, dosing will be discontinued immediately.

If a subject reports a pregnancy, we will use a specific pregnancy follow-up form to track the outcome of the pregnancy for the subject. On this form, we will document how the pregnancy diagnosis was made, whether the subject has received a medical evaluation for the pregnancy (and where), and what the outcome of the pregnancy was (e.g., miscarriage, elective abortion, live birth or stillbirth). We will also record maternal history (including congenital abnormalities or pregnancy complications) and estimated date of conception.

The pregnant participant will be followed until delivery or until the end of pregnancy (in the case of miscarriage or pregnancy termination). This will occasionally require us to follow participants after they have completed this study.

For all live births, we will document the mode of delivery, gestational delivery age, the birth weight, whether there were any health problems (infant and/or mother) at delivery, and whether the baby required a hospitalization beyond 2 days. These data will all be self-reported by the participant. We will monitor all pregnancy-related outcomes, and will request permission to obtain medical records for any participant in which adverse pregnancy-related outcomes are reported.

Any associated AEs or SAEs that occur to the mother or fetus/child will be recorded in the AE or SAE eCRF.

## 8.4 UNANTICIPATED PROBLEMS

### 8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("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); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An unanticipated problem is not necessarily an AE or SAE. Unanticipated problems that are neither an AE nor SAE, but which must be reported, are listed below in Section 8.4.2.

### 8.4.2 UNANTICIPATED PROBLEM REPORTING

Unanticipated problems (per Section 8.4.1) must be reported to the reviewing IRB, the DCOC, the sponsor, and the NIH DSMB as well as any other persons or groups noted in the DSMB charter. These problems must be reported to the reviewing IRB according to the reviewing IRB's policies and procedures and reported to local institutions according to the rules and regulations of the local institution. The reviewing IRB will report the issue to OHRP per the policies of the reviewing IRB. For the UAMS IRB, which will be the reviewing IRB for most of the sites for this study, the required reporting times are shown in the following table.

**Table 3. Unanticipated Problem Reporting**

| Unanticipated Problem | Required Reporting Time to UAMS IRB |
|---|---|
| Death or life-threatening | Immediately to IRB office or IRB Chair |
| All other events | Within 10 days of event or notification of event if non-local |

Examples of unanticipated problems that are not an AE or SAE, but which would need to be reported include:

- Breach of confidentiality
- Change in product labeling, or withdrawal from marketing, of the vitamin D used in this protocol

Typically, the report to the IRB et al. will include the following:

- Protocol identification information (title, number, etc.)
- PI name
- Detailed description of event
- Explanation of why the event is a UP
- Description of changes needed (to protocol) and/or other corrective actions taken or needed

Additional details are provided in the study-specific MOP.

### 8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

The participants will be notified only of those study-related (or potentially study-related) SAEs and unanticipated problems that may affect either the participants' willingness to continue with the study or the future health of the participant enrolled in the study. This determination can be made by any of the following: the IRB, the medical monitor, the DSMB, or the sponsor. The person or oversight body that makes the determination will inform the DCOC, which will instruct the site PIs/study coordinators to contact those participants enrolled through their site.

Contacts with participants, if necessary, will be recorded on the appropriate CRF and/or study log.

# 9 STATISTICAL CONSIDERATIONS

## 9.1 STATISTICAL HYPOTHESES

Study objective: The overall objective of this study is to determine the PK of Vitamin D supplementation in children who have asthma and are overweight or obese and therefore no formal hypothesis testing will be performed.

Part 1 Objective: Identify dosing to use in Part 2 using PK analysis on a subset of participants Part 2 Objective: Determine the effectiveness of a recommended 16-week dosing regimen to achieve a serum level of 25(OH)D greater than or equal to 40 ng/ml in vitamin D insufficient or deficient children who have asthma and are overweight/obese

The hypothesis for Part 2 will be tested using the one-group Chi-square test at the 5% one-sided significance level.

## 9.2 SAMPLE SIZE DETERMINATION

Part 1: a sample size of greater than 50 participants will be powered (greater than 80%) to target a 95% confidence interval within 60% and 140% of the geometric mean estimates of 25(OH)D systemic clearance. The sample size assumes a coefficient of variation of 50% in 25(OH)D steady-state concentration.(48, 89) Based on prior experience (90, 91, 92, 93) a sample size of 16 participants in the interim analysis will suffice to determine a recommended dose for Part 2.

Part 2: For the optimal dosing regimen defined by the interim PK-analysis, the null hypothesis that the proportion of children who achieve a vitamin D level of greater than or equal to 40 ng/ml is 50% or less at 16 weeks will be tested against the alternative hypothesis that the proportion is 75% or more at the one-sided 0.05 significance level with power of 0.90. Using the one group Chi-square test for testing a single proportion, the number of study participants required to complete Part 2 is 31 participants. To allow for an approximately 30% dropout rate (due, in part, to the COVID-19 pandemic), 63 participants will be randomized to (A) the 8,000 IU daily dose (with a 50,000 IU loading dose) regimen or to (B) the standard of care (600 IU/day) dose. The allocation ratio will be 2:1 (A:B).

## 9.3 POPULATIONS FOR ANALYSES

**PK analysis Population**: All participants with at least 1 PK sample will be included in the population PK analysis.

**Intent-to-Treat (ITT) Population:** This population includes all enrolled participants. Analysis of the ITT population will be used for all non-PK analyses.

**Safety Population**: This analysis population includes all enrolled participants who received at least one dose of study treatment.

## 9.4 STATISTICAL ANALYSES

### 9.4.1 GENERAL APPROACH

Following finalization of the Protocol, but prior to 25% enrollment, a Statistical Analysis Plan (SAP) will be issued as a separate document, which will provide detailed analytical plans for the set of analyses outlined below. All statistical analyses will be conducted following the statistical principles for clinical trials as specified in International Conference on Harmonization Statistical Principles for Clinical Trials (ICH Topic E9). Any deviations from the planned analyses will be described and justified in the final integrated clinical study report. All study data and summary tables will be presented overall and by study sites.

Descriptive statistics for continuous data will be summarized using mean and standard deviation or median and interquartile range, as appropriate. Categorical data will be summarized using frequency and percent. Any outliers detected during data review will be investigated and methods for handling outliers or data transformation will be defined in the SAP.

### 9.4.2 ANALYSIS OF THE STUDY ENDPOINT

The study does not include an efficacy endpoint. The primary objective is to conduct a population PK analysis.

A population PK analysis will be performed to characterize the PK of vitamin D in overweight/obese children using the software Nonlinear Mixed Effects Modeling (NONMEM, version 7.2). Different structural PK models (e.g., 1 and 2 compartment) with linear or non-linear (e.g., Michaelis-Menten) kinetics will be fitted to the concentration-time data of 25(OH)D. Between-participant variability on model parameters and different residual error models will be tested. Model development will be guided by goodness of fit plots, plausibility of parameter estimates, and reduction in inter-individual variability for structural and residual error parameters, as well as objective function and shrinkage values.

Covariate evaluation will be performed to examine the relationship between model parameters and demographic factors (e.g., age, weight status, ethnicity) and co-administered medications, as applicable. More detail about the weight status measures used in the model will be included in the SAP. Appropriate covariates will be incorporated into the model using a standard forward inclusion-backward elimination approach. Standard model diagnostic plots and procedures will be used to evaluate model appropriateness. Model validation will be performed using visual predictive check and bootstrapping.

Using the final PK model and parameter estimates, serum concentration-time profiles of 25(OH)D will be simulated for each participant at varied dosing regimens. The percentage of participants with serum 25(OH)D greater than or equal to 40 ng/ml during a desired time period will be calculated. The best dosing regimen will be determined as the one resulted in serum 25(OH)D greater than or equal to 40 ng/ml in a majority (i.e.

75%) of participants. It is possible that the doses outlined and evaluated in this study will not end up being the dose chosen for a larger randomized, controlled, double-masked intervention trial. Creation of a PK model will determine the optimal dosing strategy in Part 2 to achieve serum vitamin D levels greater than or equal to 40 ng/ml.

### 9.4.3 ANALYSIS OF THE PART 1 ENDPOINT

This analysis was done including participants randomized in part 1. Dose finding was done using the PK model and parameter estimates, serum concentration-time profiles of 25(OH)D was simulated for each participant at varied dosing regimens. The percentage of participants with serum 25(OH)D greater than or equal to 40 ng/ml during a desired time period was calculated. The best dosing regimen was determined as the one resulted in serum 25(OH)D greater than or equal to 40 ng/ml in a majority (i.e. 75%) of participants. It is possible that the doses outlined and evaluated in this study will not end up being the dose chosen for a larger randomized, controlled, double-masked intervention trial. Creation of a PK model determined the optimal dosing strategy in Part 2 to achieve serum vitamin D levels greater than or equal to 40 ng/ml. The participants who achieved a vitamin D level of greater than or equal to 40 ng/ml were estimated along with the corresponding 95% confidence interval for each dose regimen in Part 1. A one-group test of proportion will be used to analyze the overall proportion of participants with 25(OH)D greater than or equal to 40 ng/ml at 16 weeks among participants treated with the final recommended dose in Part 2.

### 9.4.4 ANAYLSIS OF PART 2 ENDPOINTS

The participants who achieve a vitamin D level of greater than or equal to 40 ng/ml will be estimated along with the corresponding 95% confidence interval for each dose regimen in Part 1. A one-group test of proportion will be used to analyze the overall proportion of participants with 25(OH)D greater than or equal to 40 ng/ml at 16 weeks among participants treated with the final recommended dose in Part 2.

### 9.4.5 SAFETY ANALYSES

All AEs recorded during the study period will be coded with Medical Dictionary for Regulatory Activities (MedDRA) Version March 2018. All participants enrolled in the study will be evaluated for safety. Summaries of AE will be tabulated for the following types:

- Number (%) of participants with any AE or AR
- Number (%) of participants with any SAE or SAR
- Number (%) of participants withdrawn from treatment due to AE

All summaries will be reported overall and by study site. Additional analyses and summaries may be derived if deemed appropriate and will be further described in the SAP.

### 9.4.6 BASELINE DESCRIPTIVE STATISTICS

Baseline and demographic characteristics will be summarized overall and by study site. Summary statistics will be applied for both continuous and categorical variables. For continuous measures, descriptive measures will include number of non-missing values, mean, standard deviation, median, minimum, and maximum. For categorical variables, descriptive statistics will include frequency counts and percentages by category.

### 9.4.7 PLANNED INTERIM ANALYSES

An interim PK analysis was performed after a minimum of 16 participants (at least 4 in each arm) completed PK sampling at Week 20 (visit 7) in Part 1 of the study. The interim PK analysis provided an optimal dosing regimen for Part 2.

Results of the Part 1 analysis were presented to the DSMB along with the recommended new dose. Enrollments at the new dose will begin after DSMB approval.

### 9.4.8 SUBGROUP ANALYSES

Minimal subgroup analyses will be performed.

### 9.4.9 TABULATION OF INDIVIDUAL PARTICIPANT DATA

A detailed description of participant disposition will be provided. The number of enrolled participants will be tabulated overall and by study site and presented as counts and percentages. Additionally, the number of participants either completing or discontinuing the study will be summarized using counts and percentages.

### 9.4.10 EXPLORATORY ANALYSES

At each study visit, ACT (or c-ACT) and ASDs will be computed to monitor asthma control. The presence and number of severe asthma exacerbations (as defined by the need for oral or parenteral corticosteroids), unscheduled asthma-related healthcare utilizations, and asthma-related hospitalizations will be collected. General estimating equations will be used to assess changes in the number of exacerbations, ASDs and the ACT (or c-ACT) score over time.

Baseline and 16-week inflammatory biomarkers (leptin plus TNFα, IL-2, IL-6, IL-10 and IL-17 (cytokine panel)) will be collected to assess changes on vitamin D supplementation. Participants who opt for venipuncture for vitamin D levels (rather than fingerstick) will have blood drawn for inflammatory markers at more times. Total 25(OH)D, changes in 25(OH)D, changes in individual biomarkers, and asthma outcomes will be explored.

## 9.5 MISSING DATA

To compare the proportion of participants that achieve the serum 25(OH)D levels greater than 40 ng/ml target among the standard of care group and the optimal PK dose group, differences in sample proportions will be evaluated by chi-square or Fisher's exact test as appropriate. Additionally, proportion difference with the 95% confidence interval will be obtained.

Participants will be defined as a responder or non-responder at the 16-week visit according to the serum level criteria (i.e., achieve a serum 25(OH)D of greater than or equal to 40 ng/ml at 16 weeks). Participants who withdraw prematurely will be considered non-responders in the primary analysis.
To investigate the effect of missing data on the proportion of response endpoint, a sensitivity analysis will be carried out using a multiple imputation approach. Additionally, all participants who do not complete the recommended 16-weeks dosing regimen will be regarded be excluded from the per-protocol analysis population.

# 10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

## 10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

### 10.1.1 INFORMED CONSENT PROCESS

#### 10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials will be submitted to the IRB with this protocol:

- Product information
- Diary card
- Informational brochure/flyer for participants
- Informed consent form (including the assent portion)

#### 10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that starts before a participant agrees to be a part of a study and the process continues throughout the individual's study participation. Parents/guardians of the participating children under the age of majority, participants that are at or over the age of majority, and emancipated minors will be asked to read and review the consent form. Children under the age of majority, but who are also 7 years of age or older, will be provided with a separate assent form. Both the consent and assent forms will be approved by the IRB.

The investigator or designee will explain the research study to the participant(s) (i.e., parents/guardians for children under the age of majority, children under the age of majority, participants at the age of majority, and emancipated minors). The investigator or designee will answer any questions that may arise. The verbal explanation will be provided in terms the participant(s) can understand. The explanation will state the purposes, procedures, and potential risks of the study and describe their rights as research participants. Participants will have the opportunity to carefully review the written forms and ask questions before signing. The participants will be given the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The parent/guardian of underage children, participants who have reached the age or majority, and emancipated minors will sign the informed consent document prior to any procedures being done specifically for the study. Unemancipated minors who are 7 years old or older will sign the separate assent form.

Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. They will be informed that their medical care and their children's medical care will not be adversely affected if they decline to participate in the study. A copy of the informed consent and assent documents (if separate form used) will be given to the participants/parents/guardians for their records.

Assent is a process for children who have not reached the age of majority. The age at which assent is required is dependent on central IRB (i.e., the UAMS IRB) and any local site requirements for relying sites. All sites will be using the UAMS IRB as the IRB of record; per the UAMS IRB, the age of assent is 7 years old. All children who are 7 years old or older must agree to be participants in the study. Simply not disagreeing does NOT constitute assent.

The informed consent process, as well as the child's assent, will be conducted and will be documented in the source documents (research or medical record of the participant). The documentation will include, but is not limited to: (a) the title of the study, (b) the date the participant entered into the study, (c) the name of the PI, (d) the name of the site investigator, (e) the name of the person(s) obtaining the informed consent and assent, and (e) a statement that the participant or legally authorized representative received a copy of the signed form. Additional documentation that is recommended, but not required, includes: (a) list of who else was present during the process, (b) type of questions asked by the participant, (c) details that demonstrate the participant understands the information, and (d) other specific details related to that case.

Both parental consent and the children(s)' assent - as well as records of the process of obtaining the consent and assent - will be maintained in the source documents.

### 10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended at 1 or more sites if the reviewing IRB does not authorize renewal at the time of continuing review. Potential problems include, but are not limited to, information not received in time for continuing review to occur before the 1-year authorization period is up or issues that need to be address prior to re-authorization. The study may also be suspended or stopped per any stopping/suspension specifications in the DSMB charter. Early termination may be permanent if there is sufficient cause. Written notification, documenting the reason for study suspension or termination, will be provided, directly or indirectly, by the suspending or terminating party to the following as applicable: study participants, PI, site investigators, reviewing IRB, local IRBs, the NIH, the ISPCTN DCOC, and the OHRP. Persons and offices notified shall include those specified in the study MOP, the reviewing IRB's policies and procedures, and the SMART IRB policies and procedures. When study participants are contacted, they shall be informed of any changes to the study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

If the study was temporarily suspended, the study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the DSMB, the reviewing IRB, the local IRB(s) (when applicable), the funding agency, and/or the sponsor (ISPCTN DCOC).

### 10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator. This documentation includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. Clinical study sites will permit access to such records.

The PI will ensure that the use and disclosure of protected health information (PHI) obtained during this research study complies with the Federal Privacy Regulation. In the U.S., the Health Insurance and Portability and Accountability (HIPAA) Privacy Rule applies. The rule provides U.S. federal protection for the privacy of PHI sent to or collected in the U.S. for the purposes of this research by implementing standards to protect and guard against the misuse of individually identifiable health information of participants participating in clinical trials. “Authorization” is required from each research participant (i.e., specific permission granted by an individual to a covered entity for the use or disclosure of an individual’s PHI). A valid authorization must meet the implementation specifications under the applicable Federal Privacy Regulations. The relevant privacy authorization will either be combined with the informed consent form (ICF) or will be provided as a separate document. If the privacy authorization is presented as a separate form, a copy of the signed form will be given to the participant/participant’s legal guardian. The HIPAA authorization, in addition to the ICF, will be approved by the IRB of record.

Records will be maintained as required by the privacy and security rules promulgated by HIPAA (Health Insurance Portability and Accountability Act; Title 45 of the Code of Federal Regulations Part 164). The following links direct the reader to the rules: *https://www.hhs.gov/hipaa/for-professionals/security/index.html* and *https://www.hhs.gov/hipaa/for-professionals/privacy/index.html* (accessed by KLS 06/12/17).

Certain bodies/institutions may need to review information, including the participants, for any of the following reasons: to process information, to ensure compliance with the protocol and other applicable requirements (such as the policies and procedures of the reviewing IRB). Institutions/bodies that may have access to the participants' information include:

- UAMS IRB
- the IRB for the site through which the participant is enrolled
- OHRP
- DCOC for the ISPCTN
- NIH

Individuals with access to study records will be:

- overall-study PI
- site investigators
- research coordinators
- data managers at participating site(s)

### 10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

During the conduct of the study, an individual participant can choose to withdraw consent to have their data used.

### 10.1.5 KEY ROLES AND STUDY GOVERNANCE

| **Protocol Co-Chair** | |
|---|---|
| *Name, degree, title* | Brian O'Sullivan, MD<br>Professor, Pediatrics |
| *Institution* | Geisel School of Medicine at Dartmouth College |
| *Address* | *100 Hitchcock Way, Manchester, NH 03104* |
| *Phone* | 603-695-2790 |
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| **Protocol Co-Chair** | |
| *Name, degree, title* | J. Marc Majure, MD<br>Suzanne T. Miller Chair in Pediatric Pulmonology |
| *Institution* | *University of Mississippi Medical Center* |
| *Address* | *2500 N. State St., Jackson, MS 39216* |
| *Phone* | *601-984-5205* |
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| **Protocol Co-Chair** | |
|---|---|
| *Name, degree, title* | Laura James, MD, FAAP<br>Associate Vice Chancellor for Clinical and Translational Research |
| *Institution* | *Arkansas Children's Research Institute* |
| *Address* | *1 Children's Way, Little Rock, AR 72202* |
| *Phone* | *501-296-1100* |
| *Email* | *jameslaurap@uams.edu* |
| **Statistician** | |
| *Name, degree, title* | Songthip Ounpraseuth, PhD<br>Associate Professor, Biostatistics |
| *Institution* | *University of Arkansas for Medical Sciences* |
| *Address* | *4301 W. Markham St., Little Rock, AR 72205* |
| *Phone* | *501-686-7233* |
| *Email* | *stounpraseuth@uams.edu* |
| **Duke Coordinating Center (DCC) PI** | |
| Name, degree, title | Jason Lang, MD, MPH<br>Associate Professor, Allergy, Immunology, and Pulmonary Medicine |
| Institution | *Duke Clinical Research Institute* |
| *Address* | *203 Research Drive, Durham, NC 27710* |
| *Phone Number* | *919-684-9590* |
| Email | *jason.lang@duke.edu* |
| **Data Coordinating and Operations Center (DCOC) PI** | |
| Name, degree, title | Jessica Snowden, MD<br>Associate Professor, Pediatric Infectious Diseases |
| Institution | *Arkansas Children's Research Institute /*<br>*University of Arkansas for Medical Sciences* |
| *Address* | *13 Children's Way, ACRI Slot 512-35, Little Rock, AR 72202* |
| *Phone Number* | *501-364-4693* |
| Email | *jsnowden@uams.edu* |
| ***Medical Monitor*** | |
| Name, degree, title | Rachel Greenberg, MD, MB, MHS<br>Assistant Professor, Pediatrics |
| Institution | *Duke University School of Medicine* |
| *Address* | *300 West Morgan St, Suite 800, Durham, NC 27701* |
| *Phone Number* | *(919) 668-4725* |
| Email | *rachel.greenberg@duke.edu* |

The clinical trial outlined in this protocol is part of the ISPCTN, a branch of the ECHO program supported by the NIH. The data coordination, technical instruction, data standards, quality control and assurance, and operational coordination for the clinical trial protocol outlined here (and for the ISPCTN overall) is provided by the DCOC. The work of ISPCTN is governed by the Steering Committee, which includes representatives from all 17 state awarded clinical trial sites, as well as representatives from the DCOC and the NIH. Overseeing the work of the Steering Committee is the NIH ECHO Office, as well as an executive Leadership Committee. This clinical trial protocol was completed by a team of content experts and DCOC staff and was reviewed by the members of the Airways Working Group within the ISPCTN. Further review was completed by the NIH Protocol Review Committee as well as a DSMB.

### 10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise, including pediatrics, PK, and biostatistics. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet on a regular basis, per the DSMB charter, to assess safety and efficacy data of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the NIH and the IND/sponsor.

The role of the Medical Monitor (MM) is to provide input on safety considerations, evaluate safety trends, and to provide oversight throughout the life cycle of the clinical research, in accordance with the approved protocol. This role includes review and monitoring of safety events on a regular basis, advising the protocol investigators on trial-related medical questions or problems, as needed, and to review cumulative participant safety data and make recommendations regarding the data to the Data Safety Monitoring Board. The Medical Monitor will remain blinded to treatment assignment during safety event review, unless unblinding is warranted to optimize management of an adverse event or for other safety reasons.

### 10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial complies with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by a member of the DCOC clinical operations staff or their designee.
- Monitoring will be planned to be conducted on site or remotely at least once during the course of the study and if needed for cause.

- Details of clinical site monitoring are documented in the Clinical Monitoring Plan, which will be included in the MOP. The plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

Independent audits of the monitoring system will not be conducted by the ISPCTN DCOC.

### 10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection (if applicable), documentation, and completion. Each site will follow the trial-specific MOP and any additional written site-specific processes, as applicable. These processes may include, but not necessarily be limited to: (a) procedures for recruitment, (b) data collection, entry, review and submission processes, (c) roles and responsibilities of site personnel, and (d) training methods for study staff.

Following the DCOC specific processes on monitoring, the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, the trial-specific MOP, site-specific processes, the [ICH] E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1), and applicable regulatory requirements.

Quality control (QC) procedures for the database and DCOC-maintained records will be implemented in accordance with the MOP, DSMB charter, and applicable DCOC and site-specific processes. Information about any data anomalies will be communicated to the site(s) for clarification/resolution. There will be electronic field checks, database listings, and quality control reports implemented to monitor data quality.

The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the DCOC and inspection by local and regulatory authorities. Issues uncovered during Quality Assurance (QA), QC, or monitoring activities will be addressed by making simple corrections or doing a root cause analysis, followed by instituting Corrective and Preventative Action (CAPA), as appropriate and as described in the MOP.

Vendors performing study activities, such as specimen processing, will also be evaluated prior to study start to ensure they have in place standard operating procedures. Quality checks will be implemented to ensure they are following the trial-specific MOP, their standard operating procedures and the protocol.

## 10.1.9 DATA HANDLING AND RECORD KEEPING

### 10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

The data and workflow for the study will be described and documented in a formal data management plan. The data management plan and associated documentation will specify all operations performed on data from origination to database lock, including detailed descriptions of source documentation, CRFs, instructions for completing forms, data handling and record keeping procedures, procedures for data monitoring, and reconciliation procedures and coding dictionaries to be used, if applicable. The data management plan will also describe the specific data collection and management responsibilities required of the sponsor, PI, the award site, clinical site(s), laboratory/laboratories (if applicable), and the DCOC The data management plan contents will be consistent with those described in the Good Clinical Data Management Practices (GCDMP). Components of the data management plan documenting operations performed on the data will be provided to the PI for review and approval prior to implementation.

Data collection is the responsibility of the clinical trial staff at the individual sites under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents must be completed using standard good documentation practices (attributable, legible, contemporaneous, original, and accurate).

Hardcopies of any data recorded on paper CRFs or study visit worksheets/assessment forms will be provided for use as source documents for recording data for each participant enrolled in the study. Data recorded in eCRFs derived from source documents must be consistent with the data recorded on the source documents.

Clinical data (including demographics, physical examinations, and intervention-specific questionnaires) and applicable clinical laboratory data will be entered into a 21 CFR Part 11-compliant data capture system provided by the ISPCTN DCOC at the UAMS. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Data submitted from sites to the DCOC will not be de-identified since the data will include date of birth and may include other information that could possibly be linked back to the participant. Data will be de-identified before sharing externally.

### 10.1.9.2 STUDY RECORDS RETENTION

Throughout the course of the study, all sites will retain a copy of the source documents on site in accordance with current site-specific medical record storage procedures. All study documents must be retained in accordance with local and/or federal regulations, whichever is most stringent. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

### 10.1.10 PROTOCOL DEVIATIONS

Protocol deviations are any instances in which the protocol, or any applicable DCOC or site-specific processes or applicable regulations are not followed as written. Deviations are not allowed; all deviations shall, therefore, be recorded in the study source documents. Whenever a deviation occurs, there must be an assessment of the severity and risk of the deviation. Depending on the results of the assessment, either a corrective action or a simple, 1-time correction shall be implemented. A corrective action institutes a process designed to keep that specific problem from happening again. Typically, a determination of the root cause of the problem is completed in order to develop the most appropriate corrective action plan.

The specific methods for handling protocol deviations will be provided in the study-specific MOP and/or site-specific processes.

### 10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- **NIH Public Access Policy**, which ensures that the public has access to the published results of NIH-funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

- **ISPCTN Publications and Presentations Policy**, which ensures accurate, responsible, and efficient communication of findings from ISPCTN clinical trials. The ISPCTN Publications and Presentations Policy has been approved and ratified by the ISPCTN Steering Committee, which includes representatives from all awarded clinical trial sites, as well as representatives from the NIH and the DCOC.

- This trial will be registered at ClinicalTrials.gov**,** and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this trial may be requested from other researchers by contacting Dr. Jeannette Lee at the DCOC.

### 10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH ECHO Office has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

## 10.2 ABBREVIATIONS

| | |
|---|---|
| 1,25D3 | 1,25 hydroxy-vitamin D3 |
| 25(OH)D | 25-hydroxy-vitamin D |
| ACT | Asthma Control Test |
| AE | Adverse Event |
| AR | Adverse Reaction |
| ASD | Asthma Symptom Days |
| BMI | Body Mass Index |
| c-ACT | Child-Asthma Control Test |
| CAMP | Childhood Asthma Management Program |
| CAPA | Corrective and Preventative Action |
| CAP | College of American Pathologists |
| CBC | Complete Blood Count |
| CFR | Code of Federal Regulations |
| CLIA | Clinical Laboratory Improvement Amendments |
| CMP | Clinical Monitoring Plan |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRF | Case Report Form |
| DCC | Data Coordinating Center |
| DCOC | Data Coordinating and Operations Center |
| DSMB | Data Safety Monitoring Board |
| ECHO | Environmental Influences on Child Health Outcomes |
| eCRF | Electronic Case Report Forms |
| ED | Emergency Department |
| FDA | Food and Drug Administration |
| GCDMP | Good Clinical Data Management Practices |
| GCP | Good Clinical Practice |
| HIPAA | Health Insurance Portability and Accountability Act |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| ICS | Inhaled Corticosteroid |
| IL | Interleukin |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |
| ISPTCN | The IDeA States Pediatric Clinical Trials Network |
| ITT | Intention-To-Treat |
| LABA | Long-acting Beta-agonist |
| LSMEANS | Least-squares Means |
| LTRA | Leukotriene Receptor Antagonist |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MOP | Manual of Procedures |
| NCT | National Clinical Trial |
| NHANES | National Health and Nutrition Examination Survey |

| | |
|---|---|
| NIH | National Institutes of Health |
| OHRP | Office for Human Research Protections |
| PHI | Protected Health Information |
| PI | Principal Investigator |
| PK | Pharmacokinetic(s) |
| PTH | Parathyroid Hormone |
| QA | Quality Assurance |
| QC | Quality Control |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SAR | Serious Adverse Reaction |
| SoC | Standard of Care Comparator Group |
| SMART IRB | Streamlined, Multisite, Accelerated Resources for Trials IRB Reliance platform |
| SOA | Schedule of Activities |
| TBD | To Be Determined |
| TNFα | Tumor Necrosis Factor Alpha |
| UAMS | University of Arkansas for Medical Sciences |
| ULN | Upper Limit Normal |
| UP | Unanticipated Problem |
| US | United States |
| USP | United States Pharmacopeia |
| VIDA | Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma |
| VDORA | Vitamin D Oral Replacement in Asthma |

## 11 REFERENCE

1 Schoenmakers I, Jones KS. (2018) Pharmacology and Pharmacokinetics. In D. Feldman, Vitamin D, Vol. 1: Biochemistry, Physiology, and Diagnostics (4th ed., pp. 635-661). London, England: Elsevier.

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