# SITE Protocol

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# 1. ABOUT THIS PROTOCOL

The purpose of this Protocol is to establish the framework for the Vit-D Kids Asthma Study, including defining the hypothesis, aims, and outcome measures; identifying members of the research team; describing potential risks and discomforts associated with participation and the importance of informed consent; outlining a system for safety monitoring, including tracking adverse events; and specifying a model for statistical analysis.

Additions, deletions and other modifications will be made by the Data Coordinating Center (DCC), in consultation with the PI and co-PIs. The expectation is that study personnel will maintain electronic and hard copies, for quick and easy reference.

# 2. BACKGROUND AND SIGNIFICANCE

Asthma is a major public health problem in the United States[1] and worldwide[2]. Severe disease exacerbations account for the majority of costs attributable to asthma in the United States[3].

Vitamin D is an essential nutrient with significant immuno-modulatory effects[4,5]. The observation that vitamin D deficiency and asthma share risk factors such as urban residence[6,7], obesity[8,9], and African American ethnicity[10,11] has generated significant interest in exploring a link between these two conditions (recently reviewed in detail by Celedón and colleagues[12]).

Results of experimental studies and genetic association studies of the vitamin D receptor (VDR)[12-14] have motivated observational studies of vitamin D and asthma in humans. These observational studies have differed in study design, sample size, and assessment of vitamin D status, which may explain their seemingly conflicting findings[12]. Although there is insufficient evidence of a causal association between vitamin D status and asthma *per se*, reports of an inverse association between maternal intake of vitamin D during pregnancy (or cord blood vitamin D levels) and childhood wheeze[15-18] have motivated ongoing clinical trials of vitamin D supplementation during pregnancy for primary prevention of asthma in the U.S. and Denmark[12].

In addition to a potential role in the primary prevention of asthma, there is considerable interest in assessing whether vitamin D protects against or reduces asthma morbidity. We found that vitamin D insufficiency or deficiency (defined as a 25[OH]D level <30 ng/ml (75 nmol/L)) was present in 175 (28%) of 616 children with asthma in Costa Rica[19], in whom serum vitamin D level was inversely associated with total IgE, eosinophil count, hospitalizations for asthma, use of anti-inflammatory medications and airway hyper-responsiveness[19]. To follow up on those results, Brehm et al. conducted a longitudinal study of serum vitamin D and severe asthma exacerbations (defined as at least one hospitalization or visit to the Emergency Department for asthma) in 1,024 North American children with mild to moderate persistent asthma [20]. In that study, vitamin D insufficiency or deficiency (a 25[OH]D level < 30 ng/ml (75 nmol/L)) at baseline was associated with increased risk of severe asthma exacerbations during four years of follow-up. The magnitude of the observed association was greater in children who did not receive inhaled corticosteroids (ICS) and who had vitamin D insufficiency than in children who received ICS but had vitamin D insufficiency or in those who did not receive ICS but had sufficient levels of vitamin D. This finding and others[12] suggest that vitamin D enhances steroid responsiveness.
Further evidence that vitamin D may protect against severe exacerbations is given by two small trials in children and the VIDA trial in adults. A 6-month trial of vitamin $D_3$ (500 IU/day) as adjuvant to ICS in 48

Polish children with newly diagnosed asthma[21] found no difference in 25(OH)D level between treatment groups. However, children in the intervention arm were less likely to have 25(OH) D level that decreased during the trial, and there were fewer children with an "exacerbation" in the vitamin D (4, 17%) than in the placebo (11, 46%) arm (P<0.05). A 6-month trial of vitamin D (2,000 IU/day) was conducted in 100 Indian children aged 5-13 years with moderate/severe asthma: 82 completed follow up[22]. At the 6-month visit, children in the intervention arm had a lower disease severity and fewer "exacerbations" (14 or 28% vs. 30 or 60%, P=0.01), and lower ICS requirements than those in the placebo arm[22]. These trials are inconclusive because of: inclusion of children with normal/unknown vitamin D level[21,22]; no data on treatment[22] or adherence[21,22]; no[21]/unknown[22] occurrence of severe exacerbations, and low vitamin D dose[21].

The six-month VIDA trial of vitamin D (100,000 IU, followed by 4,000 IU/day) to prevent treatment failure in 408 adults with symptomatic persistent asthma ($FEV_1$ ≥50% of predicted) on ICS and vitamin D insufficiency reported no effect on the primary outcome (treatment failure)[23]. However, there was a 37% reduction in the overall exacerbation rate (0.26 person/yr) in the vitamin D arm compared with the placebo (0.40 person/yr) arm (95% CI for HR=0.39-1.01, P=0.05). Moreover, when the 157 vitamin $D_3$-treated subjects who achieved a 25(OH)D ≥30 ng/ml (75 nmol/L) (78% of 201 subjects in the intervention arm) were compared to 207 subjects on placebo, the risk of first exacerbation was 43% lower in the vitamin D (11%) than in the placebo (19%) arm (95% CI for HR=0.33-0.99, P=0.05). Compared with subjects on placebo, those who achieved vitamin D sufficiency had overall rates that were also 40% and 50% lower for treatment failure (95% CI=0.4-0.9, P=0.03) and exacerbations (95% CI =0.3-0.8, P=0.01), respectively. Of note, the rate of increment in 25(OH)D from baseline to 12 weeks was associated with a reduction in the overall rates of treatment failure and exacerbations, emphasizing the need to achieve rapid correction of vitamin D insufficiency in future trials, such as the one proposed. Among all subjects, those on vitamin D required an overall lower dose of budesonide for asthma control (by 14.9 μg/day) than those on placebo (Bonferroni-corrected P =0.03). Given negative results for the primary outcome (treatment failure) and secondary outcomes such as lung function, VIDA is inconclusive. The 6-month VIDA trial was likely too short to adequately examine severe exacerbations, and the time needed to achieve sufficiency further accentuated this. Moreover, adults may have airway remodeling (reducing anti-inflammatory effects of vitamin D), and severe exacerbations were not defined by current standards. Further studies (particularly in children) are needed.

## 3. STUDY DESIGN

This is a 48-week randomized, double-masked, controlled trial of vitamin $D_3$ (4,000 IU/day) to prevent severe asthma exacerbations in 400 high-risk children (aged 6 to 16 years) who have vitamin D insufficiency (serum 25(OH)D <30 ng/ml (75 nmol/L)) and are well-controlled on low-dose ICS at the end of a run-in period. After stratification by race/ethnicity and study site, using a permuted-blocked strategy, participants will be randomized in a 1:1 ratio to either 4,000 IU/day of vitamin D3 or placebo and then monitored, through clinic visits and phone calls. Participants will be recruited starting February 2016, and all randomized participants will be followed for 48 weeks. Based on our randomization goals, the final children will be randomized by September 2019, and will complete their last visit in early September 2020.

## 3.1. Hypothesis and Specific Aims

We hypothesize that vitamin D supplementation reduces the incidence of severe asthma exacerbations in high-risk (so defined due to a recent exacerbation) children aged 6-16 years with vitamin D insufficiency. We further hypothesize that this protective effect results from reducing the severity of viral infections and enhanced response to ICS.

The primary specific aim is to determine whether high-dose vitamin $D_3$ supplementation (4,000 IU/day) is superior to placebo in preventing severe asthma exacerbations in high-risk school-aged children who have vitamin D insufficiency and who are on ICS for mild to moderate persistent asthma.

The secondary specific aims are to determine whether, among high-risk school-age children with vitamin D insufficiency, vitamin $D_3$ supplementation of 4000 IU/day is superior to placebo in:

- Preventing severe asthma exacerbations resulting from viral infections
- Reducing the daily dose of ICS, as well as the average cumulative dose of ICS, by the end of the trial

## 3.2. Outcome Measures

The primary outcome of the proposed trial will be severe asthma exacerbations, which will be defined following the most recent American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines[24], as the occurrence of either:

1) Use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days; OR
2) A hospitalization or ER visit because of asthma, requiring systemic corticosteroids.

Secondary outcome measures are:

1) Severe asthma exacerbations resulting from viral infections
2) Reduction in ICS dose at visit 6
3) Average cumulative dose of ICS at the end of the trial

## 3.3. Questionnaires

Eight questionnaires will be administered to the parents of participating children throughout the study (see Figure 1): 1) respiratory/general health (slightly modified from one used by the Collaborative Study of the Genetics of Asthma and used in the Genetics of Asthma in Costa Rica Study [GACRS][25]), 2) household characteristics (modified from ISAAC[26] and previously used in the GACRS), 3) the subset of questions related to asthma therapy from the pediatric version of the asthma therapy assessment questionnaire (ATAQ)[27], 4) the child or original version of the Asthma Control Test® (C-ACT[28] or ACT[29]) based on age, 5) the pediatric version of the Asthma Quality of Life® (PAQLQ)[30] and 6) the Checklist of Children Distress Symptoms (CCDS)[31]. Data obtained from these questionnaires will include a detailed history of asthma (e.g., age of onset of symptoms, hospitalizations, visits to the ED or urgent care, medication use, symptom triggers), asthma severity and control, past medical history (including gastroesophageal reflux and allergic diseases [allergic rhinitis, sinusitis, or eczema]), family history of asthma and allergies, and immunotherapy. In addition, data will be obtained on indicators of socioeconomic status (household income, employment status, marital status, paternal and maternal education), pet exposure (past and current), and household characteristics.

## 3.4. ANTHROPOMETRY

We will measure weight, height, and calculate BMI in all participants at visits 2 and 9. We will measure waist and hip circumferences according to standardized protocols, and we will assess body composition using bioelectrical impedance analysis (BIA). BIA is a method that measures the body's resistance and reactance and allows the calculation of total body water, total body fat, and total lean mass.

# 4. FUNDING/SUPPORT

This study is funded by the National Heart, Lung and Blood Institute of the National Institutes of Health ((NIH 1U01HL119952-01A1).

# 5. STUDY ORGANIZATION AND LEADERSHIP

## 5.1. Study Principal Investigators

The study's principal investigators (PIs) are **Juan Carlos Celedón, MD, DrPH** and **Dr. Stephen Wisniewski, PhD**. Dr. Celedón is Chief of Pediatric Pulmonology, Allergy and Immunology, and the Niels K. Jerne Professor of Pediatrics at the University of Pittsburgh. A physician-scientist, Dr. Celedón is involved in research, clinical activities and mentoring at Children's Hospital of Pittsburgh and the University of Pittsburgh. He has a broad background in respiratory and genetic epidemiology, and has had formal education and training in genetics and epidemiology, along with significant administrative research experience. He laid the groundwork for the Vit-D Kids Asthma Study by leading or participating in other NIH-funded studies of childhood asthma; publishing on observational studies linking vitamin D insufficiency and severe asthma exacerbations, comprehensive reviews on vitamin D and asthma, and severe asthma exacerbations in children; conducting a pilot study to determine optimal dosing of vitamin D to correct vitamin D insufficiency in children with asthma; and establishing several multidisciplinary and multicenter research collaborations.

Dr. Wisniewski a Professor of Epidemiology, Associate Vice-Provost for Planning, and Advisor to the Dean of the Graduate School of Public Health for special projects, all at the University of Pittsburgh. In the context of this study, he is the Co-Director of the Epidemiology Data Center (EDC) at the University of Pittsburgh Graduate School of Public Health, and the Principal Investigator and Director of the Data Coordinating Center.

## 5.2. Clinical Coordinating Center

The study will be conducted at four clinical centers in Pittsburgh, Saint Louis, Cleveland and Boston under the auspices of a Clinical Coordinating Center (CCC) chaired by Dr. Celedón, PI of the Pittsburgh site. The CCC will arrange for bi-weekly conference calls including the PIs (rotating attendance on a weekly basis, so that each PI attends a call every eight weeks) and study coordinators at all sites, to discuss recruitment and protocol issues. The CCC will also serve as the liaison to the sites' IRBs, preparing and submitting applications and modifications and addressing concerns, and ensure compliance with HIPAA regulations.

## 5.3. Data Coordinating Center

The Epidemiology Data Center (EDC) of the Graduate School of Public Health at the U. of Pittsburgh, will serve as the Data Coordinating Center (DCC). Under the direction of Dr. Stephen Wisniewski, co-director of the EDC, the DCC will develop and maintain systems for data management, study monitoring, quality control, and data analysis. In addition, the DCC will handle regulatory and administrative functions, including scheduling meetings, conference calls, and web-conferences for team members; identify milestones; prepare reports for the DSMB, NHLBI, and other agencies; draft and update this protocol, train and certify study personnel; coordinate the publications process; and facilitate internal and external communication by creating and maintaining a public website, as well as a password-protected intranet, where study-related data and other information can be shared among members of the team.

Quality control will begin with a two-day workshop to be conducted jointly by the CCC and DCC, at which study protocols will be distributed and carefully explained to the field staff. Bi-weekly conference calls with the DCC and the PIs/coordinators of the CCC sites will be scheduled to address any concerns regarding collection and quality of data.

Throughout the duration of the study, the DCC will conduct biannual site visits to monitor the quality of data collection processes, conduct Source Document Verification; assure the reporting and documentation of adverse events, and assess security of confidential records and informed consent procedures. Site visit reports will be sent to the PI and included in DSMB reports. In addition, during these contacts, the monitor will: check and assess the progress of the study; review study data collected; identify any issues and address their resolution; and review the storage of samples. This will be done in order to verify that data are authentic, accurate, and complete, the safety and rights of subjects are being protected, and that the study is being conducted in accordance with the currently approved protocol (and any amendments) and all applicable regulatory requirements.

## 5.4. Site Principal Investigators and Responsibilities

In addition to heading up the Vit-D Kids Asthma Study, **Dr. Celedón** is the PI for the Pittsburgh site.

The PI for the Washington University (U.) at Saint Louis (St. Louis) site is **Leonard Bacharier, MD**. Dr. Bacharier is the Donald B. Strominger Professor of Pediatrics, Clinical Director of the Division of Pediatric Allergy, Immunology & Pulmonary Medicine, and Unit Co-Leader of the Pediatric Patient Oriented Research Unit at Washington U. in St. Louis. Dr. Bacharier has been an active investigator in several NIH-funded asthma programs, including the NHLBI's Childhood Asthma Management Program (CAMP) and Childhood Asthma Research and Education (CARE) Network programs. He is currently Co-Principal Investigator (PI) of the NHLBI's AsthmaNet St. Louis site, PI of the St. Louis site of the NIAID's Inner City Asthma Consortium, and PI for the CCC of the NHLBI's Vitamin D Antenatal Reduction Trial. His major research interest is asthma in early life, including factors related to disease inception along with novel therapeutic strategies for the disorder. This has resulted in over 115 scientific articles and 17 book chapters.

The PI of the Boston Children's Hospital (Boston) site is Wanda Phipatanakul, MD, MS, a Professor of Pediatrics at Harvard Medical School. Dr. Phipatanakul has a long-standing history of recruiting for pediatric asthma clinical trials of all ages. Her databases include thousands of eligible young children interested in studies, including children attending the BCH Allergy/Asthma Clinic for allergy skin testing. Dr. Phipatanakul has had success recruiting study participants from this Asthma/Allergy Clinic, which during this past year saw nearly 5,000 children with asthma. Her Center has always met or exceeded

recruitment goals in pediatric clinical trials, such as the NHLBI-funded AsthmaNet. Dr. Phipatanakul has authored or co-authored over 130 peer-reviewed manuscripts on asthma and allergies.

The PI of the Rainbow Babies and Children's Hospital (Cleveland) site is Kristie Ross, MD, MS, an Assistant Professor of Pediatrics at Case Western Reserve University and the Clinical Director of the Division of Pediatric Pulmonary Medicine, Allergy and Immunology, and Sleep Medicine at Rainbow Babies and Children's Hospital. Dr. Ross has successfully served as an investigator in the NIH funded AsthmaNet asthma clinical trials network, the Inner City Asthma Consortium, the Severe Asthma Research Program, and many industry sponsored clinical trials in asthma, cystic fibrosis, and sleep medicine. She currently serves as the co-lead of the Clinical Studies Core of a translational program project grant in airway biology, overseeing clinical protocol development and implementation of all clinical studies across the three projects.

The PI of the Cincinnati Children's Hospital and Medical Center (CCHMC) site is Theresa Guilbert, MD, MS, a Professor of Pediatrics at the University of Cincinnati and the Associate Director of the Asthma Center at Cincinnati Children's Hospital. Dr. Guilbert has 18 years of experience in clinical and epidemiologic research. Dr. Guilbert was a key Co-Investigator in the NHLBI-funded AsthmaNet and Severe Asthma Research Program networks, where she served in the steering committees. She is currently the site PI for a multi-center NIH-funded trial to determine if IgE monoclonal antibody biologic therapy will prevent asthma development in preschool-aged children.

The PI of the National Jewish Health (Denver) site is Ronina Covar, MD, an Associate Professor of Pediatrics at the University of Colorado. Dr. Covar has ample experience as a Co-Investigator and site Principal Investigator in NIH-funded clinical trials. She served as the Medical Director and Co-Investigator for the Denver site of the Childhood Asthma Management Program (CAMP, from 1999 to 2014) and the Childhood Asthma Research Education network (CARE, from 2004 to 2012), and as a Co-Investigator for the Denver site of AsthmaNet (2010-2018).

As PIs of the St. Louis, Boston, Cleveland, Cincinnati, and Denver sites, Drs. Bacharier, Phipatanakul, Ross, Guilbert, and Covar will supervise training of study personnel, data collection, quality control of the data collected, transition of study personnel, human subjects protection (including communications with the IRB, the DCC, the FDA and the NHLBI staff, as appropriate), and communications with the DCC. They will participate in biweekly phone calls of the Steering Committee for the trial, weekly meetings of each site for the CCC, and (on a rotating basis with the PIs at the other sites) the bi-weekly conference calls of the coordinators for the study sites.

Please note that the study previously had another study site at the University of California in San Francisco (UCSF), led by Dr. Michael Cabana. The UCSF study site was terminated from conducting new randomizations in March of 2017, due to insufficient subject recruitment. Six participants had been randomized at UCSF prior to termination, and those participants completed the trial in August of 2017.

## 5.5. Co-Investigators

**Erick Forno, MD, MPH,** Assistant Professor in Pediatrics and Medicine, University of Pittsburgh, was a key member of the team that designed and implemented the pilot study that provided some of the preliminary data for the Vit-D Kids Asthma Study. His research career has focused primarily on the epidemiology of asthma, with a specific interest in the etiology of severe asthma exacerbations. Dr. Forno will assist Dr. Celedón in training study personnel, supervising data collection and quality control

of the data collected, human subjects protection (including communications with the IRB, the DCC, the FDA and the NHLBI staff, as appropriate), and communications with the DCC. He will participate in biweekly phone calls of the Steering Committee for the trial, weekly meetings of the Pittsburgh site for the CCC, and monthly meetings with the DCC.

**Nadia Boutaoui, PhD**, Research Assistant Professor in Pediatrics, University of Pittsburgh and Laboratory Director, Division of Pulmonary Medicine, Allergy and Immunology, Children's Hospital of Pittsburgh, is a molecular biologist/geneticist with an interest in understanding the effect of genetic, epigenetic and environmental exposures on human health, particularly on respiratory diseases such as asthma. She has extensive technical experience in molecular biology, genetics and epigenetics (including DNA/RNA extraction, genotyping, gene expression studies and pyrosequencing), and in measuring intermediate phenotypes of asthma, such as serum total and allergen-specific IgE. She will supervise shipment and processing of all blood samples sent from the study sites to the Pittsburgh site, as well as processing of all samples (blood and nasal epithelium) collected at the Pittsburgh site.

## 5.6. Consultants

**Augusto A. Litonjua, MD, MPH**, Associate Professor of Medicine, Harvard Medical School, Associate Physician, Brigham and Women's Hospital, and Associate in Medicine, Beth Israel Deaconess Medical Center, is the Co-PI of the Vitamin D Antenatal Asthma Reduction Trial (VDAART) and an expert in asthma epidemiology, with a particular focus on the role of vitamin D in the pathogenesis of asthma.

# 6. STUDY POPULATION

Recruitment will be carried out by clinical centers in Pittsburgh, Cleveland, St. Louis and Boston.

## 6.1. Inclusion Criteria

Inclusion criteria to be eligible for run-in:

- 6 to 16 years old
- Physician-diagnosed asthma for at least one year
- At least one severe asthma exacerbation in the previous year[1]
- Use of asthma medications (daily controller medication [ICS or leukotriene inhibitor] or inhaled $\beta_2$-agonist [at least three days per week]) for at least six months in the previous year[2]
- Vitamin D insufficiency (i.e., serum vitamin D (25(OH)D level <30 ng/ml (75 nmol/L))
- $FEV_1$ ≥70 % of predicted

1 Use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days; OR a hospitalization or ER visit because of asthma, requiring systemic corticosteroids. In other words, most asthma exacerbations treated with oral or IV steroids would be considered severe.

2 For at least 6 months in previous year (not necessarily consecutive), used an Asthma controller (inhaled corticosteroid or leukotriene inhibitor) on a daily basis OR an inhaled β2-agonist at least three days per week. Common controller medications: Flovent (fluticasone), Pulmicort (budesonide), Advair, Asmanex (mometasone), Symbicort, Qvar (beclamethasone), Singulair (montelukast). Common inhaled β2-agonists: albuterol (Ventolin, ProAir, Proventil), Xopenex.

- Positive BDR (i.e., increase in $FEV_1$ ≥8% from baseline after inhaled short acting beta agonist [recently shown to be a better predictor of asthma in school-aged children than a cutoff of 12%[32]]) or increased airway responsiveness to methacholine ($PC_{20}$ ≤8 mg/ml if not on ICS or $PC_{20}$ ≤16 mg/ml if on ICS)
- Study protocol (i.e., age-appropriate dose of Flovent and no other asthma controller medications) approved by the child's regular doctor
- Parental consent and child's assent to participate in the study.

Additional inclusion criteria applied after the run-in period, to be eligible for randomization:

- Adherence with ICS and study medication (≥75% use) during the run-in period
- Willingness to be randomized and complete study

## 6.2. Exclusion Criteria

Exclusion criteria for entering the run-in period:

- Serum calcium >10.8 mg/dl
- Serum 25(OH) D <14 ng/ml (35 nmol/L)[3]
- Chronic respiratory disorder other than asthma[4]
- Severe asthma[5]
- Hepatic/renal disease, rickets, malabsorption, or other diseases that would affect vitamin D metabolism
- Current smoking, or former smoking if ≥5 pack-years
- Immune deficiency, cleft palate (not including the occult submucosal variant) or Down's syndrome[6]
- Treatment with anticonvulsants or ≥1,000 IU/day of vitamin $D_2$ or $D_3$
- Chronic oral corticosteroid therapy[7]
- Inability to perform acceptable spirometry (defined as meeting Grade A, B, or C level)
- Use of investigational therapies or participation in trials 30 days before or during the study[8]
- Participant is currently breast feeding an infant
- Pregnancy
- Weight less than 10 kg
- Plans to move out of the clinical site area in the next year

Additional exclusion criteria applied after the run-in period:

- Any severe asthma exacerbation during the run-in period
- Need for asthma medications other than ICS and p.r.n. rescue inhalers during the run-in period

3 Children with a vitamin D level lower than 14 ng/ml will be referred to a pediatric endocrinologist for further evaluation.

4 Examples include bronchiectasis, cystic fibrosis, lung transplant, bronchopulmonary dysplasia

5 Intubation for asthma at any time OR ≥3 hospitalizations for asthma in previous year OR ≥6 severe asthma exacerbations in previous year

6 These conditions may increase the risk of respiratory infections.

7 Steroids taken regularly used to control asthma symptoms, and not simply to treat an asthma exacerbation.

8 Investigational therapies include drugs that are not FDA approved, and which are being studied in a clinical trial. A clinical trial is defined by studies that provide children with an intervention (including both drug and non-drug). Clinical studies that do not have an intervention are allowed.

## 6.3. Reconsideration for Inclusion after Run-In Period

A child who had a severe asthma exacerbation during the run-in period may be eligible for inclusion in the study if he/she has no severe asthma exacerbations after completion of a second (new) run-in period. See sections 12.2 through 12.4 for discussion of re-screening criteria.

## 6.4. Rationale for Selecting Children with a Severe Asthma Exacerbation

Children who have had at least one severe asthma exacerbation in the prior year are at increased risk for subsequent severe disease exacerbations. Participants will thus be selected on the basis of having had a severe asthma exacerbation in the prior year.[24]

# 7. STUDY MEDICATIONS

According to the 2007 guidelines developed by the NAEPP (National Asthma Education and Prevention Program) Expert Panel Report 3, 176 mcg total daily dose (88 mcg twice a day) of fluticasone is an acceptable low dose of ICS for children aged 6-11 years, and 220 mcg total daily dose (110 mcg twice a day) of fluticasone is an acceptable low dose of ICS for children 12 years and older. Children who are currently on these age-appropriate doses will continue on this medication. Children on a different dose, or on a different ICS, will be switched to fluticasone, which will be dosed according to their age (88 mcg inhaled twice a day for children 6-11 years, and 110 mcg bid for children 12-16 years). Children who turn 12 after randomization will continue the standard dose on which they were randomized.

In the absence of moderate or severe asthma exacerbations, participants may have their dose of ICS reduced by 50% if the following criteria are met at visit 6 (halfway through the Trial Phase):

- ACT score greater than 19
- Both pre-bronchodilator FEV1 and FEV1/FVC ≥80% of predicted
- Use of ≤4 puffs of a rescue inhaler per week
- ≤1 day per month with asthma symptoms preventing full participation in usual daily activities
- Clinician's judgment regarding adequate asthma control

Participants who develop a moderate to severe asthma exacerbation while on a reduced dose of ICS will be put back on their original dose of ICS.

The vitamin $D_3$ and placebo formulations will be in gel cap form and manufactured by Pharmavite (Mission Hills, CA). The gel caps will be manufactured using the same practices as for the currently marketed drug. Since these doses of vitamin $D_3$ are not FDA-approved for an asthma indication, this study is operating under an Investigational New Drug (IND) protocol, with monitoring by the FDA.

Data provided to the Data and Safety Monitoring Board (DSMB) will be labeled Treatment A or B. If the DSMB feels that knowledge of treatment groups is necessary for safety or data quality purposes, then the DCC will provide a code for study groups. Treatment assignment for individual subjects will be revealed only in the event of a medical emergency in which unmasking could change treatment. The Independent Safety Monitor (ISM) may unmask treatment for a participant. Unmasked participants will complete any remaining protocol visits.

## 7.1. Rationale for Vitamin D3 Dose and Treatment Duration

The safe upper intake for vitamin $D_3$ was set at 3,000 IU/day for children aged 4-8 years and as 4,000 IU/day for children ≥9 years by an IOM panel in 2011[33]. A dose of 4,000 IU/day of vitamin $D_3$ was well tolerated in our pilot study, and used in studies of atopic dermatitis (NCT00789880) and maternal supplementation (NCT00920621). Moreover, vitamin $D_3$ doses of 5,500 and 11,000 IU daily for 20 weeks were well tolerated In relatively vitamin D-replete men (mean serum 25(OH)D=28 ng/ml (70 nmol/L))[34]. The dietary reference intake (DRI) of vitamin $D_3$ or the desired thresholds of serum 25(OH)D for non-skeletal effects are unknown. If the anti-infective or steroid-sparing effects of vitamin $D_3$ are dose-dependent, *using a low dose could result in a Type II error*. To reduce this concern, children aged 6 to 16 years will be randomized to receive either 4,000 IU of vitamin $D_3$) or placebo once daily for 48 weeks. If our proposed intervention dose is effective, dose-response studies can then be done to determine the lowest effective dose. The proposed *dose and duration of vitamin $D_3$ supplementation are safe* and would optimize vitamin D status while allowing time to accrue a sufficient number of exacerbations in study participants.

## 7.2. Rationale for Placebo in the Control Arm

To examine whether vitamin D supplementation, at a low dose of 200 IU/day, could increase vitamin D levels bordering 30 ng/ml (and thus lead to levels ≥30 ng/ml), we conducted a pilot trial of 24 children ages 6 to 14 years who had a vitamin D level <30 ng/ml, in which 8 children with vitamin D insufficiency received 200 IU/day of cholecalciferol for 8 weeks. Two of those children achieved a level ≥30 ng/ml at 4 weeks, and one of these children still had a level ≥30 ng/ml at 8 weeks. Therefore, supplementation with doses as low as 200 IU/day could lead to a false negative result if in fact a level ≥30 ng/ml suffices to prevent severe asthma exacerbations, as suggested by our prior work. This provides a scientific rationale for using placebo in the control arm.

Vitamin D supplementation is not indicated for children with vitamin D levels between 20 ng/ml and 29 ng/ml, thus justifying the use of placebo in this group of participants. The 2011 IOM Panel recommended vitamin D supplementation for children whose vitamin D levels are known to have vitamin D deficiency (a vitamin D level lower than 20 ng/ml), but did not recommend routine screening of vitamin D levels in healthy (asymptomatic) children. Moreover, the 2016 Global Consensus Recommendations on Prevention and Management of Nutritional Rickets do not advise routine measurement of vitamin D levels in healthy school-aged children[35]. Hence, most U.S. children with vitamin D deficiency are not screened for a low vitamin D level and do not receive any treatment, since they are often asymptomatic (due to the complex interplay between vitamin D deficiency and calcium intake in maintaining serum calcium concentrations and bone integrity)[35].

Current evidence suggests that a vitamin D level below 12-13.6 ng/ml (30-34 mmol/L) may be the critical threshold at which rickets could occur, with higher risk among children in whom deficiency (particularly at vitamin D levels below 10 ng/ml) is sustained over time (i.e. chronic deficiency)[35]. We are excluding children with vitamin D (25(OH)D levels <14 ng/ml (35 nmol/L)) from this trial, and our pilot study and recently published studies suggest that the vast majority of participants will not develop severe vitamin D deficiency (a vitamin D level <10 ng/ml) while on placebo. Therefore, using placebo is ethically acceptable for children with vitamin D levels between 14 ng/ml and 19 ng/ml, as there is no current indication for routine screening of children for vitamin D deficiency, and thus the risk to study participants is minimal and no greater than that encountered in daily life by healthy community-dwelling children. Moreover, we will provide dietary counseling to parents of study participants, while closely monitoring all participants for levels <14 ng/ml (35 nmol/L), which approach severe vitamin D deficiency

(25(OH)D (defined as < 10 ng/ml (25 nmol/L)) and rickets (see below).

The Pediatric Endocrine Society recommends a vitamin D intake of 400 IU/day for all children [36]. More recently, the IOM panel recommended a DRI of vitamin $D_3$ for children aged 6-14 years of 600 IU/day[33] . The IOM's recommendations were endorsed by the American Academy of Pediatrics in 2012, superseding the 2008 recommendations.[37] The average dietary intake of vitamin $D_3$ for school-aged children is 400 IU/day[33] (a dose used in multi- vitamins), which will prevent rickets. Parents of all participants enrolled in the trial will be provided with a list of foods that are rich in vitamin D, and that are part of a balanced diet that contains 400 IU/day of vitamin $D_3$, at the randomization visit (v3).

To further ensure the safety of participating children in the placebo group, we will provide additional dietary counseling to the parents of participating children whose vitamin D level is less than 14 ng/ml (35 nmol/L) at any study visit. Should the vitamin D level of these participants remain below 14 ng/ml (35 nmol/L) at the next scheduled safety testing (16 weeks later), they will be removed from the study medication and referred to a pediatric endocrinologist for further evaluation, while also providing the participant's parent or guardian with her/his vitamin D level at the time of referral. Should the vitamin D level of these participants be below 10 ng/ml (25 nmol/L) at any study visit, the participant will be removed from the study medication and referred to a pediatric endocrinologist for further evaluation, while also providing the participant's parent or guardian with his/her vitamin D level at the time of referral.

# 8. POTENTIAL BENEFITS

The response to available controller therapy for asthma (e.g., ICS) is variable, and there is a need to identify new treatments that can be used (alone or together with existing therapies) to prevent severe asthma exacerbations. This protocol will determine whether the addition of vitamin D to ICS reduces the risk of severe asthma exacerbations in children with asthma. If vitamin D supplementation were found to be effective, there would be important benefits for pediatric patients with asthma. Because we estimate the risks associated with this protocol to be low, we judge the potential benefit/risk ratio associated with this work to be highly favorable.

Participating children and their parents will be provided with background information regarding asthma. The results of tests conducted in participating children will be communicated to their primary care physicians (with parental approval). The child's parent or legal guardian will receive a letter with the research vitamin D level results at the end of the study or, if applicable, at the time of participant withdrawal from the study.

# 9. RISKS AND DISCOMFORTS

## 9.1. Procedures

1) Phlebotomy: the potential risks of venipuncture are minimal and include hematoma at the skin site, minimal pain of venous puncture, and, rarely, fainting. The approximate amount of blood drawn at each visit is shown below. Children weighing less than 10 kg will not be eligible for this study.

| Visit number | Specimens | Total blood (approximate) |
|---|---|---|
| Visit 1 (week -5) | Serum vitamin D + calcium | 3 ml |

| | | |
|---|---|---|
| Visit 2 (week -4) | None | 0 ml |
| Visit 3 (week 0) | Serum for biobanking, IgE (total and allergen specific), and vitamin D (10 ml)<br>CBC with differential (4 ml)<br>Blood DNA (10 ml) | 28 ml |
| Visit 4 (week 8) | None | 0 ml |
| Visit 5 (week 16) | Serum vitamin D (3 ml) | 3 ml |
| Visit 6 (week 24) | None | 0 ml |
| Visit 7 (week 32) | Serum vitamin D (3 ml) | 3 ml |
| Visit 8 (week 40) | None | 0 ml |
| Visit 9 (week 48) | Serum for biobanking, IgE (total and allergen specific), and vitamin D (10 ml)<br>CBC with differential (4 ml)<br>Blood DNA (10 ml) | 28 ml |
| Unscheduled visit for elevated UCa/UCr | Serum calcium | 3 ml |

2) Spirometry: the risks of spirometry are minimal and include lightheadedness from repeated spirometric maneuvers (blows) and (rarely) precipitation of bronchospasm, which would be treated by appropriate on-site medical and nursing personnel. Inhalation of either albuterol or levalbuterol (Xopenex) (to assess bronchodilator responsiveness) can cause tachycardia and tremulousness, which are short-lived and non-life threatening. We will use Xopenex for children who use this as their rescue inhaler. Other children will receive albuterol.

3) Methacholine challenge testing (MCT): the major risk of MCT is severe bronchospasm. As a precaution, participants will not undergo MCT if their $FEV_1$ is <70% of predicted. A trained technician will perform all MCT, and medications and equipment will be available at the study site to treat any major episodes of bronchospasm. Of note, we have not had any ED visit or hospitalization related to MCT in over 2,000 procedures conducted in children of school age for other studies of asthma, while using this protocol. More commonly, MCT can sometimes be associated with symptoms, including feeling of chest tightness, cough, or wheezing, which are of very short duration and can be promptly reversed or relieved by the bronchodilator (e.g. albuterol) administered at the end of the test.

4) Questionnaire administration: the only possible risk in questionnaire administration involves the social-psychological risk resulting from inadvertent disclosure of medical history information. To minimize this risk, questionnaires will be administered electronically whenever possible, using a secure data management system created by the DCC.

5) Genetic testing: Information about a child´s participation and results from the study will not be placed in the participant´s medical records. Participants will not be informed about any individual genetic data to be obtained from this study.

6) Measurement of serum vitamin D and calcium, and urine collection for measurement of calcium/ creatinine ratio: There are no known risks.

7) Nasal samples: The is a small risk of minor bleeding with nasal scrapings. We use a small (1/2 teaspoon) amount of lidocaine spray (not an injection) in each nostril to reduce discomfort. Temporary redness, stinging, and swelling may occur at the application site.

8) Break of confidentiality (overall risk): participant data will be identified by a unique study number assigned to each child, and entered into the Data Management System through a password-protected website.

9) Anthropometry: there are no known risks.

## 9.2. Study Intervention: Vitamin D

The current (2011) dietary reference intake (DRI) of vitamin D (600 IU/day) in children and adolescents is intended to promote skeletal health.[33] The recommended dietary allowance of vitamin $D_3$ or the desired thresholds of serum 25(OH)D for maximizing non-skeletal (e.g., immune-regulatory, anti-infective) effects of vitamin D are unknown. Should non-skeletal benefits of vitamin $D_3$ be dose-dependent, using an insufficient dose could result in a Type II error. This is particularly relevant to vitamin D levels shown to be potentially relevant to severe asthma exacerbations, which are higher (≥30 ng/ml (75 nmol/L) in studies conducted by our group, see Background) than the minimal vitamin D level accepted for adequate musculoskeletal health (≥20 ng/ml (50 nmol/L)).

The 2011 guidelines from the Institute of Medicine state that 3,000 IU/day is the highest intake (upper limit) of vitamin $D_3$ that children aged 4 to 8 years can consume without adverse effects such as hypercalcemia.[33] For children older than 8 years, the current upper limit of vitamin D intake is 4,000 IU/day. In a randomized one-year trial of weekly placebo vs. daily vitamin $D_3$ in 340 Lebanese children aged 10 to 17 years, daily intake of 2,000 IU of vitamin $D_3$ was well-tolerated and increased serum 25(OH)D levels from 15 ± 7 ng/mL to 36 ± 22 ng/mL (37 ± 17 nmol/L to 90 ± 55 nmol/L).[38] A dose of 2,000 IU/day of vitamin $D_3$ was also safely used in a six-week study of infants and toddlers with hypovitaminosis.[39] A dose of 4,000 IU/day of vitamin $D_3$ has been used in studies of allergic diseases such as atopic dermatitis (NCT00789880, available from: www.clinicaltrials.gov) and in an ongoing study of vitamin D supplementation during pregnancy (NCT00920621, available from: www.clinicaltrials.gov). Pooled data from vitamin D trials in adults have shown that doses of vitamin D up to 10,000 IU/day are safe.[40] In healthy men with mean serum 25(OH) D approaching an adequate level (28 ng/ml (70 nmol/L)), vitamin D supplementation with 5,500 IU/day and 11,000 IU/daily for 20 weeks was well tolerated.[34]

We propose to test a high but safe dose of vitamin $D_3$ for this trial. If our proposed dose is beneficial, then subsequent dose-response studies can be considered to determine the lowest effective dose. Since the proposed dose of vitamin $D_3$ (4,000 IU/day) is not FDA approved, this study is operating under an Investigatonal New Drug (IND) protocol, with monitoring by the FDA.

Urinary calcium to creatinine ($U_{Ca}/U_{Creat}$) ratios will be measured periodically for early detection of vitamin D toxicity. While others have found a dose of 4,000 IU/day vitamin D to be safe and effective at raising 25(OH)D levels, we will monitor $U_{Ca}/U_{Creat}$ ratios, as this is the earliest abnormality detected and most non-invasive way of monitoring for hypervitaminosis D[41].

Parents of participants will be given a flyer with instructions to have the child drink an adequate amount

of fluids on the day prior to collection of the urine sample (i.e. 5-8 glasses of water, depending on the child's age and gender). Given that different thresholds for a "normal" $U_{Ca}/U_{Creat}$ have been reported among school-aged children[42-45], we chose the $U_{Ca}/U_{Creat}$ ratio at or below the 95$^{th}$ percentile for the first 68 participants randomized into the study as the upper limit of "normal" values (0.26 mg/mg). If an elevation of the $U_{Ca}/U_{Creat}$ (≥0.27 mg/mg) is detected, parents of study participants will be told to stop the study medication and go to the Clinical Center as soon as possible (ideally, the following day) for a repeat measurement of $U_{Ca}/U_{Creat}$. To exclude dehydration as a potential cause of the abnormal $U_{Ca}/U_{Creat}$, parents of participants will be instructed to have the child drink at least two glasses of water or a non-caffeinated beverage prior to providing their urine sample at the Clinical Center. If the repeat $U_{Ca}/U_{Creat}$ is normal, the participant will be called and asked to restart the study medication.

As an additional safety measure, we measure vitamin D levels at the same time as UCa/UCr ratios. If the vitamin D level is >100 ng/ml (250 nmol/L), or if the second $U_{Ca}/U_{Creat}$ is elevated, a serum calcium will be measured in the same sample as that used for the vitamin D measurement. If the serum calcium is normal, the participant will continue the study medication. If the serum calcium is >10.8 mg/dl, the participant will be withdrawn from study treatment.

Vitamin D levels >150 ng/ml (374 nmol/L) will be considered toxic, regardless of other measurements.[46] Participants above this level will stop the study medication.

Children in whom the study medication needs to be discontinued because any of the reasons stated above (i.e. hypercalcemia or a vitamin D level >150 ng/ml) will be referred to a pediatric endocrinologist or a pediatric nephrologist within 24 hours, and will remain in the study for follow-up visits.

## 9.3. Study Intervention: Placebo

There is a risk that vitamin D deficient participants will not get enough vitamin D. The average dietary intake of vitamin $D_3$ for school-aged children is 400 IU/day[33] (a dose used in multi- vitamins), which will prevent rickets. Parents of all participants enrolled in the trial will be provided with a list of foods that are rich in vitamin D, and that are part of a balanced diet that contains 400 IU/day of vitamin $D_3$. Thus, we will encourage dietary intake of at least 400 IU/day for all participants.

Children with very low vitamin D levels (<14 ng/ml (35 nmol/L)) will not be allowed to participate in this study (see above). We will be monitoring vitamin D levels throughout the study. To further ensure the safety of participants in the placebo group, additional dietary counseling will be provided to parents of participating children whose vitamin D level is less than 14 ng/ml (35 nmol/L) at any study visit. If the vitamin D level of these children remains below 14 ng/ml (35 nmol/L) at the next safety testing (e.g. their serum 25(OH) is 14 ng/ml or 35 nmol/L on two consecutive measurements), such children will be removed from the study medication and referred to a pediatric endocrinologist for further evaluation.

Should the vitamin D level of a participant be below 10 ng/ml (25 nmol/L) at any study visit, the participant will be referred to a pediatric endocrinologist for further evaluation, while also providing the participant's parent or guardian with his/her vitamin D level at the time of referral. Due to intention to treat principles, participants who are removed from the study medication due to a low vitamin D level or rickets will still be followed for the remainder of the study visits.

All children whose vitamin D level is below 20 ng/ml (49.9 nmol/L)) at the end of the clinical trial will be referred to their pediatrician (if their level is between 14 ng/ml and 19 ng/ml) or a pediatric

endocrinologist (if their level is below 14 ng/ml) for further evaluation.

If a participant meets criteria for referral to a pediatrician or a pediatric endocrinologist (as outlined above) but decides to withdraw from the study or is lost to follow up, we will still conduct such referral and provide the participant's parent or caretaker with his/her vitamin D level at the time of referral. Should the participant move to another city, we would still contact the participant's parent or guardian by phone and mail, both to provide them with the child's vitamin D level and to recommend that the child be referred to a pediatrician or a pediatric endocrinologist for treatment. In such instances, we will also recommend that the child's new primary care provider contacts us at their earliest convenience.

# 10.INFORMED CONSENT

There are two informed consent forms: one for an optional prescreen and the other for the main study (see appendix). In both cases, consent will be sought from the parent/guardian and assent from the child, after a thorough discussion of potential risks and benefits (see above).

# 11.ADVERSE EVENTS

## 11.1. Definition and Documentation

There is a risk of adverse events occurring over the course of the study. A clinical adverse event will be defined as any unintended worsening in the participant's signs or symptoms, whether or not study-related. This will include any side effect, injury, or sensitivity reaction, as well as any intercurrent event. A laboratory adverse event will be defined as any clinically-important worsening in a test variable which occurs during the study, whether or not it is drug-related. An adverse event will be deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events will include any experience that is fatal, life-threatening, or permanently disabling; requires or prolongs inpatient hospitalization; or is a cancer, or overdose. Serious adverse events that we are specifically monitoring in this study include hospitalization for asthma, life-threatening asthma exacerbations (requiring invasive or noninvasive mechanical ventilation), hypercalcemia, hypervitaminosis D (serum levels >150 ng/ml (375 nmol/L)), and severe vitamin D deficiency (serum level < 10 ng/ml (25 nmol/L)). We will screen for adverse events at every patient contact, which occurs on a monthly basis. We also instruct study participants to notify us if they receive non-routine medical care.

Documentation of an adverse event will be recorded on the Adverse Event Report Form and will include the following information: Description of the condition, dates of condition, seriousness (as defined above), expectedness, relatedness to study drug, event outcome, relationship of the adverse event to the study medication(s), and severity of the event.

## 11.2. Adverse Events Unrelated to Asthma

Adverse events due to concurrent illnesses other than asthma may be grounds for withdrawal if the illness is considered significant by the study investigator or if the participant is no longer able to effectively participate in the study. Participants experiencing minor intercurrent illnesses may continue in the study, provided that the nature, severity, and duration of the illness are recorded and that any unscheduled medications required to treat the illness are also recorded. Examples of minor intercurrent

illnesses will include upper respiratory infections, urinary tract infections, and gastroenteritis. Medications will be allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

## 11.3. Adverse Events Related to Asthma: Asthma Exacerbation

Severe asthma exacerbations are the primary outcome variable for the study. Since participants have previously had a severe asthma exacerbation, it is anticipated that moderate to severe asthma exacerbations will occur. Asthma exacerbations and related complications (pneumothorax, pneumomediastinum, mechanical ventilation, etc.) will be documented as adverse events. Safety net procedures, including visits and frequent telephone contacts, should identify participants who experience a severe asthma exacerbation (the primary outcome) during the study.

A **severe asthma exacerbation** (the primary endpoint of the study) during the run-in period is an exclusion criterion. A child may be rescreened after the exacerbation has resolved but if there is another severe asthma exacerbation during the new Run-In Period, he/she will not be eligible for the study.

Study participants are instructed to contact their regular asthma provider in the event of worsening asthma symptoms. After they have received care, they are then instructed to notify study personnel of an asthma exacerbation. Study personnel will then determine the severity of the exacerbation based on the following criteria:

**A moderate asthma exacerbation** will defined by the occurrence of at least one of the following[24]:

***At home:***

1. An increase in prn rescue inhaler ≥8 puffs per 24 hours over baseline use for 48 hours.

***At study visits:***

1. $FEV_1$ <80% of baseline pre-bronchodilator value
2. Use of additional inhaled ICS for asthma treatment by the study or treating physician
3. An ACT or c-ACT score ≤ 19

A **severe asthma exacerbation** will be defined as per the most recent American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines [24], as the occurrence of either:

1. Use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days; OR
2. A hospitalization or ER visit because of asthma, requiring systemic corticosteroids.

According the ATS/ERS asthma task force, the term “mild exacerbation” has no adequate definition, and should not be used. Any change in symptoms or medication use that does not meet the criteria for a moderate or severe asthma exacerbation will be considered as loss of asthma control.

## 11.4. Treatment Algorithm for Asthma Exacerbations

Study participants will be provided with a patient information card that contains information on how to manage a worsening of asthma symptoms at home (***Initial Treatment, below***). If these measures are not effective, or if the child’s parent or guardian is concerned about how to proceed, then they will be instructed to proceed to their asthma provider or the emergency department. The wallet card will

contain recommendations for treatment of asthma exacerbations (***Physician's Office or Emergency Room Treatment, below)***. We will ask that the card be provided to the child's asthma provider or ED physician at the time of treatment, but the asthma provider or ED physician may use his/her best judgment on how to treat the child.

***1. Initial Treatment for Loss of Asthma Control***

Patients who have increased symptoms may use up to three treatments of 2-4 puffs of their rescue inhaler by MDI/spacer, 20 minutes apart, for 60-90 minutes. If there is good response, they may continue their rescue inhaler every 4 hours for up to 24-48 hours. If the participant does not improve within 48 hours, they will be classified as non-responders, and treated as per Section 2 below. For safety reasons, all participants will also be asked to contact their usual provider of asthma care.

If there are persistent symptoms (such as wheezing and/or coughing) without respiratory distress that have not improved after the first 60-90 minutes of therapy, they should contact their asthma provider urgently for further instruction. After communicating with their asthma provider, we ask that study participants also notify study personnel.

If there is a poor response to treatment, with marked wheezing and/or respiratory distress, the patient should seek care in an emergency department for further management.

***2. Rescue Algorithm for Progressive Moderate or Severe Asthma Exacerbations***

Participants not responsive to the rescue algorithm will be managed according to the following rescue algorithms. Rescue algorithms are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma[47] and prior ACRN trials[48]. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

***3. Physician's Office or Emergency Room Treatment***

***3a. Physician's office***

If the child needs treatment in a physician's office, patients should initially be given up to 3 doses of short acting beta agonist by nebulizer (2.5mg) or MDI/spacer (2-4 puffs) every 20 min for the first 60-90 minutes. Depending on their response, a course of oral corticosteroids (see below) should be considered.

***3b. Emergency department treatment***

Patients will be assessed by history and physical examination (level of alertness, auscultation, use of accessory muscles, heart rate, respiratory rate, and oxygen saturation). For a moderate asthma exacerbation, up to 3 doses of rescue inhaler by MDI/spacer or nebulizer should be given in the 1st hour. Oral corticosteroids should be given if there is no immediate response. If, upon repeat assessment, there are still moderate symptoms, rescue inhaler can be given every 60 minutes, and treatment can be continued every 1-3 hours, provided there is improvement. A decision regarding patient disposition (home or hospital admission) should be made within 4 hours of treatment.

If there is a good response to treatment, no respiratory distress and a normal physical exam, the patient can be discharged home to continue the rescue inhaler every 4 hours and complete a 3-5 day course of oral corticosteroids (see below). However, if there are severe symptoms on repeat assessment, with accessory muscle use and chest retractions, a nebulized short acting beta agonist should be given (hourly or continuously) with nebulized ipratropium. At this time, if there is an incomplete or poor response, a decision should be made for hospital admission.

For a severe asthma exacerbation, short acting beta agonist via nebulizer or MDI should be given (every 20 minutes or continuously for one hour) in addition to nebulized ipratropium and oral corticosteroids.

If symptoms become moderate and are improved on repeat assessment, short acting beta agonists can be given every 60 minutes, and treatment can be continued every 1-3 hours, provided there is still improvement. A decision regarding patient disposition (home or hospital admission) should be made within 4 hours of treatment. However, if there are continued severe symptoms upon repeat assessment, with accessory muscle use and chest retractions, nebulized short acting beta agonists should be given hourly or continuously. At this time, if there is an incomplete or poor response, a decision should be made for hospital admission.

**Prednisone treatment**

The recommended dose of prednisone is 2 mg/kg/day in single or 2 divided doses (a maximum of 60 mg per day) for 3 to 10 days. Typically a prednisone burst is given for 3-5 days.

## 12. CLINICAL MANAGEMENT AND DATA COLLECTION

Figure 1. Summary of Study Protocol

| **Screening and Run-in** | | | **Trial Phase** | | | | | |
|---|---|---|---|---|---|---|---|---|
| **V1 Screening** Target N=700 | **V2** | **V3 Randomization** Target N=400 | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** Target N=340 |
| Week -5 | Week -4 | Week 0 | Week 8 +/- 7 days | Week 16 +/- 7 days | Week 24 +/- 7 days | Week 32 +/- 7 days | Week 40 +/- 7 days | Week 48 +/- 7 days |
| Informed consent (screening) | Informed consent (trial) | Adherence | Adherence | Adherence | Adherence | Adherence | Adherence | Adherence |
| Screening questionnaire | Dispense run-in study meds | Dispense study meds | Dispense study meds | Dispense study meds | Dispense study meds | Dispense study meds | Dispense study meds | Physical examination, anthropometry |
| | Physical examination, anthropometry | Serum total and allergen-specific IgE, nasal epithelial samples | | | | | | Serum total and allergen-specific IgE, nasal epithelial samples |
| | RHQ, HHQ, ACT, ATAQ, DSEQ | ACT, ATAQ, Fitzpatrick skin type, CCDS and PAQLQ | s-RHQ, ACT and ATAQ | s-RHQ, C-ACT and ATAQ | s-RHQ, ACT and ATAQ | s-RHQ, ACT and ATAQ | s-RHQ, ACT and ATAQ | s-RHQ, ACT, ATAQ, and PAQLQ, CCDS, DSEQ |
| | Spirometry/ BD | Spirometry/BD | Spirometry | Spirometry | Spirometry | Spirometry | Spirometry | Spirometry / BD |
| | Nasal blow± | U Ca / UCrt | | U Ca / Ucrt | | U Ca / Ucrt | | U Ca / Ucrt |
| Serum 25(OH)D | *MCT | 25(OH)D | | 25(OH)D | | 25(OH)D | | 25(OH)D |
| Serum calcium | **Urine pregnancy test | VDSEQ | | | | | | VDSEQ |
| | | CBC | | | | | | CBC |

ICS=inhaled corticosteroids, RHQ=respiratory health questionnaire, HHQ=household questionnaire, VDSEQ=vitamin D intake/sun exposure questionnaire, ACT=asthma control test (child or standard), PAQLQ=pediatric asthma quality of life, CCDS=Checklist of Children's Distress Symptoms, BD=bronchodilator, MCT=methacholine challenge testing (to be done only in children without BD response), CBC=complete blood count with differential **To be done in girls of child-bearing age prior to MCT, Uca/Ucrt=urinary calcium/creatinine ratio. ±For assessment of viral infections, §For RNA extraction

### 12.1. Recruitment (Visit 0)

Recruitment will be conducted at several locations at each study site, which may include emergency departments, pulmonary clinics, and general pediatric clinics. In addition, information will be available on the study website (http://www.vitdkidsasthma.pitt.edu) and ClinicalTrials.gov, to reach a broader population.

A Referral Log (REF) will be used to track the age and gender of every child whose family is approached about the study or contacts one of the study sites (e.g., through TrialSpark). If the family agrees to further contact, a Contact Form (CON) will be completed and forwarded to the local study coordinator for follow up.

### 12.2. Screening (Visit 1, optional)

In some cases, referrals will be prescreened based on a subset of the initial inclusion and exclusion criteria. Prescreening will be optional. When prescreening occurs, it may be conducted by non-study personnel at recruitment sites. A special consent form has been developed for this purpose. If the child passes the prescreening, blood will be drawn to measure serum vitamin D and calcium. Results will be not available immediately, so the Prescreening Form (PSC) will be sent to the local study coordinator, along with the Contact Form (CON), if not previously sent. The study coordinator will record the blood test results and follow up with the family, as appropriate.

If the serum calcium and vitamin D levels are consistent with inclusion/entry criteria, the child will be invited back for Visit 2. If the vitamin D level is too high for inclusion, but was drawn in the summer or fall, the child will be invited back for a re-screening in the winter, with a repeat serum vitamin D level.

### 12.3. Run-In Period (Visit 2)

Screening will be conducted only by trained study personnel using the Screening Form (SC), and will include a review of all initial and exclusion criteria, questionnaires, and spirometry. If prescreening did not take place, blood will be drawn at this visit, to measure serum vitamin D and calcium. In these cases, spirometry will be performed before the serum vitamin D and calcium levels are available.

If the child's bronchodilator responsiveness (BDR) on the spirometry is negative, the child will be asked to return for a methacholine challenge test (MCT) within two weeks. Note that MCT will only be performed if the child's serum vitamin D and calcium levels fall within the acceptable ranges for the study, as defined in the Protocol.

Families of children who pass the screening will complete several questionnaires (see Figure 1, above) and then begin a month-long Run-In Period, to make sure the child is willing and able to take gel caps (all containing placebo) and use Flovent, as directed. If the child is not already taking an age-appropriate dose of Flovent and/or is taking additional asthma medications, study personnel will consult with the child's regular doctor about implementing these changes, using data collected during Visit 2. Approval by the child's regular doctor will be documented on the Run-In Preparation Form (RUN).

Adherence to the run in medication (placebo) will be monitored using both an electronic MEMS cap (which records bottle openings) and gel cap count. Flovent inhalers have a built-in counter. Children and

their families will be informed that adherence to the gel cap (placebo) and ICS protocols will be monitored. During the four weeks of the run-in period, research coordinators will perform weekly reminder phone calls to study participants.

### 12.4. Eligibility Determination and Randomization (Visit 3)

At the end of the Run-In Period, final inclusion/exclusion criteria will be reviewed, including adherence to the study protocol and willingness to be randomized, using the Eligibility Form (ELIG). Participants who do not meet adherence criteria may be offered a second chance to demonstrate adherence. Participants who are ineligible for other reasons (e.g. had an exacerbation during run-in) may be placed on a list for re-screening at a later date.

If participants are found to be eligible, several questionnaires will be administered (see Figure 1, above) and participants will be randomized, based on a double-blinded assignment, to receive either 4,000 IU of Vitamin $D_3$ or a placebo during the 48-week Trial Phase (see below).

Randomization will be stratified on race/ethnicity and study site, using a permuted-blocked strategy. Study coordinators will execute a randomization module in the Data Management System that requires entry of stratification variables. The Data Management System will send an email to the site's research pharmacist announcing the treatment assignment. The pharmacist will prepare masked study medication bottles with vitamin D3 or placebo, labeled according to local requirements.

Dr. Wisniewski will not unmask treatment assignment for interim analysis. Information will be provided to the Data and Safety Monitoring Board (DSMB) as Treatment A and Treatment B. Treatment assignment will be revealed only in the event of a medical emergency in which unmasking could change treatment. The Independent Safety Monitor (ISM) may unmask treatment for a subject. Unmasked subjects will complete any remaining protocol visits. Randomized participants will undergo blood draws for DNA, serum bio-banking, serum vitamin D level, and total and allergen specific IgE (*B. germanica* and *D. pteronyssinus*). We will collect urine for calcium and creatinine. Nasal epithelial cells will be collected at the Pittsburgh site only.

### 12.5. Trial Phase (Visits 4-9, and follow up phone calls)

The Trial Phase will last 48 weeks, with in-person visits and phone calls alternating monthly. According to the visit schedule (see Figure 1, above), questionnaires will be administered; blood will be collected for serum vitamin D levels, and urine will be collected for calcium and creatinine. Spirometry tests will be performed, with bronchodilator administration at visit 9. In addition, adherence with study medication (as determined by MEMS cap and gel cap count) will be measured, and additional study medication and Flovent will be dispensed.

At the half-way point (Visit 6), the study team will decide whether it is appropriate to reduce the child's ICS dose by 50%, per the Protocol (see section 7).

Throughout the Trial Phase, if there is evidence of a moderate or severe asthma exacerbation (see section 11.3), the site coordinator will provide an appropriate referral (e.g., to the child's primary care physician or the nearest medical emergency facility) and make arrangements to follow up again by phone as well as at the next scheduled visit. Children who are determined to have a severe asthma

exacerbation will be seen by the research staff within 72 hours. At this acute exacerbation visit we will collect a nasal blow for a respiratory viral panel, perform spirometry, and administer several questionnaires. This asthma exacerbation visit will occur for up to three distinct severe exacerbations that a child has during the study. Children who have three severe asthma exacerbations will be withdrawn from the study drug but continue to complete study visits.

### 12.6. Protocol Violations

Protocol violations are defined as departures from accepted clinical research practices, study protocol, and/or Vit-D-Kids Asthma procedures that pose a risk to participant safety, adversely affect data quality and the integrity of the major scientific goals of the study, and/or involve a significant and repeated breach of participants' privacy. Protocol violations include (but are not limited to):

- failure to obtain informed consent appropriately
- enrollment and/or randomization of ineligible participants
- dispensation of study medications incorrectly or not at all
- failure to follow the protocol safety monitoring plan
- breaches of confidentiality resulting from lost, misplaced, or stolen study documents (e.g., a completed visit packet is missing)

By the nature of their definition protocol violations are considered the most serious class of departure from the study protocol.

### 12.7. Protocol Deviations

Protocol deviations are defined as departures from a study protocol or Vit-D-Kids Asthma methods of procedure that do not pose a risk to subject safety, do not adversely affect the integrity of the major scientific goals of the study, and do not involve a significant and repeated breach of participant privacy. Protocol deviations include (but are not limited to):

- failure to obtain appropriate source documentation
- failure to achieve appropriate Vit-D-Kids Asthma certification prior to performing procedures
- mistimed procedures (e.g., performing a lab test outside the window outlined in the protocol)
- omission of protocol and/or Vit-D-Kids Asthma procedure elements that do not affect participant safety
- failure to carry out study procedures in the appropriate order, when applicable
- submission of an outdated version of a data collection form
- submission of participant identifying information (e.g., name, address, phone number, social security number) to the DCC, CCC, or Gern lab. Repeated violations will be classified as a protocol violation.

All protocol violations and deviations will be reported at the time of discovery using the Protocol Deviation form in the data management system. PD reports will be reviewed at regular meetings of the DCC (weekly), the Steering Committee (monthly), and the DSMB (bi-annually). The DCC will monitor accumulated violations and deviations to identify repeated departures from study protocol either by a particular study person, or within a particular site, and will report to the Steering Committee for determination of remedial action, which could include required recertification and / or clarification of study procedures in the Manual of Operations.

# 13. SPECIMEN HANDLING

Blood and urine samples collected during the screening process (Visits 1-2) will be processed and tested locally and study personnel at each site will enter results directly into the electronic data management system (DMS). Nasal blows will be sent to the laboratory of James Gern at the University of Wisconsin for processing, and results will be sent to the DCC in electronic format. Urine collected during the trial phase (for calcium and creatinine) will be processed and tested locally, and study personnel at each site will enter results directly into the electronic DMS. Blood collected for vitamin D at regularly scheduled visits during the trial phase will be aliquoted locally and then sent to Children's Hospital of Pittsburgh of UPMC for testing. Additional serum vitamin and calcium results (e.g. to check for toxicity when the urine calcium creatinine ratio is elevated) will be accessed only by a study staff member who will not have any contact with participants. Preprinted labels with unique bar code identifiers will be provided for samples that will be shipped.

# 14. DATA MANAGEMENT

## 14.1. Electronic Data Entry

The Data Coordinating Center will create electronic versions of the forms, make them available for clinical personnel to use through a secure, web-based system, and assign permissions based on respondents' respective roles in the study.

## 14.2. Data Quality

The Data Coordinating Center will import all study data into SAS, a powerful statistical program and data management tool. This database load will happen on a daily basis, with each extraction of the raw data archived in case there is a need to refer back to it later on.

The DCC will identify missing data, range violations, and logical and chronological discrepancies, and work closely with the study team to resolve these concerns in a timely manner to ensure that the study yields the highest quality data for analysis.

## 14.3. Data Analysis

The primary outcome of this trial will be time to a severe asthma exacerbation. The main analysis (using the intent-to-treat principle) will be conducted using a Cox proportional hazards regression model fit to the time to a severe asthma exacerbation and adjusted for race/ethnicity and study site (as randomization will be stratified on these variables). If there is an unexpected significant imbalance in other relevant baseline covariates between treatment groups despite randomization, those covariates will be considered for inclusion in the multivariate analysis.

The secondary outcomes will be time to a viral-induced severe asthma exacerbation, reduction in dose of ICS at or after visit 6, and reduction in the average cumulative dose of ICS during the trial. The analytical approach to time to viral-induced severe asthma exacerbation will be similar to that for the primary outcome. An analysis of covariance model will be used for the analysis of the difference in average cumulative dose of ICS between the vitamin D and placebo groups at the end of the trial, with

treatment group as the primary explanatory variable, adjusting for race/ethnicity and study site. Logistic regression will be used for the analysis of the proportion of participants achieving a reduction in ICS dose at or after visit 6. As with the other analyses, the logistic regression models will be adjusted for race/ethnicity and study site.

Exploratory analyses will allow for multiple severe asthma exacerbations or viral-induced exacerbations to be included per participant. This repeated measures Cox proportional hazards regression model can adjust for correlation among multiple responses in a participant.

Additional exploratory analyses will be undertaken to identify potential baseline modifiers of the effect of the intervention (vitamin D) on severe asthma exacerbations. Selected baseline characteristics (all measured prior to randomization) to be examined will include those most likely to modify the effect of vitamin D on severe asthma exacerbations: race/ethnicity, study site, vitamin D deficiency (25(OH)D < 20 ng/ml (50 nmol/L)), BMI-z score and atopy. The approach to identification of effect modifiers will depend on the outcome and follow the same analytical strategy as that described for primary and secondary outcomes. A separate analysis will be conducted for each potential modifier of interest. Each model will include the main effects of the treatment group and the potential modifier (e.g. atopy) on the outcome, as well as a two-way interaction term.

All pre-specified hypotheses will be tested individually at the $\alpha$=0.05 level of significance, using a conservative two-sided alternative hypothesis (even when the stated hypothesis is one-sided). More specifically, results from the analyses of the effect of vitamin D on our primary and secondary outcomes will not be adjusted for multiple comparisons, since they have been defined a priori and are limited in scope. Findings from the exploratory analyses (e.g. testing for modification of the effect of vitamin D on our primary/secondary outcomes by selected baseline variables) will be adjusted for the number of models tested (e.g., using False Discovery Rate methods), and testing for effect modification will be limited to the variables specified above to reduce the potential for false positive results. In addition, findings from any exploratory analysis will be reported as such.

To prevent or minimize the amount of missing data, careful data collection, data management and QC procedures will be implemented. These measures may include training and certification of all study personnel, careful design of data collection forms, clear and thorough documentation of all study procedures, the implementation of a data management system that minimizes the probability of data entry errors, and reporting procedures that help track participants in longitudinal studies.

## 14.4. Privacy and Data Security

Procedures will be implemented to maintain the security and confidentiality of participant data, including assigning unique study identifiers rather than using names or other identifying information.

Limited identifying information necessary for implementation of the study will be recorded, specifically, the participant's date of birth and dates of clinical visits and medical events that occur. Upon study closure, the DCC ~~will de-identify these data by converting dates to the number of days relative to delivery. …~~will de-identify these data by employing a standard date-shifting algorithm to all dates in a participant's record.

In keeping with IRB-approved consent procedures, participants' records will be destroyed seven years after completion of the study. Thereafter, only de-identified data will be available for use in publications, presentations, etc.

Databases will be backed up nightly, with previous versions archived for reference.

## 14.5. Data Sharing

De-identified data will be made available to the National Heart, Lung and Blood Institute of the National Institutes of Health, as appropriate, for the purposes of study monitoring.

# 15.DATA AND SAFETY MONITORING

## 15.1. Clinical Coordinating Center

The Clinical Coordinating Center (CCC) will be chaired by Dr. Celedón at the University of Pittsburgh School of Medicine. The CCC will help prepare applications to each site IRB, address any IRB concerns, assure compliance with HIPAA regulations, and arrange regularly scheduled conference calls so that clinical staff from both sites can discuss recruitment, retention, adherence, successes and challenges.

## 15.2. Data Coordinating Center

The Data Coordinating Center (DCC) will provide scientific oversight of the trial regarding the collection, management and analysis of study data. In particular, the DCC will:

a. Create an electronic Data Management System
b. Develop procedures and monitor quality
c. Assume responsibility for reporting, statistical design and analysis
d. Support communication among study personnel through a password-protected intranet

## 15.3. Steering Committee

The Steering Committee will include Dr. Celedón of the CCC, Dr. Wisniewski of the DCC, Dr. Ross (site PI for Cleveland site), Dr. Bacharier (PI of the St. Louis site), Dr. Phipatanakul (PI of the Boston site), and representatives from NIH. Most scientific decisions will be made by the Steering Committee, with input from co-investigators, other members of the research team, and the Data and Safety Monitoring Board. The steering committee will conduct conference calls on a biweekly basis. Research coordinators from all sites will have a separate bi-weekly conference call.

## 15.4. Data and Safety Monitoring Board

The National Heart, Lung, and Blood Institute (NHLBI) has appointed a data and safety monitoring board (DSMB) composed of biostatistician(s), ethicist(s), and experts in clinical trials and pediatric asthma. This DSMB board (known as VITEL) oversees four NHLBI-funded trials. The first meeting of the DSMB to discuss this trial occurred on October 23rd, 2015. To avoid any appearance of conflict of interest, the DSMB members have no involvement in the study, vested interest in its outcome, ties to the study investigators (e.g., from the same institution and/or history of extensive collaboration), or financial ties

to any commercial concerns likely to be affected by the study's outcome. The full list of DSMB members is included as an appendix to this protocol.

During the initial discussion of this trial, the VITEL DSMB formulated its operating procedures, the Data and Safety Monitoring Plan. Procedural issues included: the Board's meeting frequency; the types and formats of reports it will receive, the policy on whether and how the members may be unmasked; what interim data (if any) may be released to the study investigators (e.g., overall adverse event rate); and how minutes will be taken and distributed. Trial review issues include: study progress, including an assessment of data quality (monitored monthly); outcomes and adverse events data, including out-of-range laboratory results (ongoing monitoring); any pertinent new information (monitored every 6 months); study procedures designed to protect the privacy of the participants and the confidentiality of the data (monitored weekly); interim analysis and final conclusions evaluating benefit-to-risk ratio of study participation. Data and Safety Monitoring Plan reports will be submitted yearly to the site IRBs at the time of renewals.

Before initiation of the trial, the DSMB conducted a conference call with Dr. Celedón (of the CCC) and Dr. Wisniewski (of the DCC) to review the study protocol (particularly the specific outcome definitions), halting rules, the interim and final analysis plan, the procedures for recording and reporting SAEs, and the monitoring proposal (including draft shells of reports and tables). The informed consent document/process was inspected to ensure that all required elements are included in language understandable to a typical study participant to be enrolled in the trial.

The DSMB will meet periodically throughout the study and again after the final analysis has been completed. In no instance will more than 12 months elapse between DSMB reviews of cumulative safety data after the first participant has enrolled. DSMB meetings may occur by teleconference. The DSMB will monitor the study according to the guidelines specified in the study protocol and the operating procedures established at the initial meeting, unless the DSMB determines during the course of the trial that modification of the guidelines is in the best interest of the study and its participants. Such a decision may be based on new information that emerges during the course of the study (e.g., publication of the results of a similar trial), realization of inappropriate initial study assumptions, or the occurrence of an unanticipated scenario. The DSMB responsibilities conclude when the study is completed, data have been verified, and the initial findings (encompassing the primary and secondary aims) have been published. We anticipate ancillary research to be conducted using specimens and data collected during the trial. Manuscripts derived from this research will be made available to the DSMB for as long as it is still in service.

The DSMB Chair will set the meeting agenda, which will usually include Open and Closed Sessions. The DSMB members, along with Drs. Celedón and Wisniewski, will participate in the Open Session, at which data concerning study conduct and aggregate safety data are discussed. Any safety and efficacy data analyzed by treatment arm will be discussed only in the Closed Session. It is critical that information presented in the Closed Session not be revealed to the study investigators, except as explicitly authorized by the DSMB to Dr. Wisniewski. Study investigators will remain masked to the interim data since knowledge of emerging trends between treatment arms may influence participant enrollment, management and evaluation, thus compromising the study. The format and reporting requirement of unmasked data was discussed and agreed upon by the VITEL DSMB. In general, Dr. Wisniewski will prepare study data reports and send them to DSMB members and the NHLBI at least 7 days prior to the meeting. These reports will contain the most up-to-date data permitted by the timeframe necessary for

Dr. Wisniewski to prepare and review the analyses. Interim data reports will usually consist of two parts, corresponding to the Open and Closed Sessions of the DSMB meeting. Only the DSMB members and appropriate NHLBI staff, including statistician, executive secretary, and others as instructed**,** will receive copies of the Closed Session report. At the completion of the meeting, the members of the DSMB will be instructed to destroy any printed documentation.

The Open Session report will focus on study participant accrual and demographics, data completeness, other study performance measures, any new information on the intervention or disease/disorder that may affect the outcome of the trial, and a list of publications or presentations. The Closed Session report will divide study participants according to cumulative data or coded treatment assignment (e.g., Treatments A vs. B), comparing participant demographics and baseline characteristics, rates of and reasons for treatment discontinuation and loss to follow-up, rates of SAEs, and, if an interim efficacy analysis is scheduled, rates of efficacy outcomes (depending on the DSMB operating procedures).

Dr. Wisniewski will work with Dr. Celedón to prepare a report addressing specific concerns he anticipates the DSMB will have regarding the conduct of the study. This will be distributed to DSMB members, along with the Open Session report. Likewise, Dr. Wisniewski's report for the Closed Session will usually contain an assessment of the progress of the trial, including recommendations on whether it should be terminated or modified. Interim data reports will generally include the following types of information, although only the Closed Session data reports will include comparisons by treatment group:

- Monthly and cumulative accrual, overall and by site, compared with targets
- Baseline characteristics, overall and by treatment group
- Completeness and quality of data collection forms
- Status of enrolled participants, overall and by treatment group
- Participants' off-protocol treatments
- Compliance of study sites with eligibility criteria and other protocol requirements
- Participant adherence to the treatment regimen, overall and by treatment group
- Outcome rates by treatment group, if an interim efficacy analysis is scheduled
- Individual SAEs by participant ID number and a table of event-specific cumulative rates, overall and by treatment group

Dr. Wisniewski will work to ensure all of the information presented at the DSMB meetings remains confidential. Each report will be marked as such. No interim treatment outcome information will be presented to site investigators or to the scientific community. At the conclusion of each DSMB meeting, the members of the DSMB will be instructed to destroy their printed documentation. The DSMB will then submit recommendations to the NHLBI regarding the continuation of the trial. The NHLBI will generate reports summarizing the DSMB recommendations and send the reports to the study sites to be sent to the local IRBs.

## 15.5. Institutional Review Board

To protect research participants, this study will comply with all policies and procedures established by Institutional Review Boards (IRBs) at the University of Pittsburgh, Rainbow Babies and Children's Hospital in Cleveland, Washington University at St. Louis and Boston Children's Hospital. Modifications will be submitted for review prior to being implemented.

## 15.6. Clinical Trial Site Monitoring

Drs. Celedón and Wisniewski have extensive experience in all areas of clinical trial oversight, including developing quality assurance benchmarks, enforcing quality control, and monitoring protocol-specific activities through ongoing reporting structures and on-site evaluations. The DCC will be responsible for generating site-specific reports on recruitment and follow-up; quality control reports, which include the quality of data received; participant follow-up adherence data, which include missed visits; and participant characteristics. The DCC will also monitor the study sites to ensure that all regulatory procedures are met. This will include assuring that all study personnel (including those hired after the trial begins) have completed required training and received certification to conduct human subjects research.

## 15.7. Reporting of Serious Adverse Events

Reporting to the IRB:
A report will be sent to the Institutional Review Board within 24 hours of learning of any unexpected serious adverse events associated with the research intervention. Unexpected adverse reactions of moderate severity in association with the research interventions will be reported to the Institutional Review Board within 5 days. Generated safety reports will be submitted to the Institutional Review Board within 30 days of their receipt.

Reporting to the DSMB:
Reports of all SAEs, irrespective of relatedness and expectedness**,** will be sent to the chair of the DSMB and NHLBI staff within 48 hours. In addition, monthly line-item reports of all SAEs and a 6-month report of all adverse events will be submitted to the DSMB. The following elements will be reviewed under the Data and Safety Monitoring Plan: study progress, including an assessment of data quality (monitored monthly); outcomes and adverse events data, including out-of-range laboratory results (ongoing monitoring); any pertinent new information (monitored every 6 months); study procedures designed to protect the privacy of the participants and the confidentiality of the data, i.e., data and charts are stored in locked area, data are recorded to protect the identity of the participant (monitored weekly); interim analysis and final conclusions evaluating benefit-to-risk ratio of study participation. Data and Safety Monitoring Plan reports will be submitted yearly to the Institutional Review Board at the time of renewals.

Reporting to the FDA:
We will notify the FDA in a written IND safety report, of any adverse experience associated with the use of the drug that was both serious and unexpected. These reports will be filed as soon as possible, and no later than 15 calendar days after we determine that an SAE occurred, and 7 days for fatal or life threatening events.

## 15.8. Withdrawal and Medication Discontinuation

Withdrawal is defined as termination from the study without further follow-up. This is distinct from discontinuation of study medications (discussed later in this section). Withdrawal may be initiated at any time by the participant, his/her parent/guardian, a healthcare provider, a study team member, or another party. If this occurs, the reason will be documented on the Withdrawal Form (WD). We will complete a withdrawal visit (with the same procedures as visit 9) if the child and family are willing. Otherwise no further data will be collected from that child. However, data already collected for study purposes, including biological samples, will be used. If a child misses either two consecutive follow-up visits or three non-consecutive follow-up visits, he/she will be withdrawn from the study.

Note that an adverse event due to a concurrent illness other than asthma (section 11.2) may be grounds for withdrawal if the illness is considered significant by the study investigator or if the participant is no longer able to effectively participate in the study.

Participants experiencing minor intercurrent illnesses may continue in the study provided that the nature, severity, and duration of the illness are documented, and that any unscheduled medications required to treat the illness are also documented. Examples of minor intercurrent illnesses include upper respiratory infections, urinary tract infections, and gastroenteritis. Medications will be allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

Participants experiencing a severe asthma exacerbation during the run-in period will be deemed ineligible for the study, but offered the opportunity to reapply after a period of at least 4 weeks following resolution of the event. If another exacerbation occurs during the subsequent run-in period, the child will be deemed ineligible and excluded from participation.

Once randomization has occurred, intention-to-treat principles will apply; withdrawn participants will be analyzed using available data according to the treatment to which they were randomized. Participants who experience three (3) or more severe asthma exacerbations will have their study medication discontinued, but will continue to be monitored through clinic visits and phone calls.

## 15.9. Medical Monitors and Independent Safety Monitor

Dr. Michael Cabana (who previously served as a site investigator at UCSF) will serve as the medical monitor for all study sites. The medical monitor will review every serious adverse event to confirm study relatedness, unexpectedness, and appropriateness of follow-up care provided.

The Independent Safety Monitor (ISM) is a clinician with clinical trial expertise, and who is not involved in the study. The ISM will adjudicate conflicts between the medical monitors regarding characterization of serious adverse events. The ISM may unmask treatment for a participant in order to determine whether the outcome was related to the study drug.

# 16.STATISTICAL ANALYSIS

## 16.1. Power Analysis

*Primary Aim*

While we will make concerted efforts to retain all of the 400 participants, we conservatively assume that up to 30 (15%) of the 200 children within each group will drop out or be lost to follow-up. Thus, we expect that ≥170 children in each group (vitamin D vs. control) will be retained in the study. We also conservatively assume an incidence of severe asthma exacerbations of 40% in the control group over the follow-up period. Under these assumptions and a two-sided alpha of .05, we will have 88% power to detect a reduction in the rate of severe asthma exacerbations from 40% in the control group to 24% in the intervention group (a 16% reduction). Under the same assumptions, we will have 79% power to detect a reduction in the rate of severe asthma exacerbations from 40% to 26% (a 14% reduction). If the incidence of severe asthma exacerbations in the control group were only 30%, we would have ≥90% power to detect a significant difference between the study groups if the rate of severe asthma exacerbations in the vitamin D group were 15% (a 15% difference in rates).

Our statistical power is shown comparing proportions of severe asthma exacerbations to illustrate that we can detect realistic important clinical differences. In fact, we will have somewhat greater statistical power, given that the primary statistical analyses will employ time to event methods.

*Secondary aims*

The number of participants to be included in the study is defined by the sample size calculations for the primary aim. Calculations for the secondary aims are based on the sample size for the primary aim and the size of the effect that can be detected is estimated.

Viral-induced severe asthma exacerbations: Using the same assumptions as above, while also assuming that 80% of the children in the placebo group who have at least one severe asthma exacerbation will have at least one viral-induced severe asthma exacerbation confirmed as viral-induced at Dr. Gern's laboratory, we will have ≥80% power to detect a reduction in the rate of viral-induced severe exacerbations from 25% (30% of 80% in the placebo group) to 13% in the vitamin D group.

Reduction of ICS dose: On the basis of the assumptions above regarding sample size and type I error, we will have ≥80% power to detect: 1) an increment in the proportion of children in whom their ICS dose is halved from 50% in the placebo group to 64% in the vitamin D group, or 2) an increment in the proportion of children in whom their ICS dose is halved, from 70% in the placebo group to 83% in the vitamin D group. For the comparison of the average cumulative dose there is at least 80% power to detect and effect size of 0.13, assuming that that the correlation between the covariates in the analysis of covariance model (site, race/ethnicity) and the average cumulative dose is 0.2.

## 16.2. Outcome Analyses

The primary outcome of this trial will be time to a severe asthma exacerbation. The main analysis (using the intent-to-treat principle) will be conducted using a Cox proportional hazards regression model fit to the time to a severe asthma exacerbation and adjusted for race/ethnicity and study site (as randomization will be stratified on these variables). Should there be an unexpected significant imbalance in other relevant baseline covariates (see above) between treatment groups despite randomization, those covariates would be considered for inclusion in the multivariate analysis.

The secondary outcomes will be time to a viral-induced severe asthma exacerbation, and reduction in

the ICS dose and average cumulative dose during the Trial Phase. Our analytical approach to time to viral-induced severe asthma exacerbation will be similar to that outlined for our primary outcome. An analysis of covariance model will be used for the analysis of the difference in average cumulative dose of ICS between the vitamin D and placebo groups at the end of the trial, with treatment group as the primary explanatory variable, adjusting for race/ethnicity and study site. Logistic regression will be used for the analysis of the proportion of participants achieving a reduction in ICS dose at or after v6. As with the other analyses, the logistic regression models will be adjusted for race/ethnicity and study site.

Exploratory Analyses will allow for multiple severe asthma exacerbations or viral-induced exacerbations to be included per participant. This repeated measures Cox proportional hazards regression model can adjust for correlation among multiple responses in a participant (using SUDAAN Software for the Statistical Analysis of Correlated Data, Release 9.0, Research Triangle Institute 2004).

Additional exploratory analyses will be undertaken to identify potential baseline modifiers of the effect of the intervention (vitamin D) on severe asthma exacerbations. Selected baseline characteristics (all measured prior to randomization) to be examined will include those most likely to modify the effect of vitamin D on severe asthma exacerbations: race/ethnicity, study site, deficient (< 20 ng/ml (50 nmol/L)) 25(OH)D (comprising approximately 45% of children in our pilot study and 53% of adults in the VIDA trial), BMI-z score and atopy. The approach to identification of effect modifiers will depend on the outcome and follow the same analytical strategy as that described for our primary and secondary outcomes. A separate analysis will be conducted for each potential modifier of interest. Each model will include the main effects of the treatment group and the potential modifier (e.g. atopy) on the outcome, as well as a two-way interaction term.

## 16.3. Multiple Comparisons

All pre-specified hypotheses will be tested individually at the $\alpha$=0.05 level of significance, using a conservative two-sided alternative hypothesis (even when the stated hypothesis is one-sided). More specifically, results from the analyses of the effect of vitamin D on our primary and secondary outcomes will not be adjusted for multiple comparisons, since they have been defined a priori and are limited in scope. Findings from our exploratory analyses (e.g. testing for modification of the effect of vitamin D on our primary or secondary outcomes by selected baseline variables) will be adjusted for the number of models tested (e.g., using False Discovery Rate methods), and testing for effect modification will be limited to the variables specified above to reduce the potential for false positive results. In addition, findings from any exploratory analysis will be reported as such.

## 16.4. Missing Data

Investigators at the DCC are well aware of the problems caused by missing data, and subscribe to the credo that the best solution to these problems is the *elimination or reduction* of missing data. To prevent or minimize the amount of missing data, careful data collection, data management and QC procedures must be implemented. These measures may include, among others, training and certification of all study personnel, careful design of data collection forms, clear and thorough documentation of all study procedures, the implementation of a data management system that minimizes the probability of data entry errors and reporting procedures that help track participants in longitudinal studies.

However, it is prudent to acknowledge that some loss to follow-up and some missing data may occur in any study. In this case, it is critical to understand what events or factors are responsible for data that are missing. That is, are the missing data: (1) missing completely at random (MCAR), (2) missing at random (MAR) or (3) non-ignorable. By knowing how the data are missing (e.g., MAR), the most appropriate analytic approach can be selected. To understand how those individuals with missing data differ from those without missing data, comparisons of the baseline characteristics and the last observed measurement of participants with missing data will be made to those without missing data and tests for MAR and MCAR will be applied when applicable.

Because of the possibility of drop-outs and other missed visits, there will be some missing data. The statistical models and analyses that are planned for the primary and secondary outcomes assume that the data are missing-at-random (MAR). Because likelihood-based methods will be applied, MAR data still yield valid estimates. In the unlikely event that the MAR assumption is violated, we would use alternative approaches such as pattern-mixture modeling.

## 16.5. Interim Data Analysis and Safety Analysis

There will be three aspects of the interim analysis. First, for efficacy, the Lan and Demets approach will be utilized to conduct the interim efficacy analysis. This approach is preferable over other approaches because it is flexible to the number of interim analyses conducted during the course of the study. The Lan and DeMets approach requires the use of a spending function to allocate the type I error to the interim analysis. The O'Brien and Fleming spending function which minimizes the type I error allocated to the interim analysis, saving the vast majority for the primary analyses will be used. For the proposed interim analysis, assuming that 50% of the study will be completed, it is estimated that the type I error allocated to the interim analysis will be 0.005, leaving 0.048 of the type I error for the primary outcome analysis

Concurrent with the interim analysis, a futility analysis will be conducted. The conditional power, the power available to detect the projected intervention effect size based on the observed overall event rate, will be calculated. A conditional power lower than 0.3 would be used to trigger a discussion with the DSMB regarding futility.

In addition to the interim efficacy analysis and futility analysis, a safety analysis will be conducted at each DSMB meeting. Two aspects of safety will be conducted, one for deficiency and one for toxicity. The rates of severe vitamin D deficiency (defined as a serum vitamin D level <10 ng/ml) observed at any time during follow-up will be compared between the two treatment arms using a chi-square or Fisher's Exact test. The same approach will be carried out to compare the rates of toxicity (defined as a serum vitamin D level >150 ng/ml or a serum calcium level >10.8 mg/dl), and multivariate linear regression (adjusting for age) will be used to compare $U_{Ca}/U_{Creat}$ ratios. A subgroup analysis will be conducted among those a baseline vitamin D levels of 10-14 ng/ml. In this instance, a Fisher's Exact test will be used to compare the proportion of participants with severely deficient levels at any time during follow-up. A separate subgroup analysis will be conducted among those with a baseline vitamin D level of 26-30 ng/ml. In this instance, a Fisher's Exact test will be used to compare the proportion of participants with vitamin D toxicity at any time during follow-up and a two-sample t-test or linear regression (as above) will be used to compare mean $U_{Ca}/U_{Cr}$ ratios.

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