ASTHMANET

Vitamin D add-on therapy enhances corticosteroid responsiveness in Asthma (VIDA)

Study Protocol

Version 21.1

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A study to determine if the addition of vitamin D is superior to placebo in vitamin D insufficient asthma patients with persistent symptoms on a low dose of inhaled corticosteroid

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I. PROPOSED TRIAL SUMMARY

This is a randomized, double-blind parallel group trial that will enroll individuals 18 years and older who have vitamin D insufficiency and asthma with persistent symptoms despite low-dose inhaled corticosteroid (ICS). Participants on low-dose inhaled corticosteroid will be randomized to add-on therapy with either placebo (ICS+placebo) or high-dose vitamin D (100,000 IU load followed by 4,000 IU/day) (ICS+D) for a 28-week period. During the inhaled corticosteroid-stable phase (phase I, weeks 5-17), participants will remain on low-dose inhaled corticosteroid. During the inhaled corticosteroid-taper phase (phases IIa and IIb), participants will taper their inhaled corticosteroid by 50% at two time-points (at 12 weeks (phase IIa, weeks 17-25) and 20 weeks (phase IIb, weeks 25-33)) post-randomization. We will determine if the addition of vitamin D reduces the likelihood of treatment failure when compared to placebo during both the inhaled corticosteroid-stable and inhaled corticosteroid-taper phases of the study. Given the high prevalence of both vitamin D insufficiency and asthma, this trial has high potential to impact daily asthma management.

II.BACKGROUND AND RATIONALE

Despite advances in the understanding of the pathogenesis and treatment of asthma, asthma morbidity remains high and patient adherence with current therapy remains moderately low (1, 2). Recent studies have highlighted the fact that even though inhaled corticosteroids are the preferred therapy for persistent asthma in children and adults (3), optimal asthma control is often not achieved even with increasing doses of medications (4) and there is significant variability in response to inhaled corticosteroid.

A. Variability in response to corticosteroids in asthmatic patients

Corticosteroids, inhaled and oral, are "the most potent and effective anti-inflammatory medication currently available" for asthma (3). Nevertheless, there is significant variability in response to corticosteroid therapy. This variability has been most notable in severe persistent asthma demonstrated by lack of adequate asthma control and improvement in FEV_1 (5-8). Such clinical corticosteroid refractoriness has been related to altered corticosteroid cellular sensitivity. Potential explanations for this phenomenon include reduced number of corticosteroid receptors, reduced corticosteroid receptor binding, increased corticosteroid receptor expression and increased nuclear transcription factors (9-15).

Malmstrom et al. first noted the finding of inter-individual variation in response to inhaled corticosteroid compared to oral montelukast in subjects with mild-moderate asthma (16). While the response to inhaled corticosteroid in terms of change in FEV_1, on average, was approximately twice of that obtained with montelukast there was significant variability. Fifty percent of subjects on inhaled corticosteroid had improvement in FEV_1 by at least 11%. However, 27% had no change and 21% had a decrease in FEV_1 ranging from 1 to 30%. Furthermore, in the Gaining Optimal Asthma controL (GOAL) study (4), despite escalation of inhaled corticosteroid dose in patients with asthma of different

severities, 30% or more of the subjects did not achieve the pre-specified endpoint of well-controlled asthma.

The Asthma Clinical Research Network (ACRN) and the Childhood Asthma Research and Education (CARE) Network further explored variability in responsiveness to inhaled corticosteroid in both adults and children. Szefler et al. compared relative effects of two inhaled corticosteroids over a 24-week period on responsiveness measured as percent change in FEV_1 or airway responsiveness (17). Of the 12 subjects studied with one inhaled corticosteroid preparation, 5 had a good response as measured by a >15% increase in FEV_1, 5 had a poor response (<5% increase) and 2 had a marginal response. Similar inter-patient variability of response was observed with the second inhaled corticosteroid preparation and near-maximal effects occurred with low-medium dose of both inhaled corticosteroids. In a study to predict response to inhaled corticosteroid efficacy, Martin et al. noted that subjects who had no response to initiation of inhaled corticosteroid in terms of FEV_1 improvement did not benefit from maintenance inhaled corticosteroid therapy (18). Those subjects who were characterized as inhaled corticosteroid responders maintained their asthma control (ACQ 0.74 ± 0.12), whereas those placed on placebo had worse asthma control (ACQ 1.23 ±0.13, $p = 0.007$). Baseline predictors of favorable inhaled corticosteroid response included a lower baseline FEV_1 and greater maximum albuterol reversibility. In a separate study, Szefler et al. used a double-blind cross over design to study within-subject variability to inhaled corticosteroid and montelukast in childhood asthma (19). As in the adult studies, a substantial majority (60%) of subjects did not respond to inhaled corticosteroid as defined by a 7.5% increase in FEV_1 over an 8-week interval. In the childhood asthma study, a favorable response was associated with greater allergic-based airway inflammation (exhaled nitric oxide, eosinophilic cationic protein), however the predictive value of baseline variables was low and was not replicated in the adult study (18). These studies indicate that a significant proportion of adult and children with asthma do not achieve a beneficial response to the current asthma standard of therapy, inhaled corticosteroid, and a better understanding of the reasons for this

lack of response and potential interventions to enhance clinical corticosteroid response are needed (as included in the current proposal).

B. Rationale for choosing vitamin D insufficiency as a possible cause of inter-patient variability in response to inhaled corticosteroid

The main role of vitamin D in the body is for calcium and phosphorous homeostasis. However, a more extensive role for vitamin D was suggested by the discovery of the near ubiquitous distribution of expression of the vitamin D receptor (VDR) and the extrarenal production of active vitamin D in almost all tissues and cells, including immune cells such as macrophages and T lymphocytes (20). $1,25(OH)_2D$ acts as an immune system modulator, preventing excessive expression of inflammatory cytokines, increasing the 'oxidative burst' potential of macrophages, and production of the antimicrobial peptide cathelicidin (21, 22). In addition, vitamin D suppresses Th1 cell production of interleukin (IL)-2 and interferon (23). These events decrease inflammation by altering the capacity of antigen-presenting cells to activate Th1-cells and favor the induction of T-regulatory (T-reg) cells (24, 25) (20, 23, 26). In a mouse model of inflammatory bowel disease, absence of the VDR accelerated intestinal inflammation (27). Biologic data suggests that **low vitamin D levels create a pro-inflammatory state** that may contribute to the asthma phenotype and lack of response to conventional asthma therapy. Vitamin D signaling pathways and receptor polymorphisms (20, 28, 29) may have effects on the Th1-Th2 imbalance (20, 23, 26), smooth muscle contraction (30, 31), airway inflammation, corticosteroid and prostaglandin regulation, and airway remodeling all of which can impact asthma control.

A specific role of IL-10, a cytokine with anti-inflammatory and immune suppression activities, has been proposed as a link between corticosteroid resistance and vitamin D insufficiency. Corticosteroids enhance the production of IL-10 by stimulated T cells from corticosteroid sensitive but not resistant cells (32). Co-culture of naïve CD4+ T cells with vitamin D and dexamethasone induces the

development CD4+ IL-10 producing T cells and more importantly this combined treatment restores the IL-10 response to corticosteroid in CD4+ T cells from corticosteroid resistance asthmatics (33, 34), providing a rationale to use vitamin D to restore corticosteroid responsiveness. Xystrakis et al. in a proof of concept study studied 3 individuals with corticosteroid resistance and demonstrated enhancement of corticosteroid-induced production of IL-10 from CD4+T cells *in vitro* after ingestion of vitamin D (34). These biologic studies suggest that vitamin D supplementation as proposed in the current study may restore corticosteroid response.

C. Vitamin D level thresholds

Vitamin D status, reflective of total body stores, is usually estimated by measuring plasma 25(OH)D levels. To achieve the maximal efficiency of vitamin D-induced intestinal calcium transport, the serum 25(OH)D concentrations must be at least 30 ng/mL (35). Thus, it is safe to say that vitamin D insufficiency corresponds to levels below 30 ng/ml and sufficiency corresponds to levels of 30 ng/ml and above (36-38). The current dietary recommendations for vitamin D intake, based on the assumption that young and middle-aged adults are more likely than older adults to be exposed to sunlight, are as follows: 400 IU/d for children and adults to age 70 years and 600 IU/d for those older than 70 years (39). To achieve sufficient levels, a minimum intake of 700 to 1000 IU/d was determined in groups of young and older adults exposed to sunshine; however, it has been shown that obese, pregnant and renal patients require about 1000-2000 IU/d (40). The Food and Nutrition Board guidelines specify 2000 IU/d vitamin D_3 as the highest vitamin D intake that healthy adults can consume without risking hypercalcemia but multiple studies show that optimal 25(OH)D level can be achieved safely during winter in adult patients with a dose of 4000 IU (40). The current study includes only individuals with asthma and vitamin D insufficiency (<30 ng/ml).

D. Evidence for increasing prevalence of vitamin D insufficiency

Vitamin D is a nutrient and a pro-hormone largely regulated by environmental factors. The major source of vitamin D is from sunlight. Human skin will produce vitamin D in response to the sun's UV rays. However, increased time spent indoors, use of more clothing (41, 42), obesity (43), use of sunscreen (44), latitude, season of the year, and race have a dramatic influence on the cutaneous production of vitamin D_3 causing an increased prevalence of vitamin D insufficiency. Approximately 1 billion people worldwide have vitamin D insufficiency (25(OH)D 20-30 ng/ml) or deficiency (25(OH)D ≤20 ng/ml) (45). In the southern US during winter, vitamin D insufficiency varies from 12% in white males to 75% in African American women (46, 47). Vitamin D deficiency could be as high as 54% of homebound older adults, 84% of elderly black women, and 40% of pregnant African Americans and their neonates (35, 45, 47, 48). The group at greatest risk includes black men and women living in the northern latitudes during all seasons (49). Vitamin D insufficiency has also been reported in association with many diseases, including but not limited to cancer, heart disease, stroke, hypertension, autoimmune diseases, diabetes, depression, chronic pain, osteoarthritis, osteoporosis, muscle weakness, muscle wasting, birth defects, autism, and periodontal disease (45, 48).

E. Studies linking vitamin D with asthma outcomes

A correlation between the increasing prevalence of vitamin D insufficiency with the "asthma epidemic" has been suggested (50). Animal model studies have shown that vitamin D is critical for fetal immune system development and that it regulates lung growth *in utero* (51, 52). These findings have been confirmed in clinical studies on pregnant women demonstrating an inverse association between vitamin D intake during gestation and wheezing in their children during the first years of life (53, 54). Post-hoc analysis of serum samples of asthmatic children from the CAMP study showed that ~35% of patients had levels of vitamin D that were <30ng/mL, and that these children had lower lung function and greater risks for exacerbations than those with levels ≥30 ng/mL (55). A second analysis

in Costa Rican children with asthma likewise found that a log_{10}–unit increase in vitamin D levels was associated with reduced odds of: hospitalization (OR=0.05, 95% CI=0.004–0.71, P=0.03), use of anti-inflammatory medications (OR=0.18, 95% CI=0.05–0.67, P=0.01), and increased airway responsiveness (PD_{20} ≤ 8.58 μmol (OR=0.15, 95% CI=0.024–0.97, p = 0.05)) (56). In addition, vitamin D levels were inversely associated with total IgE and eosinophil counts. Further evidence suggests that this relationship is not limited to children, since an analysis of subjects >20 years of age from the National Health and Nutrition Examination Survey conducted from 1988 to 1994, found a similar association between vitamin D consumption and serum levels with lung function (57).

F. Studies linking vitamin D with respiratory infections and asthma exacerbations

Vitamin D deficiency may be related to the seasonal variation in symptomatic respiratory infections (58, 59) even in southern climates where, despite greater potential for cutaneous synthesis, low vitamin D status is common (60-62). This may be particularly relevant in early childhood, wherein vitamin D deficiency has been associated with the incidence and severity of lower respiratory tract infections, particularly those leading to admission to an intensive care unit (63-66). Mechanistic studies have shown that vitamin D treatment increases β-defensin production (67, 68), alters MHC, CD14 & TLR expression (69-71), and enhances T regulatory cell suppression (34, 72). Interestingly, treatment of human airway epithelial cells with 1, 25-dihydroxy-vitamin D reduces respiratory syncytial virus triggered production of interferon-β and CXCL10 without leading to enhanced viral replication (73). As the majority of asthma exacerbations in adults and children are mediated by viral infections, the AsthmaNet Vitamin D protocol offers a unique opportunity to test the following secondary hypothesis that would provide a mechanistic link to how Vitamin D supplementation could alter the risk of exacerbations.

Limited clinical trial data exist regarding the impact of vitamin D supplementation on respiratory tract infections. Post-hoc analysis of a 3 yr trial of vitamin D supplementation in 208 African American women evaluated self-reported incidence of colds or influenza symptoms (74, 75). Twenty-six women in the placebo group reported infection compared to 8 in the vitamin D group ($p < 0.002$). These authors followed these observations with a prospective randomized controlled trial evaluating 2,000 IU/day vs placebo for 12 weeks in 162 adults from New York (76). There were no significant differences between the two groups in terms of the frequency or severity of self-reported upper respiratory tract symptoms. However, there were several limitations to this study. First, was the shorter duration of the prospective trial (12 weeks). Second, only 4% of the prospective trial participants were African American. Third, vitamin D insufficiency was not an inclusion criteria such that 25% of the participants had serum 25-OH-vitamin D levels > 30 ng/mL. Neither study documented the presence of respiratory viruses. Recently, a randomized, placebo controlled trial of cholecalciferol 1,200 IU/d was performed in 324 children (ages 6-15 yrs) showing a protective effect against the incidence of respiratory illnesses caused by influenza A (OR 0.58, 95% confidence interval 0.34-0.99, $p = 0.04$) (77).

These data support the hypothesis that vitamin D supplementation can improve corticosteroid responsiveness, as measured by lung function, and reduce treatment failures in asthmatics with vitamin D insufficiency as proposed in the current study. Preliminary data from the Childhood Asthma Management Program (CAMP) Study showed that a significant proportion of asthmatic children across the U.S. had low levels of vitamin D and low vitamin D levels were associated with increased risk for severe asthma exacerbation (78). These preliminary data suggest an association between vitamin D deficiency and asthma morbidity and severity. Thus far, a limited number of experimental and epidemiologic studies suggest that vitamin D may reduce the inflammation associated with asthma; therefore, this proposed randomized, placebo-controlled trial will help determine the role of vitamin D supplementation in asthma control.

III. HYPOTHESES TO BE TESTED IN THIS TRIAL

A. Primary Research Hypothesis

In individuals 18 years and older with persistent asthma who remain symptomatic despite low dose inhaled corticosteroid and are vitamin D insufficient (<30 ng/ml), the addition of vitamin D is superior to placebo in reducing treatment failures.

B. Secondary Research Hypotheses

(1) In individuals 18 years and older with persistent asthma who remain symptomatic despite low dose inhaled corticosteroid (ICS) and are vitamin D insufficient, the addition of vitamin D (ICS+D) is superior to placebo in achieving improved lung function in subgroups of interest – those who are overweight/obese and African-Americans.

(2) In individuals 18 years and older with persistent asthma who remain symptomatic despite low dose inhaled corticosteroid (ICS) and are vitamin D insufficient and have reduced corticosteroid responsiveness (defined by lung function change after oral corticosteroids), the addition of vitamin D (ICS+D) will be superior to placebo in improving lung function measures.

(3) In individuals 18 years and older with persistent asthma who remain symptomatic despite low dose inhaled corticosteroid (ICS) and are vitamin D insufficient, the addition of vitamin D (ICS+D) will result in less severe and less frequent colds than placebo.

C. Primary Outcome Measure

Treatment failure is a well-defined asthma outcome reflecting overall asthma control that has been used previously in multiple clinical trials (79, 80). In addition, the ATS and ERS Statement on standardizing endpoints for clinical asthma trials (81) defines moderate exacerbations as events that

require additional treatment to prevent progression to severe exacerbation. This endpoint has clinical utility given that clinical practice guidelines advise that exacerbations should be recognized and treated before they become severe (81). Treatment failure as defined in the current proposal and prior trials (79, 80) is consistent with the ATS/ERS definition of a moderate exacerbation – a deterioration in symptoms and/or lung function with increased rescue bronchodilator use that lasts 2 days or more (81).

Treatment failure status will be defined as the occurrence of one or more of the following:

At-home measurements:

1. Pre-bronchodilator PEF ≤65% of baseline on any 2 of 3 consecutive scheduled measurements.

   Baseline during the run-in period is defined as the PEF from baseline, prebronchodilator spirometry at visit 1, converted to liters/minute, for those who are not required to do spirometry at visit 2. For those who complete spirometry and methacholine challenge at visit 2, baseline PEF is the PEF from baseline, prebronchodilator spirometry at visit 2, converted to liters/minute. Baseline during the oral corticosteroid (OCS) response and post-randomization periods is defined as the average prebronchodilator AM PEF value recorded during the last two weeks of the run-in (last 14 AM PEFs prior to the visit, including the morning of visit 3).

2. An increase in PRN levalbuterol use of 8 or more puffs per 24 hours over baseline use for a period of 48 hrs.

   Baseline during the run-in period is defined as self-reported average daily use of albuterol/levalbuterol during the 14 days prior to visit 2. Baseline in the OCS response and post-randomization periods is defined as average daily use of levalbuterol during the

last two weeks of the run-in (last 14 days prior to visit 3). One nebulizer use is equivalent to two puffs of levalbuterol. Excludes use of levalbuterol for pre-medication for exercise.

In-clinic measurements:

3. Pre-bronchodilator FEV_1 values on 2 consecutive sets of spirometric determinations which are $\leq$ 80% of the baseline pre-bronchodilator value obtained at Visit 3.

   A participant will meet this criterion if he or she experiences pre-bronchodilator FEV_1 values at two consecutive visits during the post-randomization period (e.g., Visits 5 and 6) that are ≤80% of the baseline value.

   The participant will also meet treatment failure criteria if the following set of circumstances occurs:

   If the pre-bronchodilator FEV_1 value at a visit is ≤80% of the pre-bronchodilator value obtained at visit 3, and the participant does not meet other treatment failure criteria, the participant should be given levalbuterol (≥6 puffs in one hour) to assess the degree of reversibility in his/her airflow obstruction. These values must be reported to the physician responsible for the care of the participant on that day. If the physician determines that the participant's response to the bronchodilator is satisfactory, and the participant's clinical condition is stable, the participant may continue in the study, as usual, provided he or she returns to the study site in 24-96 hours for repeat spirometry. In addition, the site coordinator or designee shall telephone the participant every 24 hours to assess his or her condition. No additional provocative procedures (e.g., methacholine challenge, sputum induction) scheduled for that study day should be performed. At the additionally scheduled visit within the next four days, the repeat spirometric pre-bronchodilator FEV_1 value must be >80% of the pre-bronchodilator value obtained at visit 3; if not, the participant will be considered a treatment failure at that time. If spirometric values are within the acceptable range, all procedures for the previously scheduled

visit shall be performed, and the participant will continue on his/her study medications, as usual.

Note that this part of the criterion may also be applied at visit 4 (end of OCS response period). While unlikely, if the participant meets treatment failure criteria during the OCS response period, he or she will be ineligible for randomization at visit 4.

Note: The FEV_1 criterion will not be applicable during the run-in period (visit 2-visit 3) due to lack of a consistent baseline FEV_1 during that period.

4. Any use of additional inhaled or oral/parenteral corticosteroids related to the treatment of the participant's asthma by the study or treating physician.

5. Need for emergency treatment at a medical facility that is related to, or complicated by, the participant's asthma and which results in systemic corticosteroid treatment or hospitalization for an acute asthma exacerbation.

6. Participant refusal to continue study drugs because of lack of satisfaction with treatment.

7. Physician clinical judgment for safety reasons.

D. Secondary Outcome Measures and Baseline Characterization Variables

(1) *Lung function measures*. FEV_1 (liters and % predicted), maximum percent response to bronchodilator (180-360 mcg levalbuterol) and airway hyperresponsiveness as measured by methacholine PC_{20}. As noted previously (18), baseline FEV_1 and bronchodilator reversibility may be predictive of subsequent inhaled corticosteroid response.

(2) *Asthma symptoms.* Symptom frequency will be assessed by e-diary at each study visit and 2-week recall at visit 3 to determine randomization eligibility (inclusion criterion #2 below). The Asthma Symptom Utility Index (ASUI), a validated measure of asthma symptoms, will be used as a

quantitative assessment of asthma symptoms during the study (82).

(3) *Asthma control*. Asthma-control days (ACD) are days recorded in daily e-diary without any asthma symptoms or rescue levalbuterol use. *Rescue-free days* are days recorded in daily e-diary without levalbuterol use. Asthma control will also be assessed by the Asthma Control Test (ACT) (83).

(4) *Exacerbations.* Exacerbations of asthma will be assessed in all participants during all study phases. Exacerbations are defined by meeting criteria for treatment failure AND one or more of the following: (i) Failure to respond within 48 hours to treatment failure rescue algorithm; (ii) FEV_1 <50% of baseline on 2 consecutive measurements (18); (iii) FEV_1 <40% of predicted on 2 consecutive measurements (18); (iv) use of ≥ 16 puffs of "as needed" β-agonist (levalbuterol) per 24 hours for a period of 48 hours; (v) experiencing an exacerbation of asthma in the opinion of study investigator or personal physician; and (vi) use of oral/parenteral corticosteroids due to asthma.

(5) *Asthma-specific quality of life* (QOL). QOL will be measured in all participants using an asthma-specific instrument, Asthma Bother Profile (ABP) (84).

(6) *Impairment.* Impairment from asthma, in terms of productivity loss and activity impairment, will be assessed using an asthma-specific version of the Work Productivity and Activity Impairment questionnaire (WPAI:Asthma) (85).

(7) *Pharmacogenetics.* Potential genetic modifiers of response to corticosteroids and vitamin D and associated metabolic pathways (20, 28, 29) will be evaluated in exploratory analysis.

(8) *Vitamin D levels.* Initial and post-randomization vitamin D levels will be compared to above asthma outcomes. The area under the curve (AUC) for vitamin D levels will be calculated for all individuals and compared to asthma control, quality of life and physiologic measures. In addition, an analysis will be performed restricted to those individuals who achieved sufficient vitamin D levels (>30 ng/ml) during the trial compared to those that did not.

(9) *Corticosteroid responsiveness.* Change in lung function in corticosteroid unresponsive and

responsive individuals will be evaluated. Corticosteroid-responsive airflow obstruction will be defined as a ≥5% improvement in FEV_1 following systemic corticosteroids.

(10) *Total inhaled corticosteroid dose.* Cumulative inhaled corticosteroid received dose during all study phases (phases I, IIa and IIb) will be calculated and compared to asthma control, quality of life and physiologic measures.

(11) *Colds.* In all individuals experiencing a cold during the trial, the potential impact of vitamin D on respiratory tract infections will be evaluated. The frequency and severity of colds, as reflected by the peak Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21) score (86) during the cold, will be compared between those in the vitamin D treatment group to those receiving placebo. Secondary analysis of the severity of the cold will evaluate the effect of the intervention on day 1 and 2 WURSS-21 scores.

(12) *Airway inflammation.* Response to vitamin D based on presence of airway inflammation (≥ 3% sputum eosinophils) at baseline (visit 3) and change over time in airway inflammation.

(13) *Stress.* Levels of perceived stress will be assessed using an adapted version of the Perceived Stress Scale (PSS) (87) in all individuals.

(14) *Sinonasal disease*. Rhinitis and sinusitis symptoms will be assessed using a Sinonasal Questionnaire (SNQ) (88) in all individuals.

IV. PROTOCOL OVERVIEW

A. Protocol design

This is a randomized, double-blind, parallel group trial (**Figure 1**) in participants with persistent asthma symptoms (despite the use of low dose inhaled corticosteroid) and low 25(OH)D levels (<30 ng/ml) to determine if the addition of high-dose vitamin D for 28 weeks is superior to placebo. Individuals on low-dose inhaled corticosteroid will be randomized to add-on therapy with either placebo (ICS+placebo) or high-dose vitamin D (100,000 IU load followed by 4,000 IU/day in adults) (ICS+D) for a 28-week period. During the inhaled corticosteroid-stable phase (phase I, weeks 5-17), individuals will remain on low-dose inhaled corticosteroid. During the inhaled corticosteroid-taper phase (phases IIa and IIb), individuals will taper their inhaled corticosteroid by 50% at two time-points (at 12 weeks (phase IIa, weeks 17-25) and 20 weeks (phase IIb, weeks 25-33)) post-randomization.

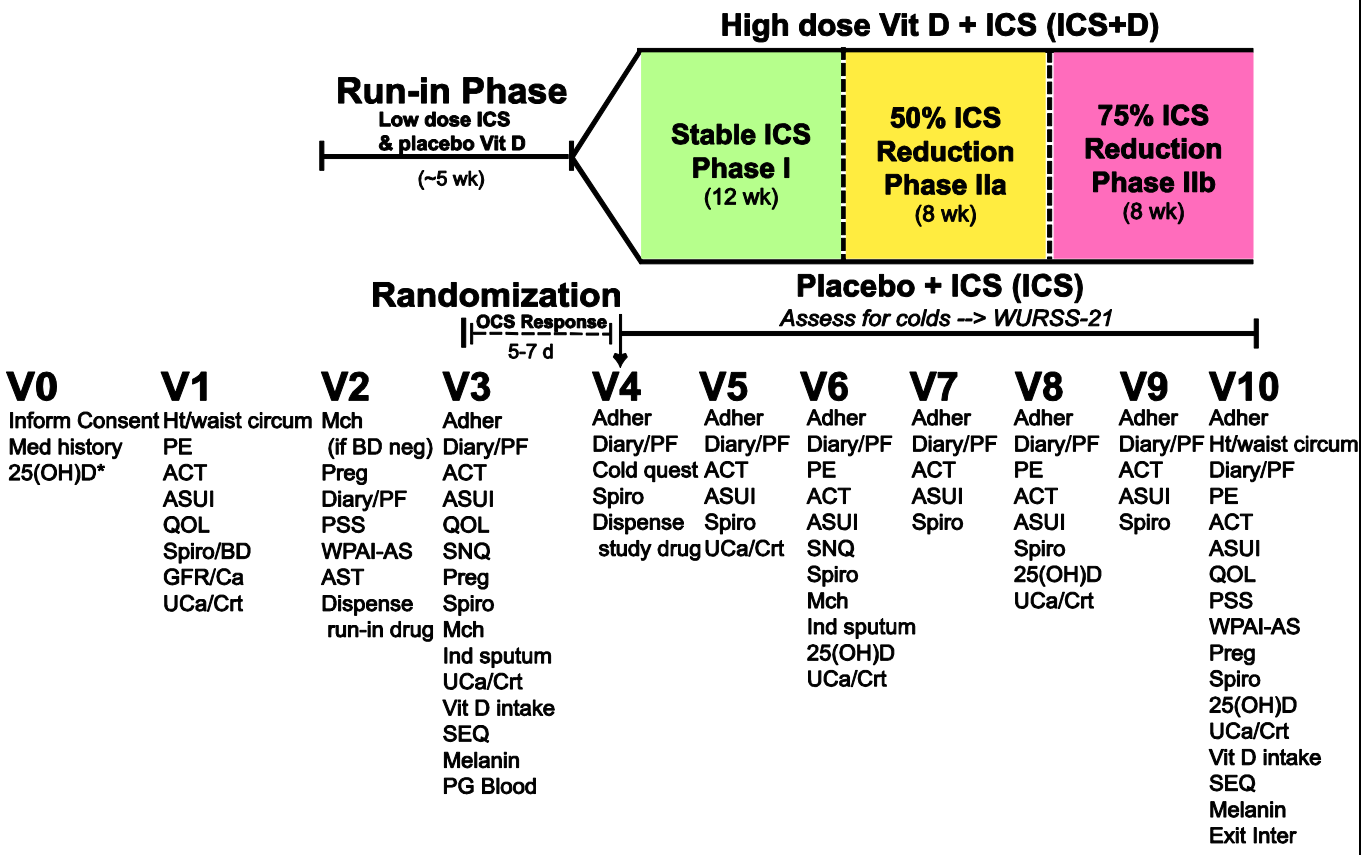

Fig 1. VIDA study design. Study visits V0-10 include: informed consent, medical history (hx)/physical exam (PE), height (Ht) and waist circumference, diary and peak expiratory flow measurements (PF), asthma control test (ACT), Asthma Bother Profile quality of life questionnaire (QOL), Asthma Symptom Utility Index (ASUI), Perceived Stress Scale (PSS), Sinonasal Questionnaire (SNQ), allergy skin test (AST), spirometry (spiro), maximum bronchodilator reversal (BD), urine pregnancy test (preg), methacholine challenge (Mch), vitamin D level (25(OH)D), glomerular filtration rate/serum calcium (GFR/Ca), urine calcium/creatinine (UCa/Crt) ratio, asthma-specific Work Productivity and Activity Impairment questionnaire (WPAI-AS), adherence (adher), vitamin D dietary intake questionnaire (vit D intake), induced sputum (ind sputum), pharmacogenetics (PG) blood, sun exposure questionnaire (SEQ), skin pigmentation (melanin), and exit interview (inter). Oral corticosteroids (OCS) are given for 5-7 d at the end of the run-in phase to assess corticosteroid responsiveness. The three phases of the study post-randomization are stable inhaled corticosteroid (ICS) dose (green), 50% inhaled corticosteroid dose reduction (yellow), and 75% of run-in inhaled corticosteroid dose reduction (red). The Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21) is completed during a cold (cold questionnaire (cold quest) is distributed at visit 4). Additional visits may be necessary to evaluate for treatment failures or exacerbations.

B. Randomization

The randomization scheme will be stratified according to clinical center partnership because differences among clinical sites typically yield a large amount of variability. Within each partnership

the randomization will be stratified based on BMI (BMI ≤ 25 vs. BMI > 25) and race (African-American vs. other).

C. Adherence

Adherence with the study drug, including capsules and inhaled medications, will be assessed during the run-in phase with the use of a medication event monitoring system (MEMS) for the capsules and dosimeter (DOSER, Newmed Corp.) for MDI. We will require all participants to demonstrate adherence with at least 75% of their prescribed inhaled medication and single-blind placebo vitamin D capsules during the run-in phase (at visit 3) in order to remain in the study and undergo baseline measurements. This will select a cohort of participants who are much more likely to be adherent with the remainder of the study, as our experience with other studies has demonstrated.

V. STUDY POPULATION INCLUSION AND EXCLUSION CRITERIA

A. Inclusion criteria for entry

All participants will meet ALL of the following inclusion criteria:

1. Males or females age 18 or greater (at visit 0);

2. Physician-diagnosed asthma for at least previous 12 months (at visit 0);

3. Asthma confirmed by: (a) β-agonist reversibility of FEV_1 ≥12 % following 180 mcg (4 puffs) levalbuterol at visit 1 OR (b) methacholine FEV_1 PC_{20} ≤ 8 mg/ml if not receiving an inhaled corticosteroid or ≤ 16 mg/ml if receiving an inhaled corticosteroid at visit 2. Source documentation for PC_{20} from an AsthmaNet methacholine challenge completed within 6 months of visit 2 will be accepted;

4. Stable asthma controller therapy (inhaled corticosteroid or leukotriene modifier only) dose for past 2 weeks (at visits 0, 1, and 2);

5. FEV_1 ≥ 50% of predicted (evaluated at visits 1, 2 and 3);

6. Vitamin D level of less than 30 ng/ml at visit 0;

7. Ability to provide screening and baseline information at visits 0 and 1;

8. Ability and willingness to provide informed consent at visit 0;

9. Experienced no more than one treatment failure in the VIDA run-in or OCS response periods on previous enrollments;

10. For women of childbearing potential: not pregnant, non-lactating, and agree to practice an adequate birth control method (abstinence, single barrier methods or combination barrier and spermicide, or hormonal) for the duration of the study (at visit 0);

11. If intranasal steroids might be needed, willingness to take a single agent at a stable dose throughout the trial, starting prior to or on enrollment in the run-in period at visit 2.

B. Exclusion criteria for entry

All participants will be excluded if they meet ANY of the following exclusion criteria at visit 0:

1. Taking vitamin D supplements containing > 1000 IU/day of vitamin D or supplements containing >2500 mg/day calcium;

2. Chronic oral corticosteroid therapy;

3. Chronic inhaled corticosteroid therapy > 1,000 mcg of fluticasone daily or the equivalent;

4. New allergen immunotherapy within the past 3 months or anticipated changes to an ongoing immunotherapy regimen. Stable allergen immunotherapy for at least the past 3 months is acceptable.;

5. History of physician-diagnosed nephrolithiasis or ureterolithiasis;

6. History of life-threatening asthma requiring treatment with intubation and mechanical ventilation within the past 5 years;

7. Use of concomitant medications that alter vitamin D metabolism – phenytoin, phenobarbital, cardiac glycosides; or absorption – orlistat, cholestyramine, colestipol; or those that interfere with study endpoints (see Appendix A)

8. Impaired renal function (GFR < 30 ml/min) at visit 1;

9. Asthma exacerbation within past 4 weeks requiring systemic corticosteroids (evaluated at visits 0, 1, and 2);

10. Respiratory tract infection within past 4 weeks;

11. Chronic diseases (other than asthma) that in the opinion of the investigator would prevent participation in the trial or put the participant at risk by participation, e.g. chronic diseases of the lung (other than asthma), heart, liver, kidney, endocrine or nervous system, or immunodeficiency;

12. History of smoking (cigarettes, cigars, pipes, marijuana or any other substances) within the past 1 year or > 10 pack years total;

13. Use of investigative drugs or enrollment in intervention trials in the 30 days prior to screening or during the study;

14. Any condition or compliance issue which, in the opinion of the investigator, might interfere with participation in the study;

15. Serum calcium greater than 10.2 mg/dl on entry (at visit 1);

16. Urine calcium/creatinine ratio greater than 0.37 (urinary Ca and Creat in mg) (at visit 1); and

17. More than 8 weeks elapsed between visit 0 (screen) and visit 2 (evaluated at visit 2).

C. Inclusion criteria for randomization

All participants must meet ALL of the following criteria after the run-in at visit 3 (required for randomization at visit 4):

1. Successful completion of the run-in period, including: a) Completed at least 10 of the last 14 days of diary entries and peak flows in the e-diary PEF device, and b) Received treatment with inhaled corticosteroid and single-blind placebo vitamin D for 75% of the days between visit 2 and 3 confirmed by DOSER and MEMS electronic monitoring;

2. Asthma symptoms on at least two days or one night per week on average over the last 2 weeks as recorded on participant e-diary;

3. No hospitalization or urgent medical care visit for asthma during the run-in period;
4. No oral corticosteroid use during run-in period;
5. No need for any additional controller medication for asthma symptoms;
6. No treatment failure during the run-in period; and
7. Urine calcium/creatinine ratio ≤0.37 (urinary Ca and Creat in mg) at visit 3.

Eligibility for randomization will be evaluated at visit 3 so that the systemic corticosteroid used to define corticosteroid responsiveness does not influence the entry criteria. Randomization and the dispensation of double-blind study medication will not occur until visit 4. While not expected, participants who experience a treatment failure during the OCS response period (visit 3-visit 4 interval) will be ineligible for randomization at visit 4.

D. Rationale for Enrollment Restricted on serum 25(OH)D concentrations

Our primary hypothesis relates to the potential benefit of Vitamin D_3 added to inhaled corticosteroid in individuals with symptomatic asthma, and while there is debate about the most clinically-meaningful cut point for serum 25(OH)D (89), 30 ng/mL corresponds to the serum 25(OH)D concentration proposed by many as the lower end of the optimal range for both skeletal and other outcomes (36-38). Therefore, we will restrict enrollment in this study to individuals with asthma and serum 25(OH)D concentrations <30 ng/mL. It is not anticipated that this cut point will impair recruitment based on data from NHANES 2000-2004, which suggest that ≥ 70% of the US population has a serum 25(OH)D concentration <32 ng/mL (90).

VI. PROTOCOL DETAIL AND VISIT STRUCTURES

A. Overview of study

All participants in the VIDA trial will undergo a brief screening visit at visit 0 to obtain informed consent, a brief medical history and a 25(OH)D level (**Figure 1**). Results of the 25(OH)D level will be made available to the AsthmaNet Data Coordinating Center (DCC) within one week of shipping date. If participants have a low vitamin D level (<30 ng/ml), they will be invited back for visit 1 procedures. To avoid introducing potential bias, the participant, clinic personnel, and DCC staff involved in day-to-day operations of the trial will be kept blinded to the actual vitamin D level. During visit 1 a complete medical history will be taken and the diagnosis of asthma will be confirmed with spirometry and bronchodilator reversibility. A complete physical exam will be completed. Asthma control will be assessed using a variety of questionnaires. Body mass index (BMI, kg/m^2) will be calculated from obtained height and weight in all participants; waist circumference and other body measurements will be measured. These data will be utilized to assess if any of these covariates influence asthma control. In addition, each participant will undergo measurement of U_{Ca}/U_{Creat}, serum creatinine (to calculate eGFR) and serum calcium at visit 1.

Methacholine bronchoprovocation will be performed at visit 2 if the participant did not demonstrate bronchodilator reversibility at visit 1. If the participant met either reversibility or PC_{20} criteria, he (she) will then be provided open-label low dose inhaled corticosteroid with a DOSER and vitamin D-like single-blind placebo study capsules with a MEMS monitor to use for a four week run-in period. Participants will complete questionnaires and undergo allergy skin testing.

At visit 3, if adherence and other study criteria are met, participants will be considered eligible to continue in the study. Questionnaires will be completed and baseline skin pigmentation measured. For eligible participants, a methacholine bronchoprovocation and sputum induction will be performed.

Eligible participants will receive additional open-label low-dose inhaled corticosteroid and single-blind placebo vitamin D-like capsules. All eligible participants will start oral prednisone 40 mg/d for 5 + 2 days (see below). In addition, DNA from whole blood will be obtained for future genotyping studies (pharmacogenetics, PG blood) and plasma will be banked for future proteomic analysis.

At visit 4, participants will undergo spirometry to establish corticosteroid responsiveness. Participants will be randomized in a 1:1 allocation to: a) high dose vitamin D (100,000 IU load followed by 4,000 IU/day) plus low dose inhaled corticosteroid (ICS+D), or b) placebo vitamin D-like capsules plus low dose inhaled corticosteroid (ICS). All participants will receive open-label low dose inhaled corticosteroid and double-blind vitamin D capsules. At visit 5 U_{Ca}/U_{Creat} ratios will be monitored for hypervitaminosis D. At subsequent visits 6, 8 and 10, 25(OH)D and U_{Ca}/U_{Creat} ratios will be monitored for hypervitaminosis D (91). Treatment failure criteria will be thoroughly evaluated at each clinic visit, and participants will be asked to contact the clinical site between visits if they experience symptoms of treatment failure. During the treatment phase, the inhaled corticosteroid dose will be held stable for 12 weeks (Phase I), followed by a 50% inhaled corticosteroid reduction for 8 weeks (Phase IIa), and then a 75% inhaled corticosteroid reduction for the final 8 weeks (Phase IIb) (see below). A second methacholine bronchoprovocation will be performed in all participants at visit 6. A second sputum induction will be performed at visit 6 in 200 participants who provided an adequate sputum sample at visit 3. Throughout the treatment phase, participants will be assessed for colds and the WURSS-21 administered when a participant gets a cold. Exit interview will be conducted at visit 10.

B. Corticosteroid taper phases

At visit 6, participants will undergo tapering of their open-label inhaled corticosteroid dose if stability criteria are met (similar to those used in prior ACRN trial (80)). The purpose of corticosteroid tapering is to test the hypothesis that the addition of vitamin D may allow sparing of corticosteroid dose in participants with stable asthma. Furthermore, we will determine if the addition of vitamin D

reduces the likelihood of treatment failure when compared to placebo during both the inhaled corticosteroid-stable and inhaled corticosteroid-taper phases of the study. Stability criteria include: 1) No significant asthma exacerbation (see criteria in X.C), 2) no more than one treatment failure post-randomization, and 3) no treatment failure in last two weeks. At visit 6, participants will have their inhaled corticosteroid reduced by 50%. For example, for those participants taking ciclesonide 80 mcg/puff two puffs twice daily, the dose will be reduced to two puffs once daily. At visit 8, participants will undergo a second tapering of their open-label inhaled corticosteroid dose if stability criteria are again met. The dose will be reduced again by 50% or a total 75% reduction of the open-label inhaled corticosteroid dose during the run-in phase. For example, those participants who achieved a 50% taper in Phase IIa will have the ciclesonide 80 mcg/puff further tapered to one puff daily. For those participants who did not taper in Phase IIa but now meet stability criteria, they would taper their inhaled corticosteroid dose by 50%.

If participants meet criteria for treatment failure status, the dose of inhaled corticosteroid will be doubled (for treatment failures occurring during Phase II, the dose will be increased to the dose the participant was receiving prior to initiation of the most recent steroid taper (i.e. returned either to Phase I or Phase IIa dosing, as appropriate)) and continued for 7 days (see X.D Adjustment of Trial Medications). If, after 7 days, the treatment failure episode is clinically-resolved, the corticosteroid stable dose (for treatment failures during Phase I) or pre-failure tapering dose (for treatment failures during Phase II) of inhaled corticosteroid will be resumed and study visits will continue as per protocol. If an exacerbation or second treatment failure occurs during the post-randomization phase of the study, no further inhaled corticosteroid dose tapering will be attempted.

If participants experience a treatment failure event that also meets the criteria for a significant asthma exacerbation, dosing of inhaled corticosteroid depends on the phase in which the event occurred. If the exacerbation occurs during Phase I, the dose of inhaled corticosteroid will be doubled (to 200% of the inhaled corticosteroid stable dose) for 7 days. If, after 7 days, the event is clinically-

resolved, the dose will be reduced back to the corticosteroid stable dose for the remainder of the study, and visits will continue as per protocol. If the exacerbation occurs during Phase II, the dose of inhaled corticosteroid will be increased to the corticosteroid stable dose and maintained for the remainder of the study. Additional treatment for exacerbations is allowed at the treating physician's discretion.

C. Detailed visit structure

Visit 0 (pre-screen)

The goal of this visit is to explain the study to potential participants, obtain informed consent, collect a brief medical history, and obtain a vitamin D level.

Procedures Performed:

Informed consent

Brief medical history

Serum 25 (OH) D

Visit 1 (screen) (must occur prior to visit 2 and within 8 weeks of visit 0)

The goal of this visit is to confirm the diagnosis of asthma by bronchodilator reversibility testing (12%) with levalbuterol (4 puffs) as part of a maximum reversal test.

Procedures Performed:

Complete medical history

Physical examination (long)

Height, weight; waist, hip, neck measurements (anthropometrics)

Asthma Control Test

Asthma Symptom Utility Index

Asthma Bother Profile

Spirometry

Maximum Reversibility Testing with levalbuterol

Serum creatinine (to calculate GFR) and calcium measurements

Urine calcium / creatinine ratio measurement

Visit 2 (must occur within 8 weeks of visit 0), Study Week 0

The purpose of this visit is to perform additional study procedures and to begin run-in phase medications. For participants who did not meet the bronchodilator reversal criterion at visit 1, spirometry and the methacholine bronchoprovocation procedure will be performed to confirm participant eligibility before proceeding further.

Procedures Performed:

Perceived Stress Scale

Asthma-specific Work Productivity and Activities Impairment Questionnaire

Urine Pregnancy Test (if female of child-bearing potential and undergoing Methacholine Bronchoprovocation)

Spirometry (only for participants undergoing methacholine challenge)

Methacholine Bronchoprovocation

Allergy Skin Testing

Diary and Peak Flow Meter Dispensation and Explanation of Procedures

Run-in Drug Dispensation and Proper Use Explanation (open-label low-dose inhaled corticosteroid and single-blind Placebo Vitamin D)

Visit 3, Week 4 (+7 day window)

The purpose of this visit is to assess participant adherence with run-in medications and study procedures and to perform additional study procedures. Eligibility assessment for randomization is made at this visit.

Procedures Performed:

Review of Participant Adherence with Run-in Medications

Asthma Control Test

Asthma Symptom Utility Index

Asthma Bother Profile

Sinonasal Questionnaire

Pregnancy Test (in all females of child-bearing potential)

Spirometry

Methacholine Bronchoprovocation

Induced Sputum

Vitamin D Dietary Intake Questionnaire

Sun Exposure Questionnaire

Skin Pigmentation (Melanin) Measurement

Diary and Peak Flow Review

Eligibility Assessment

Dispensation of Open-Label Prednisone Supply (for OCS response at visit 4)

Dispensation of low dose inhaled corticosteroid and single-blind placebo vitamin D

Blood Draw for DNA Isolation (can be drawn at V3 or V6 as an option)

Urine Calcium / Creatinine Ratio measurement

Visit 4, Week 5 (-2 day window)

The purpose of this visit is to assess participant adherence with study procedures, determine the response to the 5 + 2 days of prednisone and to perform study randomization procedures. Participants will be scheduled to return to the clinical site on day 5, 6, or 7 of prednisone in order to provide oral corticosteroid response data. In extenuating circumstances, if a participant cannot return by day 7 of prednisone, allowances will be made for the participant to return for visit 4 within 2 weeks of visit 3. In cases where more than 7 days have elapsed between visit 3 and visit 4, data will not be used for corticosteroid response analyses. If a participant cannot return for visit 4 within 2 weeks of visit 3, he or she will be terminated from the study and can consider re-screening at a later date.

Procedures Performed:

Review Participant Adherence with study medications and oral prednisone

Diary and Peak Flow Review

Cold Questionnaire (WURSS-21) Dispensation and Explanation

Spirometry for oral corticosteroid response

Randomization

Dispensation of Randomized Study Drugs (open-label 100% inhaled corticosteroid dose and blinded Vitamin D/placebo)

Dispensation of Rescue Prednisone

Telephone contact, Week 8

Review participant's use of Study Medications, Answer Questions, and Assess for Treatment Failures (participant will be scheduled for treatment failure visit, if appropriate)

Visit 5, Week 11 (-5/+5 day window)

Continuing treatment with 100% inhaled corticosteroid dose and Vitamin D / placebo.

Procedures Performed:

Asthma Control Test

Asthma Symptom Utility Index

Review of Participant Adherence with Study Medications

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

Urine calcium/creatinine ratio measurement

Telephone contact, Week 14

Review participant's use of Study Medications, Answer Questions, and Assess for Treatment Failures (participant will be scheduled for treatment failure visit, if appropriate)

Visit 6, Week 17 (-5/+5 day window)

End treatment with 100% inhaled corticosteroid dose and Vitamin D/placebo.

Procedures Performed:

Asthma Control Test

Asthma Symptom Utility Index

Sinonasal Questionnaire

Review of participant adherence with Study Medications

Diary, Cold Questionnaire and Peak Flow Review

Physical Exam (short)

Spirometry

Treatment Failure Assessment

Methacholine Bronchoprovocation

Urine Pregnancy Test (if female of child-bearing potential and undergoing methacholine bronchoprovocation)

Induced Sputum

Serum 25 (OH) D measurement

Urine calcium/creatinine ratio measurement

Dispensation of Randomized Study Drugs (open-label 50% inhaled corticosteroid dose and blinded vitamin D/placebo)

Visit 7, Week 21 (-5/+5 day window)

Continuing treatment with 50% inhaled corticosteroid dose and Vitamin D/placebo

Procedures Performed:

Asthma Control Test

Asthma Symptom Utility Index

Review of participant adherence with Study Medications

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

Dispensation of Randomized Study Drugs

Visit 8, Week 25 (-5/+5 day window)

End treatment with 50% inhaled corticosteroid dose and Vitamin D/placebo.

Procedures Performed:

Asthma Control Test

Asthma Symptom Utility Index

Review of Participant Adherence with Study Medications

Physical examination (short)

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

Serum 25 (OH) D measurement

Urine calcium/creatinine ratio measurement

Dispensation of Randomized Study Drugs (open-label 25% inhaled corticosteroid dose and blinded Vitamin D/placebo)

Visit 9, Week 29 (-5/+5 day window)

Continuing treatment with 25% inhaled corticosteroid dose and Vitamin D/placebo.

Procedures Performed:

Asthma Control Test

Asthma Symptom Utility Index

Review of Participant Adherence with Study Medications

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

Dispensation of Randomized Study Drugs

Visit 10, Week 33 (-5/+5 day window)

This visit will conclude the study.

End treatment with 25% inhaled corticosteroid dose and Vitamin D/placebo.

Procedures Performed:

Height, weight; waist, hip, neck (anthropometrics)

Physical examination (long)

Asthma Control Test

Asthma Symptom Utility Index

Asthma-specific Work Productivity and Activities Impairment Questionnaire

Asthma Bother Profile

Perceived Stress Scale

Review of Participant Adherence with Study Medication

Diary, Cold Questionnaire and Peak Flow Review

Urine Pregnancy Test (for all women of child-bearing potential)

Spirometry

Treatment Failure Assessment

Serum 25 (OH) D Measurement

Urine calcium/creatinine ratio measurement

Vitamin D Dietary Intake Questionnaire

Sun Exposure Questionnaire

Skin Pigmentation (Melanin) Measurement

AsthmaNet Satisfaction Questionnaire

Exit Interview

Exit visit (in those participants who have been randomized and are withdrawing consent or meet drop-out criteria (i.e., more than two treatment failures))

Asthma Control Test

Review of Participant Adherence with Study Medication

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

Serum 25 (OH) D Measurement

Exit Interview

Treatment failure or Exacerbation Visit

This visit will be performed if a participant experiences treatment failure or exacerbation conditions between regularly-scheduled study visits and needs to be seen at the clinical site. Procedures to be performed include:

Physical Examination (long)

Asthma Control Test

Review of Participant Adherence with Study Medication

Diary, Cold Questionnaire and Peak Flow Review

Spirometry

Treatment Failure Assessment

D. Study Procedures

Pulmonary Function Testing. All participants will undergo spirometry at visits 1 and 3-10. Spirometric studies are performed in accordance with ATS recommendations (92) and AsthmaNet manuals of procedure (MOP). All participants will undergo methacholine bronchoprovocation at visits 3 and 6 [and visit 2 if no evidence of bronchodilator responsiveness at visit 1] in accordance with ATS recommendations (93) and AsthmaNet MOP. A positive response is considered a drop in the FEV_1 from post-diluent FEV_1 ≥20%.

Corticosteroid-responsiveness phenotype. Many patients with moderate to severe asthma (with bronchial hyperresponsiveness) do not demonstrate corticosteroid reversibility likely due to airway remodeling (6, 94). All participants will be characterized as corticosteroid responsive or nonresponsive based on reversibility of airflow obstruction at visit 4 following systemic corticosteroid provided at visit 3. Corticosteroid-responsive airflow obstruction will be defined as a ≥5% improvement in FEV_1 after the administration of 5 + 2 days of prednisone 40 mg/day measured at visit 4 compared to the baseline value at visit 3. If the participant's FEV_1 does not improve by at least 5%, they will be considered to have corticosteroid-nonresponsive airflow obstruction. We have chosen to define the corticosteroid responsive phenotype at visit 3 so that all participants will have completed 4 weeks of run-in medication (confirmed by adherence monitoring). Furthermore, we have used a cut-off of 5% change in FEV_1 based on our experience with systemic corticosteroid response (18). However, we will perform additional subset analysis using the median split, 7.5% and 10% change in FEV_1 as alternate

cutoffs [as they have been used to define inhaled corticosteroid response (17, 19)], as well as the proportion of participants who achieve normal lung function as defined by an FEV_1 ≥85% predicted.

Bronchodilator reversibility phenotype. Participants will undergo spirometry with bronchodilator reversibility testing and maximal bronchodilation as defined by the maximal achievable FEV_1 after levalbuterol (up to 360 mcg or 8 puffs). Participants will withhold medications as per the AsthmaNet MOP. Bronchodilator reversibility has been shown to predict subsequent inhaled corticosteroid response (17-19) and will be evaluated as a covariate potentially influencing the primary endpoint.

Sputum induction. Sputum induction is a relatively simple, repeatable and noninvasive method of collecting airway secretions. Cellular and biochemical analyses of induced sputum samples collected from asthmatic and non-asthmatic subjects have revealed differences in markers of eosinophilic inflammation and bronchovascular permeability in an asthmatic population. This procedure will allow us to assess for the effect of vitamin D on airway inflammation and potential biologic mediators of vitamin D's effect, such as increasing cathelicidin levels (22). An induced sputum sample will be collected at visit 3 in all participants following inhalation of hypertonic saline according to the AsthmaNet MOP. A second induced sputum will be performed at visit 6 in 200 participants who have provided an adequate sputum sample at visit 3. Sputum total and differential cell counts will be counted on May-Grünwald Giemsa-stained cytospins at each center. Cell free supernatant will be frozen and assessed for cathelicidin levels. Analysis will be performed based on presence of airway inflammation at baseline and change over time in airway inflammation (comparing visit 3 to 6 in those participants with acceptable samples at both time points).

Allergy Skin Testing. Skin prick testing of standard allergens will be performed at visit 2 per AsthmaNet MOP, including at a minimum:rat, grass mix, tree mix, mountain cedar, weed mix, mite mix, cockroach mix, mouse, penicillium/alternaria/aspergillus/cladosporium (mold mix), cat, dog, peanut, egg(whole), cow milk with positive and negative control. Food allergens will not be applied if a participant reports ever having had an anaphylactic reaction to a given food product. All allergens will

be applied with the Multi-Test II device using standard preparations from Greer Laboratories (Lenoir, NC). After 20 minutes, the outer contour of the wheal reaction is outlined using a fine felt-tip pen, and the result is expressed as the mean of the lengths of the longest diameter and the perpendicular line through its center. A skin prick test is considered positive if the mean length at 20 minutes after the skin prick is at least 2 mm larger than the size of the negative control. Medications need to be held prior to the allergy skin test include: first generation antihistamines (3 days), second-generation antihistamines (7 days), topical nasal antihistamines (5 days), non-SSRI antidepressants (3 days), and H_2 blockers (3 days) as per AsthmaNet MOP.

Asthma symptoms and control. Asthma symptoms will be assessed by diary recall of asthma-control days (ACD) and rescue-free days (RFD), at all visits and by using a standardized questionnaire (ASUI) in all participants at Visits 1, 3 and 5-10. ACD are days recorded in daily diary without any asthma symptoms or rescue levalbuterol use. RFD are days recorded in daily diary without levalbuterol use. The Asthma Symptom Utility Index (ASUI) provides a validated quantitative assessment of asthma symptoms during the prior two weeks (82). *Asthma control* will be assessed by the Asthma Control Test (ACT) (83) in all participants at Visits 1, 3, 5-10 and at additional treatment failure visits scheduled between regular clinic visits.

Asthma-specific quality of life questionnaires (QOL). QOL will be measured in all participants at Visits 1, 3 and 10 using an asthma-specific instrument, the Asthma Bother Profile (ABP). The Asthma Bother Profile is a 22 item measure designed to assess adult patient perception of the asthma experience and distress, as well as patient perception of their asthma management. This asthma quality of life measure is unique among currently available measures in its emphasis on the psychosocial impact of asthma, including items measuring perceived bother, mood, fear, social relations and financial impact.

Sinonasal Questionnaire (SNQ). Sinusitis and rhinitis are very common in asthma and may lead to poorly controlled asthma. We will explore the effect of vitamin D on sinusitis and rhinitis using the

sinonasal questionnaire (SNQ) at visits 3 and 6. The SNQ is a sensitive, specific and reproducible instrument to screen for sinonasal disease (88).

Vitamin D Intake Questionnaire. Participant dietary intake of vitamin D (and other vitamin supplements) will be assessed at visits 3 and 10 using a modified food frequency questionnaire (95). Assessment of the responses to these questionnaires will help account for dietary sources of vitamin D in addition to the study intervention.

Sun Exposure Questionnaire (SEQ). We will collect UV exposure history using a modification of the UV exposure questionnaire that is used in the Nurses' Health Study (Appendix C) (96) at visits 3 and 10. Briefly, this questionnaire will inquire about UV exposure information such as average hours per dayspent outdoors in summer and winter, and sunscreen and tanning salon habits.

Asthma-specific Impairment. Impairment from asthma, in terms of productivity loss and activity impairment, will be assessed using an asthma-specific version of the Work Productivity and Activity Impairment questionnaire (WPAI:Asthma) in all participants. The WPAI:Asthma has been previously validated in a severe difficult-to-treat asthma population in The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study (85). WPAI:Asthma questionnaire will be measured at visits 2 and 10.

Cold Questionnaire. In all individuals experiencing a cold during the trial, the potential impact of vitamin D on respiratory tract infections will be evaluated. The frequency and severity of self-reported colds, as reflected by the peak Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21) score (86) during the cold, will be compared between those in the vitamin D treatment group to those receiving placebo. Instructions on how to complete the WURSS-21 at the onset of cold symptoms will be reviewed at visit 4 and reinforced at each subsequent visit. Secondary analysis of the severity of the cold will evaluate the effect of the intervention on day 1 and 2 WURSS-21 scores.

Stress Questionnaire (PSS). Psychological stress is a well known co-morbidity in asthma that

occurs as a result of having chronic respiratory symptoms that limit activity and reduce quality of life (97-99). Moreover, psychological stress is also a risk factor for asthma associated with lower FEV_1, reduced asthma control and asthma-related quality of life, increased exhaled nitric oxide levels and higher serum IgE levels (100-102). These psychological stress-related associations may be mediated via mechanisms involving up-regulation of inflammatory cytokines and increased cortisol production via activation of the hypothalamic-pituitary axis, which can lead to corticosteroid resistance. This has been shown in vitro by the reduced capacity of corticosteroid to suppress interleukin-6 production (103, 104). Levels of perceived stress will be assessed at visits 2 and 10 using an adapted version of the Perceived Stress Scale (PSS) (87). This is a short 10-question instrument that has been validated in adults.

Skin Pigmentation (Melanin). Participants will have the degree of pigmentation/melanin measured at visits 3 and 10 using the Smart Probe 400 (IMS, Inc, Milford, CT). This is a spectrophotometer device that measures degrees of pigmentation on a continuous scale from 0 to 100, 0 being absolute black and 100 being absolute white. Each participant will have pigmentation measurements recorded from the exposed forearm, upper inner arm, forehead and abdomen, with two readings averaged and recorded at each location (Appendix D).

U_{Ca}/U_{Creat} ratios. Urinary calcium to creatinine (U_{Ca}/U_{Creat}) ratios will be measured at visits 1, 3, 5, 6, 8 and 10. While others have found the 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 (91). If a U_{Ca}/U_{Creat} is greater than 0.37 (urinary Ca and Creat in mg), the participant will be instructed to increase hydration and a repeat measurement will be obtained 30 days later. If the repeat U_{Ca}/U_{Creat} is elevated, then the participant will be instructed to hold study drug and have a serum calcium and 25(OH)D level obtained. If either the calcium or 25(OH)D is elevated, the participant will stop study drug. If the calcium and 25(OH)D are normal, then the participant may resume study drug.

Measurement of serum 25(OH) vitamin D – Liaison assay and quality control and blinding considerations. Participants will have peripheral blood collected for vitamin D level at visits 0, 6, 8 and 10. The DiaSorin LIAISON® 25OH vitamin D assay is a fully automated system utilizing a direct competitive chemiluminescence immunoassay for the quantitative determination of 25OH vitamin D in serum or plasma. This method has equal specificity to both D2 and D3 forms and provides a total vitamin D result. The DiaSorin LIAISON® 25OH vitamin D method is the only fully automated FDA cleared method available today. This assay maintains both inter and intra precision of less than 8% across the linear range. Linearity and recovery, which demonstrate an assay's accuracy, are both excellent with a mean recovery of 102% and an R-squared value for linearity of 0.99. The assay has a measuring range of 4.0 to 150 ng/mL. The results will be expressed in terms of 25-(OH)D and vitamin D insufficiency is defined as level <30 ng/ml. Toxic levels will be considered ≥120 ng/ml (105). Participants above this level will stop blinded study capsules and return to the clinical site for a repeat test as soon as possible. If the second vitamin D level is ≥120 ng/ml, the participant will stay off study capsules for the remainder of the study and will continue to be seen for follow-up visits (intention-to-treat). If the repeat vitamin D level is normal, the participant will resume taking study capsules. The LIAISON® 25 OH Vitamin D TOTAL Control Set is used as assayed quality control samples to monitor the accuracy and precision of the DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay.

In order to preserve the integrity of the blinding of the vitamin D/placebo study drug and to avoid the introduction of biases related to an assessment of the severity of a participant's baseline level of vitamin D insufficiency/deficiency, study participants, clinical personnel, and DCC staff who are directly involved in the day-to-day operations of the trial will not have access to the actual assay results from the screen visit or follow-up visits. The DCC will implement a reporting process such that site coordinators will be notified of the eligibility status of each screened participant (eligible or ineligible). If a follow-up vitamin D level (from visit 6, 8, or 10) is in the toxic range, an e-mail will be autogenerated through the AsthmaNet database and sent to the site coordinator and Principal

Investigator notifying them of the toxicity (but not giving them the specific assay results) so that appropriate actions can be taken, as outlined above. This scenario is expected to occur rarely, if at all, in this trial.

VII. DRUG SUPPLIES

A. Study drugs

The low dose inhaled corticosteroid will be ciclesonide (Alvesco®) 80 mcg/puff two puffs twice daily (total 4 puffs/day). The inhaled corticosteroid will be tapered to two puffs once daily in the AM in Phase IIa and one puff daily in the AM in Phase IIb (see VI.B). The rescue inhaled short-acting bronchodilator will be levalbuterol (Xopenex®). Ciclesonide and levalbuterol will be provided for the study by Sepracor, Inc.

Vitamin D_3 100,000 IU and 4,000 IU will be administered in capsules prepared by BIO-TECH Pharmacal, Inc. with matching placebo capsules. BIO-TECH Pharmacal, Inc. is one of 12 soft gelatin capsule manufacturers in the US and has provided study medication for other clinical trials. Because these doses of vitamin D_3 are not FDA approved for an asthma indication, an IND has been obtained from the FDA (#110227).

B. Rationale for Vitamin D_3 Dose and Treatment Duration

Multiple dosing strategies are available to raise serum 25(OH)D, and there is not unanimity with regard to optimal dosing strategies for achieving optimal vitamin D concentrations (106). We have chosen vitamin D_3 (cholecalciferol) rather than vitamin D_2 (ergocalciferol), as studies suggest it has higher bioavailability and efficacy, (107-109) and with regard to optimal dosing, a recent publication from Aloia and colleagues (106) provides the basis for the vitamin D_3 dosing strategy chosen for adults in this study. These investigators enrolled subjects with serum 25(OH)D concentrations <32 ng/mL (<80 nmol/L) in a six-month long oral vitamin D_3 supplementation study and determined that oral intake of 3800 IU/d was the optimal dosing algorithm to achieve threshold serum 25(OH)D concentrations >32 ng/mL (80 nmol/L). Importantly, neither BMI nor percentage body fat significantly

influenced the response to vitamin D in this study, and there was no evidence of toxicity (hypercalcemia, hypercalciuria, serum 25(OH)D >220 nmol/L) over the 6 month intervention period. Additionally, data from their study suggested that peak serum 25(OH)D concentrations were achieved at 9 weeks in the vast majority of participants, (106) suggesting that our 28 week treatment period is more than adequate to allow maximum achievable 25(OH)D concentrations to be achieved. Data from a clinical trial of vitamin D supplementation in 3 ethnic groups (African Americans, Caucasian, Hispanics) led by Dr. Bruce Hollis demonstrated that supplementation with vitamin D_3 4,000 IU/day provides the most rapid and consistent increase in 25(OH)D levels in all ethnic groups to a mean of 45 ng/ml and eliminated all seasonal variation in circulating 25(OH)D levels (40). Data from other vitamin D supplementation studies reveal a flat curve for vitamin D dose vs. 25(OH)D response up to 10,000 IU/day (110). Lastly, other trials have demonstrated no effect of systemic corticosteroid on vitamin D metabolism (111, 112).

The pharmacokinetics of a single dose of 100,000 IU D_3 has been studied in 30 participants (113). The average increase in 25(OH)D level was 14.9 ng/mL with a 28 day area under the curve of 387 ng/mL, but two subjects never reached a level of 30 ng/mL. This dose is consistent with that obtained from assumptions made from a recently established formula derived from a loading dose trial in 208 subjects (114). Additionally, loading doses as high as 500,000 IU D_3 have been observed to be safe in elderly subjects (115) though a recent study (116) of a single dose 500,000 IU D_3 suggests there may be an increased risk of falls and fractures (a very different study population than the current VIDA study). Similarly, a loading regimen of 50,000 IU three times a week for a month of ergocalciferol (D_2) is uniformly safe and ensures rapid serum 25(OH)D levels > 40 ng/mL in nursing home residents (117), however, ergocalciferol is up to 13 times less potent than cholecalciferol. In the absence of a loading dose, daily maintenance doses of D_3 have also been studied. In 138 subjects with a mean baseline 25(OH)D level of 19.9 ng/mL, the optimal dose to get the most patients above 30 ng/mL was calculated to be 4,600 IU/day (106). Therefore, based upon the above data as well as input from

vitamin D experts (Drs. Bruce Hollis, Neil Binkley, Augusto Litonjua, Carlos Bernal-Mizrachi), we have chosen for those participants randomized to the vitamin D arm to receive a loading dose of 100,000 IU D_3 orally followed by 4,000 IU D_3 orally each day. We expect this regimen to achieve serum concentrations of 25(OH)D level between 40-100 ng/mL in all participants. This minimum target level of 40 ng/mL also ensures levels above the threshold established for entry criteria (30 ng/mL) and accounts for the intrinsic variability of the 25(OH)D measurement due to the absence of standard calibrators (118).

Although the safe tolerable upper intake level for vitamin D was set at 2,000 IU/day in 1997 by the National Academy of Sciences Institute of Medicine for adults and children greater than 1 year of age, (119) recent analyses indicate that this level could safely be updated to 10,000 IU/d, well above our chosen daily dose of 4,000 IU/day (110, 120, 121). As a benchmark, this same (4,000 IU/d) dose has been employed by other investigators in studies in related diseases such as atopic dermatitis (e.g. by the NIAID-funded Atopic Dermatitis and Vaccinia Immunization Network) (122) and an ongoing maternal supplementation study (123).

VIII. POWER ANALYSIS AND STATISTICAL METHODOLOGY

A. Power Analysis

In order to have 90% power to detect a hazard ratio of 1.8 from a Cox regression model (a reduction in treatment failure rate from 40% in the control group to approximately 24-25% in the intervention group during the randomized treatment period), a sample size of 200 participants per group is required. This calculation assumes an overall alpha level of 0.05, a 2-sided test, and 15% withdrawal (Power Analysis and Sample Size (PASS) Software 2008).

B. Statistical analysis methods

The primary outcome of time from randomization to treatment failure will be evaluated during both the inhaled corticosteroid-stable and inhaled corticosteroid-taper phase of the trial. The pattern of treatment failure in the intervention and control groups will be visually described using the Kaplan-Meier method. The primary analysis will be a Cox proportional hazards regression model with time to treatment failure, from randomization, as the outcome and treatment group as the covariate of primary interest. Because we suspect that the hazard ratios to compare between the two groups may be different between the steroid-stable and steroid-taper phases of the trial, we will include taper status as a participant-specific time-dependent covariate as well as the interaction between taper status and treatment. If there is no clinically significant evidence of a treatment by taper interaction (defined below), then the interaction term will be removed from the model and the primary analysis p-value will correspond to the one-degree-of-freedom test of the treatment group main effect. If there is clinically significant evidence of a treatment by taper interaction, then there will be three Bonferroni adjusted p-values corresponding to the three one-degree-of-freedom contrasts for treatment effect within each level of the taper effect (stable, 50% taper and 75% taper).

The interaction effect will be evaluated by clinical, rather than statistical, significance because there is relatively low power for the standard two-degrees-of-freedom test of interaction. Clinical significance will be assessed by calculating the pair-wise ratios of the treatment effect hazard ratios for the three

levels of the taper effect, and determining them to be clinically significantly different if the ratio of the hazard ratios does not fall within 0.8 to 1.25 (124). If only one of the three pairwise hazard ratio comparisons is clinically non-significant, then the taper levels corresponding to that comparison will be collapsed and the analysis will proceed as if there were only two taper levels. Under the null hypothesis of no treatment effect in any level of taper, this approach has a type-1 error rate of 5%. Under the alternative hypothesis of no interaction between treatment and taper, and with common treatment effect specified in section VIII.A., this approach has 90% power. Under the partially true alternative hypothesis of no treatment effect within two of the taper levels, but with treatment effect as specified above in the other taper level, this approach has a type-1 error rate no greater than 5% for the null taper levels and power of 84% for the non-null taper level. An adjusted model will be evaluated which will include the factors upon which the randomization was stratified: center, BMI (overweight/obese status), and race (African-American status). A secondary adjusted model will also consider the inclusion of the following baseline covariates that are of potential importance: gender, age, baseline 25(OH)D level, baseline sun exposure, baseline skin pigmentation level, FEV_1 % predicted, OCS responsiveness, bronchodilator reversibility, and methacholine responsiveness (PC_{20}) measured on a continuous scale and transformed with the $\log_2$ transformation. We will also evaluate the effect of seasonality on the study results by including season as a time-varying covariate in the adjusted model. The primary analysis will follow the intent-to-treat principle.

Vitamin D responder analysis: an exploratory analysis will be performed related to the primary hypothesis which will compare only those participants who reach sufficient levels of 25(OH)D in the treated group (≥30 ng/ml) to those participants in the control group, with respect to time to treatment failure as described above.

Multiple treatment failure analysis: an exploratory analysis will be performed related to the primary hypothesis which will allow for multiple treatment failures to be included for each participant. This repeated measures Cox proportional hazards regression model can adjust for the correlation that

exists among multiple responses within a participant (using SUDAAN Software for the Statistical Analysis of Correlated Data, Release 9.0, Research Triangle Institute 2004).

Corticosteroid dose analysis: a secondary analysis will be performed to compare the corticosteroid dose between the two treatment arms. The actual dose taken by each participant at each visit will be evaluated across the trial in a RM-ANOVA model. Least squares mean dose will be compared between the two treatment groups. In addition, the percentage of participants who achieve 50% ICS taper will be compared between the two treatment arms, as well as the percentage who achieve 75% ICS taper (via chi-square or Fisher's exact tests). To determine the potential effect of ICS dose on the time to treatment failure, the actual ICS dose will be added to the Cox regression model in two different ways: (1) as a time-varying covariate, and (2) as an average actual ICS dose taken throughout the trial, and the ICS dose by treatment interaction term will be evaluated.

Comment: Based on the ACRN SLIC trial (81), we believe that the proportion of participants who are unable to taper will be <5% in both treatment groups. While we assume that this is the case, we will also be testing this assumption in the above analysis. However, in the event that our assumption is incorrect and differential ability to taper exists between the treatment groups, those who are unable to taper will have already experienced the primary outcome of first treatment failure based on the stability criteria, and the primary analysis will be protected.

The effect of the intervention on the secondary outcomes will be characterized by the change in each measure during each of the study phases. Repeated measures analysis of variance models (RM-ANOVA) will be fit to the measurements to evaluate the change over time from baseline (Visit 3 – to allow for stabilization of control on 4 weeks of low dose inhaled corticosteroid) to the end of the inhaled corticosteroid-stable phase, as well as the change over time during the inhaled corticosteroid-taper phase. These changes over time will be estimated and compared between the treatment groups with the specification of appropriate contrast statements, and a modified auto-regressive correlation structure will be specified to reflect the longitudinal design. Repeated measures analysis of

covariance models (RM-ANCOVA) will also be fit to the secondary outcomes as discussed above, with potential adjustment for the baseline covariates listed above. The secondary outcome measuring time to exacerbation will be evaluated using survival analysis methods as described for the primary outcome.

The secondary hypothesis to evaluate the effect of the intervention within overweight participants and African Americans will be addressed by fitting RM-ANOVA models to the lung function measures within those participants with BMI ≥ 25 and within African Americans. As described above, the models will compare the change in lung function measurements over the treatment period between the intervention groups. A RM-ANCOVA model will also be fit within each of these subgroups to compare the change in lung function measures between the intervention and control groups with adjustment for the appropriate baseline factors included above. Assuming we will have approximately 50% of randomized participants in the overweight category, and approximately 30-40% African Americans, we will have the following power to detect the following effect sizes to address this secondary hypothesis.

Power estimates assuming an overall alpha=0.05, 2-sided tests, 15% withdrawal, and using change in FEV_1(L) as the lung function measure with standard deviation = 0.375 from the ACRN MIA trial

Subset	**Sample size**	**Power**	**Effect Size**
Overweight/obese	100 per group (50%)	80% 85% 90%	0.16 L 0.17 L 0.19 L
African American	80 per group (40%)	80% 85% 90%	0.18 L 0.19 L 0.21 L
	60 per group (30%)	80% 85% 90%	0.21 L 0.23 L 0.24 L

Additional exploratory analyses will be performed for this secondary hypothesis which include estimating the treatment effect within those who are **not** overweight/obese, and among those who are **not** African-American, and evaluating the corresponding tests of interaction within the RM-ANOVA

framework.

The secondary hypothesis to evaluate the effect of the intervention among those participants who have reduced corticosteroid responsiveness will be addressed by fitting RM-ANOVA models to the lung function measures within those who meet criteria for reduced corticosteroid responsiveness defined by lung function change after oral corticosteroid. Corticosteroid-responsive airflow obstruction will be defined as a ≥5% improvement in FEV_1 after the administration of 5 + 2 days of prednisone 40 mg/day measured at visit 4 compared to the baseline value at visit 3. If the participant's FEV_1 does not improve by 5%, they will be considered to have corticosteroid-nonresponsive airflow obstruction. Because there may not be consensus on the best criteria to use to define corticosteroid responsiveness, we will perform additional exploratory analyses considering the following alternative cutoffs: median split, 7.5% change in FEV_1, 10.0% change in FEV_1, as well as defining those with FEV_1 >85% of predicted after the corticosteroid burst as responders. RM-ANCOVA models which adjust for relevant covariates will also be evaluated. If we expect to find approximately 40-50% of randomized participants with reduced corticosteroid responsiveness, we will have 80-90% power to detect a difference in the change in FEV_1 between the two treatment groups in the range of 0.16 L to 0.21 L as shown in the table above. An additional exploratory analysis will be performed for this secondary hypothesis which includes estimating the treatment effect within those who **are** corticosteroid responsive, and evaluating the corresponding test of interaction within the RM-ANOVA framework. A second exploratory analysis for this hypothesis will involve treating corticosteroid responsiveness as a continuous measure, and assessing its association with change in lung function in the repeated measures models.

The secondary hypothesis to evaluate the effect of supplementation with Vitamin D on the severity of colds will be addressed by fitting a RM-ANOVA model to the peak WURSS-21 cold scores during each cold, allowing for multiple colds within each participant. In addition to evaluating the average peak cold score from each cold, the average score on day 1 and day 2 will also be compared between

the intervention and control groups. Additional models will be fit to the peak, day 1, and day 2 cold scores with adjustment for center and other important baseline covariates. Assuming that the incidence of colds is similar in our study to that seen in the ACRN PAX trial (125), a sample size of 200 per group could produce approximately 65 participants per group who experience at least one cold (32.4%). We will have at least the following power to detect the following effect sizes, assuming that the power in our analyses will be increased by the inclusion of multiple colds for each person.

Power estimates assuming an overall alpha=0.05, 2-sided tests, and assuming a standard deviation = 32 from the ACRN PAX trial

Subset	**Sample size**	**Power**	**Effect Size**
Participants with self-reported colds	65 per group (32.4%)	80% 85% 90%	16 17 18

The cold scores at Day 1 and Day 2 will also be analyzed, and had similar estimates of standard deviation in the PAX trial.

Vitamin D dose analysis: a secondary analysis will be performed to evaluate the effect of received Vitamin D dose on the primary and secondary outcomes. To determine the potential effect of Vitamin D dose on the time to treatment failure, the received dose will be evaluated in a Cox regression model in two different ways: (1) as a time-varying covariate, and (2) as an average actual Vitamin D dose taken throughout the trial. To determine the potential effect of Vitamin D dose on the secondary outcomes, the received dose will be evaluated in a RM-ANOVA model for each outcome.

C. Missing data

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. Although not expected, if it appears that the MAR assumption is not reasonable, then nonignorable statistical analyses, such as pattern-mixture modeling, will be applied.

D. Interim analyses and data monitoring

A pre-specified stopping rule is not necessary for this trial, and a formal interim analysis of efficacy data is not planned. The AsthmaNet Data and Safety Monitoring Board (DSMB), however, will be monitoring all of the safety data throughout the course of the trial and will be notified within 72 hours of any serious adverse events (SAEs) that occur.

IX. RISKS AND BENEFITS

A. Risks and Benefits of study procedures

Venipuncture: Blood samples will be obtained by venipuncture of an antecubital vein to determine vitamin D levels, serum calcium levels, renal function, and for DNA extraction for future genotyping studies.

Risks: The risks of venipuncture are minimal. The possible risks include bruising and/or infection at the site of the venipuncture and vasovagal episodes experienced by the blood donors. Pressure will be applied to the venipuncture site to prevent bruising. Aseptic technique will be used to prevent infection. Blood will be obtained while the donors are in a seated position and medical and nursing personnel will be available at the study sites to treat and manage vasovagal episodes.

Benefits: The vitamin D levels, serum calcium levels and renal function tests are necessary to determine if the participants meet the inclusion and exclusion criteria for the study. Vitamin D levels obtained during the study will be used to monitor for toxicity (hypervitaminosis). The DNA isolated for future genotyping studies will provide important insight into potential genetic modifiers of responses to vitamin D and inhaled corticosteroid.

The potential benefits justify the potential risks.

Pulmonary function testing (spirometry)

Risks: Spirometry will be performed to determine the participants' pulmonary function. The risks of spirometry are minimal. The possible risks include precipitation of bronchospasm and light-headedness from repeated blowing attempts. Medical and nursing personnel and medications will be available at the study sites to treat and manage bronchospasm. Inhalation of a short

acting beta-2 adrenergic agonist (levalbuterol) will be used to assess reversibility. The possible risks of inhaled beta-2 adrenergic agonists include tachycardia and hand tremors. These side effects are non-life threatening and are short-lived.

Benefits: Spirometry with assessment of reversibility to a short acting beta-2 adrenergic agonist will be used to determine if the participants meet the inclusion criteria for this study. Spirometry will be used during the study to monitor for treatment failure.

The potential benefits justify the potential risks.

Methacholine inhalation challenge: Methacholine challenge will be used to assess airway hyper-responsiveness.

Risks: The major risk of methacholine challenge is the induction of severe bronchoconstriction. As a precaution, participants will not undergo methacholine challenge if their FEV_1 is less than 55% of predicted or 1.0 liter. Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any bronchoconstriction episodes.

Benefits: There are two benefits to this procedure. First, for the participants who do not demonstrate a 12% improvement in FEV_1, a positive methacholine challenge would allow them to meet one of the inclusion criteria for this study. Second, the comparison of the baseline and end of treatment methacholine challenge results will allow for an assessment of the effect of study drug treatment on airway hyperresponsiveness.

The potential benefits justify the potential risks.

Induced sputum: Sputum will be induced with hypertonic saline to collect an airway sample and to assess for airway inflammation.

Risks: Like any bronchoprovocation challenge, sputum induction can provoke bronchospasm and

warrants close supervision during its performance.

Benefits: There are no direct benefits to the participant. This procedure will allow us to assess for the effect of vitamin D on airway inflammation and potential biologic mediators of vitamin D's effect, such as increasing cathelicidin levels in the airway.

The potential benefits justify the potential risks.

Allergy skin testing: Epicutaneous (prick skin) testing will be used to determine IgE sensitivity to areoallergens.

Risks: Possible risks include itching at the site of the skin tests, and systemic allergic reaction to the allergens tested. The allergic reactions range from mild (urticaria) to moderate (wheezing) to severe (upper airway angioedema, anaphylaxis). Medical and nursing personnel, medications and equipment will be available at the study sites to treat and manage any allergic reactions.

Benefits: Allergy skin testing results will be used to determine if there is a difference in response to vitamin D treatment between atopic and non-atopic participants.

The potential benefits justify the potential risks.

Evaluation of corticosteroid responsiveness: Participants will be given a seven day course of prednisone (40 mg/day) to determine corticosteroid responsiveness.

Risks: There are few risks involved with a short course of oral corticosteroids. These risks include mood changes, increased hunger, and rarely hyperglycemia.

Benefits: This test will allow for the assessment of the effect of Vitamin D treatment on corticosteroid responsive participants compared to corticosteroid nonresponsive participants.

The potential benefits justify the potential risks.

Determination of skin pigmentation: The degree of skin pigmentation will be assessed the Smart Probe 400 spectrophotometer.

Risks: There are no apparent risks to this procedure.

Benefits: This test will allow for the assessment of the effect of skin pigmentation of vitamin D levels.

The potential benefits justify the potential risks.

Urine collection: Urine will be collected to determine urinary calcium to creatinine ratios.

Risks: There are no risks associated with urine collection.

Benefits: This test will allow for the monitoring of participants who might develop hypervitaminosis D during the study.

The potential benefits justify the potential risks.

B. Risks and Benefits of Study Drug

Risks with Vitamin D: The current study includes only asthmatics with low vitamin D_3 levels (less than 30 ng/ml) and normal serum calcium levels (less than 10.2 mg/dL). Eligible participants will be randomized in a 1:1 allocation to: a) high-dose Vitamin D_3 (100,000 IU load followed by 4,000 IU/day) plus low-dose inhaled corticosteroid (ICS+D), or b) placebo Vitamin D-like capsules plus low-dose inhaled corticosteroid. A recent publication from Aloia and colleagues (106) provides the basis for the vitamin D_3 dosing strategy chosen for adults in this study. These investigators enrolled participants with serum 25(OH)D concentrations <32 ng/mL in a six-month long oral vitamin D_3 supplementation study and determined that oral intake of 3800 IU/d was the optimal dosing algorithm to achieve threshold serum 25(OH)D concentrations >32 ng/mL. Importantly, neither BMI nor percentage body fat significantly influenced the response to vitamin D in this study, and there was no evidence of

toxicity (hypercalcemia, hypercalciuria, serum 25(OH)D > 220 nmol/L) over the 6 month intervention period. The Institute of Medicine Food and Nutrition Board guidelines specify 2000 IU/d vitamin D_3 as the highest vitamin D intake that healthy adults can consume without risking hypercalcemia but multiple studies show that optimal 25(OH)D level can be achieved safely during winter in all patients with a dose of 4000 IU (40, Aloia). As a benchmark, this same (4,000 IU/d) dose has been employed by other investigators in studies in related diseases such as atopic dermatitis (e.g. by the NIAID-funded Atopic Dermatitis and Vaccinia Immunization Network) (122) and an ongoing maternal supplementation study (123). Since these high doses of vitamin D_3 are not FDA approved, an IND has been obtained (#110227). Vitamin D levels ≥ 120 ng/ml will be considered toxic (105). Participants above this level will stop blinded study medication (i.e., blinded vitamin D/placebo) and return to the clinical site for a repeat test. If the second vitamin D level is ≥ 120 ng/ml, the participant will stay off the blinded capsules for the remainder of the study, be referred to their primary care physicians for close monitoring and follow up, and will remain in the study for follow-up visits. If the repeat vitamin D level is <120 ng/ml, the participant will resume taking blinded study medication.

Urinary calcium to creatinine (U_{Ca}/U_{Creat}) ratios will be measured at visits 1, 3, 5, 6, 8 and 10. While others have found the 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 (91). If a U_{Ca}/U_{Creat} is greater than 0.37 (urinary Ca and Creat in mg), the participant will be instructed to increase hydration and a repeat measurement will be obtained 30 days later. If the repeat U_{Ca}/U_{Creat} is elevated, then the participant will be instructed to hold blinded study drug (i.e., vitamin D/placebo) and have a serum calcium and 25(OH)D level obtained. If either the calcium or 25(OH)D is elevated, the participant will stop study drug. If the calcium and 25(OH)D are normal, then the participant may resume study drug. Under intention-to-treat, participants will remain in the study for follow-up visits, even if blinded study drug is stopped.

Benefits of Vitamin D: Asthmatics participating in this study may or may not achieve any benefits. There may be some benefit involved with closer monitoring of subtle changes in their asthma control, being seen by study personnel on a frequent basis, and by methods established to ensure compliance with medications.

Risk/benefit analysis of Vitamin D: Because not all asthmatics respond adequately to inhaled corticosteroids, there is a need to identify such asthmatics and investigate potential treatments which can be used alone or in conjunction with an inhaled corticosteroid. This protocol will determine whether the addition of vitamin D provides asthma control and reduces treatment failures in vitamin D insufficient symptomatic asthmatics. If vitamin D supplementation were found to be effective when used in this manner, important benefits for asthma patients would be anticipated. 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.

X. ADVERSE EVENTS

A. Definition and reporting

Participants are at risk of developing adverse events during study enrollment. A clinical adverse event is any unintended worsening in the structure (signs) or function (symptoms) of the body, whether or not considered to be study-related. This includes any side effect, injury, or sensitivity reaction, as well as any intercurrent event. A laboratory adverse event is any clinically-important worsening in a test variable which occurs during the course of the study, whether or not considered to be drug-related. An adverse event is deemed serious if it suggests a significant hazard, contraindication, side effect, or precaution. Serious adverse events include any experience that is fatal or life-threatening, is permanently disabling, requires or prolongs inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

Documentation of an adverse event will be recorded on the Clinical Adverse Event Report Form and will include the following information: Description of the condition, dates of condition, treatment of condition (medications, doses, dates), whether hospitalization or emergency treatment was required, treatment outcome, relationship of the adverse event to the study medication(s), and severity of the event.

B. 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 include acute rhinitis, sinusitis, upper respiratory infections, urinary tract infections, and gastroenteritis. Medications are allowed for treatment of these conditions in accordance with the judgment of the responsible study physician.

C. Adverse Events Related to Asthma: Treatment Failure and Asthma Exacerbation

Since participants have persistent symptomatic asthma and inhaled corticosteroid therapy will be reduced in study phases IIa and IIb, it is anticipated that asthma treatment failures will occur. Safety net procedures, including visits and frequent telephone contacts, are in place to identify participants who experience a treatment failure (the primary outcome) or asthma exacerbation during the study.

Between in-person study visits (as described above), participants will be contacted by telephone by the clinic coordinator to assure that they are continuing to participate appropriately in the study protocol, to answer any questions that may arise, and to assure that their asthma is under adequate control, as assessed by the participant. The coordinator will attempt to determine whether the participant is showing signs of treatment failure using criteria identified below. If it is determined that the participant fulfills criteria for treatment failure or asthma exacerbation, they will be advised to visit the study center within 24 hours for evaluation and initiation of treatment as specified herein. Otherwise, arrangements will be made for the participant to have telephone contact or return to the clinic at the next regularly scheduled interval.

If, between phone contacts or in-person visits, a significant asthma exacerbation has occurred, the participant should contact the clinic coordinator and/or be evaluated at the study site or the nearest medical emergency facility as quickly as possible.

Treatment failure status will be defined as the occurrence of one or more of the following:

1. Pre-bronchodilator PEF ≤ 65% of baseline on any 2 of 3 consecutive scheduled measurements;

2. An increase in PRN levalbuterol use of ≥ 8 puffs per 24 hrs over baseline use for 48 hrs;

3. Pre-bronchodilator FEV_1 ≤ 80% of the baseline on 2 consecutive measurements (see section III.C for further details);

4. Additional inhaled or oral/parenteral corticosteroid due to asthma;

5. Need for emergency treatment at a medical facility that is related to, or complicated by, the participant's asthma and which results in systemic corticosteroid treatment or hospitalization for an acute asthma exacerbation;

6. Participant refusal to continue study drugs because of lack of satisfaction with treatment; or

7. Physician clinical judgment for safety reasons.

Although all participants with an asthma exacerbation will also meet the criteria outlined for treatment failure above, asthma exacerbations are more severe episodes of acute worsening, defined by meeting criteria for treatment failure AND one or more of the following:

1. Failure to respond within 48 hours to treatment failure rescue algorithm

2. FEV_1 <50% of baseline on 2 consecutive measurements (18)

3. FEV_1 <40% of predicted on 2 consecutive measurements (18)

4. Use of ≥ 16 puffs of "as needed" β-agonist per 24 hours for a period of 48 hours

5. Experiencing an exacerbation of asthma in the opinion study investigator or personal physician

6. Use of oral/parenteral corticosteroid due to asthma

D. Adjustments of Trial Medications and Rescue Algorithms during Treatment Failures and Asthma Exacerbations

Participants who develop treatment failure during the run-in period or OCS response period (pre-randomization) will be terminated from study enrollment and managed as clinically-indicated, with treatment based on clinical standard and initiated by/in accordance with the participant's usual asthma care provider. Once the treatment failure has resolved for at least two weeks, the participant may be rescreened for entry into the study.

Participants who experience two treatment failures during the run-in period or OCS response period (one each during two separate enrollments) will not be allowed to participate in the study further. This will enrich the study population for individuals who are likely to remain stable during the stable inhaled corticosteroid dose phase (Phase I) with the bulk of treatment failures likely to occur during the inhaled corticosteroid tapering phase (Phases IIa and IIb).

For participants who meet criteria for treatment failure during the post-randomization phases of the study, the dose of inhaled corticosteroid will be doubled and continued for 7 days. For treatment failures occurring during Phase I, the dose will be twice the inhaled corticosteroid stable dose. For treatment failures occurring during Phase II, the dose will be increased to the dose the participant was receiving prior to initiation of the most recent steroid taper (i.e. returned either to Phase I or Phase IIa dosing, as appropriate). If, after 7 days, the treatment failure episode is clinically-resolved, the corticosteroid stable dose (for treatment failures during Phase I) or pre-failure tapering dose (for treatment failures during Phase II) of inhaled corticosteroid will be resumed and study visits will continue as per protocol. If an exacerbation or second treatment failure occurs during the post-randomization phases of the study, no further inhaled corticosteroid dose tapering will be attempted.

For participants who experience a treatment failure event that also meets the criteria for a significant asthma exacerbation, dosing of inhaled corticosteroid depends on the phase in which the

event occurred. If the exacerbation occurs during Phase I, the dose of inhaled corticosteroid will be doubled (to 200% of the inhaled corticosteroid stable dose) for 7 days. If, after 7 days, the event is clinically-resolved, the dose will be reduced back to the corticosteroid stable dose for the remainder of the study, and visits will continue as per protocol. If the exacerbation occurs during Phase II, the dose of inhaled corticosteroid will be increased to the corticosteroid stable dose and maintained for the remainder of the study; no additional dosing tapers will be attempted. Additional treatment for exacerbations is at the treating physician's discretion (see Section 2 below).

1. Treatment Failure Rescue Algorithm

Home Care

Treatment failures will be recognized by the criteria above and managed as in previous ACRN trials (80). Participants will be educated to recognize treatment failures as early as possible to facilitate prompt treatment and to lessen morbidity.

Participants who recognize increased symptoms and/or a fall in PEF to ≤65% baseline will use levalbuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min, if needed, and then every 4 hours, or less, if needed. Participants will be instructed to use the "Rescue inhaler" for treatment.

If the PEF does not increase to >65% baseline or if symptoms are not improved after the first 60-90 min of therapy, the participant should contact the investigator, their primary physician or seek care in the emergency department. Failure of levalbuterol to control or maintain PEF >65% of baseline may trigger further action.

Adjustment to Trial Medication

If participants meet criteria for treatment failure status, home care measures will be instituted as described above and in previous trials (80). For treatment failures during Phase I, the dose of inhaled corticosteroid will be doubled (i.e., to 200% of the inhaled corticosteroid stable dose) and continued

for 7 days. For treatment failures during Phase II, the dose of inhaled corticosteroid will be doubled to the dose the participant was receiving prior to initiation of the most recent steroid taper (i.e. returned either to Phase I inhaled corticosteroid stable dose or Phase IIa dosing, as appropriate) and continued for 7 days. If the participant does not improve within 48 hours, he or she will be classified as a non-responder and treated as per Section 2 below.

For safety reasons, all participants will be seen within one week of the day they have been categorized as achieving treatment failure status to ensure appropriate response to inhaled corticosteroid dose escalation. If participants meet the criteria for an asthma exacerbation, they will be classified as non-responders and treated as per Section 2 below. If, after 7 days, the treatment failure episode is clinically-resolved, the Phase I inhaled corticosteroid stable dose (for treatment failures during Phase I) or tapering dose of inhaled corticosteroid (for treatment failures during Phase II) will be resumed and study visits will continue as per protocol.

If a second treatment failure occurs during the post-randomization phases of the study, the above treatment algorithm will be applied, with the exception that no further inhaled corticosteroid dose tapering will be attempted.

2. Rescue Algorithm for Asthma Exacerbations and Treatment Failure Non-responders

Participants who are not responsive to the treatment failure rescue algorithm or those who develop asthma exacerbations 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 (3) and prior ACRN trials (80). Levalbuterol and oral prednisone are the principal medications for rescue management and participants will be instructed in their use for home management. Oral prednisone will be used if alteration of inhaled corticosteroid does not resolve the

exacerbation. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

Home Care

Asthma exacerbations will be recognized by criteria described above. Participants will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Participants who recognize increased symptoms and/or a fall in PEF to ≤65% baseline will use levalbuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min if needed and then every 4 hours, or less, if needed. Participants will be instructed to use the "Rescue inhaler" for treatment.

If the PEF does not increase to >65% baseline or if symptoms are not improved after the first 60-90 min of therapy, the participant should contact the investigator, their primary physician or seek care in the emergency department. Failure of levalbuterol to control or maintain PEF >65% baseline may necessitate the use of oral steroids (see below).

Physician's Office or Emergency Room Treatment

Participants will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the participant's PEF and/or FEV_1 are less than 25% predicted or if the participant shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.

When treated in the physician's office or the hospital emergency room, participants should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEF increases to >65% baseline after the first 60-90 min, the participant can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy.

If symptoms persist and PEF remains ≤65% baseline, nebulized albuterol should be continued as often as every 20 min at the discretion of the treating physician. Oral or parenteral corticosteroids should be considered (60 mg prednisone orally; methylprednisolone 60 mg iv bolus). Monitoring of PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding participant disposition. The participant should restart their inhaled corticosteroid at least at the Phase I (corticosteroid stable) dose.

If PEF increases to >65% baseline within 4 hours, the participant can be discharged to continue treatment at home. Home treatment should include an 8-day course of prednisone (see below).

If PEF remains >40% but ≤65%, an individualized decision should be made to hospitalize the participant for more aggressive therapy or to continue therapy at home with a course of prednisone.

If PEF is <40% baseline after repeated albuterol treatments, the participant should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

Prednisone Treatment

In this protocol, prednisone will be used when treatment failures and acute exacerbations cannot be controlled by albuterol therapy and alteration of inhaled corticosteroid. Indications for prednisone therapy include the following:

- For follow-up management after discharge from the physician's office, emergency room, or hospital for an acute exacerbation.

- For home management when asthma symptoms remain clinically significant for 48 hours or longer and the participant is taking ≥ 16 puffs of levalbuterol plus alteration of inhaled corticosteroid.

- When PEF falls to <50% baseline despite levalbuterol and inhaled corticosteroid treatment.

The recommended dose of prednisone used during an acute exacerbation shall consist of 60 mg as a single dose every day for 3 days, followed by a 10 mg/day taper over the next 5 days. The decision to initiate or to continue a course of prednisone beyond 8 days is left to the discretion of the physician.

E. Study Center Visits Following Treatment Failures or Exacerbations

For safety reasons, all participants will be seen within one week from the date of the treatment failure or exacerbation. Trial drugs will be continued during a treatment failure or an exacerbation unless the treating physician considers it appropriate to suspend such therapy until the event resolves. Following this "safety" visit, subsequent protocol visits will continue in accordance with the visit schedule established at trial entry.

F. Criteria for Achieving Dropout Status

Participants may be assigned "drop-out" status during the double-blind treatment period if they become pregnant or if they withdraw consent. In addition to the usual reasons for withdrawing consent (moving to another region, inconvenience, etc.), it will be noted if a participant withdraws consent due to lack of satisfaction with the quality of asthma control. At this visit, study termination procedures will be conducted, all study medications stopped, and the participant will begin a treatment plan at the direction of the examining physician.

G. Criteria for Withdrawal from Study Based on Treatment Failures or Asthma Exacerbations

Participants experiencing any treatment failure during the run-in or OCS response period will be terminated from study participation and allowed to re-screen one additional time after a period of 2 or more weeks following resolution of the event.

Once randomization has occurred at visit 4, intention-to-treat principles will apply. As treatment failure is the primary outcome, and since treatment failure criteria are consistent with a less severe worsening of asthma than experienced during an exacerbation, participants will not be terminated from study participation until more than two treatment failures or exacerbations have occurred. Once achieving the third treatment failure or exacerbation event, the participant will be withdrawn from the blinded phase of the trial and will be seen in the AsthmaNet clinic for a final study exit visit.

Should asthma exacerbations become too severe following randomization, the AsthmaNet principal investigator may at any time elect to drop the participant from further study participation. Study termination procedures will be completed. Any complication resulting from an asthma exacerbation (pneumothorax, pneumomediasternum, mechanical ventilation, etc.) will be recorded as an adverse event in addition to the documentation also recorded for the significant asthma exacerbation.

H. Adverse Events as Outcome Variables

Treatment failures will serve as the primary outcome variable during this study. Additionally, during exacerbations, the following variables will be recorded and used as additional outcome measures: number of participants having an asthma exacerbation, corticosteroid use and total dose, hospitalizations, emergency department visits, and unscheduled physician/clinic visits.

XI. RECRUITMENT STRATEGIES

A. Brigham and Women's Hospital - Boston, Boston, MA

The Boston Center has used a variety of recruitment methods to meet and exceed recruitment goals of previous ACRN studies.

Over the past five years, we have compiled an internal database of approximately 1500 individuals with asthma who are interested in participating in asthma studies. All of these individuals contacted us and expressed interest about asthma studies within the past year and have been evaluated by our staff for participation in ongoing and future asthma clinical research studies. About 23% of the subjects in our database identify themselves as being African American. Based on the most recent NHANES data available, approximately 75% of the United States population is Vitamin D deficient. Therefore, we will assume that about 50% to 75% of our subjects may be Vitamin D deficient.

The Boston site actively recruits subjects using a variety of external media. All methods are IRB-approved and include postcard mailings to area zip codes, newspaper advertisements, and broadcast e-mails and internet postings

Brigham and Women's Hospital has introduced a new clinical research tool called the BWH Research Patient Database Registry (RPDR) that allows researchers with proper IRB approval to query the hospital's patient database for potential research subjects. We recently queried this system and identified approximately 30,000 patients with a diagnosis of asthma. With permission from their primary care physician, patients may be contacted about current asthma research. Access to the physician database further expands our capability to recruit asthmatic patients of differing severities.

B. Northwestern University, University of Chicago, Stroger Hospital of Cook County, Chicago, IL

The Chicago Center has several academic and community sites from which to recruit patients into this adult study. We will use recruitment tools and techniques that have been successful in our previous American Lung Association-Asthma Clinical Research Centers (ALA-ACRC) studies that enrolled patients with similar inclusion-exclusion criteria. These tools and techniques include asthma patient registries maintained at Northwestern University, Rush University and Northwestern University (total of approximately 1,000 patients with asthma), collaborations with community sites through our respective CTSA programs and local lung health agency (Respiratory Health Association of Metropolitan Chicago [RHAMC]), and advertisements. The IRB approved advertisements include flyers posted in physician offices and clinics, as well as advertisements on Chicago's public bus and train system and in local newspapers.

Northwestern University has recently created an Enterprise-Wide Data Warehouse for all clinical data from our respective hospitals and clinics. This now permits us to identify and characterize a larger number of patients with a diagnosis of asthma. Some of these patients already agreed to be contacted for participation in clinical research studies. Others can be contacted through their primary care physicians after IRB approval of the protocol and plan to contact patients. A similar approach has been developed for COPD studies at the University of Chicago and will be adapted for studies in asthma. Additional details about subject recruitment opportunities and success are available in our original AsthmaNet application.

The adult asthma populations at Northwestern University, Stroger Hospital/Rush University and the University of Chicago are 15-25%, 55-65% and 50-65% African-American. In previous asthma studies, the percentage of enrolled patients who are African-American is typically between 35 and 50%. We anticipate, based on epidemiological data, that about 50% of all potential enrollees will be

vitamin D insufficient and even a greater % of African-Americans. We plan to use our IRB-approved asthma research registries to pre-screen potential research subjects for vitamin D insufficiency.

C. National Jewish Asthma Research Center, Denver, CO

There are over 400 unique asthma subjects that have participated in research studies conducted at the Denver Center. Many of these subjects have been through various medication studies and bronchoscopies with lavage/biopsies. Their FEV_1s range from 30-110% of predicted. The Denver site typically recruits a population that is approximately 20% African-American. Additionally, based on data obtained from subjects participating in the BASALT and TALC ACRN studies at the Denver center, 50% of subjects are anticipated to have 25(OH)D serum concentrations of < 30 ng/mL.

The following sites will also support recruitment for this AsthmaNet study:

1. *Denver Health Medical Center* – Dr. Ivor Douglas, Head of Pulmonary Medicine, is supporting efforts of the Denver Center by helping to recruit from the asthmatic subject population at the Denver Health Medical Center. This is a large county hospital which serves a large Hispanic and African-American population.

2. *Denver Veterans Administration Hospital* – Dr. Carol Welsh, Pulmonary faculty member, will support this grant. The VA hospital has a large outpatient clinic of patients with asthma, and these patients will be serve as an additional recruitment base.

3. *Denver Kaiser Permanente HMO* – Dr. Christopher Bates is the Director of Pulmonary Medicine and Dr. John Williams is the Director of Allergy at Kaiser. The Kaiser outpatient clinic has been actively involved in supporting research at National Jewish in the past by referring subjects, and their groups will continue to play an active role in clinical research support.

D. Duke University, Raleigh-Durham, NC

In Durham County, African-Americans make up 39% and Hispanics 11% of the population. Asian Americans together represent approximately 3% of the population and 5% are listed as “other”. The prevalence of asthma in Durham is 10%, but in middle school students it is 15%, and 27.4% in African-American students. Since the inception of the Duke Asthma, Allergy and Airway Center in 2004, and at its present location in 2006, we have screened over 1,000 subjects for participation in our NIH sponsored and industry-sponsored studies in asthma. We have extensive experience in the recruitment of African-Americans into airway studies, as Dr. Sundy, the Duke co-investigator has enrolled over 1,000 African-American subjects in genetic studies over the last three years.

The Duke Asthma, Allergy and Airway Center is a multidisciplinary center created to deliver state-of-the-art clinical care and perform research in allergic and airway diseases. Pediatric and adult patients are evaluated and it contains two body plethysmographs, and capability to perform methacholine challenge, bronchoscopy, laryngoscopy, exhaled nitric oxide, induced sputum and exercise testing, both for clinical and research purposes. The Center also houses the NIH/NHLBI Asthma Clinical Research Center and the American Lung Association Asthma Clinical Research Center. There are four clinical coordinators performing primarily adult studies directed by Dr. Monica Kraft with assistance from Drs. Loretta Que and Njira Lugogo. The center sees approximately 8,000 adult patient visits per year and provides a rich environment from which to recruit subjects, particularly those with severe asthma, for clinical studies.

To recruit subjects outside the Center, we employ several strategies that include the following: radio advertisement, television advertisements, direct email to undergraduate and graduate students (via permission from the Deans of both schools), and advertising on Craig’s List, Facebook and Google. We have found the emails to students have been particularly productive, as has Craig’s List and specific campus newspapers, the latter targeted to students at Duke and the University of North

Carolina, Chapel Hill, located eight miles from Duke. We also advertise at other surrounding research and educational institutions, including North Carolina State University, Meredith College, National Institute of Environmental and Health Sciences (NIEHS) and North Carolina Central University. Lastly, our center has a website (http://aaac.duhs.duke.edu) where we describe our trials. We also advertise on the Duke Clinical Trials website (http://www.dukehealth.org/clinicaltrials).

In addition to advertising strategies, we have gone directly to our primary care colleagues and specialty practices for assistance. The staffs of Dr. Kraft and Sundy already cooperate in recruiting subjects directly from the Duke Asthma, Allergy and Airway Center and from our primary care physicians in the Duke-Durham community. We are also partnering with an allergy practice (Dr. Peter Bressler), and a pulmonary practice (Dr. Allen Hayes) affiliated with Duke and with NIEHS (Dr. Stavros Garantziotis). We at the Duke Asthma, Allergy and Airway Center, are in the midst of establishing a formal partnership with key primary care providers in Durham to identify diseases of high risk in Durham (asthma and COPD) and working with them directly to design strategies that will alter outcomes. This relationship is through the Duke CTSA grant; Monica Kraft is the PI of this endeavor; we feel that these ongoing relationships will increase our recruitment directly from our primary care providers, both within and outside Duke.

Lastly, we have developed HIPAA and IRB-approved database for our Center to capture clinical data from patients who have received clinical care at our Center, participated in our trials, or answered our advertisements. This database allows searching for subjects with specific qualifications for participation in clinical trials, and also for hypothesis generation. Overall, we feel we have and will continue to employ numerous strategies to recruit asthma subjects insufficient in vitamin D for VIDA.

E. Washington University, St. Louis, MO

Washington University School of Medicine (WUSM) actively recruits participants using a variety of external media. All methods are IRB-approved. These include newspaper advertisements in the local

and minority newspapers, the University newspaper, posting flyers throughout the medical school campus, and the university website called "Volunteer for Heath" (VFH). The overall purpose of VFH is to match interested volunteers with current clinical research projects at the medical school. The current VFH database includes over 25,000 participants that have provided information that can be used to screen for studies. The service maintains an office onsite at the entrance of Barnes-Jewish Hospital (main teaching hospital of WUSM), a high-volume telephone line, and a website (http://vfh.wustl.edu). The service will therefore be ideal for recruiting subjects for VIDA.

The *Recruitment Enhancement Core (REC)* is a unique service that WUSM offers to help sponsors and PI's maximize the attainment of enrollment goals. A dedicated staff of five recruitment specialists consult with Drs. Castro and Bacharier to develop and implement a unique recruitment plan for each new protocol in AsthmaNet. This plan will include targeted, strategic activities tailor-made to recruit and retain eligible participants for trials such as VIDA. Such activities include querying the VFH database, reviewing patient charts, calling on high volume community practitioners to generate referrals, generating study interest though local support and advocacy groups and attending appropriate health fairs. Additionally, the REC will work with our AsthmaNet staff to provide pre-screening services on all potentially eligible participants. Finally, the REC will participate in community outreach efforts designed to build trust and promote the importance of clinical trial participation in order to deliver a more diverse participant base. It is the REC goal to provide hard-hitting, targeted recruitment efforts early on in the clinical trial process in order to meet our enrollment goals as quickly as possible.

The *Center for Community Based Research (CCBR)* as part of the WUSM CTSA improves relationships between community members, health care providers, community organizations, and researchers. The CCBR Recruitment and Retention group is housed in a community site known as HealthStreet which makes research more accessible to underrepresented populations (apart from the medical campus). HealthStreet offers opportunities for community members to learn about medical

research, sign up to be in a research study, and referrals to other needed services. Community Health Outreach Workers (CHOW) go to all areas of the inner city in vans to educate about our services. Since May 2008, our CHOWs have contacted 792 interested individuals of which 72% are African American. These individuals will be ideal for the current VIDA proposal given the high prevalence of Vitamin D insufficiency in African Americans.

Over the past 16 years, the St. Louis site has compiled an internal database of more than 1,300 individuals with asthma who are interested in participating in asthma studies. All of these individuals have contacted us and have expressed an interest in participating in an asthma study. These individuals have been evaluated by our staff for participation in ongoing and future asthma clinical research studies.

F. University of Pittsburgh, Pittsburgh, PA and Case Western Reserve University, Cleveland, OH

The University of Pittsburgh site will recruit through a number of areas. The Asthma Institute has a growing database of about 200 asthmatics of all severity levels who have been initially characterized and are available for research studies. This is a mixed racial/ethnic database. We have recently hired a full time recruiter, as well. In addition, the Falk clinic sees about 2000 additional asthmatic patients. We have an established relationship with Dr. Stephen Thomas who has built relationships with several family practice clinics in the Pittsburgh area which serve primarily minority populations. We are planning “asthma days” at these clinics. In addition, Dr. Fernando Holguin and Shean Aujla currently staff an “Asthma bus” which travels to various Pittsburgh neighborhoods and provides asthma care to minority children. However, these children are often brought in by their parents, many of whom also have asthma. We will make information on the AsthmaNet protocols readily available and recruit subjects when interest is shown. Beyond these sites, we will work with Dr. David Skoner and Debbie Gentile at Allegheny Hospital to recruit this VIDA study population from their adult asthma clinical trials database. Finally, our co-investigators at Case Western (who primarily also serve as a

pediatric site) also deal with the parents of many of their children with asthma. They feel comfortable recruiting parents of children in their clinical research operation to participate in this clinical trial as well. In addition, they care for patients up to age 25 years in their outpatient clinics and inpatient wards. They have a research database that dates back to 1999 and a clinical database with over 4000 patients that dates back to 2006. They use these databases extensively in recruiting patients. Although many former patients are now young adults, they consented to allow the Pediatric Pulmonologist to maintain their information in these databases so that they could participate in any future clinical trials involving young adults with asthma. Finally, we will also initiate efforts at both print and electronic advertising through the Asthma Institute web site, Facebook, newsletters and traditional newspaper advertising. We are very optimistic that with this combined approach, we will have no difficult reaching our recruitment goals.

G. University of California, San Francisco, CA

Study population: Beginning in 2008, the UCSF Center collectively undertook a major e-recruitment initiative, transforming our traditional recruitment system, based in telephone and paper flyer recruitment methods, into a dynamic, web-based recruitment platform that has since reduced recruitment costs while simultaneously improving contact-to-enrollment efficiency. Potential participants access a recruitment questionnaire via the new e-recruitment system by clicking on a link embedded in an e-community bulletin board (e.g. Craigslist.org, ClinicalTrials.gov) or by entering the web address posted on our paper flyers. Alternatively, potential subjects may still call the center and have a clinical coordinator complete the recruitment questionnaire with them over the phone.

The recruitment questionnaire is specifically tailored to the respondent based on disease category (asthma, COPD, healthy, CF, etc.) and further individualized based on the respondent's specific answers to questions requiring follow-up information. Potential subjects complete the questionnaire, which is spread across several easy to use screens. The questionnaire begins with a brief consent,

which informs respondents of the risks associated with any potential loss of privacy and the electronic and physical security measures in place to reduce these risks. All questionnaire language is written to an 8th grade reading level; respondents may choose to discontinue at any point and all partial data will be destroyed.

Once a potential participant has completed the questionnaire, the e-recruitment platform screens each questionnaire against a list of inclusion and exclusion criteria, which has been programmed into the system for each Center study. Clinical coordinators may then log-on through a secure web portal to retrieve a list of potential participants meeting their pre-specified study criteria.

Subject Characterization: The UCSF center's methods for characterizing subjects conform to national guidelines (e.g. spirometry), to widely accepted custom (e.g. methacholine challenge), or to its own standards as the center developing the method (e.g. sputum induction and analysis). We have adopted standardized questionnaires for assessing asthma symptom severity, asthma control, and asthma-related quality of life. We have additionally developed questionnaires on asthma history, patterns of health care utilization, and domestic exposure to allergens.

The UCSF recruitment database currently stores phenotypic information on >7,000 potential subjects of a variety of ethnic backgrounds (64% Caucasian, 13% African American, 7% Hispanic, 10% Asian and 6% other).

In addition to community advertising, subjects are recruited, especially those with severe asthma, from clinical programs overseen by UCSF faculty at Moffitt, S.F. Veteran's Administration, S.F. General, and Mt. Zion Hospitals. The faculty is responsive to approaches from colleagues conducting clinical trials and there has been collaboration with the Division of General Internal Medicine to recruit for specific protocols. This Division follows approximately 18,000 patients, of whom 8% (2,683) have a primary or secondary diagnosis of asthma (ICD-9 493.00, 493.01, 493.10, 493.11, 493.20, 493.21). Of these asthmatic patients, 48% are White, 20% Asian/Pacific Islander, 10% Latino, 16% African American, and 1% Native American. Sixty-four percent are female.

Determination of vitamin D deficiency: A UCSF IRB-approved "asthma characterization" protocol permits administration of questionnaires, pulmonary function testing, and blood draw for laboratory analysis. From pilot studies we have conducted, we anticipate that the proportion of asthmatics screened deficient or insufficient for 25(OH)VitD will range from 20-40%, depending on race and ethnicity.

H. University of Wisconsin, Madison, WI, and Aurora Sinai Medical Center, Milwaukee, WI

The Allergy, Asthma and Pulmonary Research Program at the University of Wisconsin maintains a file of potential subjects with mild to severe asthma who are interested in future research participation. These individuals have been screened and/or participated in previous asthma studies. The following information is maintained: birth date, gender, ethnic background, pulmonary function test results, age of asthma diagnosis, childbearing status, concurrent medical history, and asthma and non-asthma medications. This database of subjects will be used as the primary source of recruitment for this protocol.

In order to maintain successful recruitment and continue to build our database, the Madison center has utilized a variety of recruitment and marketing strategies. We have a strong community-based referral system in place. In order to have a representative sample, we diligently work to get minorities in our studies, especially African Americans.. We work with several surrounding clinics and those within the University of Wisconsin's system to provide referrals to us by using a FAX referral system. For example, we get referrals from the U.W. Allergy Clinic subject population as well as the U.W. Sports Medicine Clinic, U.W. Student Health, and UW clinics through out the city of Madison. In addition, we work with UW Northeast Family Medical Center that serves a large minority population, especially African Americans. In addition, Madison is among the most northern AsthmaNet Centers, with sufficient sun exposure to maintain normal vitamin D levels occurring only about a third

of the year. According to previous research performed by our collaborators, around 70% of Caucasians and 90% of African Americans and Hispanics will have vitamin D levels of twenty-five (OH), which would meet the entry criteria of 30 ng/mL or less.

Our department has a Marketing Committee that meets on a regular basis to continually develop recruitment and marketing strategies that have applicability to a wide range of inclusion and exclusion criteria. We have targeted various populations, especially minority groups. We developed a network television advertisement with The Madison television station The CW (a company that is part of CBS and Warner Brothers). The CW keeps viewer demographic statistics so we can place the advertisements at appropriate times and target them to particular populations. In addition, we participate in health fairs and other community events to promote our studies. We schedule our health fair participation to enhance interactions with minority and hard to reach populations. For example, we have had booths at Boys and Girls Club of Madison and in local churches. Each year for World Asthma Day, we set up educational/promotional booths throughout the community for the entire week.

Three times a year, the department sends out a web-based survey to all the students on campus, in which we gather information about their asthma and allergies. We get approximately one thousand responses each semester and approximately six hundred in the summer. In addition, we recruit participants via U.W. Human Subjects committee-approved newspaper, radio or other media advertisements. We advertise in minority publications, such as The Madison Times newspaper and Umoga, which are publications targeted to African Americans and La.Communidad, which is targeted to Hispanics. Also, The U.W. University Hospital and Clinics now has electronic medical records and we are exploring different strategies to be able to query for potential participants. Collectively, these strategies have proven to provide efficient ways to recruit and target subjects who meet specific criteria, such as asthma severity and minorities.

The Aurora Health Care system has sites in more than 90 communities throughout eastern Wisconsin, including 13 hospitals and over 100 clinics and 3,400 physicians affiliated with it. Aurora Sinai Medical Center is Aurora Health Care's most centrally, urban hospital, located in Milwaukee. This metro area has a population of approximately 1,500,741 residents, with over 384,591 of those residents reporting a race other than white.

The Center for Urban Population Health (CUPH), a collaboration between Aurora Health Care, UW-Madison, and UW-Milwaukee employees a team of researchers, coordinators and outreach staff that collaborate across projects and have established connections across the Milwaukee community. Clinical recruitment consists of patients, primarily from within the Family Medicine clinics, and has expanded to include other selected outpatient medical clinics, hospitals, and emergency departments. In addition, community recruitment efforts have included targeting Milwaukee Public Schools, neighborhood organizations and community groups, community clinics, as well as local universities, such as UW-Milwaukee, Marquette University and Alverno College. CUPH has also hired a new Research Ambassador who will be instrumental in seeking out new community and clinical resources to increase recruitment efforts beyond those already developed.

Successful recruitment strategies incorporate a wide range of culturally appropriate media specific to the communities targeted for recruitment. Within the Aurora system, the use of the employee-based intranet (I-Connect) allows for an inexpensive, yet far reaching form of advertisement. Additionally a data request to identify potential subjects using the entire Aurora Health Care systems electronic record system will allow a diverse recruitment pool . Thereby postcards summarizing the project are sent to specific individuals meeting initial inclusion criteria identified from this data request. CUPH has established a number of working relationships with Milwaukee community organizations supporting needs specific to African American and Hispanic populations which will increase the diversity of recruitment efforts.

I. Atlantic Coast Consortium, Wake Forest University Health Sciences Center, Winston-Salem, NC, North Carolina Clinical Research, Raleigh, NC, University of Virginia, Charlottesville, VA, Emory University, Atlanta, GA

The Cloverdale Clinical Research Center at Wake Forest University Health Sciences and the Center for Human Genomics, Winston-Salem, NC, maintains a screening database of approximately 1,200 subjects with asthma. These are subjects who have called our clinic expressing interest in participating in asthma research studies. The following information is maintained on these subjects as it is obtained: gender, age, ethnic background, medical history, asthma history, skin testing results, exhaled breath condensate results, exhaled NO results, methacholine challenge testing results, pulmonary function, sputum induction results, bronchoscopy results, chest x-ray results, and medication usage. Should additional subjects be needed beyond this database of potential subjects, we continuously advertise for potential subjects using television, radio, and newspaper and flyer advertising (all advertising is IRB approved), as well as recruitment from the Wake Forest University Health Sciences Pulmonary and Allergy Clinics through our Primary and Sub-Investigators.

With reference to the VIDA trial inclusion criteria, the Wake Forest clinical site for the Severe Asthma Research Program has extensively characterized 350 not severe and severe asthmatic subjects who are immediately available for participation in the VIDA trial (78% of subjects would be classified with mild to moderate asthma based on their FEV_1 % predicted). Our SARP cohort is enriched for two important subgroups of interest in the VIDA trial, 37% of the cohort is African American and 75% are overweight or obese with a BMI >= 25. Preliminary unpublished data show that 100% of our AA subjects and 50% of our Caucasian subjects are vitamin D deficient with levels < 30 ng/ml that would qualify them as potential study subjects for the VIDA trial.

Our other primarily adult AsthmaNet site, North Carolina Clinical Research, Raleigh, NC, led by Craig LaForce, MD, maintains an asthma database of nearly 1,000 asthmatic subjects which span the severity ranges from mild to severe, with nearly 30% being African American. While we do not have

data on the vitamin D levels for these subjects, we assume that the vitamin D levels for these subjects would mirror those observed in Winston-Salem.

Although the University of Virginia site is predominantly a pediatric AsthmaNet site, Dr. W. Gerald Teague, MD has established links to adult patients with asthma through the Division of Adult Allergy and Immunology (Larry Borish, MD). Therefore, we anticipate limited participation in adult asthma studies, as deemed acceptable by Dr. Stephen Peters at the primary adult site at Wake Forest University.

Emory, like the University of Virginia, is primarily a pediatric AsthmaNet site. However, there is a large population of adults with asthma in the Atlanta area that is easily accessed through local pulmonology and allergy subspecialty practices. We also have established links with the Division of Pulmonary and Critical Care Medicine at Emory University (Cherry Wongtrakool, MD). Thus, Emory has had no difficulty in recruiting both adults and children for asthma clinical trials (i.e., through the American Lung Association's Asthma Clinical Research Centers Network and the NHLBI Severe Asthma Research Program). Emory will, therefore, participate in adult asthma studies as deemed acceptable with Dr. Stephen Peters at the primary adult site at Wake Forest University.

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XIII. APPENDICES

A. Appendix A. Exclusionary and Withhold Medications

Table 1. Drugs/Substances to be withheld throughout the study	**Washout prior to Visit 1 and 2**
Inhaled Steroids, except as provided in study	None
Leukotriene modifiers (e.g., montelukast, zafirlukast, zileuton)	None
Oral Steroids, except as provided in study	≥ 6 weeks
Cromolyn/Nedocromil	≥ 1 week
Oral beta-adrenergic agonists	≥ 1 week
Beta-adrenergic blockers	≥ 2 weeks
Inhaled beta-adrenergic agonists (intermediate-acting, e.g., albuterol, terbutaline, metaproterenol, pirbuterol, bitolterol), except as provided in study	≥ 6 hours
Long-acting Inhaled beta-adrenergic agonists (e.g., formoterol, salmeterol)	≥ 24 hours
Short-acting anticholinergics (e.g., ipratropium)	≥ 6 hours
Long-acting anticholinergics (e.g., tiotropium)	≥ 72 hours
Short-acting theophylline (e.g., Slophyllin tablets)	≥ 12 hours
Long-acting theophylline (e.g., Theo-Dur, Slo-BID)	≥ 24 hours
Ultra long-acting theophylline (e.g., Theo-24, Uniphyl)	≥ 48 hours
Macrolide antibiotics (chronic use)	≥ 4 weeks
MAO inhibitors	≥ 4 weeks
Colestipol	≥ 1 week
Orlistat	≥ 1 week
Cholestyramine	≥ 1 week
Cardiac glycosides	≥ 1 week
Phenytoin	≥ 1 week
Phenobarbital	≥ 1 week
Table 1. Drugs/Substances to be withheld throughout the study	**Washout prior to Visit 0**
Vitamin D supplements (including cod liver oil) containing >1000 IU/day	≥ 6 weeks
Calcium supplements > 2,500 mg/day	≥ 6 weeks
Table 1. Drugs allowed during study, but must be withheld prior to skin testing	**Washout prior to Visit 2 skin test**
Antihistamines (except for topical) • 1st generation • 2nd generation • Nasal antihistamines	 ≥ 3 days ≥ 7 days ≥ 5 days

Table 1. Drugs allowed during study, but must be withheld prior to pulmonary function and/or methacholine challenge	Specified time period
Levalbuterol (study rescue drug)	≥ 6 hours
Oral decongestants (pseudoephedrine and others)	≥ 48 hours
Nasal decongestants (oxymetazoline and others)	≥ 6 hours
Oral antihistamines (chlorpheniramine and others)	≥ 48 hours
Nasal and ophthalmic antihistamines (azelastine nasal and others)	≥ 6 hours
Methylxanthine-containing foods or beverages (e.g., coffee, tea)	≥ 6 hours
Alcohol-containing foods or beverages	≥ 6 hours

1

B. Appendix B. Modified Food Frequency Questionnaire (FFQ)

1) Do you take vitamin D supplements or multivitamins that include vitamin D on a regular basis (most months)? Yes/No
    a. If yes, provide name _____
    b. If yes, number of capsules/tablets taken per day
    c. If yes, amount of vitamin D per capsule/tablet
2) Do you take cod liver oil on a regular basis (most months)? Yes/No
    a. If yes, how many teaspoons or capsules per day on average? _____
3) How many servings (8 oz glass) of milk do you consume on average?
    a. Number of servings per day _____
    b. Number of servings per week _____
    c. Does not regularly consume _____
4) How many servings of salmon (4 oz equivalent) do you consume on average?
    a. Number of servings per week _____
    b. Number of servings per month _____
    c. Does not regularly consume ____
5) How many servings of sardines (equivalent of a 3.7 oz can) do you consume on average?
    a. Number of servings per week _____
    b. Number of servings per month ____
    c. Does not regularly consume _____

C. Appendix C. Sun Exposure Questionnaire (SEQ)

Note: A modified version of this questionnaire will be administered at visit 10 to ask specifically about sun exposure over the 7 months the participant spent on study medication.

1. In summer, during your leisure time, how much time do you normally spend in the sun

	< 1 hr a day	1 to 2 hrs per day	2 to 3 hrs per day	3 to 4 hrs per day	≥ 4 hrs a day
The last 3 years	☐1	☐2	☐3	☐4	☐5

2. In winter, during your leisure time, how much time do you normally spend in the sun

	< 1 hr a day	1 to 2 hrs per day	2 to 3hrs per day	3 to 4hrs per day	≥ 4 hrs a day
The last 3 years	☐1	☐2	☐3	☐4	☐5

3. In summer, how much do your activities (playing, day sports, spectator sports, gardening, walking, etc.) take you outside

	Not that often	A moderate amount	Quite a lot	Virtually all the time
The last 3 years	☐1	☐2	☐3	☐4

4. In winter, how much do your activities (playing, day sports, spectator sports, gardening, walking, etc.) take you outside

	Not that often	A moderate amount	Quite a lot	Virtually all the time
The last 3 years	☐1	☐2	☐3	☐4

5. When outside in summer, how often do you use a sunscreen or make sure you are 'covered up'

	Never / rarely	Occasionally	Most of the time	Always / almost always
The last 3 years	☐1	☐2	☐3	☐4

6. In the last 3 years, have you ever used a sunlamp or a tanning bed at a tanning salon? ☐1 Yes ☐0 No

If Yes, how often?

☐1 At least once a week

☐2 Less than once a week, but at least once a month

☐3 Less than once a month, but more than two times a year

☐4 Less than or equal to two times a year

D. Appendix D. Melanin Recording

SmartProbe 400 "L" measurements from four body areas. "L" is a measure of lightness or darkness (0=black, 100=white).

Body Area	First L Reading	Second L Reading
Upper Inner Arm	0-100	0-100
Outer Forearm	0-100	0-100
Exposed Forehead	0-100	0-100
Abdomen/Stomach	0-100	0-100

E. Appendix E. Protocol Table.

Visit	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**TF**[***]
Week	Pre-Scr	Scr	0	4	5	11	17	21	25	29	33	
Window (regular/extended)(Days)				+3/+7	-2/+7	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	
Randomization					X							
Steroid inhaler dose taper (if stable)							X		X			
Informed consent	X											
Brief medical history	X											
Full medical history		X										
Long physical exam		X									X	X
Short physical exam							X		X			
Height/weight/BMI		X									X	
Body measurements (waist, hip, neck)		X									X	
Melanin test				X							X	
Allergy skin testing[++]			X									
Genetics blood sample [**]				X								
Serum vitamin D	X						X		X		X	
Serum calcium		X										
Serum creatinine/eGFR		X										
Urine calcium and creatinine ratio		X		X		X	X		X		X	
Urine pregnancy test			X[*]	X			X				X	
Spirometry		X	X[*]	X	X	X	X	X	X	X	X	X
Maximum bronchodilator reversal		X										
Methacholine challenge			X[*]	X			X					
Sputum induction				X			X[+]					
Cold questionnaire (WURSS-21) distribution/instruction					X							
Asthma Control Test (ACT)		X		X		X	X	X	X	X	X	X
Asthma Bother Profile (QOL) (ABP)		X		X							X	
Asthma Symptom Utility Index (ASUI)		X		X		X	X	X	X	X	X	
Asthma-Specific Work Productivity and Activities Impairment Questionnaire (WPAI:Asthma)			X								X	
Home Environment Questionnaire (HEQ)		X										
Household Socio-Economic Information questionnaire (HOUSEHOLD_SEI)		X										
Perceived Stress Scale (PSS)			X								X	

Visit	0	1	2	3	4	5	6	7	8	9	10	TF***
Week	Pre-Scr	Scr	0	4	5	11	17	21	25	29	33	
Window (regular/extended)(Days)				+3/+7	-2/+7	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	±3/ ±5	
Sinonasal Questionnaire (SNQ)				X			X					
Sun Exposure Questionnaire (SEQ)				X							X	
Vitamin D Intake Questionnaire				X							X	
Review diaries				X	X	X	X	X	X	X	X	X
Review medication use				X	X	X	X	X	X	X	X	
Satisfaction questionnaire											X	
Prednisone burst				X								
Treatment failure assessment				X	X	X	X	X	X	X	X	X
Dispense e-diary/PEF meter			X									
Dispense run-in medications (open-label ICS and single-blind vit D capsules)			X	X								
Dispense rescue prednisone supply					X							
Dispense randomized medications (open-label ICS and double-blind vit D capsules)					X	X	X	X	X	X		

* At visit 2, participants who did not show adequate bronchodilator reversal at visit 1 will complete spirometry, a urine pregnancy test (for women of child-bearing potential), and a methacholine challenge test.

** The genetics blood sample may be postponed until the time of the vitamin D blood test at visit 6.

*** Additional visits to the clinical site will be required if the participant experiences treatment failure conditions between regular study visits.

+ Approximately 200 people will undergo sputum induction at visit 6. Only those whose sputum sample at visit 3 was deemed adequate through lab analysis will be eligible to have the sputum induction at visit 6.

++ If washouts required for skin testing at visit 2 are not met, the skin test may be deferred to a future visit.