Official Title: Daily Vitamin D for Sickle-cell Respiratory Complications (ViDAS-2)

ClinicalTrials.gov ID: NCT04170348

Version 4: 06/29/2022

3. Introductory Statement: This Phase 2 clinical trial, ViDAS-2, will **determine if *daily* oral vitamin D can reduce the risk of respiratory complications in children with sickle-cell disease.** Sickle-cell disease, an orphan disease affecting an estimated 100,000 individuals in the United States,[1, 2] is an inherited red blood cell disorder with acute vaso-occlusive complications and chronic multi-organ damage resulting from hemolysis-induced endothelial dysfunction and vasculopathy.[3, 4] **Respiratory complications are the leading cause of morbidity and of death**.[5, 6] Our earlier ViDAS-1 clinical trial provided evidence that *monthly bolus* oral vitamin D_3 supplementation in children with sickle cell disease reduced the annual rate of respiratory complications by more than 50% but only after a year of treatment and with no significant difference between vitamin D_3 given as standard- (12,000 IU/mo) or high- (100,000 IU/mo) dose therapy (Figure 1). The observed reductions are both statistically significant (P<0.0001) and clinically important. Respiratory infections or asthma attacks that would have no lasting effects in individuals with no sickle hemoglobinopathy can trigger severe or even fatal manifestations in those with sickle cell disease.

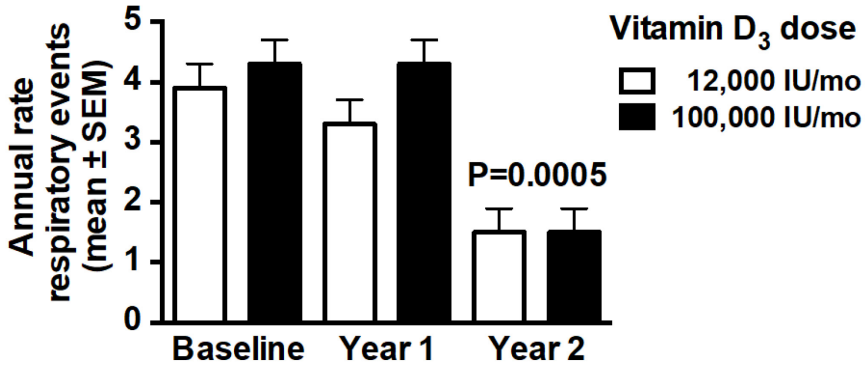

Figure 1: Annual rates of respiratory events (infections, exacerbations of asthma, and the acute chest syndrome) in children with sickle cell disease treated with no supplementation during a baseline year and monthly oral doses of vitamin D_3 given under observation during Years 1 and 2.

Vitamin D, in addition to its role in calcium and bone homeostasis, is a multifunctional regulator of both innate and adaptive immune responses.[7-11] **The scientific premise of the proposed clinical trial is that circulating concentrations of vitamin D_3, the parent compound, are the principal determinant of the anti-infective and immunomodulatory effects of supplementation**.[12-14] With *monthly bolus* oral vitamin D_3, the parent compound is cleared from the circulation within days.vxc[12, 13, 15, 16] In our earlier clinical trial *monthly bolus* oral vitamin D_3, neither the standard- nor high-dose treatments would produce sustained increases in circulating vitamin D_3, potentially explaining the uniformity of response to the two treatments. Apparently, a year of treatment with bolus dosing was needed to permit sufficient amounts of vitamin D_3 to accumulate within cells of the immune system and produce the observed reductions in the rates of respiratory events. Our hypothesis is that *daily* oral vitamin D_3 will rapidly reduce the rate of respiratory complications during Year 1. **We propose a 2-year controlled, double-blind, randomized Phase 2 clinical trial comparing the efficacy in reducing the rate of respiratory events in sickle-cell disease of *daily* oral vitamin D_3 (3,333 IU/d) with *monthly bolus* oral vitamin D_3, (100,000 IU/mo) as a control.** Baseline concentrations of 25-hydroxyvitamin D in our population (mean ~14 ng/mL) guide the choice of the high-dose daily treatment for the proposed trial and are too low to permit inclusion of a placebo control.

This Phase 2 clinical trial, designed to be a minimal risk study, has three specific aims:

(1) to determine whether *daily* oral vitamin D_3 (3,333 IU/d) given to children and adolescents with sickle-cell disease, compared to *monthly bolus* oral vitamin D_3, (100,000 IU/mo) will more rapidly reduce the rate of respiratory events, defined as respiratory infections, exacerbations of asthma, and episodes of the acute chest syndrome;

(2) to evaluate the effects of daily oral vitamin D_3 on pulmonary function; and

(3) to examine the effects of monthly oral vitamin D_3 on immune function, using measures of T-cell effector and regulatory function and of systemic inflammation.

The results of our clinical trial could offer a simple and low-cost intervention to help avert serious respiratory complications in sickle-cell disease that could lead to important reductions in morbidity and death in children with sickling disorders and to major changes in the management of their vitamin D status.

4. General investigational plan

Acute respiratory illness is a principal cause of morbidity and mortality worldwide[17] and can be devastating for children with sickle cell disease, an inherited red blood cell disorder affecting an estimated 100,000 Americans,[3] predominantly of African ancestry. Sickle cell disease is characterized by a shortened life expectancy, hemolytic anemia, acute episodes of vaso-occlusive pain, and recurrent, chronic damage to vital organs.[3, 4] Pulmonary manifestations are common and often severe. Respiratory infections or asthma attacks that would have no lasting effects in individuals with no sickle hemoglobinopathy can trigger severe or even fatal manifestations in those with sickle cell disease.[18] For instance, children with influenza and sickle cell disease are hospitalized at a rate more than 50-fold greater than children without sickle cell disease.[19] Asthma is common, affecting 15 to 28% of children with sickling disorders.[5] Unique to sickle cell disease is a respiratory complication characterized by fever, respiratory symptoms and a new pulmonary infiltrate, known as "acute chest syndrome."[6] Commonly precipitated by respiratory infections and asthma, the acute chest syndrome is the leading cause of death in sickle cell disease.[5, 6] The pathogenesis of sickle cell lung disease is unclear, but involves microvascular occlusion, hemolysis-induced endothelial dysfunction and vasculopathy that produce a chronic inflammatory state, often exacerbated by an infectious trigger.[3, 20, 21] Compromised immunity from functional asplenia may contribute to the risks of respiratory infections and pulmonary disease.[22] Improved vaccination and penicillin prophylaxis have greatly reduced the risk of bacterial pathogens but are ineffective against viruses and atypical organisms that now predominate as risks for acute chest syndrome.[23]

Vitamin D, in addition to its role in calcium and bone homeostasis, is a multifunctional regulator of innate and adaptive immune responses and of inflammation.[7-11] Vitamin D acts, in part, through its metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$),[24, 25] which binds to the vitamin D receptor to function as a transcription factor inducing vitamin D responsive genes that are present in most if not all cells of the immune system.[26] $1,25(OH)_2D$ mediates the innate immune host response against respiratory tract pathogens by stimulating expression of cathelicidin (hCAP18/LL37), an antimicrobial peptide with activity against viral, bacterial and fungal pathogens.[27, 28] $1,25(OH)_2D$ regulates the adaptive immune system by modulating T-lymphocyte proliferation and function, and by down-regulating the inflammatory response and cytokine expression.[29] In addition to these functions of $1,25(OH)_2D$, the parent compound vitamin D itself is a potent and general mediator of endothelial stability and barrier function.[30] A recent Cochrane review of randomized clinical trials identified high quality evidence that vitamin D supplementation reduced the risk of asthma exacerbations, although too few pediatric trials were included to permit definitive conclusions for children.[31, 32] While observational studies consistently have found significant associations between low vitamin D levels and increased susceptibility to respiratory infections, randomized clinical trials examining the effects of vitamin D supplementation on the risk of respiratory infections have had conflicting findings due, in part, to differences in the baseline status of the participants, the duration of the studies and the amount and frequency of dosing.[33-36]

Children with sickle cell disease are at high risk for vitamin D deficiency due to limited sun exposure, dark skin color and poor nutrition. Our own and other observational studies have found that most children with sickle cell disease have low vitamin D status.[37, 38] Contrary to an earlier hypothesis,[39] the low levels of vitamin D in black Americans are not explained by differences in the concentration of vitamin D-binding protein.[40, 41] Considering that individuals with low vitamin D levels seem to derive the most benefit from supplementation[36] and that children with sickle cell disease have a heightened susceptibility to respiratory infections and asthma together with a greatly increased vulnerability to complications, we conducted the ViDAS-1 study, a 2-year active-controlled, double-blind, randomized clinical trial comparing high-dose oral vitamin D_3, 100,000 IU, given once a month under observation (daily equivalent 3,333 IU/day), with standard-dose oral vitamin D_3, 12,000 IU, given once a month under observation (daily equivalent 400 IU/day),[42] to determine if oral vitamin D_3 (cholecalciferol) can reduce the risk of respiratory complications in children and adolescents, 3 to 20 years old, with sickle cell disease (ViDAS-1 study). Because of the established risks of inadequate mineralization of the skeleton associated with vitamin D deficiency,[9, 42-44] a placebo group could not be included in the study design.

The ViDAS-1 trial results have been presented in our annual reports and are summarized here to clarify the rationale for the ViDAS-2 trial. Seventy sickle cell subjects, ages 3-20 years, with baseline records of respiratory events over one year before randomization, underwent screening. Sixty-two subjects with 25-hydroxyvitamin D levels 5-60 ng/mL were randomly assigned in a 1:1 ratio to oral vitamin D_3 100,000 IU (n=31) or 12,000 IU (n=31), under observed administration once monthly for two years. The primary outcome was the annual rate of respiratory events (respiratory infection, asthma exacerbation, acute chest syndrome) ascertained by use of a

validated questionnaire administered biweekly. Analysis included 62 children, mean (SD) age 9.9 (3.9) years, 32 (52%) females, predominantly HbSS (87%), with mean baseline 25-hydroxyvitamin D of 14.3 ng/mL. The mean (SEM) 25-hydroxyvitamin D concentrations during Treatment Years 1 and 2 are shown in Figure 2. A total of 291 adverse events were reported, including 55 serious adverse events in 20 subjects, most of which were expected for sickle cell disease; none were attributed to the study drug. There were 17 abnormal safety laboratory values; all abnormal laboratory values were determined to be clinically insignificant, without requiring withholding or discontinuation of study treatment.

The annual rates of respiratory events at baseline, intervention year-1, and year-2 with the high-dose were 4.34±0.35 (SEM), 4.28±0.36, and 1.49±0.37, and, with the standard-dose, 3.91±0.35, 3.34±0.37, and 1.54±0.37, respectively (see Figure 1 above). In pediatric patients with sickle cell disease, two-year monthly oral vitamin D3 was associated with a >50% reduction in the rate of respiratory illness during the second year, with similar decreases from high- and standard-dose treatment.

In summary, in children and adolescents with sickle cell disease who received monthly bolus doses of oral vitamin D_3 under observation for two years, the annual rates of respiratory events (respiratory infections, asthma exacerbations and acute chest syndrome) decreased by more than 50% during the second year of treatment, with similar reductions in the groups treated with 100,000 IU/month and with 12,000 IU/month. The treatment groups did not differ significantly with respect to pulmonary function, hand-grip strength, fever or episodes of vaso-occlusive pain. Both doses were safe with no significant differences in adverse events.

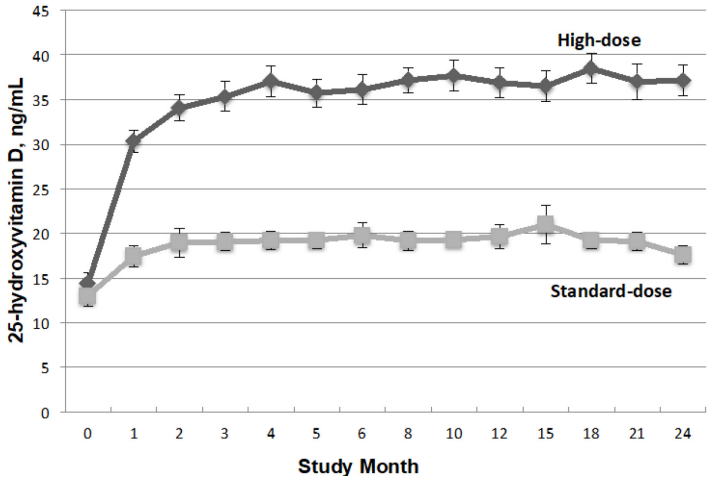

Figure 2: Mean serum 25-hydroxyvitamin D concentrations by treatment group: High-dose: 100,000 IU (2.5 mg) vitamin D3 given once a month under observation (daily equivalent 3,333 IU/day); Standard-dose, 12,000 IU (0.3 mg) vitamin D3 given once a month under observation (daily equivalent 400 IU/day).

This ViDAS-1 study is the first randomized clinical trial to examine the potential benefit of vitamin D for preventing respiratory complications in sickle cell disease. We chose monthly dosing to allow for directly observed administration and avoid non-adherence with daily dosing. We provided prolonged treatment to allow for seasonal variation. Our primary outcome measure was collected biweekly using a validated questionnaire[45] to adequately capture all respiratory events, and verified by review of medical records. Because we could not include a placebo group, given the known risks of skeletal harm with vitamin D deficiency,[9, 42-44] the decrease in the annual rates of respiratory events during Year 2 cannot be ascribed unequivocally to vitamin D supplementation. Nonetheless, no significant differences in the mean weekly rates of influenza-like illnesses in New York City during the baseline and study years (2012-2015) were found by the New York State Department of Health Influenza-Like Illness Surveillance Program (ILINet).[46] Moreover, because recruitment for the trial extended over 18 months, overlap between study participants receiving supplementation during Year 1 and 2 was considerable. For example, Year 2 treatment of patients enrolled in months 1 to 6 of the trial coincided with Year 1 treatment of patients enrolled in months 12 to 18.

In the ViDAS-1 study, the effects of standard- and high-dose vitamin D on respiratory events were statistically indistinguishable. Conceivably, these results could be interpreted as evidence that current recommendations for vitamin D supplementation are sufficient to maintain respiratory health in patients with sickle cell disease. In the

standard-dose group, the estimated mean dietary vitamin D of 228.6 IU/day, together with the monthly supplement providing about 400 IU/day, would meet the current Estimated Average Requirement for vitamin D of 600 IU/day.[9] Nonetheless, 25-OHD concentrations were at insufficient or deficient levels for skeletal health in 75% of the standard-dose group while in the sufficient range in 98% of the high-dose group.

Alternatively, both (i) the timing of the observed decreases in the annual rates of respiratory events and (ii) the uniformity in the magnitudes of the reductions with the standard- and high-dose treatments potentially could be explained by the monthly bolus dosing schedule for vitamin D used in the trial. Since the design of our study, new evidence has accumulated that circulating concentrations of vitamin D_3, the parent compound, rather than those of 25-OHD, may be vital determinants of the anti-infective, immunomodulatory and other extra-skeletal effects of supplementation.[12, 13, 30, 47] To be active, supplemental vitamin D_3 must undergo two hydroxylations, first to 25-OHD and then to the functional metabolite, 1,25-dihydroxyvitamin D, that binds to the vitamin D receptor to function as a transcription factor inducing vitamin D responsive genes. For the principal endocrine functions of vitamin D, bone mineralization together with calcium and phosphate homeostasis, the first hydroxylation takes place using a 25-hydroxylase in the liver,[24, 25, 48] producing 25-OHD that enters the circulation. The second hydroxylation occurs in the kidney, using the 1-alpha- hydroxylase CYP27B1 to yield 1,25-dihydroxyvitamin D.[24, 26] By contrast, for extra-skeletal autocrine and paracrine activities, vitamin D_3, the parent compound, enters cells more easily than 25-OHD, and both hydroxylations may take place intracellularly.[12-14] Both CYP27B1 and 25-hydroxylases are widely expressed and the vitamin D receptor is present in most, if not all, cells of the immune system.[26] In addition to these functions of $1,25(OH)_2D$, the parent compound vitamin D itself is a potent and general mediator of endothelial stability and barrier function.[30]

With a single oral bolus dose of vitamin D_3,12,000 IU, the half-life of 25-OHD is on the order of weeks while that of vitamin D_3 is about one day. With a single oral bolus dose of vitamin D_3,100,000 IU, 25-OHD remains elevated for more than 3 months while vitamin D_3 returns to near baseline in less than a week.[12, 13, 15, 16] The repeated oral bolus doses of vitamin D_3 resulted in the plateaus in 25-OHD shown in Figure 2. While we did not measure vitamin D_3 concentrations in our study, neither the standard- nor high-dose treatments would produce sustained increases in circulating vitamin D_3, potentially explaining the uniformity of response to the two treatments. Vitamin D_3 in blood seems to be in diffusional equilibrium with vitamin D_3 in adipose tissue.[12] With bolus doses, vitamin D3 in excess of use will accumulate in fat and gradually raise circulating vitamin D_3 concentrations. Apparently, a year of treatment with bolus dosing was needed to permit sufficient amounts of vitamin D_3 to circulate, accumulate within cells of the immune system, and produce the observed reductions in the rates of respiratory events. In shorter trials of bolus oral doses of vitamin D_3, insufficient durations of treatment could explain the lack of effect on respiratory infections,[36, 49] perhaps in conjunction with other possible explanations for the lack of a protective effect of bolus doses of vitamin D.[50-54]

Children with sickle cell disease typically have low vitamin D status,[37, 38] heightened susceptibility to respiratory infections and asthma, and a greatly increased vulnerability to potentially fatal complications of respiratory illness. Our study results provide evidence for a substantial protective effect of vitamin D against sickle cell respiratory complications that was manifest only after a year of bolus administration. While our results cannot prove that vitamin D was responsible for the substantial and significant reduction in respiratory illness, this hypothesis is consistent with the known facts.

The ViDAS-2 trial is designed to determine if daily oral administration of vitamin D will produce sustained increases in the concentration of circulating vitamin D_3 and provide children and adolescents with sickle cell disease with protection against respiratory infections, asthma exacerbations and acute chest syndrome. Daily supplementation with 2000–6000 IU/d vitamin D_3 results, within three weeks, in stable circulating concentrations of vitamin D_3 within the range of 10–40 ng/mL in a linear fashion.[13, 55-57] Our primary study hypothesis is that *daily* oral vitamin D_3 (cholecalciferol), 3,333 IU/d (0.083 mg/d), will reduce the annual rate of respiratory events (infections, exacerbations of asthma, and the acute chest syndrome) by 50% or more during Treatment Year 1 compared with *monthly* bolus oral vitamin D3, 100,000 IU/mo (25 mg/d) as a control.

The control treatment with monthly bolus administration should provide an estimate of the annual rates of respiratory events that would be expected with placebo while protecting against the skeletal risks associated with insufficient or deficient vitamin D levels ($25(OH)D<20$ ng/mL).[9, 42-44]

5. Investigator's brochure: Not required for a Sponsor-Investigator Investigational New Drug (IND) application.

6. Protocols:

6a. Study protocol: This ViDAS-2 study is a 2-year active-controlled, double-blind, parallel-group, randomized Phase 2 clinical trial comparing the efficacy in reducing the rate of respiratory events in children and adolescents with sickle cell disease of daily oral vitamin D3, 3,333 IU/d (0.083 mg/d) with monthly bolus oral vitamin D3, 100,000 IU/mo (2.5 mg/mo), as a control. A profile of the Phase 2 clinical trial design is shown schematically in the Figure.

The study population for the ViDAS-2 randomized trial will be recruited from among approximately 200 patients, ages 3-20 years old, followed in the Columbia University Medical Center Pediatric Sickle Cell Program. Subjects will be recruited and screened based on medical history, physical examination, and laboratory evaluation (serum 25-hydroxyvitamin D, parathyroid hormone, chemistry profile including calcium, phosphate, magnesium, BUN, creatinine, and liver function tests, urinalysis, and urinary calcium/creatinine ratio). Patients who complete the screening visit will then return and their potential inclusion in the trial will be determined by the serum 25-hydroxyvitamin D concentration:

(i) Patients found to be severely vitamin D deficient, with a screening serum 25-hydroxyvitamin D concentration below 5 ng/mL, will not be randomized but will be eligible to continue participation in the study by being assigned to treatment with daily oral vitamin D3, 3,333 IU/d (0.083 mg/d), in an open-label manner and by completing all other study procedures. Management of these patients will consist of oral administration of vitamin D_3, 100,000 IU, initially, followed by oral vitamin D_3, 100,000 IU, every other week for 2 months, and then daily oral vitamin D3, 3,333 IU/d (0.083 mg/d), thereafter for the duration of the study, with follow-up by the participant's attending physician. This schedule of vitamin D administration for treatment of vitamin D deficiency is consistent with the recommendations for Stoss therapy of vitamin D deficiency by the Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society.[42]

(ii) Patients with excessive levels of vitamin D, as determined by a screening serum 25-hydroxyvitamin D concentration above 100 ng/mL, will be excluded from participation in the trial and referred to their attending physician for follow-up and management. We do not anticipate that any of our patients will have excessive levels of vitamin D but include this provision for exclusion as a precautionary measure.

(iii) Patients with levels of vitamin D already within the range found in sun-rich environments, as determined by a screening serum 25-hydroxyvitamin D concentration of 61-100 ng/mL, will not be randomized because no supplemental vitamin D would be indicated. We do not anticipate that any of our patients will have levels of vitamin D in this range but if any are identified, they would be eligible to continue participation in the study and complete all other study procedures. They would not receive supplemental vitamin D within the study.

(iv) Patients with a screening serum 25-hydroxyvitamin D concentration of 5 - 60 ng/mL, will be randomized within a month in a gender- and age-stratified (3 to 9 and 10 to 20 years of age) manner to treatment with either (1) daily oral vitamin D3, 3,333 IU/d (0.083 mg/d) or (2) 100,000 IU (2.5 mg) oral vitamin D_3 once monthly with daily placebo.

Prior to initiation of treatment, subjects will have baseline assessments that will include: (i) pulmonary function test (limited to age above 5y/o); and (ii) blood tests for biomarkers of inflammation (complete blood count, C-reactive protein, and Th1/Th2 cytokines). Participants will be seen monthly in person or remotely in a telehealth visit for confirmation of administration of study drug,accounting of unused study drug, interval history including respiratory events and adverse event monitoring. Laboratory safety assessments will include serum albumin-corrected calcium and spot urinary calcium-creatinine ratios to be obtained immediately before study drug administration, and performed at intervals as shown in the Table (Schedule of Study Evaluations) to monitor hypercalcemia and hypercalcuria. Abnormal laboratory values will be reviewed by the Clinical Trial Safety Officer to determine clinical significance and need for intervention, as described in detail below.

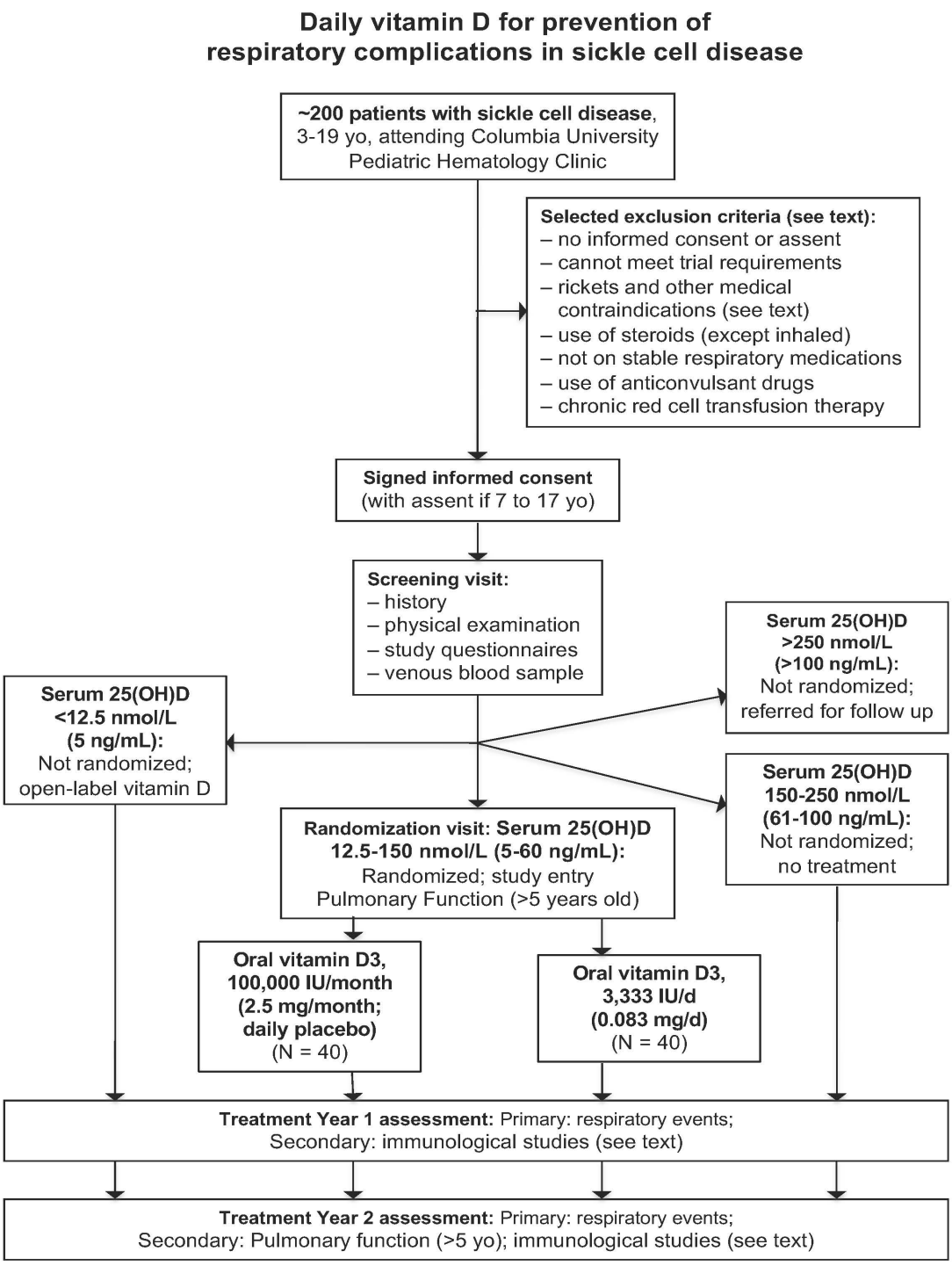

Figure: Profile of the Phase 2 ViDAS-2 clinical trial design.

Blood specimens for 25(OH)D and vitamin D_3 will be collected every third month for the first six months, then at months 6, 12, 18 and 24, stored at -112°F (-80°C) freezer, and analyzed in a single batch at the end of the study by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (*See below for Vitamin D assay*). Adverse events will be determined according to the Common Terminology Criteria of Adverse Events version 4.0. Pulmonary function tests will be repeated at 12-months and 24-months after initiation of treatment, while the biomarkers of inflammation, the health-related quality of life questionnaire and the Block Calcium and Vitamin D screener and sun exposure questionnaires will be collected every 6 months. Table 3 below outlines the schedule of study evaluations.

Primary Outcome Measure: Respiratory Events

A Respiratory Events Questionnaire, modified from established instruments,[58, 59] will be used to track respiratory events. The questionnaire, to be administered in person during monthly outpatient study visits and biweekly telephone interviews, includes questions about the following six symptoms: difficulty breathing, wheezing, fever, cough, runny nose, and sore throat. If any of the above symptoms is present, additional questions about severity and duration of symptom, medication use, visit to a medical provider, hospitalization, and missed school day/s due to illness will be asked. History of outpatient sick visits and hospital admissions will be verified by review of medical records. All completed questionnaires will be reviewed and adjudicated in a blinded fashion by a designated Investigator to determine whether a respiratory event had occurred.
(*Study Questionnaire is attached in Appendix*)

Definitions of Respiratory Events:

A "respiratory event" will be considered if at least one of the six symptoms occurred, except for fever alone without accompanying respiratory symptoms, or a runny nose accompanied by itchy/watery eyes suggestive of allergy. A new event will be counted if at least seven days had elapsed since the last counted event. Patients with prolonged respiratory symptom/s lasting more than two weeks will be evaluated by the study pulmonologist, whose final diagnosis will determine whether the symptom/s comprised a respiratory event.

"Respiratory infection" will be defined as: (i) runny nose, cough, or sore throat for at least 2 days or any of these symptoms for at least 1 day accompanied by fever, not otherwise attributed to allergy; (ii) physician diagnosed pharyngitis, otitis media, or influenza; or (iii) physician-diagnosed croup, bronchiolitis, or pneumonia. The study questionnaire was validated in our study population and found to have 80% over-all accuracy for identifying respiratory tract pathogens[60].

"Asthma exacerbation" will be defined by a physician-diagnosed acute asthma attack that required treatment with corticosteroids, emergency room treatment, or hospital admission.

"Acute chest syndrome" will be defined according to the standard definition, as an acute illness characterized by fever and/or respiratory symptoms, accompanied by a new pulmonary infiltrate on a chest x-ray.[61]

In instances when there is an overlap of these diagnoses within a single duration of symptoms, such as when respiratory infection preceded an asthma exacerbation or acute chest syndrome, or when infection or acute asthma occurred with an acute chest syndrome, the separate diagnoses will be counted as a single event.

Vitamin D assay:

Serum 25-hydroxyvitamin D2 (25-OH-D2) and 25-hydroxyvitamin D3 (25-OH-D3) will be measured and summed as total 25-hydroxyvitamin D. Assays will be performed in the Biomarkers Core Laboratory within the Irving Institute for Clinical and Translational Research using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (LC-MSMS).

Sample preparation:

25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 will be extracted from serum samples using liquid-liquid extraction. Briefly, 150uL of human serum will be spiked with 10uL of internal standard and vortexed, followed by the addition of 150uL of 0.2 M zinc sulfate. After mixing, 300uL of methanol is added followed by 750uL of hexane with vortexing in between. The mixture is again vortexed for 30 seconds followed by centrifugation at 13,000 rpm for 5 minutes. The hexane layer is transferred to LC vials, dried down under nitrogen and dissolved in 100uL of 70% methanol.

Table. SCHEDULE OF STUDY EVALUATIONS AND PARTICIPANT TIMELINE

	Screening	Randomize¶	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12	Month 13	Month 14	Month 15	Month 16	Month 17	Month 18	Month 19	Month 20	Month 21	Month 22	Month 23	Month 24
Medical history	X													X												X
Physical exam	X													X												X
Anthropometry	X													X												X
Respiratory events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oral vitamin D_3		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HRQL, Diet/Sun exposure		X						X						X						X						X
LABORATORY EVALUATIONS																										
Serum 25-OHD	X	X			X			X						X						X						X
Serum D_3		X			X			X						X						X						X
Serum Calcium, albumin	X	X			X			X						X						X						X
CMP, Mg, PO4	X							X						X						X						X
Urinalysis	X	X						X						X						X						X
Urinary Ca, Cr		X			X			X			X			X			X			X			X			X
VDBP		X												X												X
Serum PTH		X						X						X						X						X
β-hCG	X							X						X						X						X
AIRWAY EVALUATIONS (aim 2)																										
PFT		X																								X
IMMUNE SYSTEM CHANGES (aim 3)																										
T effector function																										
IL-4, IL-5, IL-13, IFNγ, IL-2		X						X						X						X						X
T regulatory function																										
IL-10, TGF-β		X						X						x						X						X
Systemic inflammation																										
CBC/plts		X						X						X						X						X
Serum CRP		X						X						X						X						X
IL-1α, IL-1β, TNFα		X						X						X						X						X

Abbreviations and explanations: Respiratory events: questionnaire for respiratory events during preceding two weeks; Oral vitamin D_3: 100,000 IU (2.5 mg) oral vitamin D_3 once monthly with daily placebo or daily oral vitamin D3 (3,333 IU/d); Diet/Sun exposure: Block Calcium and Vitamin D screener questionnaire and sun exposure questionnaire; HRQL: child and parent reported; PedsQL™ Generic Core Scale, and PedsQL™ Sickle Cell Disease Module; PFT: Pulmonary function tests; Serum 25(OH)D: Serum 25-hydroxyvitamin D concentration; Serum D_3: serum concentrations of vitamin D_3, the parent compound; Ca: calcium; CMP: comprehensive metabolic panel; PO_4: phosphate; Mg: magnesium; VDBP: vitamin D-binding protein concentration; Urinary Ca, Cr: Urinary calcium and creatinine; Serum PTH: Serum intact parathyroid hormone; β-hCG: β- Human chorionic gonadotropin; T-effector function: T-helper 1,2 cytokines interleukin (IL)-10, 4,5,13, 2 and interferon (IFN)γ. T-regulatory function by cytokines IL-10, transforming growth factor (TGF)β; Systemic inflammation assessed by CBC: complete blood count with leukocyte and platelet (plt) counts; Serum CRP: Serum high-sensitivity C-reactive protein; and assessed by IL-1α, β, tumor necrosis factor(TNF)α). For all assessments, a protocol window of ± 7 days is specified.

LC-MSMS:
LC-MSMS analysis will be performed on a UPLC-MS/MS platform comprising a triple quadruple Agilent 6410 mass spectrometer (Agilent, Santa Clara, CA) integrated to Agilent UPLC 1290 series controlled by MassHunter quantitative analysis software (version B 04.00). Chromatographic separation will be performed by injecting 10uL of the extract onto an Agilent Poroshell 120 EC-C18 column (3.0 x 50mm, 2.7 µm) maintained at 50°C. The flow rate is maintained at 500µl/min. The initial flow conditions are 30% solvent A (water containing 0.1% formic acid) and 70% solvent B (methanol containing 0.1% formic acid). Solvent B is raised to 90% linearly over 4min and to 98% in 4.1min, held for 1.4min and back to initial conditions by 5.6min with a total run time of 6.5min. The mass spectrometer is operated under multiple reaction monitoring (MRM) mode with positive electrospray ionization. MRM transitions will be m/z 413->395 for 25(OH)D_2, 401->383 for 25(OH)D_3 and 407->389 for d6-25(OH)D_3. Retention times 2.89 min for 25(OH)D_3 and 3.04 min for 25(OH)D_2. Lower limit of quantitation for the assay for both 25(OH)D_3 and 25(OH)D_2 is 1.25 ng/ml. Intra-day precision is 2.4% for 25(OH)D_2 and 3.5% for 25(OH)D_3. Inter-day precision is 8.1% for 25(OH)D_2 and 5.5% for 25(OH)D_3. Calibrators are standardized against the NIST standards.

Serum vitamin D_3, the parent compound**,** will be measured by LC-MSMS by Heartland Assays, Ames, Iowa.

STUDY DRUG

The 3,333 IU (0.083 mg) oral vitamin D_3, the 100,000 IU (2.5 mg) oral vitamin D_3, and placebo dosage forms will be custom manufactured as single batches from Tishcon Laboratories Inc, (Westbury, NY) a Good-Manufacturing-Practice (GMP) facility, in identical capsule formulations but for the concentration of vitamin D_3. The content of active ingredient will be analyzed in random samples from the batch in Tishcon Laboratories by high-performance liquid chromatography using United States Pharmacopeia methods.

For study participants randomized to daily treatment, the 3,333 IU (0.083 mg) oral vitamin D_3 will be taken daily, with the dose at monthly in-person visits administered by the Clinical Research Coordinator or directly observed during a telehealth visit. For study participants randomized to control treatment, placebo will be taken daily and the 100,000 IU (2.5 mg) oral vitamin D_3 dose will be administered by the Clinical Research Coordinator at monthly in-person visits or directly observed during a telehealth visit.

The rationale for the choice of oral vitamin D_3 dosage forms is based on the results of our earlier ViDAS-1 trial. In our study population of children and adolescents with sickle cell disease, the majority of the participants (77%) had insufficient or deficient vitamin D levels (25(OH)D<20 ng/mL),[42, 43] with an overall mean baseline 25(OH)D of 14.3 (median 14.6, range 5-28 ng/mL). Because of the established risks of inadequate mineralization of the skeleton associated with vitamin D insufficiency and deficiency,[9, 42-44] a placebo group could not be included in the study design. At the end of our earlier ViDAS-1 trial, 25(OH)D concentrations were at insufficient or deficient levels for skeletal health in 75% of those who had received 2 years of standard-dose treatment (400 IU/day vitamin D supplement together with an estimated mean dietary vitamin D of 228.6 IU/day, providing the current Institute of Medicine Recommended Dietary Allowance (RDA) for vitamin D of 600 IU/day, considered to meet the requirement of 97.5% of the population[9, 62]). By contrast, in study participants who received 100,000 IU (2.5 mg) oral vitamin D_3 monthly (daily equivalent 3,333 IU/day), 25(OH)D concentrations were in the sufficient range in 98%. Nonetheless, in the study participants receiving bolus 100,000 IU (2.5 mg) oral vitamin D_3 monthly during ViDAS-1 Treatment Year 1, the annual rates of respiratory events (respiratory infections, asthma exacerbations and acute chest syndrome) were not significantly different from the year-long ViDAS-1 baseline period with no supplemental vitamin D (baseline rate = 4.34±0.35 (SEM), Treatment Year 1 rate = 4.28±0.36). Accordingly, in the current trial, **control treatment with bolus administration of 100,000 IU (2.5 mg) oral vitamin D_3 monthly should provide an estimate of the annual rates of respiratory events that would be expected with placebo** while protecting against the skeletal risks associated with insufficient or deficient vitamin D levels (25(OH)D<20 ng/mL).[9, 42-44]

The scientific premise of the proposed clinical trial is that circulating concentrations of vitamin D_3, the parent compound, are the principal determinant of the anti-infective and immunomodulatory effects of supplementation. With *monthly bolus* oral vitamin D_3, the parent compound is cleared from the circulation within days.[55] In contrast, *daily* supplementation with 2000–6000 IU/d vitamin D_3 results, within three weeks, in stable circulating concentrations of vitamin D_3 within the range of 10–40 ng/mL in a linear fashion.[13, 55-57] The study hypothesis is that participants randomized to *daily* treatment with the 3,333 IU (0.083 mg) oral vitamin D_3 dose will develop

stable circulating concentrations of vitamin D_3 that will provide rapid protection against respiratory infection, exacerbations of asthma and the risk of acute chest syndrome. As shown in our earlier ViDAS-1 trial, during Treatment Year 1, *monthly bolus* oral vitamin D_3 with 100,000 IU (2.5 mg) oral vitamin D_3 (daily equivalent 3,333 IU/day) failed to decrease the annual rate of respiratory events compared to the baseline period with no vitamin D supplementation. The continuation of the trial into Treatment Year 2 will determine (i) if any reduction in the rate of respiratory illness with daily vitamin D_3 seen during Treatment Year 1 is sustained and (ii) if *monthly bolus* oral vitamin D_3 again reduces the rate of respiratory events by 50% or more during Treatment Year 2. To our knowledge, this study will provide the first direct comparison of the effects on respiratory illness of daily and monthly doses of vitamin D that deliver approximately equivalent amounts of vitamin D per month.

Drug Accountability: Once a randomization request has been completed and at least 2 days before the scheduled Baseline study visit), the Study Coordinator will coordinate dispensing of the study medication by the Research Pharmacy. The Research Pharmacy is responsible for determining which assignment (A or B) is active study-dose treatment (vitamin D_3 3,333 IU/capsule to take daily except during the monthly study visit when 3,333 IU/capsule x 1 capsule and placebo x 1 capsule will be administered by study coordinator on-site or self-administered under direct observation by study coordinator during telehealth visit), and which assignment is control-dose treatment (placebo capsule to take daily except during monthly study visit when vitamin D_3 50,000 IU/capsule x 2 capsules will be administered by study coordinator on-site or self-administered under direct observation vy study coordinator during telehealth visit). Study medication labels will include the prescription number, subject ID, and randomization ID as recorded in the Research Pharmacy Medication Dispensing Log. The initial dose will be administered during the Baseline study visit after all the baseline procedures have been completed. Two capsules containing either study-dose (3,333 IU/capsule x 1 capsule and placebo x 1 capsule) or control-dose (vitamin D_3 50,000 IU/capsule x 2 capsules) will be given orally. Capsules can either be swallowed or for those subjects who are unable to swallow capsules, contents of the capsules can be sprinkled in apple sauce or pudding and consumed in its entirety. Subjects will be instructed to take one capsule daily until the time of the next study visit. Subjects will be asked to count and dispose any unused capsules observed on video by study coordinator at the following monthly visit. The number of unused capsules will be recorded by the study coordinator in the Research Pharmacy Drug Return Log. These procedures will be repeated at each monthly study visit. Month 24 will serve as the Study Exit visit; study drug will not be administered or dispensed at this visit.

STUDY SUBJECTS

Inclusion criteria:

1) Diagnosis of sickle cell disease (Hb SS, Hb SC, Hb S-Beta-thalassemia)
2) Age 3-20 y/o

Exclusion criteria:

1) Patient unwilling or unable to provide written informed consent (and assent, if applicable)
2) Patient unable or unwilling to comply with requirements of the clinical trial
3) Participation in another clinical trial
4) Current diagnosis of rickets
5) History of hypercalcemia or diagnosis of any medical condition associated with hypercalcemia, including primary hyperparathyroidism, malignancy, sarcoidosis, tuberculosis, granulomatous disease, familial hypocalciuric hypercalcemia
6) Current use of corticosteroids, excluding inhaled steroids
7) Current use of anticonvulsants (phenytoin, phenobarbital, carbamazepine)
8) Therapy with thiazide diuretics or lithium carbonate
9) Known liver or renal disease
10) Patients taking medications for pulmonary complications of sickle cell disease not on a stable dose of medications, as defined by a change in medications or doses within the three months prior to study entry
11) Patients on chronic red blood cell transfusion therapy
12) Pregnancy

Subject Justification:

This study will enroll about 80 children and adolescents, ages 3-20 years, with sickle cell disease. Participation of children in the proposed research is essential to answer the research question, because pulmonary complications in sickle cell disease develop early in life. The study aims to determine whether vitamin D supplementation can reduce the risk of respiratory complications during childhood and prevent deterioration in lung function. The proposed research will predominantly include subjects of African origin, including African Americans and those from the Caribbean Islands (Dominican Republic, Puerto Rico, West Indies, Cuba, and Haiti), because sickle cell disease primarily affects people of African descent.[3]

Subject Randomization:

Within one month after screening procedures have been completed, subjects will be randomized. Using computer-generated randomization, participants with 25-hydroxyvitamin D concentrations of 5-60 ng/mL will be randomly assigned using randomly permuted blocks of size 2, 4 or 6 assignments with separate schema prepared for stratification by age (3-9 and 10-20), gender and hydroxyurea therapy. Randomization will be performed by the Research Pharmacy; all other research staff and participants will be blinded to allocation.

Subject Compensation:

Study participants will receive $40 per monthly study visit, with the exception of the following 3 study visits: 1) Baseline/Randomization visit, 2) Month 12 visit, and 3) Month 24 Study exit visit, for which participants will receive $100. The modest compensation will be provided to compensate for participant's time, transportation expense, and completion of study-required procedures. Compensation will be in the form of cash or gift card and paid in installment during each monthly visit. Participants will not be required to provide receipts for reimbursement. A total of $540 per calendar year is compensated to each subject who completes all study-related visits.

STATISTICAL ANALYSIS PLAN

Our primary study hypothesis is that *daily* oral vitamin D_3 (cholecalciferol), 3,333 IU/d (0.083 mg/d), will reduce the annual rate of respiratory events (infections, exacerbations of asthma, and the acute chest syndrome) by 50% or more during Treatment Year 1 compared with *monthly* bolus oral vitamin D3, 100,000 IU/mo (25 mg/d) as a control. As detailed above, control treatment with bolus administration of 100,000 IU (2.5 mg/d) oral vitamin D3 monthly should provide an estimate of the annual rates of respiratory events that would be expected with placebo treatment.

Our primary study analysis will examine the annual rate of respiratory events (the sum of respiratory infections, asthma exacerbations and episodes of acute chest syndrome) in children with sickle cell disease randomized in a double-blind manner to the daily and monthly doses of oral vitamin D_3. The primary statistical analysis will be by “intention to treat” and will use a semiparametric regression method to estimate the between group difference in intensity (events per subject per unit of time at risk) with the assumption that covariates have a multiplicative effect on the mean and rate functions. This approach takes account of the skewed distribution of the respiratory events. The results of this analysis will be summarized as a regression parameter estimate, with the standard error of the estimate, a chi-square test with associated p-value, and a hazard ratio with confidence limits expressing the rate difference of the daily relative to the monthly group (as described in Lin, DY, Wei, LJ, Yang, I, and Ying, Z. “Semiparametric regression for the mean and rate functions of recurrent events,” J R Stat Soc Series B Stat Methodol, 2000; 62:711–730).[63] This approach can adjust for group differences in time-independent covariates and estimate rate ratios at specific values of covariates and at specific times of follow up. The annualized rate of events and the average inter-event interval (in two-week intervals, the observation protocol) will be entered as time-independent covariates. After testing the initial hypothesis of overall group difference, we will sequentially test the between-treatment group differences in the study design enrollment criteria strata: age, gender and therapy (hydroxyurea). Secondary analyses using the same statistical framework will separately test the study hypotheses at the one-year time point. The functional form of covariates and proportional hazards assumptions will be checked for all models and cumulative hazard plots and cumulative residual plots will be used to display group differences in the evolution of events and model fit, respectively.

Subsidiary analyses will be made to examine a number of secondary study hypotheses with respect to the effects of vitamin D_3 on changes in pulmonary function and airway inflammation, and on immune system, but these comparisons have not entered into the sample size calculation. Data will be summarized as means ± standard

deviation or as counts and proportions. Study Group differences in continuous measures at baseline will be compared using independent *t* tests and differences in dichotomous categorical measures at baseline will be compared using Fisher's exact test.

Sample size

For sample size estimation, we base our estimate of the annual rate of respiratory events on the rate observed in the earlier ViDAS-1 trial in our study population, a mean annual baseline respiratory event rate of 4.1. For our estimate of the treatment effect, we use the observed reduction in annual mean respiratory event rate in the earlier ViDAS-1 trial for *monthly* bolus oral vitamin D3, 100,000 IU/mo (2.5 mg/mo), from Treatment Year 1 to Treatment Year 2 from 4.28±0.36 (SEM) to 1.49±0.37, or a decrease of about 65 percent. Using a conservative estimate of a treatment effect of 50 percent reduction in the rate of respiratory events as the minimal *clinically* significant difference to be detected, **26 subjects in each group will be sufficient to detect an effect size of this magnitude with a two-sided significance level of alpha = 0.025 and 90 percent power. Allowing for as much as a 15 percent loss of participants during the two-year study (due to clinical events, such as initiating chronic red blood cell transfusion therapy, failure of compliance or exclusion or withdrawal for any reason), then our sample size estimate is 26/0.85 = 31 participants in each treatment group, or a total of 62 participants.** Although the secondary analyses planned have not entered into the calculation of the sample size required for the proposed study, the conservative specification for the treatment effect; 90 percent power; and a generous allowance for loss of participants, should help provide adequate power for subsidiary comparisons.

Annualized Event Rate and Inter-Event-Interval (IEI)

Individual participant rates of events during the treatment phase (Treatment Years 1 and 2) will be annualized by dividing the number of days of observation by 365.25. Individual participant inter-event-interval (IEI) will be the average of the number of days between the first day of observation and the day of the first event and the number of days separating the resolution of one event to the onset of the subsequent event. The treatment phase IEI will be calculated as the average of the number of days separating the resolution of one event to the onset of the subsequent event, the number of days from randomization to the first event, and the days between the last event and final day of study participation. If a participant is in the midst of an event episode on the day of randomization, the calculation of the average IEI will start with the date the event resolved.

Treatment Outcome Analysis

All primary and secondary outcome analyses will be performed under the intent-to-treat principle. Crude estimates of group differences in the annualized event rate and average inter-event-interval during Treatment Year 1 and Treatment Year 2 will be analyzed by linear mixed models for repeated measures with fixed effects for group, time and group-by-time interaction, and an AR(1) covariance structure. Analyses of secondary outcomes for pulmonary function testing will also use this approach. Further analysis of treatment group differences will be assessed using a variation of the Cox Proportional Hazards model with a counting process input of start and stop times for each event and the robust sandwich covariance estimator of Lin.[63] This approach models hazard for recurrence under the assumption of proportional mean processes and generates hazard ratios with 95% confidence intervals and Wald Chi-Square statistics for testing the null hypothesis. We will evaluate the influence of the event rate, average IEI as covariates and calculate between treatment group hazard ratios. We will also evaluate the influence of randomization stratifying variables as covariates of the estimate of the between treatment group difference: sex (female vs male), age (child vs adolescent) and hydroxyurea use (use vs non-use). For participants in each treatment group who withdraw from the study prior to completion of the protocol, we will repeat the primary intent-to-treat analysis with the subset of participants who completed the two-year study in a "per protocol" analysis. Finally, treatment group differences in adverse events of least grade 2 severity will be assessed with chi-square or Fisher's Exact test. We will only present comparisons of events where three or more instances are reported over the two-year treatment period.

DATA AND SAFETY MONITORING

Adherence to protocol: Conduct of the clinical trial will be in accordance with this new protocol for the ViDAS-2 study in our Investigational New Drug application. This protocol includes the appointment of a Clinical Trial Safety Officer to review laboratory results as they become available, and the formation of a Data and Safety Monitoring

Board (DSMB) consisting of an expert in pediatric sickle cell disease, a biostatistician, and a Pediatric Endocrinologist with expertise in vitamin D metabolism. The DSMB will meet bi-annually.

Safety monitoring and review plan: For the first month after initiation of study treatment, the Clinical Research Coordinator will telephone each study participant (or their parent or guardian) weekly to ask about any adverse experiences or any change from baseline status. To maintain blinding of participant treatment assignment during safety monitoring, a Clinical Trial Safety Officer, a physician expert in Pediatric Endocrinology and vitamin D metabolism, will be appointed to review laboratory results as they are performed during the course of the clinical trial to detect any abnormality requiring intervention. Abnormalities that require dose adjustment or other intervention will be reported immediately to the Sponsor-Investigator and summarized for annual reports to the U.S. Food and Drug Administration (FDA), the DSMB, and the Columbia University Institutional Review Board (CU IRB).

Reporting of adverse events: All identified Adverse Events will be reported to the Sponsor-Investigator. The Sponsor-Investigator will determine whether the event is *serious* or an *Unexpected Suspected Adverse Reaction* and requires expedited reporting. Serious Adverse Events (SAEs) that meet criteria for FDA and/or CU IRB reporting, i.e., unexpected, suspected to be related to study drug (FDA requirements), or unanticipated problems (CU IRB requirement) will be reported within 24 hours to the Sponsor-Investigator for review. Other SAEs that do not meet the above criteria will be reviewed by Clinical Trial Safety Officer with reports to the Sponsor-Investigator quarterly. The Sponsor-Investigator must also report SAEs that meet criteria for FDA and/or CU IRB reporting to the DSMB promptly within one week, while other SAEs that do not meet the above criteria will be reported to the DSMB Chair quarterly. The Sponsor-Investigator is required to report to the FDA serious, unexpected, suspected adverse event within 15 days, and within 7 days if fatal. A serious, unexpected suspected adverse reaction is a serious adverse event not previously observed with the drug and that has a reasonable possibility of having been caused by it. The CU IRB requires reporting of all Unanticipated Problems promptly and not later than one week, as well as during continuing annual review. An Unanticipated Problem is defined as any incident, outcome or experience involving risks to subjects or others in any human subject research that is unexpected, related or possibly related to research participation, and suggests that the research places subjects or others at a greater risk of harm (physical, psychological, economic or social harm) than was previously known or recognized. Adverse Events that do not meet the criteria of a SAE will be reported the DSMB during biannual review.

Clinical Trial Data and Safety Monitoring Board review: The Trial Statistician and Trial Epidemiologist will prepare a report for the DSMB at the end of Treatment Year 1 and Treatment Year 2. Data will be presented in a blinded fashion and study subjects will be identified only in the case of a safety concern. Biannual (and, if needed, more frequent) safety reviews will be conducted. Consideration for early termination of the trial will include: (i) failure of enrolled participants to meet entry criteria, (ii) differences in the baseline characteristics of the two study groups (despite gender- and age-stratified randomization), (iii) excessive or unbalanced loss of study participants, and (iv) other considerations to be determined by the DSMB.

Data management and quality: The Sponsor-Investigator or a designated Study Investigator will review all completed data collection forms biannually for protocol compliance and for completeness and accuracy of data. A statement of the results of this review will be included in the annual report to the FDA Orphan Products Program, in the annual IND report to the FDA, the CU IRB, and in the biannual reports to the Clinical Trial Data and Safety Monitoring Board and.

Reviews of clinical trial progress: Progress in the conduct of the clinical trial will be reviewed at least annually and more frequently as required by the FDA and the CU IRB, and biannually by the Clinical Trial Data and Safety Monitoring Board,

Records to be kept: Case Report Forms (CRF) will be provided for each subject. Subjects will not be identified by name on any study documents. After patient and subject identifiers have been replaced by study identifiers, the results of all clinical and laboratory assessments will be recorded on Case Report Forms for each subject. In a similar manner, demographic data and data from the medical history, physical examination will be recorded on Case Report Forms. All data on the Case Report Forms will be legibly recorded in black ink or typed. Corrections will be made by striking through the incorrect entry with a single line and then entering the correct information

adjacent to it. The correction will be initialed and dated by the Sponsor-Investigator or a designated, qualified staff member. Any requested information that is not obtained as specified in the protocol will have an explanation noted on the Case Report Forms as to why the required information was not obtained. Data for the Case Report Forms will be entered into a computerized database and entered into a Columbia University Certified Environment encrypted and protected with a strong password, in accordance with Columbia University encryption policies

Confidentiality of study data: The Sponsor-Investigator will be responsible for the accumulation and maintenance of appropriate data files and the preservation of the confidentiality and security of these files. The need for strict confidentiality of all study records will be emphasized to the staff of the study. Subjects will not be identified by name on any study documents and will be identified solely by a Study Identification Number. All reports and summaries prepared by the Investigator will be presented in such a way that no individual participant can be identified. The data collected in this study will remain under the control of the Investigator and will be kept confidential with the sole exception that Hospital or Government authorities may have access to records containing the identity of study volunteers. If publications result, volunteer names will not be used. All forms, computer tapes and diskettes will be kept in locked cabinets. All computerized files will be encrypted and protected with a strong password, in accordance with Columbia University encryption policies.

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Appendix:

Pediatric Hematology
RESPIRATORY QUESTIONNAIRE (2 to 20 years old)

Subject ID#	Interviewer Name	Date

For tracking purposes only; identifiers will be removed during data collation.

Patient's Name: _____

Parent/Guardian Name: _____

Telephone Number: _____

Method of Completion: □ Clinic visit □ Telephone Interview
Source of Information: □ Parent/guardian □ Patient

Use "YOUR CHILD" when the responder is the parent/legal guardian of a minor subject.
Use "YOU" when the responder is the adult subject (≥18y) or older mature adolescent (≥15y) who is responding to the questionnaire for himself/herself as the subject.

1. Has your child (you) had **difficulty breathing** in the past 2 weeks?
   Check Yes if subject had any of the following: rapid breathing, ribs drawn in, bluish lips, noisy breathing
   Yes [ ] No [ ]

   If Yes, how long did your child (you) have the difficulty breathing? _____ days
   Start date:_____ End date:_____ (indicate "ongoing" if patient has difficulty breathing today)

2. Has your child (you) had **wheezing or whistling in the chest** in the past 2 weeks?
   Yes [ ] No [ ]

   If Yes, how long did your child (you) have the wheezing? _____ days
   Start date:_____ End date:_____ (indicate "ongoing" if patient has wheezing today)

3. Has your child (you) had **fever** in the past 2 weeks?
   Yes [ ] No [ ]

   If Yes, what was the highest temperature your child (you) had? _____ °F

   If Yes, how long did your child (you) have the fever? _____ days
   Start date:_____ End date:_____ (indicate "ongoing" if patient has fever today)

4. Has your child (you) had **cough** in the past 2 weeks?
   Yes [ ] No [ ]

   If Yes, how severe was the cough?
   [ ] Mild (not disturbing activities)
   [ ] Moderate (coughs most of the day but sleeps at night)
   [ ] Severe (causes vomiting or disturbs sleep)

   If Yes, how long did your child (you) have the cough? _____ days
   Start date:_____ End date:_____ (indicate "ongoing" if patient has cough today)

5. Have your child (you) had **runny or stuffy nose** in the past 2 weeks?
   Yes [ ] No [ ]

   If Yes, how severe was the runny or stuffy nose? [ ] A little [ ] A lot

If Yes, was the runny/stuffy nose accompanied by itchy or watery eyes?
Yes [ ] No [ ]

If Yes, how long did your child (you) have the runny or stuffy nose? _____ days
Start date:_____ End date:_____ (indicate "ongoing" if patient has runny/stuffy nose today)

6. Has your child (you) had **sore throat/hoarseness** during the past 2 weeks?
Yes [ ] No [ ]

If Yes, how long did your child (you) have the sore throat/hoarseness? _____ days
Start date:_____End date:_____ (indicate "ongoing" if patient has sore throat/hoarseness today)

*** If the subject has "**ongoing symptom/s**" at the time of **interview 2 weeks ago**, please ask a follow-up question referring to the previous interview 2 weeks ago:

Your child (you) was having "symptom/s" when we spoke to you 2 weeks ago. How many days did your child (you) have the "symptom/s"? _____ days

<u>If subject answered "NO" to all the above questions 1-6, there is no need to ask the rest of the questions below</u>. If the subject answered "YES" to any of the above questions, please proceed to the following questions:

7. Did your child (you) take any medication for the symptom/s?
Yes [ ] No [ ]

If Yes, what medication? _____

8. Did your child (you) miss school in the past 2 weeks because of the symptom/s?
Yes [ ] No [ ]

If Yes, how many days? _____days

9. Did your child (you) see a medical provider (doctor or nurse) in the past 2 weeks for the symptom/s?
Yes [ ] No [ ]

If Yes, please check who saw your child (you) or where were you seen:
[ ] Primary care provider or pediatrician (Name _____)
[ ] Hematology clinic
[ ] Emergency room

10. Was your child (you) hospitalized in the past 2 weeks because of the symptom/s?
Yes [ ] No [ ]

If Yes, how many days? _____days

<u>If subject answered "YES" to questions 9 and/or 10, please review medical records.</u> Done [ ]

Please enter DIAGNOSIS recorded in the medical record: _____

Please enter TREATMENT/S recorded in the medical record:
[ ] Antibiotic
[ ] Oxygen
[ ] Transfusion
[ ] Other: _____