

# VITAMIN D AMELIORATES ACUTE AND CHRONIC MUSCULOSKELETAL OUTCOMES IN SICKLE CELL DISEASE.

## Introduction

Sickle Cell Disease (SCD) is the most common genetic disorder in North America, afflicting over 100,000 people in the US. With improved comprehensive care, universal use of penicillin prophylaxis, vaccinations against invasive pneumococcus, and, the advent of hemoglobin F modulators such as Hydroxyurea, the median survival for SCD has improved significantly, approaching 45 – 65 years depending on genotype<sup>21,3</sup>. As these individuals with SCD live longer, they develop progressive end organ damage in addition to recurrent episodes of acute pain and persistent chronic pain that becomes their primary reason for health care utilization<sup>4, 5</sup>. Progressive bone deterioration characterized by abnormal bone mineral density, vertebral fractures and avascular necrosis (AVN) plagues nearly all individuals with SCD over time and contributes to chronic debilitating pain and increased morbidity. The lifetime fracture risk among adults with SCD approaches 30% while prevalence of AVN exceeds 80% by age 50y<sup>6,7,8</sup>. There is therefore an urgent need to develop strategies to reduce morbidity from SCD bone disease to improve long term clinical outcomes in this population.

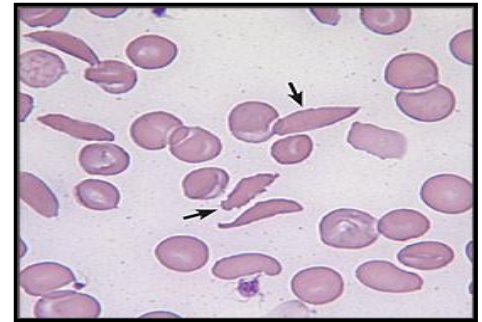


Fig 1: sickle cell peripheral smear

Vitamin D is a critical pro-hormone synthesized in skin or obtained from the diet with a primary role in regulating bone-mineral homeostasis. The major circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D). The third National Health and Nutrition Examination Survey (NHANESIII) demonstrated that 92% of African Americans in the U.S were vitamin D insufficient (25(OH)D <32ng/mL) and 42.4% have severe vitamin D deficiency (VDD) (25(OH)D <15ng/mL)<sup>9, 10</sup>. VDD causes abnormal bone homeostasis with increased bone loss, bone fragility and chronic pain. Other novel vitamin D effects include immune modulation, anti-inflammatory and anti-oxidant functions. Persons with SCD are at particular risk for VDD due to melanin skin hyper-pigmentation which blocks cutaneous vitamin D production and high rates of lactose intolerance that leads to reduced intake of vitamin D fortified dairy products<sup>16, 17</sup>. The prevalence of VDD in children with SCD varies by season between 65%-100%<sup>18</sup>. Despite established reports of VDD in SCD, an understanding of how vitamin D status impacts the musculoskeletal (MSK) phenotype of SCD is unknown. None of these reports were longitudinal nor did they include infants and toddlers. The **objective** of this research grant is to develop and validate methods to improve vitamin D status in SCD and determine the impact of improved vitamin D status on MSK health and pain phenotype (both acute and chronic) in SCD. Our **hypothesis** is that vitamin D status predicts frequent acute pain events, chronic pain and poor MSK health in SCD patients. We will test our hypothesis by utilizing two specific aims:

**[1] To demonstrate a correlation between the degree of VDD and frequent acute pain episodes, chronic pain and musculoskeletal health in SCD patients** and therefore ascertain vitamin D status is predictive of a more severe clinical pain phenotype. Our hypothesis for this aim is that VDD is associated with more frequent acute vaso-occlusion, the presence of daily chronic pain and increased bone turnover in SCD. To address this aim we will execute a cross sectional trial evaluating baseline vitamin D status and bone turnover markers in relation to pain phenotype determined by number of acute vaso-occlusive events within the preceding year and a 30 diary report to determine the presence of chronic pain.

**[2] To demonstrate that correction of vitamin D deficiency results in improved pain status and musculoskeletal health in SCD.** Our hypothesis for this aim is that correcting vitamin D deficiency will improve skeletal health using surrogate serum markers of bone formation and resorption and reduce both acute pain exacerbations and number of diary pain days in SCD. To address this aim we will conduct a prospective pilot study measuring bone turnover markers and acute and chronic pain status in SCD subjects with identified VDD and moderate to severe pain phenotype before and after correction of VDD.

## Background and Significance

### High prevalence of vitamin D deficiency and impact on health:

Vitamin D is emerging as a major health problem among individuals with chronic diseases including SCD. It plays a

critical and primary role in regulating bone-mineral homeostasis<sup>21</sup>. In infants and toddlers VDD presents as growth retardation, muscle weakness, skeletal deformities, hypocalcaemia, tetany, and seizures. Adolescents and adults with VDD present with mineralization defects, bowing, severe MSK pain and myopathy, and an increased risk of falls and fractures<sup>22-25</sup>. Several investigators have documented the role of VDD in the pathogenesis of asthma and poor lung function, periodontal disease, autoimmune disorders and several cancers such as breast, colon and prostate cancer<sup>22</sup>. Symptoms attributable to VDD begin to manifest following prolonged periods with severely low 25(OH)D levels.

*Vitamin D deficiency leads to osteoporosis, increased risk of fractures and causes pain:*

VDD in non SCD subjects causes chronic pain, myopathy osteopenia, osteoporosis, debilitating bone pain, and increased fractures. Plotnikoff and Quigley studied 150 subjects with chronic pain and found that 28% had severe VDD (25OHD  $\leq$ 8ng/mL); 93% had 25OHD levels  $\leq$ 20ng/mL. In a recent report of 33 female Africans with chronic pain and an initial diagnosis of somatization; all 33 subjects (100%) had severe VDD (mean 25(OH)D 4.5ng/mL), and 22 subjects (66.7%) had complete resolution of pain symptoms 2.8 months into a course of daily high dose vitamin D therapy<sup>26</sup>. Faraj and Mutairi also observed the complete resolution of chronic low back pain following vitamin D repletion among 299 subjects with very low 25(OH)D levels (<11ng/mL)<sup>14</sup>. These data provide a rationale to investigate the possible role of VDD in patients with SCD and chronic pain.

*VDD is extremely common in SCD:*

VDD is highly prevalent among patients with SCD. The clinical significance of VDD in SCD has not been fully elucidated and there are no epidemiologic studies to determine when VDD develops or the consequences of VDD in SCD. Lal and Adewoye independently reported VDD (25(OH)D <20ng/mL) in 74% and 100% of SCD children and adults respectively<sup>15,16</sup>. Buisson *et al* reported prevalence of VDD in children with SCD varied by season (65%-100%)<sup>17</sup>. Two other studies also report higher rates of VDD among African Americans with SCD compared to those without SCD<sup>19,20</sup>. However the contribution of VDD to the pathobiology of the pain experience in SCD is unknown. Despite established reports of VDD in most patients with SCD, evaluation of how vitamin D status impacts the clinical phenotype of SCD is not known. Data from the cystic fibrosis literature, another genetic disorder associated with chronic bone disease has indicated that VDD develops at an early age which negatively impacts the mineralization of their bone, resulting in decreased peak adult bone mass and increased risk for fractures<sup>27-30</sup>.

*Acute and chronic pain is characteristic of SCD, etiology is multi-factorial:*

Pain is a hallmark symptom of SCD, accounting for the majority of SCD disease morbidity and health care costs which averaged \$488million in the US in 1994<sup>3</sup>. The number of severe acute pain episodes per year correlates positively with disease severity and predicts early death in SCD adults<sup>31,32</sup>. The Pain in Sickle Cell Epidemiology Study (PISCES) reports over 20% adult SCD patients had daily pain and over 50% had pain at least 50% of the time. Dampier *et al* reported in pediatric SCD that 10% had daily pain, 20% had pain 20-40% of the time and pain days increased with age and female gender<sup>33</sup>. The exact biologic mechanisms responsible for and predictive of acute and chronic pain in SCD remain unclear<sup>34,35</sup>. In acute sickle crisis there is vaso-occlusion, ischemia, endothelial damage and inflammation occurs at varying severity and location among individuals regardless of genotype. Acute "crisis" are unpredictable in onset and duration with a variable response to treatment. In contrast, SCD chronic pain lasts over 2 weeks, is often subjective, and is also poorly responsive to standard therapies suggesting that there is a complex pathophysiology behind both acute and chronic pain in SCD which has been underappreciated.

*The symptoms of VDD overlap significantly with those of SCD.* It is well established that VDD presents with diffuse chronic bone pain, proximal muscle weakness and fractures. The overlap of symptoms of VDD and SCD is striking. In both disorders the pain is deep seated, dull and achy, exacerbated by weight bearing and commonly involves the lower spine, pelvis and extremity bones<sup>23,36,37</sup>. Compression spine fractures are common to both disorders. Plotnikoff *et al* studied 150 patients with non sickle chronic pain patients; 28% had severe VDD (25(OH)D  $\leq$ 8ng/mL); 93% had 25(OH)D levels  $\leq$ 20ng/mL; and *all African Americans in the study had VDD* ( $p>0.006$ )<sup>26</sup>. They concluded that *VDD is a known cause of persistent, nonspecific musculoskeletal pain and that all patients with these symptoms should be screened for VDD*. The role of vitamin D in the pathogenesis of acute and chronic pain in SCD and the potential value of screening for VDD in patients with SCD begs to be explored.

## Preliminary Studies and Supporting Data:

1. *Vitamin D deficiency is highly prevalent among SCD patients with chronic pain attending a South Eastern Comprehensive Sickle Cell Center.* We reviewed the charts of six patients at our center with sickle chronic pain syndrome (median age 16y [11-18y]; [2M:4F]; [5 HbSS, 1 HbSB+Thal]) who had 25(OH)D levels drawn as part of a chronic pain evaluation (Table 1). All subjects had severe VDD (mean 25(OH)D of  $9.16 \pm 4.11$  ng/mL); two subjects had undetectable 25(OH)D levels (<5ng/mL) and four subjects had radiographic evidence of skeletal complications. These results mimic what we have also seen in cystic fibrosis (CF) at the Emory CF center, where we have previously reported that vitamin D insufficiency correlated with increased risk of vertebral fractures<sup>25</sup>. Our preliminary data suggests a high prevalence of severe VDD in pediatric SCD patients with chronic pain and a potential correlation between vitamin D status, musculoskeletal health and chronic pain. Therefore, there is a compelling rationale to evaluate the role of VDD in SCD chronic pain.

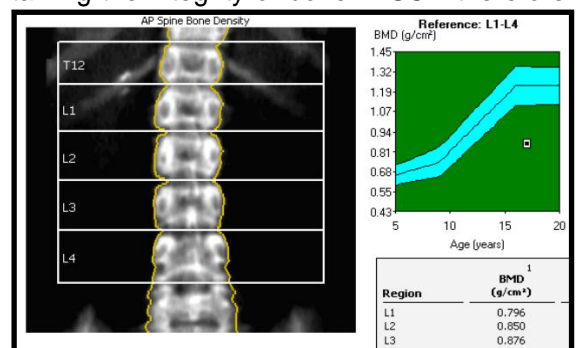
**Table 1: PRELIMINARY DATA:** Summary of vitamin D levels in 6 pediatric subjects with SCD, Children's Healthcare of Atlanta, comprehensive sickle cell clinic (unpublished data). All subjects were on daily opioids. All but subject #004 were on Hydroxyurea.

Patient	Age/Sex	Genotype, pain status	25OHD level	Chest X-ray
001	16y F	Hgb SS Chronic pain	<8	T-Spine concavity, Osteopenia
002	13y F	Hgb SS Chronic Pain, RLS	13	Normal
003	11y M	Hgb SS AVN, Short Stature	15	AVN, osteopenia,
004	16y F	Hgb SB+Thal Chronic Pain	<5	Normal
005	16y F	Hgb SS Chronic Pain	<5	Minimal end plate deformity
006	18y M	Hgb SS AVN, Chronic Pain	9	End plate deformity

AVN=avascular necrosis; T-spine = thoracic spine; RLS = restless leg syndrome

2. *Vitamin D deficiency is highly prevalent among SCD patients with osteonecrosis (AVN) in spite of disease modifying treatments or pain status.* This proposal challenges the prevailing paradigm that sickle osteonecrosis (AVN), a common consequence of SCD, is caused primarily by acute vaso-occlusion within vital nutrient arteries of the joint leading to infarction, bone necrosis, collapse and eventually chronic pain. This challenge is based on the observation that chronic hyper transfusion (CHT) and hydroxyurea (HU), two disease modifying therapies that prevent sickle vaso-occlusion do not reduce the risk of AVN in SCD. On the contrary, there are reports of new and worsening of AVN in SCD patients on HU (Rogers 1997; Kattamis 2004, Gulbis 2005) and, AVN has been reported to occur (11%) while on CHT (Osunkwo I, unpublished data). Our group recently demonstrated a 72% prevalence of AVN among children with SCD (n=39; age 1-19y) who were on HU while new joint involvement was seen in 53% of subjects (Osunkwo I, unpublished data). Incidentally, we also found that 51% of subjects with SCD and VDD also had AVN. We propose in this project that vitamin D status is critical to maintaining the integrity of bone in SCD therefore VDD and its associated increased bone turnover augments development of chronic bone disease and pain in SCD.

3. *Vitamin D depletion associated with resolution of chronic pain in a 15y with Hgb SS.* One of my patients, a 15y F, Hgb SS with frequent recurrent acute pain exacerbations, chronic headaches from pseudotumor cerebrii, and, persistent chronic pain was non responsive to standard therapeutic interventions with mean 25(OH)D level <5ng/mL. Work up revealed profound osteopenia (figure 2) and vertebral bony changes buy no obvious AVN. Treatment with high dose ergocalciferol (600,000IU over 6 weeks) was associated with complete resolution of headaches, chronic leg, back and shoulder pain within 3 months. Toxicity included mild paraesthesias and borderline serum calcium levels treated with calcium carbonate supplementation.



**Figure 2: 15y F Hgb SS, DEXA scan shows severe abnormality in BMD and chronic vertebrae changes**

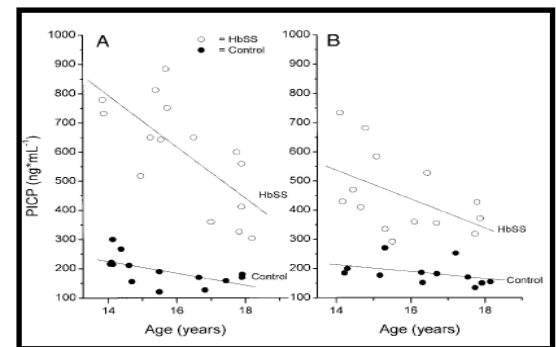
## Research Plan and Methods

**Aim #1: To demonstrate a correlation between the presence and extent of VDD and frequent acute pain episodes, chronic pain and musculoskeletal health in SCD patients.**

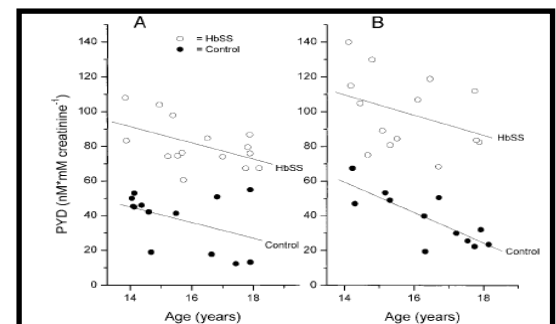
**Hypothesis of this aim:** Our hypothesis for this aim is that *VDD is associated with more frequent acute vaso-occlusion, diary pain days and an increased bone turnover in SCD compared to SCD with adequate vitamin D status.* We propose that individuals with SCD and VDD are more likely to have profound imbalances in bone remodeling measured by surrogate serum markers of bone formation and resorption and mineralization which contribute to the development of recurrent acute VOC episodes and chronic pain in SCD. To address this aim we will execute a cross sectional trial evaluating baseline vitamin D status and bone turnover in relation to pain phenotype determined by number of acute vaso-occlusive events within the preceding year and a 30 day diary report to determine presence of chronic pain.

**Rationale:** There are currently no guidelines recommending routine 25(OH)D testing or routine ergocalciferol administration to maintain adequate vitamin D status as part of comprehensive care in SCD. Furthermore, there is scant data in SCD correlating bone turnover with clinical pain phenotype in SCD even though increased bone turnover has been associated with skeletal morbidity in other chronic disease states such as thalassemia and rheumatoid arthritis (ref). If this relationship is positive, modulation of bone turnover may be exploited as a therapeutic modality to prevent acute and chronic pain in at risk individuals with SCD and VDD. Elucidating the evidence based rational for prospective vitamin D status screening in SCD is justified and timely. *It will have immense impact if vitamin D status was proven to be of benefit in modulating the pain experience in SCD.*

**Experimental strategy:** We will conduct a cross-sectional trial to determine whether vitamin D status is predictive of pain phenotype in SCD. We propose to recruit 100 patients with SCD, all genotypes from the three SCD clinics of Children's Healthcare of Atlanta which provides multidisciplinary comprehensive care to over 1,600 SCD patient's  $\leq 21$  yrs with confirmed sickle hemoglobinopathy. **Exclusion:** Chronic hypertransfusion therapy, previous high dose vitamin D treatment, active renal disease (serum creatinine  $> 1.5$  ULN), active liver disease, on greater than 10 mg of prednisone or equivalent, hypocalcaemia, within 6 months of starting other disease modifying therapy, neurological deficits precluding adequate pain assessment. Patients who have been followed by the Atlanta Sickle Cell consortium for less than a calendar year will not be eligible. After informed consent, the subject will provide a baseline blood specimen for 25(OH)D and parathyroid hormone (PTH) for vitamin D status and for bone turnover status<sup>39</sup> which will include markers of bone formation (amino-terminal procollagen propeptides of type I collagen (P1NP), osteocalcin (OC)), markers of bone resorption (bone sialoprotein (BSP), cross-linked C-terminal (CTx), cross-linked N-terminal (NTX) telopeptide of type I collagen and tartarate-resistant acid phosphatase isoenzyme 5b (TRAP)] and lastly markers of osteoclastogenesis [osteoprotegerin (OPG), receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) in form of ratio. There is data to suggest increased levels of bone formation and resorption cytokines among children with SCD compared to age matched controls<sup>40</sup>. This aim will elucidate the correlation between these markers and vitamin D status. Subjects will be asked to complete a baseline food questionnaire to estimate daily calcium and vitamin D intake levels and also given a 30day pain diary to complete. Hospital medical records will be reviewed to determine number and frequency of acute pain episodes in the preceding calendar year. A questionnaire will be administered to the subject to obtain their recall history of all acute pain events and this will be compared to the medical record data. Subjects will return to the sickle cell clinic 4 weeks after enrollment to submit pain diary.



**Fig 3<sup>40</sup>. Bone Formation - PICP v.s age in boys (A) and girls (B). \* statistically different from Hgb AA ( $p < 0.001$ )**



**Fig 4<sup>40</sup>. Bone Resorption : Urine PYD vs age in boys (A) and girls (B). \*statistically different from Hb AA controls ( $p < 0.01$ )**

**End points:** This aim will help determine whether 25(OH)D levels are predictive of more frequent acute pain events or chronic daily pain in patients with SCD. We will compare baseline 25(OH)D levels with number of acute pain events and number of reported diary pain days for the two genotype groups (SS/SB0thal and SC/SB+Thal). Bone turnover markers will also be compared across pain status. We will also determine the proportion of subjects who are profoundly vitamin D deficient (25(OH)D < 15ng/ml) versus only mildly vitamin D deficient (25(OH)D <30 ng/mL) in each of these groups.

**Interpretation/Expected Result:** Our preliminary data would lead us to expect more profound VDD among patients who have a higher number of diary pain days suggesting a role for vitamin D status in etiology of chronic pain in SCD. We also anticipate a similar finding among patients with more frequent acute pain episodes. We expect there to be a discrepancy between reported acute pain events and hospital record review as patients tend to under report pain managed at home or away from primary institution. *These results will be important preliminary data for the prospective study proposed in specific aim 2.* It will also validate anecdotal reports of the importance of vitamin D status in predicting acute and chronic pain in SCD.

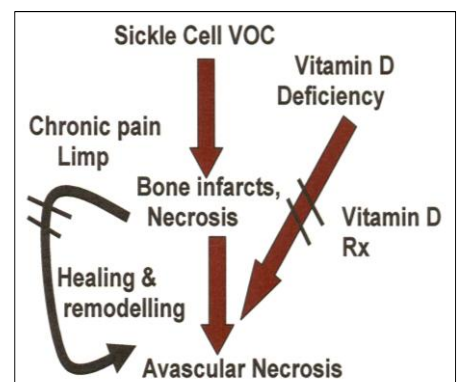
**Pitfalls/Alternative Studies:** With such a large patient population (over 1650 pediatric subjects aged <21y with over 140 clinic visits / week) and with our pilot study experience we anticipate minimal difficulties with enrollment. Relying on self report for pain history has its limitations which we attempt to address by corroborating history with detailed review of medical records. This is a cross sectional pilot and results will need to be confirmed in a prospective study (specific aim #2)

**Aim #2: To demonstrate that correction of vitamin D deficiency results in improved skeletal health and pain status in pediatric SCD.**

**Hypothesis of this aim:** Our hypothesis for this aim is that correcting VDD in SCD will improved skeletal health (using surrogate serum markers of bone turnover) and reduce both acute pain exacerbations and number of diary pain days. To address this aim we will conduct a prospective intervention study and measure bone turnover and acute and chronic pain status before and after correction of VDD in subjects with SCD.

**Rationale:** To prove the efficacy and benefit of prospective monitoring of vitamin D status in SCD there needs to be rigorous evaluation of the clinical impact of replacement therapy using objective primary endpoints. Vitamin D is critical for the maintenance of bone homeostasis. In SCD the repetitive bone ischemia deters remodeling, increases bone turnover thereby predisposing to infarcts, necrosis, osteopenia and eventually culminating in acute and/or chronic pain. The presence of VDD will amplify this process. *The major focus of this aim is to show the clinical benefit of restoring vitamin D status on the pain experience in SCD.* The hypothesis that VDD contributes to acute and chronic pain in SCD is novel and has not been reported in the literature. If our results are positive, this would lead to a phase II/III multicenter randomized trial of a simple dietary intervention to reduce pain morbidity in SCD.

**Experimental strategy:** We will recruit 40 SCD subjects all genotypes with severe vitamin D deficiency (identified from specific aim #1). Using a prospective pilot intervention trial design we will determine whether vitamin D status is predictive of pain phenotype in SCD. **Exclusion:** Given the space limitations of this application, the inclusion and exclusion criteria will not be explicitly stated here but will closely resemble those in aim #1. Subjects will undergo all study procedures outlined in aim #1.(see table 2) and complete a baseline food questionnaire to estimate daily calcium and vitamin D intake levels. They will then be given a daily pain diary to complete through out the study. Hospital Medical records will be reviewed to determine number and frequency of acute pain episodes in the preceding calendar year which will represent baseline “pain rate”. A questionnaire will be administered to subjects to obtain their recall history of all acute pain events and this will be compared to the medical record data. Subjects randomized to vitamin D will be given optimal vitamin D correction to maintain 25(OH)D levels between 30-70ng/mL using STOSS like therapy (table 2). After correction of vitamin D status, subjects will be given vitamin D 2000 IU daily (safe upper limit for



**Figure 4 mechanisms for skeletal complications in sickle cell disease**

vitamin D) to maintain normal vitamin D status. Subjects randomized to placebo will go on conventional vitamin D therapy recommended by the American Academy of Pediatric (400 IU of vitamin D daily). All subjects will be followed for 6 months. **End points:** The endpoints include percent change in bone turnover markers, pain diary days and acute pain episodes before and 6 months after therapy.

**Statistical Analysis and Power:** There are no previous studies that examine influence of vitamin D on SCD pain phenotype. Our results from this pilot study will provide essential preliminary data to prove the feasibility of detecting changes in pain related clinical outcomes with vitamin D repletion in SCD and provide us with the effect size if any of vitamin D therapy on acute and chronic pain prior to embarking on a larger scale study.

**Interpretation/Expected Result:** If our hypothesis is correct that correction of vitamin D deficiency results in improved pain status in SCD we expect to document successful repletion of 25(OH)D levels which will be associated with clinical improvement in acute and chronic pain symptoms and a reduction in markers of bone turnover in favor of bone formation not bone resorbtion. Our data will therefore support the hypothesis that 25(OH)D levels are decreased in most SCD children and levels are inversely related to bone turnover, acute and chronic pain status and skeletal complications. We also anticipate daily diary reports of pain will decrease over the course of the study and there will be fewer acute pain exacerbations compared to baseline.

**Pitfalls/Alternative Studies:** Six months of follow up was chosen because pain in VDD has been reported to resolve within 3 months following repletion therapy and because experience with pediatric pain diary has documented good adherence through six months. This study duration may be suboptimal to detect the full clinical benefit of vitamin D repletion. In addition we recognize that psychosocial factors and the placebo effect influence pain and health care utilization for pain and are potential confounders to the study. This can however, only be addressed by a larger prospective randomized study. We are also unable to control for variable intestinal absorption of vitamin D in this pilot. The results from this study will provide the background and preliminary data for an NIH grant application to fund a multi-center prospective study to determine if correcting VDD improves musculoskeletal health and the pain phenotype in SCD. This study will also provide a rational for basic investigation of the mechanisms by which VDD impacts the pathophysiology of SCD.

**Table 2: Study Procedures:** Study Procedures at Enrollment and various time points

End Points	Study Procedures	Study enrollment	Week 0	week 8	week 16	week 24
<b>Vitamin D Status</b>	25(OH)D, PTH, Ca		XX	XX	XX	XX
	Food Frequency Questionnaire		XX			XX
<b>MSK Health</b>	BSP, CTx, NTX, ICTP, TRAP-5b		XX			XX
	P1NP, OC		XX			XX
	OPG /RANKL		XX			
<b>Pain Status</b>	Daily pain diary	30 day run-in	XX	XX	XX	XX
	Medical History Review					

CTx C telopeptide; FFQ food frequency questionnaire; 25(OH) D 25 hydroxyvitamin D; MSK Health = musculoskeletal health; carboxyterminal telopeptide of type I collagen (ICTP); PTH parathyroid hormone; P1NP amino-terminal procollagen propeptides of type I collagen, OC osteocalcin; BSP bone sialoprotein; CTx cross-linked C-terminal; NTX cross-linked N-terminal telopeptides of type I collagen; TRAP-5b tartrate-resistant acid phosphatase isoenzyme 5b; OPG osteoprotegerin; RANK-L receptor activator of nuclear factor κB ligand

**SPECIFIC GOALS FOR ONE YEAR TIME FRAME**

The study proposed is a complement to my internally funded pilot study through the Aflac Pediatric Hematology and Oncology Research Group evaluating vitamin D status in SCD chronic pain. Aim 1 of this proposal is already IRB approved and enrolling subjects. We will initiate enrollment for aims #2 after obtaining IRB approval and the funding requested from the ACTSI New Investigators Grant is critical for us to perform the bone turnover assays and to conduct the pilot trial of clinical response to vitamin D. We intend to utilize the ACTSI’s Biostatistical Consulting Center for database set up, analysis of data and statistical support in preparing my extramural proposal. The Investigational Drug Pharmacy Services through ACSTI will be leveraged in areas of study initiation and drug dispensing. Laboratory sample processing, bar coding, tracking and storage will be requested from the ACTSI Laboratory Services

## REFERENCES

---

1. National Heart, Lung and Blood Institute, National Institute of Health. Sick cell anemia: Who is at risk? Available at [www.nhlbi.nih.gov/health/dci/Diseases/Sca/SCA\\_WholsAtRisk.html](http://www.nhlbi.nih.gov/health/dci/Diseases/Sca/SCA_WholsAtRisk.html) Accessed November 3, 2006
2. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med* 330(23): 1639-1644 (1994).
3. Frederec Galacteros. SCD a short guide to management Chapter 13, pp277
4. Carroll CP, Haywood C Jr, Fagan P, Lanzkron S. The course and correlates of high hospital utilization in sickle cell disease: Evidence from a large, urban Medicaid managed care organization. *Am J Hematol.* 2009 Jul 30;84(10):666-670
5. Ballas SK Pain management of sickle cell disease. *Hematol Oncol Clin North Am.* 2005 Oct;19(5):785-802,
6. Fung EB, Harmatz PR, Milet M, Coates TD, Thompson AA, Ranalli M, Mignaca R, Scher C, Giardina P, Robertson S, Neumayr L, Vichinsky EP; Multi-Center Iron Overload Study Group. Fracture prevalence and relationship to endocrinopathy in iron overloaded patients with sickle cell disease and thalassemia. *Bone.* 2008 Jul;43(1):162-8. Epub 2008 Mar 15.
7. Neumayr LD, Aguilar C, Earles AN, Jergesen HE, Haberkern CM, Kammen BF, Nancarrow PA, Padua E, Milet M, Stulberg BN, Williams RA, Orringer EP, Graber N, Robertson SM, Vichinsky EP; National Osteonecrosis Trial in Sickle Cell Anemia Study Group. Physical therapy alone compared with core decompression and physical therapy for femoral head osteonecrosis in sickle cell disease. Results of a multicenter study at a mean of three years after treatment *J Bone Joint Surg Am.* 2006 Dec;88(12):2573-82.
8. Hernigou P, Habibi A, Bachir D, Galacteros F. The natural history of asymptomatic osteonecrosis of the femoral head in adults with sickle cell disease. *J Bone Joint Surg Am.* 2006 Dec;88(12):2565-72.
9. Zadshir A, Tareen N, Martins D et al. The prevalence of hypovitaminosis D among US adults: data from the NHANES III. *Ethn Dis.* 2005 ;15(4 Suppl 5):S5-97-101
10. Nesby-O'Dell S, Scanlon KS, Bowman B et al. Hypovitaminosis D prevalence & determinants among African American & white women of reproductive age: 3rd NHANES, 1988-1994. *Am J Clin Nutr.* 2002 Jul;76(1):187-9
11. Boland R. Role of vitamin D in skeletal muscle function. *Endocr Rev* 1986;7:434-47
12. Gelrup H, Mikkelsen K, Poulsen L et al. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. *Calcif Tiss Int* 2000;66:419-24
13. de Torrente de la Jara G, Pecoud A, Favrat B. Female asylum seekers with musculoskeletal pain: the importance of diagnosis and treatment of hypovitaminosis D. *BMC Family Practice* 2006,7:4
14. *Al Faraj S, Al Mutairi K, Vitamin D Deficiency and Chronic Low Back Pain in Saudi Arabia. Epidemiology Spine.* 28(2):177-179, January 15, 2003
15. Lal A, Fung E, Vichinsky E et al. Bone mineral density in children with sickle cell anemia. *Pediatr Blood Cancer.* 2006 Dec;47(7):901-6.
16. Adewoye A, Chen T, Holick M et al. Sickle cell bone disease: Response to vitamin D and calcium. *Am J Hematol.* 2008 Apr;83(4):271-4
17. Buison A, Ohene-Frempong K, Zemel B et al. Low vitamin D status in children with sickle cell disease. *J Pediatr.* 2004 Nov;145(5):622-7.
18. Chapelon E, Garabedian M, Brousse V, Souberbielle JC, Bresson JL, de Montalembert M. Osteopenia and vitamin D deficiency in children with sickle cell disease. *Eur J Haematol.* 2009 Aug 13
19. Padmos A, Roberts G, Serjeant B, Serjeant G et al. Avascular necrosis of the femoral head in Saudi Arabians with homozygous sickle cell disease - risk factors. *Ann Saudi Med.* 1995 Jan;15(1):21-4
20. Mohammed S, Addae S, Richards J et al. Serum calcium, parathyroid hormone, and vitamin D status in children and young adults with sickle cell disease. *Ann Clin Biochem.* 1993 Jan;30 ( Pt 1):45-51.
21. Khazai N, Judd SE, Tangpricha V Calcium and vitamin D: skeletal and extraskelatal health. *Curr Rheumatol Rep.* 2008 Apr;10(2):110-7
22. Bischoff-Ferrari H, Giovannucci E, Dawson-Hughes B et al. Estimation of optimal serum concentrations of 25-OHvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84:18-28

- 
23. Holick MF. Optimal Vit D status for prevention & treatment of osteoporosis. *Drugs Aging*. 2007;24(12):1017-29
  24. Boland R. Role of vitamin D in skeletal muscle function. *Endocr Rev* 1986;7:434-47
  25. Gelrup H, Mikkelsen K, Poulsen L et al. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. *Calcif Tiss Int* 2000;66:419-24
  26. Plotnikoff G, Quigley J. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc*. 2003 Dec;78(12):1463-70.
  27. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, Guise TA, Hardin DS, Haworth CS, Holick MF, Joseph PM, O'Brien K, Tullis E, Watts NB, White TB. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab*. 2005 Mar;90(3):1888-96.
  28. Feranchak AP, Sontag MK, Wagener JS, Hammond KB, Accurso FJ, Sokol RJ. Prospective long term study of fat soluble vitamin status in children with cystic fibrosis identified by newborn screen. *J Pediatr* 1999;135:601–610.
  29. Laursen EM, Mo'lgard C, Michaelsen KF, Koch C, Mu'ller J. Bone mineral status in 134 patients with cystic fibrosis. *Arch Dis Child* 1999;81:235–240.
  30. Rossini M, Del Marco A, Dal Santo F, Gatti D, Braggion C, James G, Adami S. Prevalence and correlates of vertebral fractures in adults with cystic fibrosis. *Bone*. 2004 Sep;35(3):771-6.
  31. Ballas S, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology & prognostic significance. *Am J Hematol*. 2005;79:17–25
  32. Beyer J, Simmons L, Woods GM, Woods PM. A chronology of pain/comfort in children with SCD. *Arch Ped Adolescent Med*. 1999;153:913–920
  33. Smith W, Penberthy L, Roseff S et al. Daily Assessment of Pain in Adults with Sickle Cell Disease *Annals of Int Med*, 2008:148 (94-101)
  34. Dampier C, Ely E, Brodecki D, O'Neal P. Home management of pain in sickle cell disease: a daily diary study in children and adolescents. *J Pediatr Hematol Oncol*. 2002 Nov;24(8):643-7.
  35. Dampier C, Setty N, Stuart M et al. Vaso-Occlusion in Children With Sickle Cell Disease: Clinical Characteristics and Biologic Correlates. *JPHO Vol 26, Number 12, 2004*
  36. Beyer J, Miaskowski C, Styles L et al. Are there phases to the vaso-occlusive painful episode in sickle cell disease? *J Pain Symptom Manage*. 2005;29:392–400
  37. Wolfenden L, Judd S, Tangpricha V et al. Vitamin D and bone health in adults with cystic fibrosis.
  38. Carol L. Wagner, MD, Frank R. Greer, MD and the Section on Breastfeeding and Committee on Nutrition Prevention of Rickets and Vitamin D Deficiency in Infants, Children, and Adolescents *PEDIATRICS Vol. 122 No. 5 November 2008, pp. 1142-1152 (doi:10.1542/peds.2008-1862)*
  39. Jung K, Lein M, Stephan C, Von Hösslin K, Semjonow A, Sinha P, Loening S, Schnorr D. Comparison of 10 serum bone turnover markers in prostate carcinoma patients with bone metastatic spread: Diagnostic and prognostic implications *International Journal of Cancer*. Volume 111 Issue 5, Pages 783 – 791
  40. Buchowski M<sup>3</sup>, de la Fuente A, Flakoll P, Chen K-Y<sup>3</sup>, Turner EA. Increased bone turnover is associated with protein and energy metabolism in adolescents with sickle cell anemia. *Am J Physiol Endocrinol Metab* 280 Vol. 280, Issue 3, E518-E527, March 2001

---

## **HUMAN SUBJECTS**

### 1. Risk to Human Subjects

Human Subjects Involvement and Characteristics: The risks of participating in this study include minor pain or bruising that may occur with blood draws. Additional potential risks are related to potential excessive supplementation with Vitamin D beyond physiologic levels, resulting in elevated 25(OH)D levels and hypercalcemia. This risk will be minimized by using a modification of the STOSS protocols with vitamin D dosed by kg body weight and monitoring 25(OH)D levels prospectively. Initial loading dose of vitamin D is limited to cumulative dose of 600,000IU followed by maintenance dosing of 2000IU daily over the study period. Regular clinical assessments for signs of vitamin D toxicity will be performed. There is no evidence to suggest any immediate consequences to short term VDD however all subjects will receive at the minimum daily RDA for both vitamin D and calcium. At the end of the study all subjects will be made aware of their vitamin D status and offered replacement therapy if indicated.

### 2. Adequacy of Protection against Risk

IRB review and Patient Informed Consent: The informed consent will be obtained by the study principal investigators or her designee in the presence of at least one witness. Patients identified as eligible for the study will be invited to participate by their primary hematologist or through fliers and posters placed in the clinic. The patient and a legal guardian (if a minor) will be introduced to the study. Participants will be told their participation is completely voluntary and that they have a right to withdraw from participation at any time without any threat of losing health care services in the clinic, that their participation is completely confidential, that any data or information they supply will be kept in locked files and that no identifying information about them will be published or disseminated in any manner. Participants must give written informed consent before any study related procedures can be performed. Patients will be consented per local IRB guidelines in accordance with the Code of Federal Regulations (21 CFR 50 and 21 CFR 50.27 documentation of Informed Consent). Objectives and procedures required during the study will be discussed with the family and with the patient, while reading the informed consent. Patients older than 18 years old will sign their own informed consent statement. The legal guardian will sign the informed consent statement if the patient is a minor. Verbal and/or written assent will be obtained as is mandated by local IRB for underage children in addition to having a parent or guardian give written informed consent.

### 3. Potential Benefits of Proposed Research to Subjects and Others

Participants in this study may or may not get a direct benefit from participation. If hypothesis holds true participants may benefit from improved clinical pain. However, a potential benefit of participation may be that additional screening tests will be drawn (vitamin D and bone turnover status) and subjects who are deficient will be offered opportunity to receive vitamin D replacement treatment under specific aims #2. Patients may benefit from additional useful information obtained from the results of this study at its completion about their musculoskeletal health and nutritional status (including whether they are vitamin D deficient) and how this influences their clinical presentation.

### 4. Importance of Knowledge to be Gained

The information gained from this study will inform about the clinical effects of vitamin D on sickle cell pain experience and musculoskeletal health. It will also generate research questions for further investigation into the mechanisms of these effects

### 5. Inclusion of Women and Children and Other Underrepresented Minorities

No exclusion will be made for this study on the basis of race/ethnicity, gender, or social status of the potential subjects. It is expected that the makeup of the study population will reflect the distribution of race/ethnicity and gender of sickle cell disease patients within the greater Atlanta area. Children will not be excluded from this study. Women will be included in this study, it is expected that half of the participants will be female.

---

## Data Safety Monitoring Plan and Adverse Event Reporting

The Aflac Pediatric Hematology Oncology program already has in place a Data Safety Monitoring Board (DSMB) made up of both internal and external faculty and members of the Aflac Clinical Research Organization (CRO). They will be responsible for close monitoring of study outcomes and will receive at the minimum quarterly reports updating them as to enrollments status, adverse events and their resolution as well as confirmation of adherence to all study procedure. The DSMB will also be responsible for scheduling and executing periodic audits of the study and all study related personnel. Subjects will undergo a complete physical exam at each study visit and their parents will be queried regarding recent medical events or procedures. These events will be documented at the visit to ascertain the nature and treatment of the event including pain crisis, episode of acute chest syndrome, transfusion, any imaging and hospital admissions.

**Adverse Event Reporting:** Serious adverse event reporting will begin with events that arise after the Informed consent is signed until 30 days after the last dose of study drug.

Adverse Events are reported in real time by the Aflac CRO to the Principal Investigators of each study, DSMB, FDA, CTEP and/or pharmaceutical company. The Principal Investigator of the study prepares the AE report. This report is reviewed by the study PI and Aflac CRO, and then filed by the CRO to the appropriate regulatory agencies. A log of all AE's filed will be maintained in the Aflac CRO. Any death on study undergoes formal review by the SMC with a written record.

---

**Table 3: Vitamin D dosing schedule**

Age (y)	Vitamin D Dose	Cumulative vitamin D dose
2-6	40,000IU/week	280,000IU
7-13	80,000IU / week	480,000IU
14-21	100,000IU /week	600,000IU

*Vitamin D is formulated as a 50,000IU gel cap or a suspension of vitamin D 8000IU/ml. Doses based on suggested dosing for vitamin D replacement therapy in pediatrics by Pediatric Lexi Drugs for VDD*

---

## **ENVIRONMENT AND INVESTIGATOR**

Subjects will be recruited from the three SCD clinics of Children's Healthcare of Atlanta which provides multidisciplinary care to over 1,600 SCD patient's  $\leq 21$  yrs, the largest comprehensive Sickle Cell center in the country. There is significant enthusiasm for the use of vitamin supplements to treat sickle cell pain among our patients that has been confirmed through parent surveys at our annual education day. Our center leads nationally in patient accrual for several NIH sponsored multicenter SCD clinical trials. Dr. Osunkwo offers a track record as an investigator on several of these trials and over 14 years experience in the management of SCD. As the P.I, Dr Osunkwo, conceived this project and has assembled a multi-disciplinary team of experts who have experience in all aspects of the research proposed in this application. Dr. Tangpricha has significant expertise in clinical vitamin D nutrition for which he received his Ph.D. degree and for which topic he has several publications including several for his extensive research in the role of VDD in CF. He will be responsible for performing the vitamin D status biochemical assays (25(OH)D, PTH, CTx) in his research laboratory. Dr Ofori Acquah will be performing bone remodeling cytokine assays in his vascular biology laboratory at the Emory Children's Center. Finally, consultants on this study include Dr. Eckman, a national leader in SCD research and clinical practice who will provide his expertise on trial design and execution and is a mentor for Dr Osunkwo and Dr. Dampier a leading expert in the evaluation and management of pain in SCD who provided the pain diary tool for this study. Collectively our combined experience makes us ideally suited to conduct this study.