PURPOSE
To describe vitamin D levels among patients co-infected with HIV and Tuberculosis (TB) at a comprehensive care clinic in La Romana, Dominican Republic (DR). This study is designed in two phases: the first phase is cross-sectional and the second, an observational cohort study.

INTERVENTION
Primary Aim:

1. Cross sectional evaluation of vitamin D levels in HIV-TB co-infected individuals at baseline (defined as day of enrolment) compared to matched HIV mono-infected controls.

   Hypothesis: HIV-TB co-infected patients will have lower serum vitamin D levels compared to HIV mono-infected controls

Secondary Aim:

1. Examine the relationship of vitamin D status at baseline with outcomes including: mortality, HIV disease progression (CD4 count, viral load) in HIV-TB co-infected and HIV mono-infected individuals, and response to TB treatment (defined as clinical improvement from baseline to completion of treatment) in HIV-TB co-infected individuals.

   Hypothesis: Vitamin D status has a protective association with HIV disease progression, all-cause mortality, and response to TB treatment during follow-up in HIV-TB co-infected patients and HIV mono-infected patients.

SCIENTIFIC ABSTRACT
The purpose of this study is to describe vitamin D levels among patients co-infected with HIV and Tuberculosis (TB) in the adult and pediatric clinic population currently receiving care at Clínica de Familia in La Romana, Dominican Republic. In the first phase, we will conduct a cross-sectional evaluation of vitamin D levels in HIV-TB co-infected patients at baseline and matched HIV mono-infected patients to determine the prevalence and severity of vitamin D deficiency in the selected population. The second phase is an observational cohort study evaluating the relationship of vitamin D status at baseline with outcomes including mortality and HIV disease progression in HIV-TB co-infected and HIV mono-infected individuals and response to TB treatment in patients co-infected with HIV and TB. The data collected from this study will contribute to the knowledge base of the HIV-TB co-infection epidemic in the DR and generate hypotheses for future study. By examining the association between vitamin D levels and the clinical outcomes of patients being treated at this clinic, we will be able to improve prognosis and outcomes in TB and HIV infection in our own CUMC-associated facilities and inform the expansion of treatment initiatives in the DR and other underserved areas. In addition, our results can be used to ensure that existing treatment programs are tailored to the needs of the country and that additional treatment efforts are targeted to the appropriate populations.

LAY ABSTRACT
This study will look at and analyze the serum vitamin D levels of patients who are infected with both Human immunodeficiency virus (HIV) and Tuberculosis (TB) in the community of La Romana, Dominican Republic by determining vitamin D levels in HIV-TB co-infected patients at diagnosis and following the disease course of the patient. Our goal is to describe the level vitamin D levels at TB diagnosis of HIV-TB co-infected patients compared to the vitamin D levels of patients infected by HIV only, and evaluate disease outcomes in both groups.

Study Description
A. Study Purpose and Rationale

1. Study Purpose and Rationale
The Caribbean region, after sub-Saharan Africa, has the highest HIV prevalence in the world, with the Dominican Republic (DR) and Haiti accounting for nearly three-quarters of HIV cases in this area.[1]

Currently there are an estimated 62,000 individuals living with HIV in the DR, a prevalence of 0.8% among the adult population.[2] In addition, there are 2,700 children less than 15 years of age living with HIV in the country.[3] Conservative estimates suggest that there have been 35,000 deaths attributable to HIV/AIDS in the DR since the beginning of the epidemic in the 1980s to the present and that HIV/AIDS is currently the primary cause of mortality among Dominicans 25-44 years of age.[4] Within the DR, HIV has taken a significant toll on three key socially vulnerable groups including female commercial sex workers (CSWs), men who have sex with men (MSM), and Dominican-Haitian residents living on bateyes (underserved sugarcane plantation communities). La Romana (LR) is a province in the eastern DR, and is the region with the highest HIV prevalence in the country.[5] It is a major sugar, manufacturing, and tourism industry center, with 276,000 inhabitants, including 80,000 women aged 15 to 50 years, and an estimated 8,000 CSWs.[6,7] Many area sugarcane workers and their families live in the 126 rural bateyes in and around LR where preventative health care is almost non-existent, public service accessibility is limited, and geographic isolation prevents utilization of care even by those families with resources.[8,9] The Clínica de Familia is a family AIDS clinic in LR that has collaborated with the International Family AIDS Program at Columbia University Medical Center (CUMC) to provide free, comprehensive and HIV-specialized care. This clinic, the second largest in the country, takes a multidisciplinary approach to family-centered HIV care and has been looked to as a model for HIV care in the country.[10]

The HIV pandemic has fueled a rise in both Tuberculosis (TB) incidence and mortality, with approximately a 40% increase in incident TB cases compared to 20 years ago.[11] Today, TB is one of the leading causes of mortality in people living with HIV. It has been suggested that the ever-expanding HIV epidemic serves as the greatest challenge to controlling TB globally. HIV co-infection increases the risk of TB both by facilitating reactivation of a remote latent infection and by favoring the progression of a recently acquired infection towards active disease.[12] All HIV-infected persons diagnosed with TB, either culture-positive for TB or clinically diagnosed are considered to be HIV-TB co-infected.

At present, the adult HIV seroprevalence rate in the DR is 1.1% and an estimated 15.2% of new TB patients had HIV/AIDS. According to the WHOs Global TB report 2009, the DR has an estimated 6,764 TB cases in 2007, and the 2007 case detection rate for sputum smear-positive (SS+) cases was 66%, falling just below the WHO global target of 70%.[13] In 2011 alone, out of the 3,732 persons screened at the Clínica de Familia, there were 223 newly diagnosed HIV + patients, including 22 pediatric patients and there were 28 newly diagnosed cases of TB.[14] However, limited data regarding the demographic and clinical factors at TB diagnosis is available and even less is known regarding the disease course and response to treatment among HIV-infected individuals. This deficiency in knowledge limits the ability of treatment programs to target the subgroups in the country that have the highest risk for TB co-infection and makes it difficult for health care workers to anticipate the types of interventions (lab testing, medications, support services, etc.) needed to care for these patients.

A growing body of evidence suggests that vitamin D deficiency is associated with impaired immune function and increased risk of active TB. Vitamin D metabolites are important immunomodulatory hormones that activate monocytes and suppress lymphocyte proliferation, immunoglobulin production and cytokine synthesis[15] and vitamin D is known to suppress the intracellular growth of *Mycobacterium tuberculosis* (MTB) *in vitro.*[16] Most of the human vitamin D requirement is met by cutaneous synthesis of vitamin D during exposure to UV light.[17] Vitamin D is metabolized in the liver to form 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite and measure of vitamin D status.[18] Low vitamin D levels have been associated with a 5-fold risk for progression to TB in HIV-uninfected people in Europe and recently, low vitamin D levels have been related to disease progression of HIV-AIDS and its complications in West Africa.[19,20,21] These studies highlight the importance of adequate amounts of 25(OH)D for sustaining innate and acquired immunity against infections; however, it remains to be clarified whether vitamin D deficiency represents a marker of comorbidity or is causally related to adverse health outcomes. More information is needed to

determine if the association between vitamin D deficiency and active TB is causality (vitamin D deficiency impairs host immune response to MTB and causes susceptibility) or reverse causality (active TB causes vitamin D deficiency, due to anorexia, decreased exposure to sunlight in debilitated patients, or MTB-induced dysregulation of vitamin D metabolism).

There is a paucity of research on the relationship of vitamin D with health outcomes of HIV-TB co-infected individuals, specifically their response to TB treatment. Determination of baseline vitamin D levels may have implications for public health initiatives as well as the clinical treatment of HIV-TB co-infected patients. Improving serum vitamin D levels could potentially boost immune recovery in HIV-infected patients co-infected with TB. We propose to assess prevalence of vitamin D deficiency and insufficiency in HIV-TB co-infected individuals in LR and evaluate the possible correlation between vitamin D level and response to TB treatment. Our results can be used to ensure that existing treatment programs are tailored to the needs of the country and that additional treatment efforts are targeted to the appropriate populations.

2. Study Design and Statistical Procedure

Phase one of this study is a cross-sectional analysis describing vitamin D levels in HIV-TB co-infected individuals. Evaluations will be based on baseline vitamin D levels at TB diagnosis and compared to HIV mono-infected controls. Sample size will be determined by the number of diagnosed cases of TB during the study period. Based on historically data from the clinic we propose an estimated enrolment of 150 HIV-TB co-infected patients will be recruited during the 6 month period. The total duration of the study from recruitment to study end will be 6 months, with follow-up and endpoints at 2 and 6 months, following the conclusion of the intensive and continuation phase respectively.

We have estimated that about 50% of HIV mono-infected individuals (n= 150) will demonstrate severe (total 25(OH)D ≤25 nmol/L) to moderate (25(OH)D 25-50 nmol/L) vitamin D deficiency. This percentage is obtained from a previous study investigating vitamin D status among HIV-infected individuals in a tropical setting, as there is limited data with regard to vitamin D status in HIV-infected individuals residing in the Caribbean.

Based on the sample size of 150 participants in each group and our above estimation, the detectable difference in proportion of severe to moderate vitamin D deficiency is 17%. Calculated for chi-square analysis with 2 groups; $\alpha = 0.05$, power = 80%.

Phase two is an observational cohort study looking at TB treatment outcomes. Clinical improvement will be assessed by TBscore. This is a newly developed tool aimed at assessment of change in clinical state in patients with TB. It is based on points assigned to signs and symptoms, including cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, low BMI, and a low mid-upper arm circumference, giving patients a TBscore from 0 to 13. Changes in TBscore has been shown to detect clinical change well; a high TBscore correlates well with mortality and low TBscores correlate with favorable outcomes, cure, and completed treatment.[22] A low TBscore or fall in TBscore at treatment completion can be used as a measure of improvement.

Assuming that the minimum detectable difference in proportion will be seen in the cross-sectional analysis we can estimate that two-thirds of the HIV-TB co-infected patients will demonstrate severe to moderate vitamin D deficiency. Using an unpaired t-test we will have the power to find statistical significance if the difference is at least 34% of the SD in the change in TB score with a power of 80% and a 5% significance level.

We will collect data on the following demographic information and clinically relevant endpoints:

1) Vitamin D status will be measured at baseline, completion of the intensive phase (month 2), and completion of the continuation phase (month 6). Once the patient's vitamin D levels have been assessed at baseline, they will then be stratified into three groups: Severe vitamin D deficiency will be defined as total 25(OH)D ≤25 nmol/L, moderate as 25(OH)D between 25 and 50 nmol/L, and suboptimal as 25(OH)D between 50 and 75 nmol/L.[16]

2) Treatment outcomes will be measured by TB treatment completion defined by number of doses ingested and duration of treatment administration, resolution of symptoms, number of adverse drug reactions during treatment, new opportunistic infections, sputum culture and CXR results following completion of TB treatment

3) Mortality

If deemed appropriate once the data have been collected and described, univariate analysis will be used to assess the impact of serum vitamin D levels on response to treatment. If possible, factors significant in the univariate analysis, potential confounders and variables found significant in other models will be used to construct a multivariate logistic regression model to predict treatment failure.

3. Study Procedures

All patients presenting to Clínica de Familia who are HIV-positive are started on HAART if they are found to be eligible, per Dominican Republic national guidelines. HIV-infected patients receiving long-term care at Clínica de Familia are scheduled for monthly appointments to monitor HIV health status. Patients presenting to the clinic with signs and symptoms suspicious for TB are screened for TB with the current standard diagnostic technique regardless of their CD4 count or viral load. Signs and symptoms suspicious for tuberculosis include (pulmonary) cough for more than 15 days, hemoptysis; (extra-pulmonary) persistent fever with weight loss and fatigue, muscle pain, chills, and night sweats. These patients are evaluated with a chest x-ray (CXR) and sputum samples and cultures.

Patients with a CXR suggestive of TB are started on TB medications (isoniazid, rifampin, ethambutol, and pyrazinamide for the first 2 months – intensive phase, isoniazid and rifampin for the last 4 months – continuation phase) with dosage based on patient's weight.

Patients are required to come in daily for the first 2 months of TB treatment and every other day for the remaining 4 months of treatment.

HAART is continued regardless of simultaneous TB treatment.

Data collection

	Baseline	Intensive phase completion 2 month	Continuation phase completion 6 month
Demographic Data	X		
Patient History	X		
Physical Exam	X	X	X
5.0 mL blood sample‡	X	X	X
CXR	X	X	X
Sputum sample and culture	X	X	X

‡Minimum volume of blood needed to obtain total serum volume of 1 mL.

Demographic Data: Sex, age, ethnicity, marital status, living situation, employment status, HIV risk factors, living location and proximity to clinic and access to transportation, history of incarceration

Patient History

- Standard follow-up: approximate date of HIV diagnosis and initiation of ART, history of opportunistic infections (OIs), prior and current ART regimen, co-morbidities, adherence to medication based on physician's notes and patient report, number of visits, missed visits,

dates of visits, number of missed doses of HAART, dates of missed doses of HAART, past screening for TB, past history of TB, history of TB exposure
- Additional information: use of vitamin D supplements, dietary intake (see questionnaire), evaluation of activities of daily living (ADLs), and history of UV exposure

Physical Exam: Height, weight, BMI, evidence of OIs, developmental milestones (children)

Laboratory/Radiographic Data: blood sample (CBC, viral load, CD4 count, total lymphocyte count, serum25-hydroxyvitamin D [25(OH)D]), CXR, sputum sample and culture.

This data will be recorded in a spreadsheet without any protected health information on the data extraction tool.

4. Study Drugs or Devices
No experimental drugs or devices will be used in this study.

5. Study Instruments
No study instruments will be used in this study.

6. Study Subjects
Inclusion criteria: HIV-infected patients as defined by a positive ELISA over the age of 18 months who are diagnosed with and/or treated for TB from November 2012-November 2013 and HIV-mono infected patients matched to HIV-TB co-infected patients by age, gender, height, weight, and BMI, and stage of HIV (CD4 count, viral load).

Exclusion criteria: Patients on any additional vitamin D and calcium supplementation and those enrolled in other interventional studies.

7. Recruitment
Participants will be recruited in-person at Clínica de Familia in LR once the patient has been informed of the study by their physician who has ascertained and documented that the patient is willing to discuss the study with the investigators. HIV-TB co-infected patients will be recruited upon diagnosis of TB and HIV-mono infected controls will be recruited at a regularly scheduled clinic appointment.

8. Informed Consent Process
Informed consent will be obtained by the clinic staff at the time of TB diagnosis by use of consent form written in Spanish requiring signature and verbal explanation if needed.
Participants will be asked to provide written informed consent for the following procedures for the 6 month study: a) collection of data through questionnaires at baseline; b) collection of blood for testing of HIV viral load and serum vitamin D levels at baseline and 3, 6 month intervals; c) data abstraction from their medical records.

9. Confidentiality of Study Data
The names and personal health information of the patients will never be disclosed. Confidentiality of study subjects will be protected by ensuring that none of the collected information will be linked with individual names or other personal information.

This will be accomplished as follows:

All databases relevant to the study will be password protected, encrypted, and accessible only by study personnel. Each patient in the study will be given a unique identifier - this is called a study ID number. The study database with the demographic and clinical information will utilize this unique

study ID number to differentiate patients. The patient´s identity and clinic medical record number (MRN) will be linked with the study ID number and saved in a separate password protected file accessible only to Clínica de Familia study team in the Dominican Republic. This file will not contain any clinical information. The study database will not contain patient identifiable information, such

as their name and MRN. The birthdates of patients will be collected in order to determine the age of initiation of ART, and the patient´s birth month and birth date will be expunged from the study database once the age at ART initiation has been calculated. The key to link study patients to the study ID number will not come to CUMC and will be maintained by the Clínica de Familia study team in the Dominican Republic in a password protected electronic file that will destroyed at the end on the study. A back-up copy of the study database will be created; it will also be password protected, encrypted, and accessible only to study personnel.

10. Privacy Protections

Subject's privacy will be protected by ensuring that none of the collected information will be linked with individual names or other personal information. The only access to original patient identifiers and the coded database will be the research staff: PI, co-investigator and the student investigator.

11. Potential Risks

Risks could include loss of confidentiality of HIV or tuberculosis status; however, this is very unlikely as CU-IFAP has many years of experience in protecting the confidentiality of these patients and all data will be de-identified and maintained in locked cabinets and on a password-secured computer saved in pass-word protected files.

In addition, there are complications related to the blood draw. Including blood loss, infection or discomfort at the venipuncture site. However, the blood draw will be performed by trained nursing and physician staff thus the risk of these complications is very low.

12. Data and Safety Monitoring

Data and safety will be monitored locally to identify unanticipated problems including events, outcomes, or occurrences that are unexpected, at least possibly related to the research, and suggest an increase in risk of harm to subjects or others.

13. Potential Benefits

Study participants will receive HAART and TB treatment for the length of the study as well as regular medical attention and follow-up. With the information collected in this study, it will be easier to tailor the management guidelines to the subjects' needs. This study will also contribute to a more expanded understanding of HIV-TB co-infection in LR.

14. Alternatives

Subjects can receive standard of care management of their HIV and tuberculosis diagnosis.

15. Research at External Sites

Authorization and IRB approval at the Clínica de Familia site will be obtained through both CU and the government of Dominican Republic as per country guidelines. Approvals will be obtained from the local IRB committee and no study procedures will start until both CU and local IRB have granted approval for this study.

16. Columbia as Lead Institution

CU will serve as the lead institution for this study located in La Romana, Dominican Republic. The specific information about management of information related to safety of subjects will be provided. CU will obtain and maintain IRB approval at each site in La Romana. CU will ensure that each site follows consent procedures and utilizes consent documents approved by the designated IRB.

REFERENCES

[1] Joint United Nations Programme on HIV/AIDS. Uniting the world against AIDS. 06. Fact sheet. Caribbean. Geneva: UNAIDS; 2006 [cited 2008 Jun 10]. Available from: http://data.unaids.org/pub/GlobalReport/2006/200605-FS_Caribbean_en.pdf.
[2] Centro de Estudios Sociales y Demográficos (CESDEM). Encuesta Demográfica y de Salud 2007 : Informe Preliminar. Santo Domingo and Calverton; 2007 November.
[3] UNAIDS. Report on the global AIDS epidemic. Geneva; 2008.
[4] Health Statistics from the Americas: 2006 edition. 2006. (Accessed at http://www.paho.orgo/englihs/dd/ais/hsa2006.htm.)
[5] Monitoring the AIDS Pandemic. HIV infection and AIDS in the Americas: lessons and challenges for the future. Provisional reportMAP/EpiNet. Havana: MAP/Epidemiology Network for HIV/AIDS in Latin America and the Caribbean; 2003 [cited 2008 Feb 19]. Available from: www.mapnetwork.org/MAPLAC DraftApril9.2003%20to%20print.do
[6] Horsbrugh M. Dominican Republic. In: International Chamber of Commerce, editor. Americas review 2003/04: the economic and business report. 21st ed. London: Kogan Page; 2003. p. 241–6.
[7] United Nations Public Administration Network. E-government readiness in the Caribbean—selected indicators of basic country data. Vol. 1. New York: UNPAN; 2004 [cited 2008 Feb 19]. Available from: http://unpan1.un.org/intradoc/groups/public/documents/un/unpan017454.pdf.
[8] Brewer TH, Hasbun J, Ryan CA, Hawes SE, Martinez S, Sanchez J, et al. Migration, ethnicity and environment: HIV risk factors for women on the sugar cane plantations of the Dominican Republic. AIDS. 1998;12(14):1879–87.
[9] Crouse HL, Macias CG, Cruz AT, Wilson KA, Torrey SB. Utilization of a mobile medical van for delivering pediatric care in the bateys of the Dominican Republic. Int J Emerg Med. 2010 Aug 21;3(4):227-32
[10] Kerrigan D, Barrington C, Montalvo LM. The State of HIV/AIDS in the Dominican Republic: Guarded Optimism amidst Sustainability Concerns. In: Celentano DD, Beyrer C, eds. Public Health Aspects of HIV/AIDS in Low and Middle Income Countries: Springer Science and Business Media; 2008.
[11] H. Getahun, C. Gunneberg, R. Granich, and P. Nunn, "HIV infection-associated tuberculosis: the epidemiology and the response," *Clinical Infectious Diseases*, vol. 50, supplement 3, pp. S201–S207, 2010.
[12] Girardi E, Antonucci G, Vanacore P, Palmieri F, Matteelli A, Iemoli E, Carradori S, Salassa B, Pasticci MB, Raviglione MC, Ippolito G; GISTA-SIMIT Study Group. Tuberculosis in HIV-infected persons in the context of wide availability of highly active antiretroviral therapy. Eur Respir J. 2004 Jul;24(1):11-7.
[13] http://www.usaid.gov/our_work/global_health/id/tuberculosis/countries/lac/domrep.pdf
[14] Clinica de Familia Annual Report
[15] Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J. 2001 Dec;15(14):2579-85. Review.
[16] Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of Mycobacterium tuberculosis in a human macrophage-like cell line. Infect Immun. 1998 Nov;66(11):5314-21
[17] Holick MF (2007) Vitamin D deficiency. N Engl J Med 357:266–281.
[18] Institute of Medicine (2011) Dietary Reference Intakes for Calcium and Vitamin D (National Academy Press, Washington, DC).
[19] Douglas AS, Strachan DP, Maxwell JD (1996) Seasonality of tuberculosis: The reverse of other respiratory diseases in the UK. Thorax 51:944–946.
[20] Wejse C, et al. (2007) Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. Am J Clin Nutr 86:1376–1383.
[21] Sudfeld CR, Wang M, Aboud S, Giovannucci EL, Mugusi FM, Fawzi WW. Vitamin D and HIV Progression among Tanzanian Adults Initiating Antiretroviral Therapy. PLoS One. 2012;7(6):e40036. Epub 2012 Jun 29.
[22] Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, Sodemann M. TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scand J Infect Dis. 2008;40(2):111-20