

**Bone Loss and Immune Reconstitution in HIV/AIDS (BLIR-HIV)**

**Protocol Version 6.0**

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**Emory University School of Medicine**

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## LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

<b>Abbreviation</b>	<b>Definition</b>
AE	Adverse event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminase
ANC	Absolute neutrophil count
ART	Antiretroviral therapy
AST	Aspartate transaminase
ATV	Atazanavir
BID	Twice daily
BM	Bone marrow
BMD	Bone marrow density
BUN	Blood urea nitrogen
CBC	Complete blood count
CD40L	CD40 ligand
CLIA	Clinical Laboratory Improvement Amendments
CM	Condition medium
CRF	Case report form
CTx	C-terminal telopeptide
DAIDS	Division of Acquired Immune Deficiency Syndrome (U.S.)
DEXA	Dual-energy x-ray absorptiometry
DHHS	Department of Health and Human Services
EAE	Expedited adverse event
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorting
FDA	Food and Drug Administration (U.S)
FTC	Emtricitabine
GCP	Good clinical practice
GI	Gastrointestinal
HAART	Highly active anti-retroviral therapy
H & P	History and Physical
HEENT	Head, eyes, ears, nose, and throat
HIV	Human immunodeficiency virus
I.P.	Intraperitoneally

IRB	Institutional review board
IRIS	Immune reconstitution inflammatory syndrome
IV	Intravenous
KO	Knock out
LFT	Liver function test
LN	Lymph node
μCT	Micro-computed tomography
OHRP	Office for Human Research Protections
OI	Opportunistic infection
OPG	Osteoprotegerin
PBMC	Peripheral blood mononuclear cells
PCP	Primary care physician
PI	Protease inhibitors
PID	Patient identifier (number)
PO	By mouth
RANK	Receptor activator of NF-κB
RANKL	RANK ligand
RTI	Reverse transcriptase inhibitors
RTV	Ritonavir
SAE	Serious adverse event
SGOT	Aspartate aminotransferase
SGPT	Alanine aminotransferase
SID	Study ID
TDF	Tenofovir disoproxil fumarate
Tg	Transgenic
ULN	Upper limit of normal
WK	Week
WT	Wild type
X-HIGM	X-linked hyper-IgM syndrome

## ABSTRACT

- RATIONALE** With the increasing age of people living with HIV/AIDS, age-induced osteoporosis is likely to be compounded by HIV/AIDS and HAART-associated bone loss. Mechanistically, osteoclasts, the cells responsible for bone resorption form under the influence of the key osteoclastogenic cytokine Receptor- Activator of NF- $\kappa$ B (RANKL). The osteoclastogenic and proresorptive activities of RANKL are moderated by its physiological decoy receptor osteoprotegerin (OPG). Imbalance in the ratio of RANKL to OPG alters osteoclastic bone resorption and lead to osteoporosis. Activated T- and B-cells are a major source of RANKL, while normal physiological B-cells are a major source of OPG. T-cells regulate the production of OPG by B-cells. Thus changes in the immune system induced by HIV/AIDS and/or by HAART could affect B-cell and T-cells RANKL and OPG production. Indeed, data from our group shows that in an animal model of HIV/AIDS, the HIV-1 Transgenic rat, the development of osteoporosis is recapitulated as observed in HIV-infected patients, and B-cell OPG and RANKL production are concurrently downregulated and upregulated respectively. Furthermore, preliminary data in HIV-infected subjects suggests dramatic acute upswing in bone resorption following HAART initiation that peaks at 12 weeks and then declining. Based on these findings, we hypothesize HAART associated bone loss is driven by immune reconstitution. Because this effect of HAART is dramatic in magnitude but short in duration, we propose to apply antiresorptive agent (zoledronic acid, reclass®) to specifically spare patients from this dramatic but acute bone damage.
- DESIGN** In a prospective, blinded placebo-controlled randomized trial, treatment naïve HIV-infected subjects initiating HAART will be assigned to HAART + zoledronic acid or HAART + placebo. Serial assessment of serum levels of bone markers, cellular expression of OPG/RANKL and other cytokines, cellular immune activation markers, serum bone regulating hormones, and bone mineral density (BMD) by DXA scan will be undertaking at pre-defined time points from baseline through week 144 of HAART.
- In the primary analysis, changes in serum CTx level, BMD, and cellular OPG/RANKL expression from baseline through week 24 will be quantitated and subsequently compared between treatment arms. In addition, the impact of zoledronic acid administration on these covariates will be assessed at various study time points. The relationship between OPG/RANKL expression, immune activation, serum bone regulating hormonal levels, and bone turnover will be evaluated.
- DURATION** 144 weeks after the enrollment of the last participant. Completion of enrollment is expected within 2 years of study initiation.
- SAMPLE SIZE** 120 subjects will be screened for eligibility to enter the study, from which 94 subjects (47 per study arm) will be enrolled and followed in the study. Note that an informed consent is signed by each subject screened.
- POPULATION** Treatment naïve HIV-1 infected ambulatory men and women, age  $\geq 30 \leq 50$  years.



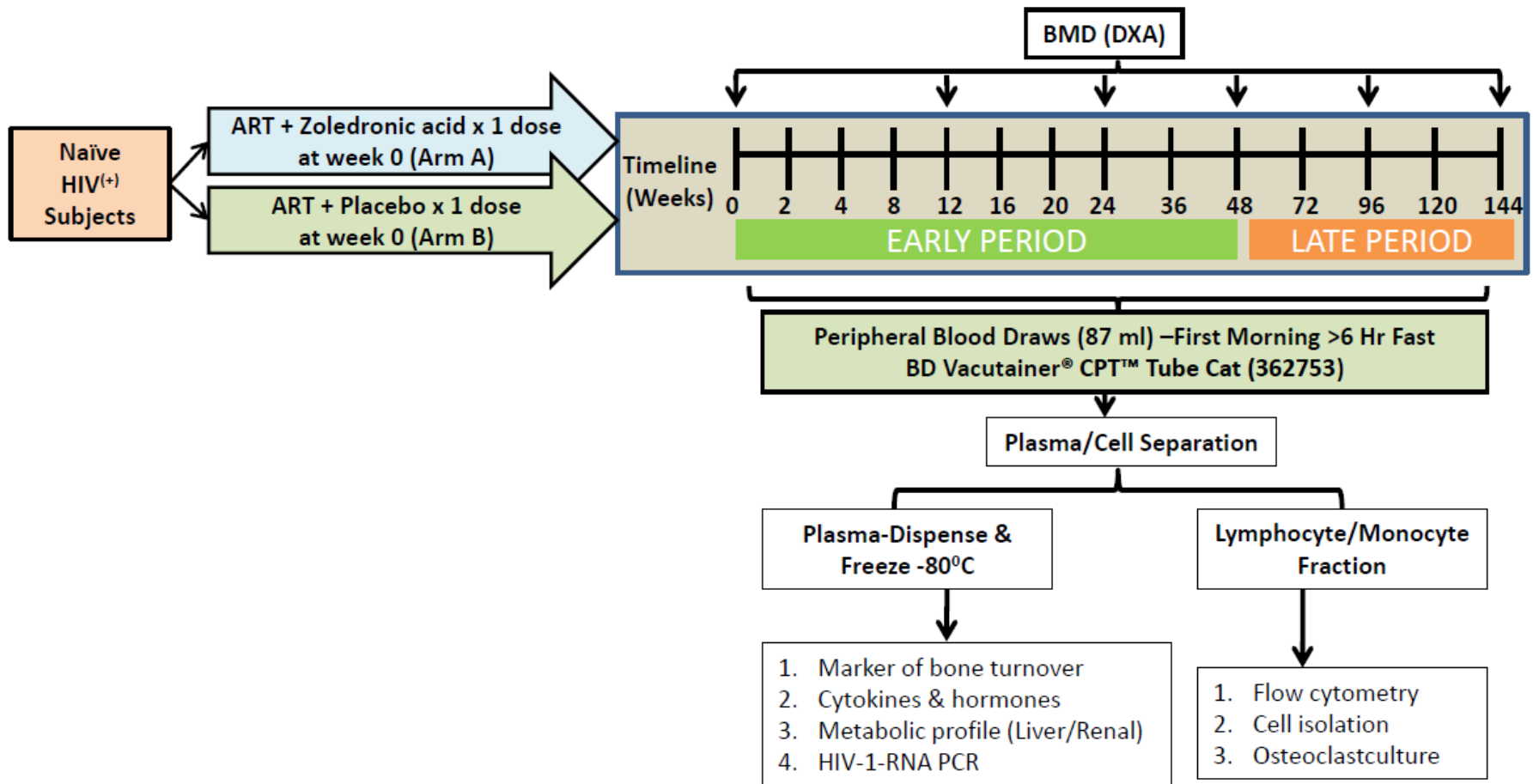
## **Lay Summary**

Other studies have shown that HIV-infection and HAART treatment lead to increased bone loss. The reason for this is not clear. Recent data from animal studies generated by our group suggest bone loss in HIV-infection may be due to imbalance between two proteins (OPG and RANKL) in the body that regulate bone structure. This study will therefore examine whether this is the case in humans. Furthermore, will examine whether bone loss resulting from HAART use is due to restoration of the immune function.

We will measure the amount of these proteins in HIV-patients starting treatment with HAART to see if they change over time. We will also examine whether changes in this protein and bone loss that occur during HAART treatment can be prevented by the bone protective medication, zoledronic acid (reclast®).

Individuals who volunteer to participate in this study will undergo thorough medical evaluation, serial blood draws for bone regulating proteins and other measurements, and total body DXA scans (DXA scan is a special type of X-ray that measures bone density). They will also begin HAART for treatment of HIV-infection and receive one dose of bone protecting medication or its saline solution placebo. The duration of the study from entry to completion will be approximately 144 weeks.

## Study Schema



## 1.0 HYPOTHESIS AND STUDY OBJECTIVES

### 1.1 Hypothesis:

We hypothesize that HAART-associated acute bone loss is mediated through either direct effect on bone cells, or through indirect effect on metabolism related to disease reversal and/or immune reconstitution, and because this effect is acute and dramatic in nature, it may be reversible by a single dose of the long-acting antiresorptive agent (zoledronic acid).

### 1.2 Primary Endpoints:

The focus of this study is to quantify the effect of HAART on bone turnover at the cellular level, to determine whether HAART-mediate bone effects through immune-reconstitution and perturbation of cellular (B and T cell subsets) balance in OPG/RANKL production, and to assess the effectiveness of antiresorptive agent in blocking HAART induced acute change in bone turnover. Because human data on cellular expression of OPG/RANKL in the setting of antiretroviral naïveté is currently limited, the study is powered to assess HAART induced acute changes in the plasma levels of markers of bone turnover.

1.2.1 To assess HAART induced acute change in bone turnover by quantitating the change from baseline to week 144 in plasma level of marker of bone resorption (CTx) among treatment naïve HIV-infected subjects treated with atazanavir based HAART regimen.

1.2.2 To quantitate the inhibitory effect of the long acting inhibitor of bone resorption (zoledronic acid) on HAART-induced change in the marker of bone resorption (CTx) at 24 weeks of HAART therapy.

### 1.3 Secondary Endpoints:

1.3.1 To quantitate the change from baseline at weeks 2, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120, and 144 in cells (lymphocyte subsets; B- and T-cell frequency and number/ml) and their production of OPG and RANKL, among treatment naïve HIV-infected subjects treated with atazanavir based HAART regimens.

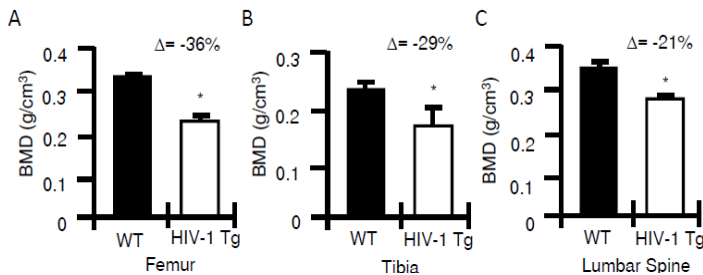
1.3.2 To evaluate the inhibitory effect of single dose zoledronic acid on HAART associated changes in other markers of bone turnover (Osteocalcin, P1NP, (bone formation markers) and TRAP5b (a marker of osteoclast activity) and BMD at appropriate study evaluation time points.

1.3.3 To quantitate the inhibitory effect of antiresorptive (zoledronic acid) on HAART-induced change in cellular (lymphocyte subsets; B- and T-cell) production of OPG and RANKL at weeks, 2, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120 and 144 of HAART therapy.

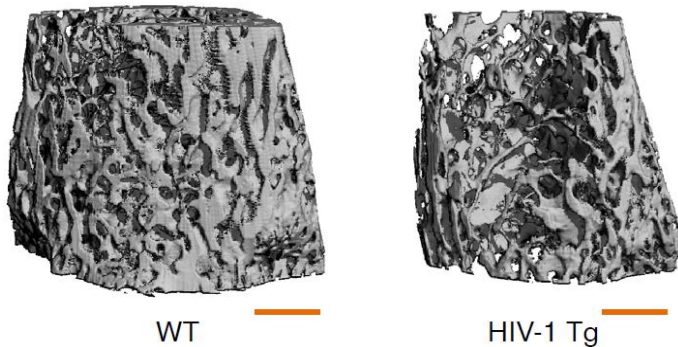
1.3.4 To correlate changes in serum, and cellular (lymphocyte subsets; B- and T-cell) expression of OPG and RANKL with markers of bone turnover and BMD at appropriate study evaluation time points.

- 1.3.5 To correlate changes in bone regulatory hormones (PTH, estrogen, testosterone, vitamin D) and inflammatory cytokines ( IL-1, IL-6,  $TNF\alpha$ ) with serum and cellular expression of OPG/RANKL, markers of bone turnover, and BMD at appropriate study evaluation time points.
- 1.3.6 To correlate changes in lymphocyte subsets; (B- and T-cell (CD4 and CD8) with bone turnover markers and BMD.

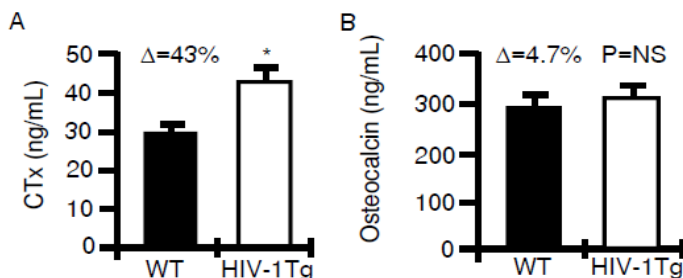
**2.0. INTRODUCTION:** Introduction of HAART has led to marked reduction in HIV/AIDS morbidity and mortality. However, serious metabolic complications including bone loss, osteoporosis and fractures (1) are now becoming increasingly common. With increasing age of people living with HIV/AIDS, age-associated osteoporosis is likely to be significantly compounded by HIV/AIDS-associated bone loss leading to an epidemic of fractures in this population. The contributions to osteoporosis of direct and indirect effects of HIV/AIDS and of HAART, and the mechanisms involved are poorly understood.



**Figure 1: HIV-1 Tg rats develop osteoporosis**  
\* $P < 0.004$  (Mann-Whitney),  $n = 5$  WT/6 HIV, Age = 15 months



**Figure 2:  $\mu$ CT Showing Three dimensional Reconstructions of femurs from WT and HIV-1 Tg rats.**



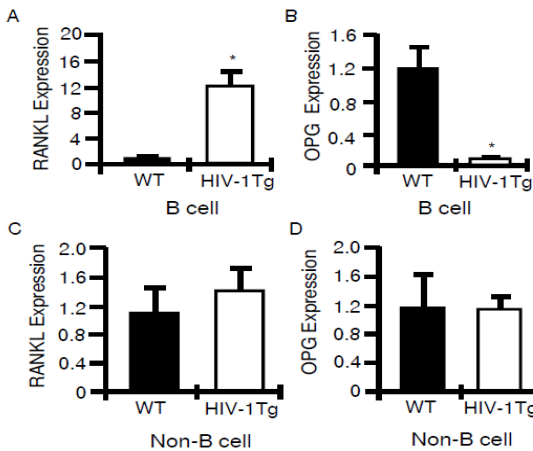
**Figure 3: Elevated biochemical markers of bone resorption in HIV-1 Tg rats.** \* $p = 0.002$  (t test),  $n = 4$  rats/group: Age = 9 months

Only recently has the depth of integration between the immune and skeletal systems begun to be appreciated. The skeleton is a dynamic organ that is continually regenerated by the process of homeostatic bone remodeling (2). Osteoclasts (OCs), the cells responsible for bone resorption, form from cells of the monocytic lineage in the bone marrow (BM) under the influence of the key osteoclastogenic cytokine Receptor- Activator of NF- $\kappa$ B (RANKL). The activity of RANKL is moderated by its physiological decoy receptor osteoprotegerin (OPG) (3). We now appreciate from both human and animal studies that any increase in the ratio of RANKL to OPG accelerates the rate of osteoclastic bone resorption leading to the development of osteoporosis. Our group recently reported that in rodents B cells are critical stabilizers of peak bone mineral density (BMD) in vivo as cells of the B lymphocyte lineage, under the control of T cells, in part through CD40/CD40 ligand (CD40L) costimulation, account for up to 64% of total bone marrow (BM) OPG concentrations, with mature B cells alone contributing 45% of total BM OPG (4). Thus, a putative mechanism of HIV/AIDS and/or HAART related bone loss may be an imbalance in RANKL/OPG production as a consequence of T-cell and/or B-cell disruption/dysregulation.

**2.1. Studies in animal model of HIV/AIDS suggest that B cells play a role in maintenance of bone homeostasis:** The HIV-1 Transgenic (Tg) rat is a model of HIV-1/AIDS that recapitulates many of the features of the human disease. Preliminary data from our group demonstrated that like their human

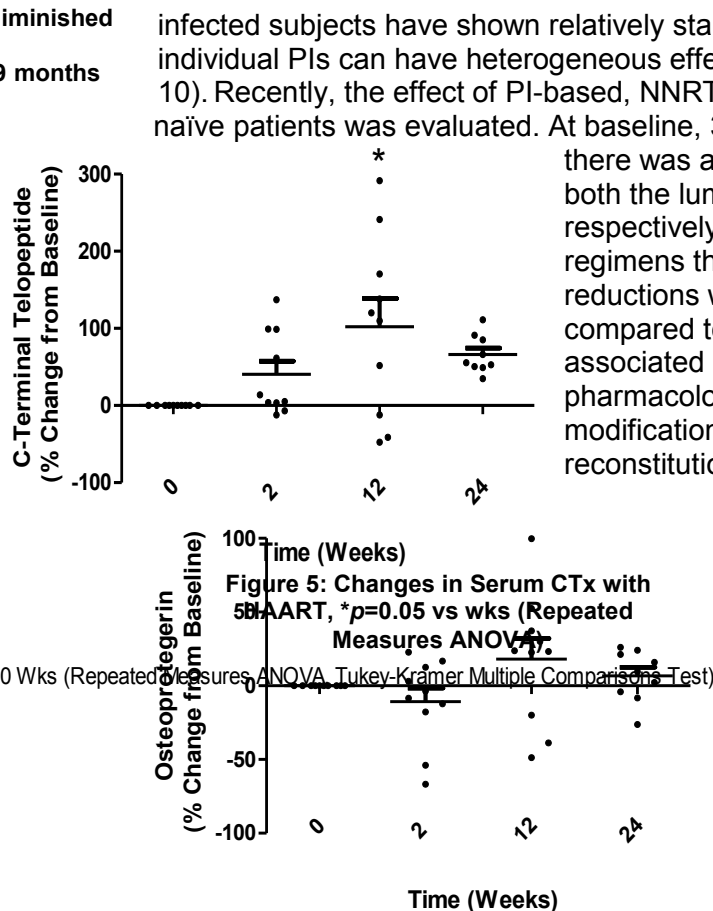
counterparts, HIV-1 Tg rats develop severe osteoporosis and display significant decrements in bone BMD (Figure 1) and in cortical and trabecular bone volume (Figure 2), as a consequence of elevated osteoclastic bone resorption (Figure 3). Mechanistically, this osteoclastic bone loss appears to be driven by enhanced production of RANKL, the key osteoclastogenic cytokine, and amplified by a decrement in production of OPG, the key physiological suppressor of RANKL activity (Figure 4). Our preliminary studies suggest that this cytokine imbalance stems from a switch in production of OPG to production of RANKL by B cells, an immune cell that we have recently demonstrated to play a critical

role in the regulation of basal bone homeostasis. Thus, our data suggest that disruption of B cells function, either due to direct viral action, or indirectly as a consequence of T cell disruption, is a critical factor in bone loss in HIV/AIDS *in vivo*.



**2.2. HAART induces bone turnover:** Besides the osteopenia associated with disruption of bone homeostasis in treatment-naïve patients, the initiation of antiretroviral therapy has been consistently associated with a 2-3% reduction in BMD over the first 48-96 weeks of therapy (5-7), suggesting that the underlying cause of osteoporotic bone changes in HIV may be multi-factorial, and may include pharmacologic, immune or viral factors. In a recent meta-analysis of cross-sectional studies, HIV-infected subjects receiving PIs had a higher prevalence of osteoporosis compared to those receiving non-PI regimens (8). By contrast, other studies of PI-treated HIV-

infected subjects have shown relatively stable or increased BMD over time (11-13), suggesting that individual PIs can have heterogeneous effects on bone, data supported by *in vitro* experiments (9, 10). Recently, the effect of PI-based, NNRTI-based, or PI/NNRTI-based regimens on BMD in 71 naïve patients was evaluated. At baseline, 31% were osteopenic and 3% osteoporotic. At week 48, there was an overall median decrease in BMD that was significant at both the lumbar spine and the femoral neck (4.1% and 2.8%, respectively). Greater decrements were noted in the PI containing regimens than in the NNRTI/NRTI regimen (14). In Gallant et al., BMD reductions were greater in those randomized to tenofovir (TDF) compared to those randomized to stavudine (d4T) (5). Whether HIV-associated osteoporotic changes are a direct consequence of the pharmacological agents themselves or a consequence of disease modification/metabolic realignments to therapy (such as immune reconstitution) or both, is presently unclear.



**Figure 5: Changes in Serum CTx with HAART, \* $p=0.05$  vs wks (Repeated Measures ANOVA, Tukey-Kramer Multiple Comparisons Test)**

**2.3. HAART stimulates a rapid increase in bone resorption and dysregulated OPG Production.** In a recent pilot study (Emory IRB protocol #IR800007234), we examined biochemical markers of bone resorption (C-terminal Telopeptide of collagen (CTx)) and serum OPG concentrations in HIV/AIDS patients ( $n=10$ ) at baseline and prospectively at 2, 12 and 24 weeks of treatment with lopinavir/ritonavir + tenofovir/emtricitabine. Within 2 weeks of HAART initiation the percentage change from baseline of serum CTx rose by an average of 50%, reaching a statistically significant 115% increase by 12 weeks of treatment (some patients reached a 300% increase in bone resorption) and slowly declined reaching 70% by 24 weeks (Figure 5). In contrast, within 2 weeks of HAART initiation OPG levels declined by 11%, consistent with enhanced bone resorption at this time point. Interestingly, by 12 weeks OPG levels had rebounded to ~18% above baseline levels, and then declined to 7% above baseline by 24 weeks (Figure 6). These data suggest that a decline in production of OPG shortly after HAART initiation may contribute to an initial wave of bone loss, potentially due to T cell and B cell activation and reconstitution, while a compensatory response to elevated bone resorption leading

**Figure 6: Changes in Serum OPG with HAART \* $p=0.18$  (Repeated Measure ANOVA)**



to realignment in OPG production, may counteract bone resorption, thus slowing bone loss during medium to long term HAART treatment, as the immune system attains a more physiological state. ***Importantly, the observation that these changes occurred within the initial 24 weeks of therapy offers a unique opportunity for the exploration of early intervention to prevent antiretroviral induced bone loss.*** Virtually all previous studies have focused on long time points, and consequently these dramatic resorptive events immediately following HAART administration have not been previously documented. The magnitude of these changes in bone resorption may have a profound impact on long term skeletal health in these patients. We thus propose a short-term (6 months to 1 year) intervention arm to provide a proof of concept that this resorptive effect can, and should be, prevented pharmacologically.

**2.4. Long-Acting Antiresorptive Agents.** Bisphosphonates including zoledronic acid are pyrophosphate analogues that inhibit bone resorption by binding to hydroxyapatite crystals. Several oral formulations of this class of compound are currently licensed for the treatment of osteoporosis. However, oral bisphosphonate use in the setting of HIV-infection may be limited by gastrointestinal side effects and contribution to an already high pill burden of HAART and other medications used for prophylaxis or treatment of opportunistic infections. Zoledronic acid on the other hand, is an intravenous third-generation, long-acting bisphosphonate that can be administered annually. This compound has been shown to improve bone density and reduce fracture risk in subjects with osteoporosis [15, 16]. Two recent studies have also demonstrated that annual zoledronate infusion was well tolerated and led to significant increases in bone density in HIV-infected individuals with osteoporosis or osteopenia [17, 18]. Denosumab is another long-acting parenteral agent that offers a novel approach to bone loss prevention. It is unique in the sense that is a fully humanized monoclonal antibody against RANKL, a cytokine, as noted above that is essential for the formation, function, and survival of osteoclasts. By binding RANKL, denosumab prevents the interaction of RANKL with its receptor, RANK, on osteoclasts and osteoclast precursors and reversibly inhibits osteoclast-mediated bone resorption [19]. Bi-annual subcutaneous administration of denosumab has been shown to be well tolerated and to reduce bone turnover and increase bone mineral density in human subjects [20 - 24]. Because HAART associated bone loss occurs early during therapy, and because zoledronate and denosumab have very long half-lives that span the window of rapid bone turnover during early phase of antiretroviral therapy, they may offer a convenient approach to aborting the rapid bone loss associated with HAART initiation in HIV-infected patients. However, in this proof-of-concept study, zoledronic acid will be used because it is currently approved in the U.S. by the Food and Drug Administration for the treatment of osteoporosis.

**2.5. Study Rationale:** This proposal therefore aims to explore in further detail the cellular mechanism of HAART-associated bone turnover among HIV-infected subjects initiating antiretroviral therapy. We plan to evaluate the pharmacologic role of HAART therapy and the potential role of immune-reconstitution following HAART on the homeostatic balance between RANKL and OPG in the serum and its production by B cells and T cells, cell types dramatically impacted by HIV-1 infection, and by HAART, and correlate these data with bio-markers of bone turnover and BMD in HIV-infected patients.

Furthermore, we will investigate whether a single parenteral dose of zoledronic acid, can prevent or slow the early rapid bone turnover seen among HIV-infected patients initiating HAART. Such finding would be significant and could potentially offer an opportunity to reverse or slow the progression of osteopenia and osteoporosis in the setting of HAART with a convenient single dose administration of this or similar agent.

Understanding, the risks to the skeleton associated with HIV/AIDS, and of HAART regimens, is imperative if we are to recognize the risk factors and treat patients appropriately to prevent premature or accelerated bone loss. With the mean survival time for HIV-infected individuals continuing to rise,

damage sustained by the skeleton due to AIDS and/or HAART is likely to lead to an epidemic of osteoporosis and bone fractures among AIDS patients in the future, as age-associated declines in BMD exacerbate an already impoverished skeleton. While it may take some time before a consensus is reached as to the chronic use of antiosteoporotic agents in a population that may have to be treated for many decades, the utilization of an acute antiresorptive treatment to rescue patients from intensive skeletal damage associated with the early phase of HAART administration, could rapidly be implemented as part of the standard of care for these patients, dramatically improving long term skeletal health.

### **3. STUDY DESIGN:**

#### **3.1. Study Overview:**

In a prospective, blinded placebo-controlled, randomized study design, treatment naïve HIV-infected subjects initiating HAART will be assigned to HAART plus zoledronic acid or HAART plus placebo. Longitudinal blood sampling (separated into plasma and PBMC) will be performed at pre-defined time points. Serial assessment of serum levels of bone markers, cellular expression of OPG/RANKL and other cytokines, cellular immune activation markers, serum bone regulating hormones, and bone mineral density (BMD) by DXA scan will be undertaken at pre-defined time points from baseline through week 144 of HAART.

#### **3.2. Subject Population and Recruitment:**

A total of 94 HIV-infected subjects (47 subjects per arm) will be recruited from the Grady Health Care System Infectious Diseases Program out-patient Clinic (Grady IDP). The Grady IDP manages over 4,500 HIV-infected patients and sees more than 50 antiretroviral naïve HIV-infected patients per month. HIV sero-positive patients may also be recruited from other areas where these populations may be found such as county health departments, private physician practices and community organizations.

#### **3.3. Study Intervention:**

##### **3.3.1. Screening:**

Study related activities will include a screening visit to determine eligibility. This visit will entail informed consenting, review of medical records, detailed medical history and physical examination. Definitive proof of HIV-infection by ELISA test, western blot and/or plasma HIV-RNA PCR (viral load) will be documented. Urine pregnancy test will be performed for female subjects with childbearing potential.

##### **3.3.2. Study Entry**

Upon confirmation of eligibility and within 60 days of the screening visit, the subject will be enrolled into the study. Thereafter 87 mL of peripheral blood will be obtained via veni-puncture of a peripheral vein and collected into EDTA tubes. EDTA-Plasma is suitable for all ELISA Endpoints required in the specific aim of this proposal.

Plasma and mononuclear cells will be recovered from collected blood samples by centrifugation using CPT tubes. Plasma will be stored at -80°C until analyzed in the



laboratory. PBMC will be processed immediately for FACS analysis and lymphocyte recovery (B and T cells) using immunomagnetic isolation procedures.

Baseline plasma HIV-1 RNA viral load, CD4 T cell counts, and safety blood parameters (including chemistry panel, liver function tests, and complete blood cell counts) will be performed. Urine pregnancy test will be performed again for female subjects with childbearing potential. Subjects will be sent to have baseline DXA scans performed at the radiology unit of the Emory University Hospital. In addition, blood sample (1 tablespoon) for DNA testing (optional) will be collected at one time point, usually at the time of enrollment.

Following antiretroviral adherence counseling by trained and experienced medication counselors, subjects will be blindly randomized to one of two study arms;

- (a) HAART: Atazanavir (ATV)/ritonavir (RTV) + tenofovir (TDF)/emtricitabine (FTC) plus zoledronic acid
- (b) HAART (ATV/RTV + TDF/FTC) plus placebo

### 3.3.3. Follow-up/safety Data

Follow-up visits will occur at weeks 2, 4, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120, and 144. During each visit, targeted physical examination driven by signs and symptoms will be performed. This will include vital signs: temperature, pulse, respiratory rate, blood pressure, weight, and height.

At each visit, 87 mL of peripheral blood will be obtained via veni-puncture of a peripheral vein and collected into EDTA tubes following an overnight fast. Plasma and mononuclear cells will be recovered from collected blood samples by centrifugation using ACCUSPIN SYSTEM-HISTOPAQUE-1077 tubes. Plasma will be stored at -80°C until analyzed in the laboratory. PBMC will be processed immediately for FACS analysis and lymphocyte recovery (B and T cells) using immunomagnetic isolation procedures. DXA scan will also be performed at weeks 12, 24, 48, 96 and 144.

As per standard of care at the Grady IDP, at weeks 4, 16, 24, 36, 48, 72, 96, 120, and 144 safety blood parameters, plasma HIV-1 RNA viral load, and CD4 T-cell counts will be performed and also at permanent study discontinuation.

In addition, during each study visit, adherence to HAART treatment (as determined by pill counts or by using a previously validated questionnaire [25], and recording of adverse clinical events will be performed.

Subjects with virologic failure will be evaluated with HIV-1 genotype and for change in antiretroviral treatment as per standard of care at the Grady IDP. Virologic failure for the purpose of this study will be defined as a confirmed plasma HIV-1 RNA level >1000 copies/mL 16 weeks after randomization and before week 24 or >200 copies/mL 24 weeks or more after randomization.

## 4 **SELECTION AND ENROLLMENT OF SUBJECTS**

### 4.1 Inclusion Criteria

- 4.1.1 HIV-1 infection, as documented by any licensed serologic test and confirmed by a western blot or by a positive plasma HIV-1 RNA performed by any laboratory that has a CLIA certification.
- 4.1.2 Meets Grady IDP clinical criteria for antiretroviral therapy initiation, and subject and his/her provider are agreeable to subject initiating therapy with a regimen consisting of ATV/RTV + FTC/TDF as part of his/her routine HIV management.
- 4.1.3 Ambulatory men and women age  $\geq 30 \leq 50$  years.
- 4.1.4 Ability and willingness of subject or legal guardian/representative to give written informed consent.
- 4.1.5 ARV drug-naïve (defined as  $\leq 10$  days of ART at any time prior to entry).
- 4.1.6 Screening HIV-1 RNA  $\geq 1000$  copies/mL obtained within 90 days prior to study entry by any FDA-approved test for quantifying HIV-1 RNA at any laboratory that has a CLIA certification.
- 4.1.7 Laboratory values obtained within 90 days prior to study entry.
  - Absolute neutrophil count (ANC)  $\geq 500/\text{mm}^3$
  - Hemoglobin  $\geq 8.0$  g/dL
  - Platelet count  $\geq 40,000/\text{mm}^3$
  - Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase  $\leq 3 \times \text{ULN}$
  - Total bilirubin  $\leq 2.5 \times \text{ULN}$
  - Calcium  $\geq 8.0$  mg/dL
  - Serum vitamin D level  $\geq 12\text{ng/mL}$
  - CrCl  $\geq 50$  mL/min as estimated by the Cockcroft-Gault equation.
- 4.1.8 Absence of non-HIV related active immunological or bone disorders such as;
  - Bone marrow or organ transplantation
  - Inflammatory bowel disease (ulcerative colitis, crohn's disease)
  - Multiple Myeloma
  - Osteogenesis imperfect
  - Osteomalacia
  - Osteosarcoma
  - Paget's disease
  - Postmenopausal osteoporosis
  - Rheumatoid arthritis
  - Systemic lupus erythmatosus
  - Thyroid disorders (hyper/hypothyroidism)
- 4.1.9 Contraception requirements
  - 4.1.9.1 Female Subjects of Reproductive Potential:  
Female subjects of reproductive potential, who are participating in sexual activity that could lead to pregnancy, must agree to use at least

one reliable method of contraception while participating in the study.

Acceptable methods of contraception include:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based contraceptive (must contain at  $\geq 35$  mcg of ethinyl estradiol)

#### 4.1.9.2 Female Subjects Who Are Not of Reproductive Potential

Women who are not of reproductive potential or whose male partner(s) has azoospermia are eligible to start study drugs without requiring the use of contraceptives. Any statement of self-reported sterility or that of her partner's must be entered in the source documents.

NOTE: Acceptable documentation of lack of reproductive potential is the woman's self-reported history of surgical sterilization, menopause, or male partner's azoospermia.

## 4.2 Exclusion Criteria

- 4.2.1 The patient is pregnant or breast feeding
- 4.2.2 The patient has physical or biochemical evidence or a medical history of malignancy.
- 4.2.3 The patient is currently (within the past 8 weeks) taking any systemic medication with known influence on the immune or skeletal system (e.g. immune modulation therapy, glucocorticoids, steroid hormones, other bisphosphonates).
- 4.2.4 The patient has osteoporosis defined as T-score  $< -2.5$  at the hip, or spine, or history of osteoporotic fracture.
- 4.2.5 The patient has prior or current use of zoledronic acid (reclast®)
- 4.2.6 The patient has a recent (within the past 6 months) or planned (within the next 6 months) invasive dental procedure.
- 4.2.7 The patient has any condition that, in the opinion of the investigators, would compromise the subject's ability to participate in the study.
- 4.2.8 The patient has a serious illness requiring systemic treatment and/or hospitalization until subject either completes therapy or is clinically stable on therapy, in the opinion of the investigators, for at least 7 days prior to study entry.
- 4.2.9 The patient has discontinued, for at least 30 days prior to randomization, any medications (per protocol) that are prohibited with study ARV's and zoledronic acid.
- 4.2.10 The patient is currently imprisoned or involuntarily incarcerated in a medical facility for psychiatric or physical illness.

Exclusion criteria are primarily centered on immunological aspects with bone related aspects secondary. This is because in our model immunological function is proximal to bone function. Consequently, use of vitamin D or calcium supplementation will not be exclusion criteria, but will be added as covariates in our analysis.

#### 4.3 Study Enrollment Procedures

- 4.3.1 Prior to implementation of this study, the protocol and protocol consent form will be approved by the Emory University institutional review board (IRB) and the Grady Health System Research Oversight Committee. Once a candidate for study entry has been identified, details will be carefully discussed with him/her. The candidate will be asked to read and sign the approved protocol consent form that was approved by the Emory University IRB prior to undergoing any study procedures. A copy of the signed informed consent form will be given to the subject, and the original copy will be kept in subject's record.

### 5 **STUDY TREATMENT**

#### 5.1 Regimens, Administration, and Duration

##### 5.1.1 Regimens

Subjects will be randomized to one of the following two treatment arms:

ARM A: Atazanavir (ATV) 300 mg PO QD + Ritonavir (RTV) 100 mg PO QD + Tenofovir (TDF) / Emtricitabine (FTC) 200/300 mg PO QD plus single dose Zoledronic acid 5 mg in a 100 mL ready to infuse solution.

ARM B: ATV 300 mg PO QD + RTV 100 mg PO QD + TDF/FTC 200/300 mg PO QD plus single Zoledronic Acid 5mg placebo in 100 mL ready to infuse solution.

##### 5.1.2 Administration and Dispensing

5.1.2.1 ATV will be administered orally as one 300 mg capsule once daily with food and is to be taken with RTV 100 mg once daily.

5.1.2.2 TDF/FTC will be administered orally as one 300/200 mg coformulated tablet (Truvada®) once daily with food.

5.1.2.3 Zoledronic acid will be administered intravenously as 5 mg/100 mL infusion given over 30 minutes.

##### 5.1.3 Duration

Subjects will participate in this study for approximately 144 weeks.

#### 5.2 Study Product Formulation and Preparation

HAART (ATV, RTV or FTC/TDF) will be administered as standard of care for HIV-infection management and is not provided by the study. Key study protocol products provided by the study are listed below.

#### 5.2.1 Zoledronic acid

- Zoledronic acid (reclast®) is available in bottles (each bottle contain 5 mg/100 mL. NDC 0078-0435-61)
- Product will be kept in a secured location. It will be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

### 5.3 Pharmacy: Product Supply, Distribution, and Accountability

#### 5.3.1 Study Product Acquisition

5.3.1.1 HAART will be acquired by subject as part of their standard of care through their payor source.

5.3.1.2 Zoledronic acid/placebo is provided by Novartis

#### 5.3.2 Study Product Accountability

The Emory University Clinical Trial Unit pharmacists assigned to this protocol will be required to maintain complete records of all study products received from the supporting pharmaceutical companies and subsequently dispensed. All unused study products will be destroyed or returned after the study is completed or terminated.

### 5.4 Concomitant Medications

To avoid drug interaction and adverse events, the manufacturer's most recent package inserts of the study allowed antiretroviral agents, zoledronic acid, and the concomitant agent will be referred to whenever a concomitant medication is initiated or dose of a concurrent medication is changed.

#### 5.4.1 Required Medications

No concomitant medications are required by the protocol.

#### 5.4.2 Prohibited Medications are listed below.

<b>Table 1: Prohibited Medications</b>			
<b>Medication Category</b>	<b>Zoledronate</b>	<b>ATV</b>	<b>RTV</b>
<b>Alpha<sub>1</sub>-adrenoreceptor antagonist</b>			Alfuzosin HCL
<b>Antiarrhythmics</b>			amiodarone, bepridil, flecainide, propafenone, quinidine
<b>Antifungal</b>			voriconazole
<b>Antihistamines</b>			astemizole, terfenadine
<b>Antimycobacterials</b>		rifampin	rifampin
<b>Antineoplastics</b>		irinotecan	
<b>Ergot Derivatives</b>		dihydroergotamine, ergotamine, ergonovine, methylergonovine	dihydroergotamine, ergotamine, ergonovine, methylergonovine
<b>GI Motility</b>		cisapride	cisapride
<b>Herbal Products</b>		St. John's wort ( <i>Hypericum perforatum</i> )	St. John's wort ( <i>Hypericum perforatum</i> )
<b>HMG-CoA Reductase Inhibitors</b>		lovastatin, simvastatin	lovastatin, simvastatin
<b>Neuroleptic</b>		pimozide	pimozide
<b>PDE5-inhibitors</b>			Sildenafil* (Revatio®) only when used for the treatment of pulmonary arterial hypertension (PAH)
<b>Sedative/hypnotics<sup>1</sup></b>		triazolam, orally administered midazolam	triazolam, orally administered midazolam

#### 5.4.3 Precautionary medications may include, but may not be limited to:

<b>Table 2: Precautionary Medications</b>				
<b>Medication Category</b>	<b>Zoledronic acid</b>	<b>ATV</b>	<b>RTV</b>	<b>Clinical comment</b>
Aminoglycosides				Caution is advised when bisphosphonates are administered with aminoglycosides, since these agents may have an additive effect to lower serum calcium level for prolonged periods. This effect has not been reported in zoledronate clinical trials.
Anesthetic			meperidine	Dose increase and long-term use of meperidine with RTV is not recommended due to the ↑ concentrations of metabolite which has analgesic and/or CNS stimulant activity (e.g., seizures).
Antiarrhythmics		Amiodarone, bepridil, lidocaine (systemic), quinidine	disopyramide, lidocaine, mexilitine	Antiarrhythmics concentrations are ↑ by PIs. Caution is warranted and TDM (if available) is recommended for these agents when co-administered with PIs.
Anticoagulant		warfarin (↑ levels)	warfarin (↓ levels)	Initial frequent monitoring of the INR is indicated when warfarin is co-administered with PIs.
Anticonvulsants			↑ carbamazepine ↑ clonazepam ↑ ethosuximide  ↓ divalproex ↓ lamotrigine ↓ phenytoin	Clinical monitoring of anticonvulsant concentrations and its dose titration is recommended to achieve the desired clinical response.
Antidepressants		Trazodone, tricyclics	bupropion, nefazodone, SSRIs, tricyclics, trazodone, desipramine	If these drugs are used with a PI, the combination should be used with caution and the lowest possible dose should be prescribed initially. Serum drug concentrations should be monitored when possible and used to titrate dose to obtain the desired clinical effect. Adverse events of nausea, dizziness, hypotension, and syncope have been observed following co-administration of trazodone and RTV.
Anti-HCV agent				For treatment of hepatitis C, interferon should not exceed a dose of 10 million IU/ week, and pegylated interferon should not exceed 180µg/wk. It is recommended that participants not start hepatitis C treatment during the first year on-study; however, if treatment is started, participants will continue to be

				followed on-study and on-study treatment.
Antiemetic			dronabinol (↑ levels)	A dose ↓ of dronabinol may be needed when co-administered with RTV.
Antifungal		↑ketoconazole ↑itraconazole	↑ketoconazole ↑itraconazole	Due to the effect of RTV on ketoconazole or itraconazole, high doses (>200 mg/day) are not recommended.  Plasma concentrations of voriconazole may be ↓ in the presence of DRV/r. Voriconazole should not be administered to patients receiving DRV/r unless an assessment of the benefit/risk ratio justifies the use of voriconazole.
Anti-infective		↑clarithromycin	↑clarithromycin	Co-administration of ATV with clarithromycin ↑ clarithromycin levels, which could result in QTc prolongation. Dose reduction of clarithromycin by 50% should be considered. With DRV/r, no dose adjustment of the combination is required for patients with normal renal function. For patients with renal impairment, refer to PI package insert for appropriate dosing.
Antimycobacterial		↑rifabutin	↑rifabutin	A rifabutin dose reduction of up to 75% (e.g., 150 mg every other day or 3 times per week) is recommended when co-administered with a RTV-boosted PI.
Antiparasitics			atovaquone	Clinical significance is unknown; however, ↑ in Atovaquone dose may be needed.
Antihypertensives		felodipine, nifedipine, nicardipine, verapamil	Diltiazem, nifedipine, verapamil, Metoprolol, timolol	Antihypertensive levels are ↑. Caution is warranted and clinical monitoring of patients is recommended. A dose ↓ may be needed for these drugs when co-administered with RTV boosted PIs.  Co-administration of ATV with diltiazem resulted in a 2-fold ↑ in the steady state concentration of diltiazem. A 50% dose reduction in diltiazem may be necessary.  Caution should be used when ATV is co-administered with drugs known to increase the PR interval (e.g., diltiazem).
Bile sequestering resins				Co-administration of PIs with bile sequestering resins (cholestyramine, colestipol) are discouraged because their use can be associated with ↑ triglyceride levels,



				and their effects on antiviral drug absorption have not been studied.
Bronchodilator			↓theophylline  Long-acting beta-adrenoceptor agonist: ↑salmeterol.	↑ dosage of theophylline may be required with RTV; TDM should be considered.  Concurrent administration of salmeterol and RTV is not recommended. The combination may result in increased risk of cardiovascular adverse events associated with salmeterol, including QT prolongation, palpitations and sinus tachycardia.
Digoxin			↑Digoxin	Caution should be exercised when co-administering RTV containing regimen with digoxin, with appropriate monitoring of serum digoxin levels.
Gastric acid reducing agents		↓ ATV levels		Any drug or buffered product (e.g., antacids, H2 blockers, proton pump inhibitors) that reduces gastric acid may reduce the plasma concentrations of ATV. - Note: If co-administered with an antacid (e.g., Maalox®, Mylanta®), ATV/RTV should be administered together at least 2 hours before and 1 hour after a dose of the antacid. Since there are limited data on the effect simultaneous co-administration of both H2 blockers (e.g., famotidine, cimetidine) and TDF have on ATV levels it is recommended that whenever possible ATV/RTV should be administered at least 2 hours before and at least 10 hours after a dose of the H2 blocker. The proton-pump inhibitor dose should not exceed a dose comparable to omeprazole 20 mg and must be taken approximately 12 hours prior to the REYATAZ 300 mg and ritonavir 100 mg dose.
Grapefruit juice				Participants should avoid grapefruit juice throughout the study period, and especially within 72 hours prior to study visits when PI trough levels are obtained.

Loop Diuretics				Caution should also be exercised when zoledronate is used in combination with loop diuretics due to an increased risk of hypocalcemia.
Nephrotoxic Drugs				Caution is indicated when zoledronate is used with other potentially nephrotoxic drugs.
PDE5-inhibitors:		↑sildenafil, ↑tadalafil, ↑vardenafil.	↑sildenafil, ↑tadalafil, ↑vardenafil	Particular caution should be exercised when prescribing these agents to participants receiving RTV-boosted PI regimens. Co-administration of RTV with any of these agents is expected to substantially increase their concentrations which may cause hypotension, syncope, visual changes, and prolonged erection. When co-administered with any PI: <ul style="list-style-type: none"> <li>- Sildenafil should not exceed a maximum single dose of 25 mg in a 48-hour period.</li> <li>- Tadalafil should not exceed a maximum single dose of 10 mg in a 72-hour period when co-administered with any potent inhibitors of CYP3A4, such as RTV.</li> <li>- Vardenafil should not exceed a maximum single dose of 2.5 mg in a 72-hour period when co-administered with RTV. When co-administered with other PIs, caution should be exercised when dosing vardenafil.</li> </ul>
HMG-CoA Reductase Inhibitor		↑rosuvastatin, ↑atorvastatin	↑rosuvastatin, ↑atorvastatin	Caution should be used when taking these agents as the risk of myopathy, including rhabdomyolysis, may be increased. They should be used at the lowest possible dose with careful monitoring and dose titration, and when possible, alternative agents should be considered.
Hormonal contraceptives		↓ or ↑ethinyl estradiol,  ↓or ↑norgestimate	↓ethinyl estradiol	The effectiveness of estrogen-based contraceptives when co-administered with RTV-boosted PI regimens is unknown. Alternative or additional contraceptive measures should be used when estrogen-based oral contraceptives and RTV-boosted PI regimens are co-administered
Immuno-suppressants		↑cyclosporine, ↑tacrolimus, ↑sirolimus	↑cyclosporine, ↑tacrolimus, ↑sirolimus	TDM is recommended for immunosuppressant agents when co-administered with a RTV-boosted PI.
Inhaled Steroid: fluticasone		↑fluticasone	↑fluticasone	Concomitant use of inhaled fluticasone propionate and RTV-boosted PI may increase plasma concentrations of fluticasone, resulting in significantly

				reduced serum cortisol concentrations. Alternatives should be considered, particularly for long term use.
Narcotic Analgesic			↓methadone	Dose adjustments of methadone may be required for participants on RTV-boosted PI regimens. Participants on RTV-boosted regimens should be closely monitored for symptoms of opiate withdrawal.
Nephrotoxic agents: e.g., amphotericin B, aminoglycosides, cidofovir, acyclovir, ganciclovir, vancomycin				Co-administration of agents with nephrotoxic potential (e.g., amphotericin B, aminoglycosides, cidofovir, acyclovir, ganciclovir, vancomycin) or that are renally excreted with TDF may increase serum drug concentrations of TDF and/or increase the concentrations of the other renally excreted agents. TDF (or FTC/TDF fixed-dose combination) should be avoided in participants with concurrent or recent use of a nephrotoxic agent, in accordance with the Viread® package insert
Neuroleptics			↑perphenazine, ↑risperidone, ↑thioridazine	A dose decrease may be needed for these drugs when co-administered with RTV containing regimen.
Niacin				Niacin increases the risk of insulin resistance even in non-diabetic individuals. It should therefore be avoided as first-line therapy in participants receiving PIs.
Sedatives/ hypnotics			↑buspirone, ↑clonazepam, ↑diazepam, ↑estazolam, ↑flurazepam, ↑zolpidem	A dose decrease may be needed for these drugs when co-administered with RTV.
Steroids			Dexamethasone, prednisone	Systemic dexamethasone induces CYP3A and can thereby decrease DRV plasma concentrations. This may result in loss of therapeutic effect to DRV.
Thalidomide				In multiple myeloma patients, the risk of renal dysfunction may be increased when zoledronate is used in combination with thalidomide.

## 5.5 Adherence Assessment

Antiretroviral adherence will be assessed by pill counts during each study visit or by a brief adherence

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questionnaire at intervals during the study.

## 6 CLINICAL AND LABORATORY EVALUATIONS

### 6.1 Schedule of Events

**Schedule of Events: Table 3**

Follow up weeks →	Screening	Entry	2	4	8	12	16	20	24	36	48	72	96	120	144	Prem. D/C
<b>Clinical Parameters</b>																
Documentation of HIV-1	x															
Medical/Medication History	x															
Clinical Assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical Exam (complete)	x															
Physical Exam (targeted)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant medication		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adherence Assessment			x	x	x	x	x	x	x	x	x	x	x	x	x	x
<b>Study Specific Intervention</b>																
HAART (ATV/r + FTC/TDF)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Zoledronic acid		x														
<b>Study Specific Specimens</b>																
Fasting Blood (plasma)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Fasting Blood (PBMC)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DEXA-Scan	x					x			x		x		x		x	
<b>HIV Treatment Outcome Labs</b>																
Plasma HIV-1 RNA	x	x		x			x		x	x	x	x	x	x	x	x
CD4+/CD8+	x	x		x			x		x	x	x	x	x	x	x	x
<b>Safety Lab. Tests</b>																
Hematology (complete CBC)	x	x	x			x			x	x	x	x	x	x	x	x
Liver Function Tests (chem14)*	x	x	x			x			x	x	x	x	x	x	x	x
Serum vitamin D	x	x	x			x			x	x	x	x	x	x	x	x
Serum intact Parathyroid hormone (iPTH)		x	x			x			x	x	x	x	x	x	x	x
Calculated CrCl	x	x	x			x			x	x	x	x	x	x	x	x
Urinalysis	x	x	x			x			x	x	x	x	x	x	x	x
Pregnancy Testing	x	x							x		x		x		x	
†Stored blood for genetic testing		x														

- \*Chem 14 includes serum calcium, protein, albumin, liver function test, blood urea nitrogen, creatinine, glucose, bicarbonate, chloride, and sodium.
- Perm. D/C = premature study discontinuation
- †Blood sample for genetic testing can be collected anytime during the study visits if not collected at entry.

## 6.2 Definitions for Schedule of Events – Timing of Evaluations

### 6.2.1 Screening Evaluations

Screening evaluations will occur prior to the participant's starting any study medications.

#### Screening

Screening evaluations to determine eligibility will be completed within 60 days prior to study entry, unless otherwise specified.

NOTE: Screening plasma HIV-1 RNA will be obtained and performed locally within 90 days prior to study entry.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a screening log and entered into the study database.

### 6.2.2 Entry Evaluations

Entry evaluations will occur within 60 days of screening (unless otherwise specified) and completed prior to the initiation of study medications. Randomization will occur at the entry visit, and subjects will begin treatment within 7 days after randomization.

### 6.2.3 Post-Entry Evaluations

#### On-Treatment Evaluations

On-treatment evaluations will occur after entry. Study visits will be scheduled on the weeks listed in the Schedule of Events (Table 3) and within the windows specified below.

- The week 2 and 4 visit will occur +/- 3 days.
- The weeks 8, 12, 16, and 20 visits will occur +/- 7 days of the week indicated.
- Beginning at week 24, visits will occur +/- 14 days of the week indicated.

#### Event-Driven Evaluations

##### Confirmation of Virologic Failure Evaluations

For participants who have a sample suggesting virologic failure, a confirmatory plasma HIV-1 RNA test will be performed within 2 weeks after the initial HIV-1 RNA sample that indicated virologic failure as per standard of care at the Grady IDP. If confirmation cannot be obtained within 2 weeks, the next viral load obtained will be considered the confirmatory one. Participants who meet criteria for the first episode of confirmed virologic failure will undergo real-time genotypic resistance testing as per standard of care (not provided by the study). Participants with confirmed virologic failure will be evaluated for antiretroviral regimen change, and may continue in the study.

#### 6.2.4 Discontinuation Evaluations

##### Evaluations for Registered Participants Who Do Not Start Study Treatment

Registered participants who do not start study treatment will be discontinued from the study, and will be replaced by new participants. All CRFs will be completed and entered into the study database for the period up to discontinuation and including week 0.

##### Premature Treatment Discontinuation Evaluations

Premature discontinuation of study treatment is defined as permanently stopping all study treatment prior to study completion.

All participants who discontinue their study regimens before the end of the study will come in for a premature discontinuation of study regimen visit within 14 days after stopping their antiretroviral therapy.

##### Study Completion Evaluations

##### Final Visit

All registered participants, who either complete the study or who prematurely discontinue participation in the study, will be requested to report to the clinic to have the final visit evaluations performed.

### 6.3 Definitions of Evaluations

#### 6.3.1 Documentation of HIV

HIV-1 infection will be documented by any serologic test and confirmed by a positive western blot or plasma HIV-1 RNA performed by any laboratory that has a CLIA certification.

#### 6.3.2 Medical History

A medical history will be recorded in source documents.

Any allergies to any medications and their formulations will be documented and entered on the CRFs.

#### 6.3.3 Medication History

A medication history including HIV treatment history, current HIV treatment, and treatment history of any prescription medication taken for the treatment or prophylaxis of opportunistic infections will be recorded in source documents including start and stop dates.

All medications including prescription, non-prescription, alternative therapies, and dietary supplements taken within 30 days of the screening visit, and all medications currently taken will be recorded on the CRFs, including start and stop dates.

#### 6.3.4 Concomitant Medications

Any changes in concomitant medications since the previous visit will be recorded in the source documents and CRFs, including start and stop dates. .

#### 6.3.5 Clinical Assessments

##### Complete Physical Exam

A complete physical examination will be performed at screening only and will include at a minimum an examination of the skin, HEENT (head, eyes, ears, nose, and throat), mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; examination of the extremities; neurologic exam, and Karnofsky performance test. The complete physical exam will also include signs and symptoms, diagnoses, height, weight, and vital signs (axillary or oral temperature [Fahrenheit], pulse, and blood pressure).

##### Targeted Physical Exam

A targeted physical examination will be performed at every visit beginning at entry and is to be driven by any previously identified or new signs or symptoms including diagnoses that the participant has experienced since the last visit. It is to include weight, vital signs (axillary or oral temperature [Fahrenheit], pulse, and blood pressure).

##### Signs and Symptoms

At entry, all grades that occurred 30 days before entry will be recorded. Post-entry and at the time of confirmation of virologic failure, only signs and symptoms Grade  $\geq 3$  will be recorded. All signs and symptoms that led to a change in treatment, regardless of grade will be recorded. The source document will include date of onset and date of resolution.

Signs and symptoms will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004, which can be found on the DAIDS RCC Web site: [http://rcc.tech-res.com/tox\\_tables.htm](http://rcc.tech-res.com/tox_tables.htm).

##### Diagnoses

At screening and entry, all HIV-related and non-HIV-related confirmed and probable diagnoses will be recorded in the source documents. During the study, all confirmed and probable diagnoses made since the last visit will be reviewed and recorded in the source documents, including current status at the time of study visit.

The following will be recorded on the source documents: HIV-related diagnoses, HIV-related malignancies, AIDS-defining events, IRIS events, tuberculosis at any site, hepatitis, pancreatitis, coronary artery disease (myocardial infarction or angina), diabetes mellitus, and death. Any other diagnosis that is, in the opinion of the clinical investigators, associated with study medications, will be recorded on the source documents. The source document will include date of diagnosis and date of resolution.

##### Concomitant Medications

All changes in the following medications will be recorded on the CRFs: ARV medications, medications for treatment or prophylaxis of HIV infection or complications, interferon, ribavirin, lipid-lowering drugs, oral hypoglycemics, and insulin.



At each study visit, all concomitant medications taken since the last visit will be recorded in the source documents. For lipid-lowering drugs, oral hypoglycemics (including any drug used to treat diabetes), and insulin, the source document will include start dates and stop dates.

#### Study Treatment Modifications

All study drug modifications will be recorded, including initial doses, participant-initiated and/or protocol-mandated modifications, and inadvertent and deliberate interruptions at each visit. Any permanent discontinuation of treatment will be recorded. Treatment interruption is failure for any cause to take study drugs for more than 48 hours.

#### 6.3.6 Laboratory Evaluations

Laboratory tests will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004, which can be found on the DAIDS RCC Web site: [http://rcc.tech-res.com/tox\\_tables.htm](http://rcc.tech-res.com/tox_tables.htm).

At screening and entry, all laboratory values will be recorded. For post-entry assessments, all Grade  $\geq 3$  laboratory values will be recorded. All laboratory toxicities that led to a change in treatment, regardless of grade, will be recorded.

All the following laboratory results (regardless of grade) will be recorded on the CRFs each time they are performed, according to the protocol Schedule of Events:

- Complete blood count
- Chemistry profile (including albumin and calcium)
- Serum vitamin D
- Serum intact parathyroid hormone (iPTH)
- HIV-1 RNA PCR
- CD4+/CD8+ T-cell counts
- Creatinine
- Calculated Creatinine Clearance\*

\*(See <https://www.fstrf.org/common/utilities/calculators/ccr.html> for the Cockcroft-Gault equation).

#### Hematology

Hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelet count will be performed in real time at the local laboratory.

#### Blood Chemistries

Blood urea nitrogen (BUN), creatinine, and electrolytes (sodium, potassium, chloride, and bicarbonate) will be performed in real time at the local laboratory.

### Liver Function Tests (LFTs)

Total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase will be performed in real time at the local laboratory.

### Plasma HIV-1 RNA

Screening HIV-1 RNA will be performed locally using Abbott HIV-1 Real Time assay within 90 days prior to study entry by a local laboratory certified for that assay.

Eligibility will be determined based on the screening value. The screening value for all registered participants will be  $\geq 1000$  copies/ml.

HIV-1 RNA quantitation will be measured from entry forward in real time using the Abbott Real Time HIV-1 Monitor assay.

### CD4+/CD8+

CD4+/CD8+ counts and percentages performed by flow cytometry at a CLIA-certified or equivalent lab will be recorded in the source document and on the CRFs at each visit.

#### 6.3.7 DEXA Scan

A single, total-body DEXA scan will be performed +/-14 days at screening and at weeks 12, 24, 48, 96 and 144.

- Since food in the stomach can alter weight and body composition results, fasting 8 hours prior to the DEXA scan is preferable. If a participant must take food with medications or for medical reasons, it should be a small snack (toast or crackers) with, at most, 8 oz of fluid at least 2 hours before the scan.
- DEXA scans will be performed on the same machine using the same software for each individual participant for the duration of the study.
- Screening for pregnancy prior to the DEXA scans will be performed in compliance with the requirements of the radiology department.

#### 6.3.8 Collection of samples to be used as a source of DNA for future analyses

A Blood sample (~10mL) may be collected from each participant after their consent. Participants who consent will be asked to contribute DNA to be archived in the following manner.

Whole blood will be collected to fill a 2 x 7.5 mL plastic Sarstedt brand K3 EDTA Monovettes, following manufacturer's instructions for use (Product Number 01.1605.100). If possible, this specimen will be obtained at the entry visit or at 1 other scheduled visit if not obtained at entry. After filled with blood, Monovettes will be stored at  $-80^{\circ}\text{C}$  until analysis.

## **7 CLINICAL MANAGEMENT ISSUES**

### **7.1 General Consideration**

All participants will remain under the care of their primary care physicians (PCP) throughout the study period. Patients will undergo routine clinical and laboratory evaluations and monitoring for complications of antiretroviral therapy under the care of their PCP as per the standard of care at the Grady IDP. Parameters assessed for HIV-infected patients during routine clinic visits at the Grady IDP Clinic include general health, vital signs, complete CBC with differential, clinical chemistries, and immunologic studies (CD4+ and CD8+ lymphocyte counts). Patients' PCPs will have access to research records if considered necessary for patients' care.

### **7.2 Potential Risk**

In addition to starting antiretroviral therapy as part of subjects' standard treatment for HIV-infection, subjects will also be exposed to either a single dose of i.v. zoledronic acid. Zoledronic acid is approved by the Food and Drug Administration (FDA) for the treatment of osteoporosis and has been used with success in the setting of HIV-infection and antiretroviral therapy [15 - 19]. Subjects will be monitored closely for complications related to these agents and managed appropriately as per standard of care for managing these complications.

Subjects enrolled into this study will be exposed to the risk of needle puncture during blood sample collection. The risks associated with vein punctures are uncommon and may include small blood clots in a vein, and infection at the site of needle puncture. Other uncommon risks also include pain, bruising, soreness, and reaction to tapes at the site of needle insertion. Some people may experience a fainting spell with needle puncture. These risks occur only on rare occasions and the likelihood for serious harm is extremely low.

Bone densitometry by DXA is a completely non-invasive procedure that is routinely used for medical purposes. It utilizes extremely low levels of X-rays that is equal to or less than the natural environmental radiation the average person receives in the United States annually. The principal risk associated with a radiation dose is the possibility of developing a radiation-induced cancer later in life or harm to the fetus in a pregnant woman. There are however currently no studies that show an increase in the risk of genetic mutation in the next generation of offspring. The risk from radiation exposure from DXA scan is considered to be negligible when compared to everyday risks, thus there is consequently no credible risk of injury from this procedure.

### **7.3 Minimization of Risk**

The blood will be drawn by research personnel with experience in this procedure. Universal precautions will be employed in all instances involving human specimens and samples processed assumed infectious and blood processed under at least BSL2 conditions. Women who may be pregnant will be excluded from participation in this study because of possible effects of radiation exposure on their unborn child.

### **7.4 Toxicity Management**

Toxicities related to study intervention will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004, located at the Division of AIDS Regulatory Compliance Center Web Site: <http://rcc.tech->

[res.com/tox\\_tables.htm](#). Study related toxicity will be recorded by treatment arms, monitored closely by study team, and management by the standard care at the Grady IDP.

#### 7.5 Vitamin D Deficiency/Low Serum Calcium Levels

Serum intact parathyroid hormone, calcium and vitamin D levels will be monitored periodically as outlined in the schedule of event. Subject found to have albumin corrected calcium levels < 9.0 mg/dL will be placed on calcium replacement therapy. Similarly, subject with serum vitamin level  $\leq 20$  ng/dL will be started on vitamin D replacement therapy per standard of care.

#### 7.6 Development of Osteoporosis

DXA scans will be monitored at multiple pre-defined time points during the study. If a subject develops a T-score of  $< -2.5$  at any point after entry into the study, it will be necessary that the subject be unblinded in order that this condition can be appropriately managed. Unblinding will be conducted in a manner that keeps the study team (clinical, basic science, and statistical) blinded. Subjects will be referred for appropriate treatment by their primary care physician. Subjects treated with calcium and/or vitamin D may remain in the study and be followed per study protocol until study completion. Subjects treated with a bisphosphonate or related drugs with bone modifying effects, however, will be permanently discontinued from the study.

#### 7.7 Pregnancy

Women who become pregnant while on study will be followed for safety evaluation until the completion of all study visits. Blood draw at each visit will be limited to safety lab (chem.14, CBC, CD4 T-cell counts, HIV-1 RNA, serum vitamin D, serum intact parathyroid hormone (iPTH)) as per the standard of care for the management of pregnant patient on HAART at the Ponce Clinic and according to national (US Public Health or other) guidelines. Collection of blood for study specific end points (fasting PBMC & plasma) and DXA scan will be discontinued.

## 8 CRITERIA FOR DISCONTINUATION

#### 8.1 Permanent Treatment Discontinuation

- Requirement for prohibited concomitant medications (see section 5.4).
- Pregnancy or breast-feeding.
- Completion of treatment as defined in the protocol.
- Request by participant to terminate treatment.
- Clinical reasons believed life threatening by the clinician, even if not addressed in the toxicity section of the protocol.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the IRB, FDA or other government regulatory authorities, Office for Human Research Protections (OHRP), investigator, or pharmaceutical supporter.

#### 8.2 Premature Study Discontinuation

- Failure by the participant to attend three consecutive clinic visits.

- Request by the participant to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.
- Participant judged by the clinician to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the IRB, FDA or other government regulatory authorities, OHRP, investigator, or pharmaceutical supporter.

## **9.0 STATISTICAL CONSIDERATIONS**

### **9.1 General Design Issues**

This is a single center, prospective, blinded placebo-controlled, randomized study designed to evaluate the mechanism of HAART induced bone loss in HIV-infected individuals, and to assess if this effect of HAART on bone can be reversed by long acting inhibitors of bone resorption.

### **9.2 Summary of Endpoints**

9.2.1 Serum viremia: HIV-1 RNA PCR by commercially available Abbott HIV-1 Real Time assay.

9.2.2 Bone mineral density by DXA –scan: lumbar spine (L1-L4) and left proximal femur, femur neck and left hip.

9.2.3 Serum biochemical markers of bone formation and resorption by ELISA

- Markers of bone formation (Osteocalcin and P1NP).
- Markers of bone resorption (CTx and TRAP5b).

9.2.4 Quantitation of plasma cytokines and bone regulating hormones by ELISA.

- RANKL (Osteoclast Inducer)
- OPG (Osteoclast Suppressor)
- $\text{TNF}\alpha$  (Inflammatory Marker)
- IL-6 (Inflammatory Marker)
- IL-1 (Inflammatory Marker)
- PTH (2° Hyperparathyroid Marker)
- Vitamin D
- Testosterone
- $17\beta$ Estradiol

9.2.5 Metabolic Function Markers

- Liver function assessed by serum levels of total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase will be performed in real time at the local laboratory.
- Renal function assessed by creatinine clearance (Cockcroft-Gault equation).

## 9.2.6 Quantitation of B cell and T cell numbers and percentage in PBMC by flow cytometry.

### 9.2.6.1 T cell Subsets: (% and total/ml)

- CD3+, CD4+, CD8+, CD4+ CD25+ FoxP1+
- Memory & Naïve T cells Markers:
  - Memory (CD4+ CD45RO+ CD27+)
  - Naïve (CD4+ CD45RO- CD27+);
- CD4 Activation Markers:
  - CD4+ CD69+; CD4+ CD25+ FoxP1-
- T cell Proliferation:
  - CD3+ Ki67+; CD4+ Ki67+; CD8+ Ki67+
- T Cell Cytokine Expression:
  - CD4+ RANKL; CD4+ OPG; CD4+ TNF $\alpha$ ; CD4+ IL-17; CD4+ CD40L, CD4+ IL-4; CD4+ IFN $\gamma$ , CD4+ CD25+ FoxP1+ RANKL, CD4+ CD25+ FoxP1 OPG
  - CD8+ RANKL; CD8+ OPG; CD8+ TNF $\alpha$

### 9.2.6.2 Monocytes/M $\phi$ /OC Precursors (% and total/ml) --- CD14

- Osteoclast Precursors: (% and total/ml) --- c-Fms+ RANK+
- Monocyte Proliferation:---- CD14+ Ki67+
- Monocyte Cytokine Expression: ---- CD14 + TNF $\alpha$

### 9.2.6.3 B Cell Subsets (% and total/ml)

- CD19+, CD20+, B220
- Memory and Naïve B cells Markers
  - Memory (CD19<sup>+</sup>CD27<sup>+</sup>) &
  - Naïve (CD19<sup>+</sup>CD27<sup>-</sup>)
  - Immature transitional (CD10<sup>-</sup> CD21<sup>+</sup> CD27<sup>-</sup> B220)
  - Mature plasma cells (CD19<sup>low</sup>, CD38<sup>+</sup> CD27<sup>++</sup>)
- B cell Proliferation:
  - CD19+ Ki67+; CD21+ Ki67+
- B Cell Activation Markers:
  - CD10<sup>-</sup>, CD21<sup>low</sup> CD27<sup>+</sup>
- B Cell Cytokine Expression:
  - Memory: CD19<sup>+</sup>CD27<sup>+</sup> RANKL; CD19<sup>+</sup>CD27<sup>+</sup> OPG
  - Naïve: CD19<sup>+</sup>CD27<sup>-</sup> RANKL; CD19<sup>+</sup>CD27<sup>-</sup> OPG
  - Others: CD10<sup>-</sup> CD21<sup>+</sup> CD27<sup>-</sup> RANKL; CD10<sup>-</sup> CD21<sup>+</sup> CD27<sup>-</sup> OPG
    - Immature transitional B cells (CD10<sup>++</sup>CD21<sup>low</sup> CD27<sup>-</sup>)
    - Naïve mature B cells (CD10<sup>-</sup>, CD21<sup>hi</sup> CD27<sup>-</sup>)
    - Activated mature B cells (CD10<sup>-</sup>, CD21<sup>low</sup> CD27<sup>+</sup>)
    - Exhausted tissue-like memory B cells (CD10<sup>-</sup>, CD21<sup>low</sup> CD27<sup>-</sup>)
    - Resting memory B cells (CD10<sup>-</sup>, CD21<sup>hi</sup> CD27<sup>+</sup>)

- Short-lived plasmablasts (Ki-67<sup>+</sup>CD19<sup>+</sup>CD10<sup>-</sup> CD20<sup>-</sup>CD21<sup>low</sup> CD27<sup>++</sup>)
- Mature plasma cells (CD19<sup>low</sup>, CD38<sup>+</sup> CD27<sup>++</sup>)
- Memory subsets (CD19<sup>+</sup>CD27<sup>+</sup>B220<sup>+</sup> and CD19<sup>+</sup>CD27<sup>+</sup>B220<sup>-</sup>)

9.2.7 Quantitation of total plasma and peripheral blood B cell and T cell OPG & RANKL mRNA by real time RT-PCR and protein production by ELISA.

9.2.8 Number, frequency and quantitation of OPG and RANKL production by B cell subsets and by T cell subsets (CD4 and CD8) by intracellular staining.

9.3 Detailed Description of Endpoints:

In its current design the study is powered to detect changes in plasma levels of the bone marker CTx following HAART initiation and the inhibitory effect of bone resorption inhibitors on these changes. However, a major focus of this study is to understand the cellular (lymphocytic OPG/RANKL expression) changes associated with HIV/HAART induced bone loss and how these changes can be pharmacologically modified.

9.3.1 Primary Endpoints:

To assess HAART induced acute change in bone turnover by quantitating the change from baseline to week 24 in plasma level of marker of bone resorption (CTx) among treatment naïve HIV-infected subjects treated with atazanavir based HAART regimen.

9.3.2 To quantitate the inhibitory effect of the long acting inhibitor of bone resorption (zoledronic acid) on HAART-induced change in the marker of bone resorption (CTx) at 24 weeks of HAART therapy.

9.4 Secondary Endpoints:

9.4.1 To quantitate the change from baseline at weeks 2, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120, 144 in cellular (lymphocyte subsets; B- and T-cell frequency and number/ml) and their production of OPG and RANKL, among treatment naïve HIV-infected subjects treated with atazanavir based HAART regimens.

9.4.2 To evaluate the inhibitory effect of single dose zoledronic acid on HAART associated changes in other markers of bone turnover (Osteocalcin, P1NP, (bone formation markers) and TRAP5b (a marker of osteoclast activity) and BMD at appropriate study evaluation time points.

9.4.3 To quantitate the inhibitory effect of antiresorptive (zoledronic acid) on HAART-induced change in cellular (lymphocyte subsets; B- and T-cell) production of OPG and RANKL at weeks, 2, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120, and 144 of HAART therapy.

9.4.4 To correlate changes in serum, and cellular (lymphocyte subsets; B- and T-cell) expression of OPG and RANKL with markers of bone turnover and BMD at appropriate study evaluation time points.

9.4.5 To correlate changes in bone regulatory hormones (PTH, estrogen, testosterone, vitamin D) and inflammatory cytokines ( IL-1, IL-6, TNF $\alpha$ ) with serum and cellular expression of



OPG/RANKL, markers of bone turnover, and BMD at appropriate study evaluation time points.

9.4.6 To correlates changes in lymphocyte subsets; (B- and T-cell (CD4 and CD8) with bone turnover markers and BMD.

## 9.5 Randomization and Stratification

Subjects will be blindly assigned in equal proportions to one of the two treatment arms using permuted block randomization with stratification by screening HIV-1 RNA (<100,000;  $\geq$ 100,000 copies/mL). The purpose of the stratification is to ensure balance between treatment arms in terms of HIV-1 RNA status at screening. Additionally, to ensure balanced treatment allocation as well as balance across the three study arms in relation to age, randomization will also be stratified by subjects' age (<40;  $\geq$  40 years); and by sex (defined as sex at birth).

## 9.6 Sample Size Estimation:

As osteoclastic differentiation and activity is largely a function of the RANKL/OPG ratio we speculate that these cytokines will be related to plasma levels of the markers of bone resorption such as CTx. As there are no comparable published studies of the impact of HAART on cellular expression of bone regulating cytokines in treatment naïve HIV-infected subjects, Power estimation is based on preliminary data from a recent unpublished study from our group. In this study, we examined biochemical markers of bone resorption CTx and serum OPG concentrations in treatment naïve HIV/AIDS patients (n=9) at baseline and prospectively at 2, 12 and 24 weeks of treatment with lopinovir/ritonavir + tenofovir/emtricitabine. The observed mean serum CTx level at baseline was 3.29  $\mu$ g/L. The observed change of serum CTx from baseline to 24 weeks was 2.06  $\mu$ g/L, approximately a 60% increase (observed SD of change = 1.37  $\mu$ g/L). Because the sample size for the above referenced unpublished work is small, and as such variability around mean change is wide, we have assumed a more conservative change in serum CTx with HAART in the design of the current study. Table 4 shows sample size required to detect changes in serum CTx from baseline to week 24. Setting  $\alpha$  level at 0.05 and using a 2-sided one-sample t-test on the difference of means, a sample size of 31 subjects in the HAART arm will achieve 90% statistical power to detect a mean change of 0.85 (20% change from baseline; effect size = 0.6) in serum CTx from baseline to week 24. The standard deviation estimate for the change used in the power calculation is 1.4.

Table 4: Power needed to detect increase in CTx from baseline to week 24 of HAART in treatment naïve HIV patients

	Power	Mean change ( $\mu$ g/L)	% change	Effect size	Sample size
1	90%	0.40	10%	0.3	131
2	85%	0.40	10%	0.3	112
3	80%	0.40	10%	0.3	99
4	90%	0.55	14%	0.4	71
5	85%	0.55	14%	0.4	61
6	80%	0.55	14%	0.4	53
7	90%	0.70	17%	0.5	44
8	85%	0.70	17%	0.5	38



9	80%	0.70	17%	0.5	34
<b>10</b>	<b>90%</b>	<b>0.85</b>	<b>20%</b>	<b>0.6</b>	<b>31</b>
11	85%	0.85	20%	0.6	27
12	80%	0.85	20%	0.6	24

Similarly as shown in table 5, a sample size of 31 subjects per arm will achieve a statistical power of 90% to detect a difference of 1.20  $\mu\text{g/L}$  in serum CTx between HAART alone or HAART + zoledronic acid at week 24 of HAART ( $\alpha=0.05$ ; using a 2-sided 2-sample t-test). Observed change in serum CTx from baseline to 24 weeks (2.06  $\mu\text{g/L}$ ) was used to estimate mean change for the HAART + placebo group. Observed SD (1.4  $\mu\text{g/L}$ ) was used as an estimate of standard deviation for both groups. Given an attrition rate of 50% in due to the prolonged duration of the study, 94 total subjects may be needed to achieve the desired sample size (47 in each of 2 groups). The sample size was estimated by PASS 2008 software. No formal interim analyses are planned for this trial.

Table 5: Power estimation for mean difference in serum CTx between treatment arms

	Power	Mean increase: HAART/Placebo	Mean increase: HAART/Reclast	Detectable difference ( $\mu\text{g/L}$ )	Sample size (per group)
1	90%	2.00	2.80	0.80	66
2	85%	2.00	2.80	0.80	56
3	80%	2.00	2.80	0.80	50
4	90%	2.00	3.00	1.00	43
5	85%	2.00	3.00	1.00	37
6	80%	2.00	3.00	1.00	32
<b>7</b>	<b>90%</b>	<b>2.00</b>	<b>3.20</b>	<b>1.20</b>	<b>30</b>
8	85%	2.00	3.20	1.20	26
9	80%	2.00	3.20	1.20	23

## 9.7 Statistical Analyses:

### 9.7.1 General Analysis Consideration:

Unless otherwise specified, analyses will be carried out with an intention-to-treat (ITT) approach where subjects will be analyzed as randomized. All analyses will use a 5% type I error for significance testing and present data with 95% confidence intervals.

Continuous variables (e.g., OPG and RANKL, etc) will be summarized with standard descriptive statistics and represented graphically with displays such as scatterplots while categorical variables will be described by frequency tables. The appropriate distributional assumptions will be checked. The data will be transformed for the analysis, if necessary. Prior to implementation of simple linear regression to investigate the relationship between outcomes and predictor variables of interest, all assumptions will be assessed. Scatterplots and residual analyses will be used to help assess nonlinearity of the

relationship and the appropriateness of the assumptions of normality for each outcome and constant variance of the outcome. If these assumptions are violated, alternative methods such as data transformations (e.g., natural log) or the use of nonparametric regression such as Spearman rank correlation coefficient, kernel smoothing, spline fitting and LOESS techniques will be used.

#### 9.7.2 Baseline and cross-sectional analyses:

Mean CTx level, BMD, OPG and RANKL will be compared between groups at baseline using ANOVA to ensure comparability between groups. Standard demographic and clinical variables (viral load, CD4 count, age, sex, race, etc.) will also be compared between groups at baseline.

Cross-sectional analyses will be performed with baseline CTx level, BMD, OPG and RANKL variables. Simple linear regression will be used to investigate the relationship between these outcomes and predictor variables of interest, such as age, gender, race and BMI.

Additionally, a cross-sectional analysis will be performed comparing baseline markers of bone turnover of HIV-positive patients in this study to similar data collected from an HIV-negative cohort study (Emory University IRB Study # 00027210). Analysis of covariance (ANCOVA) will be used to compare the HIV-negative patients and HIV-positive patients. Initially, we will assume that each outcome (e.g., CTx, BMD, OPG and RANKL) can be expressed as a linear function of age (X, covariate) with possible different slopes and intercepts for each subgroup. Assuming a common linear slope model is justified by our analyses, an age-adjusted mean will be estimated for each outcome (CTx, BMD, OPG and RANKL). An age-adjusted mean of the HIV-negative or HIV-positive group will be defined as the predicted response value obtained by evaluating the regression equation for each group at the mean age for the 2 groups.

#### 9.7.3 Initial exploratory analyses of CTx versus RANKL over time, by treatment group:

The rate of change in longitudinally obtained measurements will be determined by a linear mixed effects model (a random-coefficients model) in which the regression of CTx on time is assumed to be linear, with a random intercept and slope for each patient (fit for each treatment group). The same regression model will be fit for RANKL. We expect to see a linear relationship over time for CTx and RANKL for the HAART treatment group but we not expect to see a linear relationship for the other two treatment groups. Assuming there will be a linear relationship with time for the two endpoints (CTx and RANKL) for the HAART group we will fit the random coefficients model in which the regression of CTx on RANKL is assumed to be linear with a random intercept and slope for each patient.

To estimate the correlation between the rates of change in CTx and RANKL, a bivariate linear random-effects will be fit allowing estimation of the correlation between the true underlying intercepts and slopes for CTx and RANKL.

#### 9.7.4 Change over time in markers of bone turnover and BMD, within and between groups:

Mean change in bone markers and BMD from baseline to weeks 2, 8, 12, 16, 20, 24, 36, 48, 72, 96, 120, and 144 their 95% confidence intervals will be estimated for each of the treatment arm. Change over time will be assessed using an analysis of repeated

measures data to properly account for the correlation between multiple observations from the same patient. Two commonly used models to analyze such data will be employed: the mixed-effects model and the generalized estimating equations (GEE) approach proposed by Liang and Zeger (1986). Both approaches allow for covariate adjustment. The results will be summarized with adjusted means and 95% confidence intervals for continuous outcomes. The GEE methodology can also be used to perform logistic regression of repeated binary responses within patients.

Repeated-measures analyses, using mixed linear models will be performed for bone markers (listed in section 9.2) and percent change from baseline with a means model using SAS Proc Mixed (version 9) providing separate estimates of the means by time on study (2, 8, 16 weeks etc) and treatment group. A compound symmetry variance-covariance form among the repeated measurements from each treatment group will be assumed for each outcome, and robust estimates of the standard errors of parameters (Diggle et al.) will be used to perform statistical tests and construct confidence intervals. The model-based means are unbiased with unbalanced and missing data, as long as the missing data are non-informative (missing at random – see Shih JW). Statistical tests will be 2-sided. A P value  $\leq 0.05$  will be considered statistically significant for the main effects (time effect and treatment group effect) and the statistical interaction between treatment group and time on study from the repeated measures analysis for each outcome. A Bonferroni adjustment will be used for the pairwise comparisons at each time point if there is a statistically significant interaction between treatment and time on study since the main effects are not directly interpretable in the presence of a statistical interaction. Results from these models will focus on the magnitude of the differences. Data will be summarized using adjusted means plus 95% confidence intervals and observed mean differences plus 95% confidence intervals. Since the sample sizes will be small for each subgroup we will focus the interpretation on the magnitude of the mean differences and confidence intervals.

A potential problem in any of the above analyses is missing data, as missing data may cause the usual statistical analysis of complete or all available data to be subject to bias. Every effort will be made to minimize missing data on outcomes and covariates. The required follow-up for this clinical trial is 144-weeks and we expect listed lost to follow-up (<10%) for the primary endpoints. There are no universally applicable methods for handling missing data. If missing data becomes an issue then we will conduct sensitivity analyses to encompass different scenarios of assumptions and discuss consistency or

#### 9.7.5 Amendment to Statistical Analysis:

This double-blind, placebo-controlled, 2-group parallel clinical study was originally designed as a fixed sample size ITT trial and each patient was to be followed for 144 weeks after randomization. We have decided to change the design of the study and follow all randomized subjects for the first 24 weeks at which point the study will be unblinded to study team, data analyzed and findings published. We will however continue to follow all enrolled patients as a cohort for the originally planned 144 weeks study duration.

## 10 EXPECTED OUTCOMES AND INTERPRETATION OF DATA

Based on our preliminary data in rats we hypothesis that a switch in B cell OPG to RANKL production during HIV/AIDS progression will lead to increased osteoclastic bone resorption and diminished BMD. The

expected outcome is thus that HIV-infected individuals will display diminished BMD that correlates positively with decreased total plasma and B cell OPG, and increased indices of bone resorption (CTx and TRAP5b). Diminished BMD is expected to correlate positively with increased plasma and B cell and/or T cell RANKL and the latter positively with bone resorption markers. We further anticipate that baseline CD4 T-cell counts and plasma HIV-RNA PCR (both surrogates of disease severity) will correlate positively (possible linearly) with the magnitude of bone loss and with indices of bone resorption (CTx and TRAP5b) and with plasma and B cell and/or T cell RANKL production. We expect that plasma HIV-RNA PCR will correlate negatively with plasma OPG and B cell OPG production and with total B cell (and/or T cell) number.

Based on our preliminary data in human, we also expect acute exacerbation of bone turnover following initiation of HAART. Thus pre-existing diminished BMD associated with HIV-infection will be further diminished at the onset of antiretroviral therapy. We speculate that the mechanism of HAART induced bone loss may be mediated through either direct effect on bone cells, or through indirect effect on metabolism related to disease reversal and immune reconstitution. We therefore expect HAART related bone loss to correlates positively with decreased total plasma and B cell OPG, and increased indices of bone resorption (CTx and TRAP5b). Diminished BMD in the setting of HAART is also expected to correlate positively with increased plasma and B cell and/or T cell RANKL and the latter positively with bone resorption markers. Because the effect of HAART on bone homeostasis occurs early in therapy, we anticipate a complete reversal or amelioration of this effect with long-acting inhibitors of bone resorption.

An additional outcome that is consistent with our hypothesis is that a specific B cell population or subpopulation may correlate with decreased OPG production and/or increased RANKL production. For example there is reported to be a relative increase in immature translational B cells, activated mature B cells, exhausted memory B cells and plasmablasts with HIV viremia and these cells may account for RANKL production. By contrast naïve mature B cells, resting memory B cells and CD27<sup>+</sup> B220<sup>-</sup> are diminished and could account for decreased OPG production. Alternatively, altered cell function and not absolute number could explain these differences. We may also find that specific subtypes of B cells make both RANKL and OPG or that different cell types make either RANKL or OPG. If such outcomes correlate strongly the percentage of the relevant B cell subtypes may be a useful diagnostic marker for predicting, and intervening to prevent, the future development of bone loss in HIV patients.

T cells are known to secrete RANKL and could contribute RANKL to the final state, although the decline in T cells with viremia would be expected to eventually moderate any such effect. Consequently RANKL production may correlate positively with T cell numbers but negatively with viremia. As T cells are known to regulate B cells functions, including OPG production and given their importance as a target of HIV action, our basic analysis of T cell number and function may provide important information relevant to our hypothesis and to inform future studies.

## **11 DATA COLLECTION, MONITORING AND ADVERSE EXPERIENCE REPORTING**

### **11.1 Records to Be Kept**

CRFs will be provided for each subject. Subjects will not be identified by name on any CRFs. Subjects will be identified by the participant identification number (PID) and study identification

number (SID) provided by the study Data Manager upon registration.

## 11.2 Role of Data Management

11.2.1 Instructions concerning the recording of study data on CRFs will be provided by the study Data Management Coordinator.

11.2.2 It will be the responsibility of the Data Management Coordinator to assure the quality of computerized data. This role extends from protocol development to generation of the final study databases.

11.2.3 A centralized, secured, web-based relational database for data acquisition and storage will be developed for the BLIR-HIV clinical trial. Study investigators and biostatistics personnel will work jointly to develop paper versions of Case Report Forms (CRF's) for the study. The data will be entered into electronic case report forms (eCRF) and saved in a database using web services. Microsoft's Structured Query Language (MS SQL) server 2000 database will be used for data collection and storage. All data will be maintained in a database that is fully compliant with Emory University's HIPAA policy and is subject to audit by the University Compliance Office. Data checks will be done at time of data entry and a data audit trail will track changes made to the database. Data validation including valid codes, chronological date checks, value ranges and integrity checks will be performed on eCRF as data are entered. A comparison tool will be used to ensure data quality and accuracy. Database edit checking programs will check data for data completeness of each CRF, check for validity between forms, and check whether these data were collected and key-entered in the study protocol's prescribed time frame. Data access and security rules will be established for all study personnel. Data reports will be generated each month to identify missing or extreme values using the database query engine and SAS software tools. These reports will be sent to the study coordinator who will verify data with source documents; errors will then be corrected and logged in the master database.

11.2.4 The data entry screens of the application will mimic the paper Case Report Forms for efficient data entry. The data entry forms will be GUI – based- with list boxes, radio-buttons, check boxes, and formats for data fields. The GUI-based forms will make data entry simple, fast and minimize error.

## 11.3 Clinical Site Monitoring and Record Availability

11.3.1 Clinical site monitors may be performed by the Emory University IRB, FDA or other government regulatory authorities, OHRP, or pharmaceutical supporters. Clinical research sites monitor may include the review of the individual participant records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts) to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors may also inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

11.3.2 The investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the OHRP, or the pharmaceutical supporter or the supporter's designee for

confirmation of the study data.

#### 11.4 Expedited Adverse Event Monitoring and Reporting

Adverse events (AEs) will be reported on an expedited basis at the standard level during the protocol-defined expedited adverse event (EAE) Reporting Period, which is the entire study duration for an individual subject (from study enrollment until study completion or discontinuation of the subject from study participation for any reason).

##### 11.4.1 AE Reporting

Any AE that is reported to either the investigators or their designated research associates by a study subject or by medical staff caring for the subject and which meets the criteria will be documented.

In addition, clinical investigators will monitor subjects for AEs and laboratory abnormalities during each study visit. Any AE will be reported to the Emory University IRB within 10 days of the event, and any serious adverse event (SAE) will be reported to the IRB within 24-48 hours of the event. The standard Emory IRB reporting guidelines for AE and SAE reporting, as documented at [http://www.emory.edu/IRB/guidelines\\_adverse\\_event.php](http://www.emory.edu/IRB/guidelines_adverse_event.php), will be followed.

A SAE is an adverse drug experience that results in any of the following outcomes:

- Death.
- Life-threatening situation - The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the AE were more severe or were to progress.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity - Any AE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- Congenital anomaly/birth defects - Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- Important medical events/experiences that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above, i.e., death, a life-threatening adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.



- Spontaneous and elective abortions will be reported to the Emory IRB as serious adverse events.

Severity of AEs will be rated according to the following definitions:

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Table 8. Severity of Adverse Events Rating

<u>Mild:</u>	The adverse event is transient and easily tolerated by the subject.
<u>Moderate:</u>	The adverse event causes the subject discomfort and interrupts the subject's normal activities.
<u>Severe:</u>	The adverse event causes considerable interference with the subject's normal activities and may be incapacitating or life-threatening.

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The following definitions will be used to assess the relationship of the AE to study drugs:

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Table 9. Relationship of Adverse Events to Study Medications

<u>Probably Related:</u>	An adverse event has a strong temporal relationship to study drug or recurs on re-challenge, and another etiology is unlikely or significantly less likely.
<u>Possibly Related:</u>	An adverse event has a strong temporal relationship to the study drug, and an alternative etiology is equally or less likely compared to the potential relationship to study drug.
<u>Probably Not Related:</u>	An adverse event has little or no temporal relationship to the study drug and/or a more likely alternative etiology exists.
<u>Not Related:</u>	An adverse event is due to an underlying or concurrent illness or effect of another drug and is not related to the study drug (e.g., has no temporal relationship to study drug or has much more likely alternative etiology).

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If the adverse event is in, the investigator's opinion, possibly or probably related, or not related to study drug, then an alternate etiology will be provided by the investigator.

It should however be noted that a SAE/experience is not necessarily serious, as the term severe is a measure of intensity while a serious adverse event is determined based on the aforementioned regulatory criteria.

All AEs and laboratory abnormalities will be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004, which can be found on the DAIDS RCC Web site: [http://rcc.tech-res.com/tox\\_tables.htm](http://rcc.tech-res.com/tox_tables.htm). All AEs will be followed to satisfactory clinical resolution as previously described in section 7.1.

## 12 HUMAN SUBJECTS

### 12.1 Responsibilities of the Investigator

In implementing this protocol, the investigators will adhere to the basic principles of “Good Clinical Practice” as outlined in CFR 312, subpart D, “Responsibilities of Sponsors and Investigators,” CFR 21, part 50 and CFR 21, part 56 and Section 4 of ICH Harmonized Tripartite Guideline for GCP.

### 12.2 IRB Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the Emory University IRB. A signed consent form will be obtained from the subject (or parent, legal guardian, or person with power of attorney for subjects who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this fact will be documented in the subject's record.

### 12.3 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the OHRP, or the pharmaceutical supporter or the supporter's designee.

### 12.4 Data Safety Monitoring

Summaries of deaths, AEs (Grade 3 or 4), and targeted AEs across arms as well as study conduct (in terms of off-study rates, off-treatment rates, and data completeness) will be reviewed on a regular basis by the study investigators. Any AE will be reported to the Emory University IRB within 10 days of the event, and any serious adverse event (SAE) will be reported to the IRB within 24-48 hours of the event. The standard Emory IRB reporting guidelines for AE and SAE reporting, as documented at [http://www.emory.edu/IRB/guidelines\\_adverse\\_event.php](http://www.emory.edu/IRB/guidelines_adverse_event.php), will be followed.

The tolerability of zoledronic acid in HIV infected patients on HAART was recently documented in two large randomized clinical trials [17, 18]. No SAEs or acute AEs specifically due to the study drug infusion are expected in the current study. However, additional safety monitoring will be performed annually by an independent Medical Safety Monitor. Based on the 3 year accrual expectation for the study, it is anticipated that the study will undergo 3 reviews by the Medical Safety Monitor. The first will occur approximately one year after the accrual of the first subject. The Data



Coordinating Center (DCC) will produce a safety report for the Medical Safety Monitor to review. The safety report will summarize AEs, SAEs, and lab toxicities by blinded treatment group. The DCC will work with the Medical Safety Monitor to complete a 'final assessment' following the review of each safety report. As part of the final assessment the Medical Safety Monitor will conclude 'the study can continue as no safety concerns have been identified at the time of the review' or 'the study cannot continue as currently designed'. The final assessment by the Medical Safety Monitor will be provided to the study PI who will make the findings available as appropriate to the Emory IRB, the FDA, and the NIH.

#### 12.5 Study Discontinuation

The study may be discontinued at any time by the IRB, the pharmaceutical supporter, the OHRP, or other government agencies as part of their duties to ensure that research subjects are protected.

#### 12.6 Potential Benefits of the Proposed Research to the Subjects and Others

The immediate benefit of the study to the subjects is uncertain at this time, however, osteoporotic patients may be identified and treated early. The knowledge gained in this research proposal is very important as the underlying mechanisms that cause osteoporosis in treatment naïve HIV/AIDS patients remains completely unknown. As the mean age of the HIV/AIDS population continues to trend upwards impaired bone turnover is likely to lead to an onslaught of fractures in the future as age-associated effects on bone mass combine with virally induced factors compounding bone loss. A considerable amount of pre-clinical data has been generated in mice to support our hypothesis that the B cell plays a cardinal role in protection against osteoporosis and that B cell aberrations associated with HIV effects on the immune system may be the driving force behind HIV-associated osteoporosis. However, this pre-clinical information has not yet been translated to humans. This research project is an important first step into human investigation of B cells and their role in HIV/HAART associated osteoporosis. The potential benefit of long-acting inhibitors of bone resorption in the setting of HIV/HAART related bone loss will be tested. The risk to the individual subject is minimal to moderate while the benefit to the future standard of care of HIV/AIDS patients may be very large.

### 13 PUBLICATION OF RESEARCH FINDINGS

Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical supporters and other sponsors (if requested) prior to submission.

### 14 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72, and as specified by individual carrier guidelines, e.g., Federal Express, Airborne Express, for specific instructions.

### 15 STUDY DURATION/TIMELINE

Subjects enrolled in this study will be followed for a total period of 144 weeks. We anticipate the duration of this study from beginning of accrument to completion to be 5 years.

## **16 STUDY SPONSOR**

This study is sponsored by the grant awarded by National Institute of Health (#1R01AR059364-01 and 1R01AG40013-1A1). Study medication (zoledronic acid: reclast®) is provided by Novartis.

## **17 FUTURE DIRECTION**

The fact that treatment naïve HIV-1 patients develop low BMD, compounded by treatment with Specific formulations of HAART is now well established. However, the mechanisms responsible remain undefined and enigmatic. Based on our pre-clinical preliminary studies we anticipated that we will ratify a specific mechanism underlying the bone loss present in treatment naïve HIV/AIDS patients involving an imbalance in the RANKL/OPG ratio, a direct consequence of a switch in OPG to RANKL production by B cells and possible RANKL production through activated T cells. We further hope to identify specific populations of B cells responsible for this effect. Should this be the outcome future investigations will test the utility of utilizing changes in B cell number, function or subsets, as a diagnostic test for the development of osteoporosis and for increased fracture risk in those groups of patients. Long term goals will be the restoration of bone protective immune cell populations and/or restoration of normal bone turnover, using pharmacological or other means.

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