Clinical Protocol:

A randomised open-label study comparing the safety and efficacy of ritonavir boosted lopinavir and 2-3N(t)RTI backbone versus ritonavir boosted lopinavir and raltegravir in participants virologically failing first-line NNRTI/2N(t)RTI therapy: the SECOND-LINE study

Bone and Body Comp: A sub study of the SECOND-LINE study

Version 1.0
8th February 2010

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## 1. SYNOPSIS

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<td><strong>Protocol Number</strong></td>
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<td><strong>Participating sites</strong></td>
<td>This sub-study is open to sites participating in the SECOND-LINE study.</td>
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<td><strong>Patient Population</strong></td>
<td>HIV-infected subjects enrolled into the SECOND-LINE study.</td>
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| **Treatment arms and duration** | Arm 1. Lopinavir / ritonavir + 2-3N(t)RTI  
Arm 2. Lopinavir / ritonavir + raltegravir  
Subjects will be followed for 96 weeks. |
| **Research Hypotheses** | Body Fat: HIV-infected subjects randomised into the experimental arm (LPV/r + RAL) will demonstrate greater increases in limb fat over 48 weeks than those randomised into the control arm (LPV/r + 2-3N(t)RTIs).  
Bone: HIV-infected subjects randomised into the experimental arm (LPV/r + RAL) will demonstrate smaller reductions in BMD at the proximal femur over 48 weeks than those randomised into the control arm (LPV/r + 2-3N(t)RTIs). |
| **Primary Objectives** | Body Fat: To determine the difference in mean limb fat changes (absolute and percentage change) as measured by DXA scan between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 48 weeks.  
Bone: To determine the difference in BMD changes at the proximal femur (absolute and percentage change) as measured by DXA scan between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 48 weeks. |
| **Secondary objectives** | Body Fat: To determine the mean change in regional and total body fat between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.  
To determine the percentage of study subjects with >20% alterations in limb fat over 48 and 96 weeks between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA.  
Bone: To determine and compare the mean percentage change in proximal femur and lumbar spine BMD between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.  
To determine and compare the percentage of participants with low BMD (Z-score less than -2), osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score less than -2.5) between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 96 weeks.  
To determine and compare the mean percentage change from baseline in Z-score and T-score between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to weeks 48 and 96. |
raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.

To estimate and compare changes in 10-year fracture risk using the FRAX equation between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 96 weeks.

To determine the added predictive value for fracture risk that is provided by BMD assessment using the FRAX® risk assessment tool.

To relate changes in BMD to changes in bone metabolism biomarkers (osteocalcin, CTX, OPG, RANK/L, vitamin D and parathyroid hormone), body composition (total and regional body fat) and clinical demographics.

### Trial plan

Collection of demographic and bone specific clinical information, sample collection and whole body DXA and DXA of proximal femur and lumbar spine will be performed at baseline, week 48 and week 96.

### Statistical analyses

#### Body fat:
The primary comparison will be percentage change in limb fat from baseline to w48. Secondary analyses will include between-group differences in change from baseline (to weeks 48 and 96), change between weeks 48 and 96 in total fat, trunk fat and lean mass, proportion of patients experiencing >20% reduction in limb fat (with and without corresponding changes in trunk fat) and proportion of patients meeting the definition of lipodystrophy according to the Lipodystrophy Case Definition Score.

#### Bone:
The primary between-group comparison will be percentage change proximal femur BMD from baseline to w48. Secondary analyses will include between-group differences in change from baseline (to weeks 48 and 96) and change between weeks 48 and 96 in BMD, T-score and Z score at proximal femur and lumbar spine, percentage of patients with diagnosis of low BMD (by Z-score), osteopenia and osteoporosis (by T-score) at proximal femur and lumbar spine and incident fractures.

Changes in BMD and regional and total body fat will be correlated to determine if there are relationships between changes in body fat and BMD after correction for clinical demographics and risk factors for low BMD.

Additional analyses include estimation of 10-year risk for osteoporotic fracture using FRAX® risk calculator both with and without BMD data to estimate the change in fracture risk before and after inclusion of BMD. A series of reclassification measures will then be utilised to quantify the added predictive value for fracture risk that is provided by BMD assessment.

Analysis will be primarily by intent-to-treat. A per-protocol analysis will also be performed.

Endpoints will be analysed using ANOVA methods or non-parametric equivalents with no adjustments made for multiple comparisons.

### Site requirements

DXA scan (Hologic / LEXOSS / lunar or equivalent) with software capabilities to perform both whole body and regional DXA scans.
2. INTRODUCTION

2.1. Background

The advances in the treatment of HIV-infected individuals, particularly since the introduction of highly active anti-retroviral therapy (HAART), have significantly decreased the morbidity and mortality of this infection. However, excess mortality and morbidity from non-AIDS illnesses in HIV-infected patients is of ongoing concern, with conditions such as cardiovascular disease, bone disease and abnormalities of fat and lipid metabolism prevalent in HIV-infected patients. Both HIV infection itself and long term exposure to antiretrovirals (ART) have been implicated in the pathogenesis of these common conditions; body fat abnormalities (1-4) and bone alterations (5-8) can affect as many as 50% of individuals receiving ART.

2.2. Body fat alterations

Abnormalities of fat and lipid metabolism have long been linked to use of ART. Loss of subcutaneous adipose tissue (lipoatrophy) is associated with exposure to thymidine analogue nucleoside reverse transcriptase inhibitors (tNRTI) (1-4, 9-12) whilst changes in circulating lipoproteins have been demonstrated with use of all three major classes of ART (protease inhibitors [PI], NRTI and non-nucleoside reverse transcriptase inhibitors [NNRTI]) although the pattern of changes differ between the three classes (9, 13-17).

Although exposure to PI was initially thought to drive development of lipoatrophy, subsequent data suggested a more important role for tNRTI its pathogenesis and recent data suggest little potential for the development of lipoatrophy when PI are used without NRTI in antiretroviral naïve patients (Moyle, Castle Study, EACS 2009, LBPS11/6). Similarly, use of raltegravir in antiretroviral naïve patients has not been associated with significant changes in body composition (DeJesus, ICAAC 2009 H-1571).

In patients who have been exposed to NRTI, switching from tNRTI to non-thymidine NRTI improves limb fat (18-21) albeit slowly and with incomplete restoration for many patients. Less data is available on the effect of NRTI-sparing regimens on limb fat in patients previously exposed to NRTI (21-23) with most examining the combination of a PI + NNRTI, use of which is associated with a worrying trend toward increased virological failures (24-25).
2.3. Bone alterations

Low bone mineral density (BMD) and osteoporosis are prevalent in HIV-infected patients with more osteoporosis-related fractures experienced in HIV-infected patients than the general population. Although cross sectional studies suggest this increased prevalence to be associated both with HIV infection itself in addition to a greater prevalence of traditional risk factors for low BMD in HIV-infected patients (such as smoking, low BMI and steroid use), studies in ART naïve patients initiating ART have consistently demonstrated loss of BMD over the first 48 weeks of exposure to ART, with greater loss experienced by those initiating tNRTI and PI. Little is known on the effects of exposure to integrase inhibitors and BMD.

It is unclear if this initial loss of BMD is related to exposure to ART, immune restoration or arises as a consequence of altered inflammatory responses resulting from control of HIV viraemia. If the latter is the case, then additional loss of BMD may occur in the setting of virological failure and use of second line ART.

2.4. Study rationale

The SECOND-LINE study provides an opportunity to examine if NRTI-sparing ART regimens containing integrase inhibitors (experimental arm of SECOND-LINE [LPV/r + RAL] result in greater restoration of limb fat than combinations containing NRTI (control arm of SECOND-LINE [LPV/r + 2-3N(t)RTI]) in HIV-infected patients previously exposed to NRTI.

This study also provides an opportunity to examine if additional bone loss occurs with second line treatment of HIV infection and whether NRTI-sparing ART used in second line therapy results in greater or less bone loss than use of PI/NRTI combinations (control arm of SECOND-LINE [LPV/r + 2-3N(t)RTI]).

3. RESEARCH HYPOTHESES

Body Fat:
HIV-infected subjects randomised into the experimental arm (LPV/r + RAL) will demonstrate greater increases in limb fat over 48 weeks than those randomised into the control arm (LPV/r + 2-3N(t)RTIs).

Bone:
HIV-infected subjects randomised into the experimental arm (LPV/r + RAL) will demonstrate smaller reductions in BMD at the proximal femur over 48 weeks than those randomised into the control arm (LPV/r + 2-3N(t)RTIs).

4. OBJECTIVES

4.1 Primary Objective

Body Fat:
4.2 Secondary Objectives

Body Fat:
- To determine the mean change in regional and total body fat between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.
- To determine the percentage of study subjects with >20% alterations in limb fat over 48 and 96 weeks between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA.

Bone:
- To determine and compare the mean percentage change in proximal femur and lumbar spine BMD between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.
- To determine and compare the percentage of participants with low BMD (Z-score less than -2), osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score less than -2.5) between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 96 weeks.
- To determine and compare the mean percentage change from baseline in Z-score and T-score between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART as measured by DXA from baseline to weeks 48 and 96.
- To estimate and compare changes in 10-year fracture risk using the FRAX equation between LPV/r + 2-3N(t)RTI and LPV/r + raltegravir based ART from baseline to 96 weeks.
- To determine the added predictive value for fracture risk that is provided by BMD assessment using the FRAX® risk assessment tool.
- To relate changes in BMD to changes in bone metabolism biomarkers (osteocalcin, CTX, OPG, RANK/L, vitamin D and parathyroid hormone), body composition (total and regional body fat) and clinical demographics.
5. STUDY POPULATION

There will be approximately 52 investigative centres participating in the main Second Line protocol. All sites will be offered this sub-study but only those with the available infrastructure will participate. Pregnant or breast feeding mothers are excluded from entering the main protocol as well as this sub-study. If a woman becomes pregnant during the study, they will not participate in this sub-study.

6. METHODOLOGY

6.1 Clinical Demographics and Fracture Risk

In addition to demographics collected as part of the main study protocol, the following information will be collected at baseline; country of birth, ethnicity, socioeconomic status (employment status, maximum educational grade achieved, annual income, number of dependents) previous and incident fractures (including low-impact fractures) and risk factors for osteoporosis (smoking status, family history of hip fracture, hypogonadism, last menstrual period (to estimate onset of menopause / menarche), lifetime steroid exposure, alcohol intake and secondary causes of osteoporosis), recreational drug use (including anabolic steroids), medication exposure (including exposure to heparin, opiates and oestrogen replacement) and estimates of physical activity.

At weeks 48 and 96 the following additional information will be collected; socioeconomic status (employment status, maximum educational grade achieved, annual income, number of dependents), incident fractures (including low-impact fractures) change in risk factors for osteoporosis (smoking status, family history of hip fracture, hypogonadism, last menstrual period (to estimate onset of menopause / menarche), lifetime steroid exposure, alcohol intake and secondary causes of osteoporosis), recreational drug use (including anabolic steroids), medication exposure (including exposure to heparin, opiates and oestrogen replacement) and estimates of physical activity.

6.2 DXA scan

6.2.1 Body Composition

Whole body DXA will be performed at weeks 0, 48 and 96 using the protocol specified in appendix 1. Scans should be performed within 2 weeks of the study visit.

6.2.2 Bone Mineral Density

DXA scan for BMD of the proximal femur and lumbar spine will be performed at weeks 0, 48 and 96 using the protocol specified in appendix 2. Scans should be performed within 2 weeks of the study visit.
6.3 Sample storage
The list of biomarkers will be decided upon by the Bone and Body Comp sub group of the Protocol Steering Committee upon completion of the primary comparisons. Assays will be performed batched at the end of 96 weeks. These assays will be performed using stored samples already being collected as part of the main SECOND-LINE study protocol.

7. STATISTICAL ANALYSIS

Body fat:
The primary comparison will be percentage change in limb fat from baseline to w48.

Secondary analyses will include between-group differences in change from baseline (to weeks 48 and 96), change between weeks 48 and 96 in total fat, trunk fat and lean mass, the proportion of patients experiencing >20% reduction in limb fat (with and without corresponding changes in trunk fat) and the proportion of patients meeting the definition of lipodystrophy according to the Lipodystrophy Case Definition Score. Secondary analyses will control for age, gender and baseline BMI.

Bone:
The primary between-group comparison will be percentage change proximal femur BMD from baseline to w48. Secondary analyses will include between-group differences in change from baseline (to weeks 48 and 96) and change between weeks 48 and 96 in BMD, T-score and Z score at proximal femur and lumbar spine, percentage of patients with diagnosis of low BMD (by Z-score), osteopenia and osteoporosis (by T-score) at proximal femur and lumbar spine and incident fractures.

Changes in BMD and regional and total body fat will be correlated to determine if there are relationships between changes in body fat and BMD after correction for clinical demographics and risk factors for low BMD.

Additional analyses include estimation of 10-year risk for osteoporotic fracture using FRAX® risk calculator both with and without BMD data to estimate the change in fracture risk before and after inclusion of BMD. A series of reclassification measures will then be utilised to quantify the added predictive value for fracture risk that is provided by BMD assessment.

Analysis will be primarily by intent-to-treat (defined as all study subjects who undergo randomisation and who receive at least one dose of study medication) in which subjects will be compared regardless of the treatment received. A per-protocol analysis will also be performed (defined as all subjects from the intent-
to-treat population excluding those who changed randomly assigned antiretroviral therapy for any reason other than the HIV RNA exceeding 200 copies/ml. Endpoints will be analysed using ANOVA methods or non-parametric equivalents with no adjustments made for multiple comparisons.

8. SAMPLE SIZE

Target recruitment is 100 subjects per arm.

Body fat:
From data derived from the INITIO study, inclusion of 100 subjects per arm would provide 80% power to detect a mean difference between study arms of 1 kg (SD = 2.5 kg) in limb fat and 2.4% in limb fat percentage.

Bone:
Assuming a normal age related change in BMD of 1.8% over 96 weeks (0.9% per year is standard change in adults >40 years old) with a standard deviation of 6%, inclusion of 100 subjects per arm would provide 80% power to detect a between group difference in change in BMD over 48 and 96 weeks of 1.7%, a clinically relevant difference at the lower end of that observed in clinical trials (18, 20, 22).

9. FUNDING

The sponsor, NCHECR, will fund this study.

10. REFERENCES

8. Hoy J, Hudson J, Law M, Cooper DA. Osteopenia in a randomized multicenter study of protease inhibitor substitution in patients with the lipodystrophy syndrome and well - controlled HIV viremia.


Appendix 1. Lunar DXA procedure for whole body, proximal femur and lumbar spine DXA scans.

Appendix 2. Hologic DXA procedure for whole body, proximal femur and lumbar spine DXA scans.

Appendix 3. Sample patient information statement and consent form.