Lipid changes on Protease Inhibitors in South Africa

A retrospective cross-sectional analysis comparing metabolic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Johannesburg Academic Hospital

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine.

Johannesburg, 2022

DECLARATION

I, Robyn Bruce declare that this Research Report is my own, unaided work. It is being submitted for the degree of Master of Medicine in the Department of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Signature: RH Bruce……

Date: 22/12/2022..……

Place: Johannesburg..……

CONFERENCE PROCEEDINGS/ PUBLICATIONS

1. Publications: Submitting to the South African Journal of HIV Medicine
2. Poster presentation: Planning to submit to SEMDSA 2023, Johannesburg
3. Poster presentation: Planning to submit to SA HIV Clinicians Society Conference 2023, Cape Town

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr F Mohamed, Prof F Venter and Dr S Stacey, for their valuable guidance and endless support throughout the preparation of this report.

Secondly, I would like to thank my family and husband for the continuous encouragement and patience throughout this journey.

ABSTRACT

Background

People living with HIV (PLWH) are at risk of cardiovascular (CV) disease, with few studies having assessed CV risk in Southern Africa. Protease inhibitors (PIs) are known to cause dyslipidaemia, in particular, lopinavir more so than darunavir. The WRHI 052 study by Venter et al, a randomised parallel-group open label non-inferiority phase 3 trial, at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), demonstrated that low dose darunavir/ritonavir (400 mg/ 100 mg) sustained viral load suppression equivalently to lopinavir/ritonavir (800 mg/ 200 mg). This could be a cost-effective option with improved metabolic effects.

Objective:

To assess CV risk screening in PLWH receiving two different PI based antiretroviral therapy (ART) regimens and exploring the difference in the metabolic profiles between the two PI groups.

Methods:

A retrospective sub-analysis of the WRHI 052 study. Between June 30 2016, and June 15 2017, 300 PLWH on a lopinavir/ritonavir regimen were randomized into two groups, 152 continued the lopinavir/ritonavir combination and 148 were switched to darunavir/ritonavir. The metabolic parameters of the two PI arms were compared at 24 and 48 weeks. A 2-year post study completion retrospective analysis was performed at the CMJAH HIV clinic where routine care was continued. An analysis of CV risk using the Framingham CV risk score, lipid management and achievement of low-density lipoprotein cholesterol (LDLC) goals was performed.

Results:

In the darunavir/ritonavir group, a significant decrease in total cholesterol (TC) of 7.7% at 48 weeks (95% CI, p<0.001) was observed, and the TC/HDLC ratio declined at 24 and 48 weeks by 1.7% and 3.9% respectively (95% CI, p=0.09; p=0.99). The TC increased by 0.4% at 48 weeks in the lopinavir/ritonavir group (95% CI, p=0.01) and the TC/HDLC ratio increased by 3.3% and 0.9% at 24 and 48 weeks (95%CI, p=0.04 and p=0.12). There was no statistical significance between the median differences and median percentage difference in TC/HDLC ratios between PI groups. LDLC decreased significantly in both PI groups at 24 and 48 weeks, however there was no statistical significance between PI groups. Weight and consequently body mass index (BMI) increased over time with a consistent trend in both groups and genders. Due to weight gain, the percentage of participants with a normal BMI declined from 41.5% at baseline to 33% and 35% at 24 and 48 weeks. Eighty-three percent of all participants had dyslipidaemia with 22% receiving lipid lowering therapy. Eighty-five percent of all participants had a low Framingham CV risk score with 52% achieving target LDLC.

Conclusion

TC and TC/HDLC ratio improved when switched to darunavir/ritonavir. Significant weight gain was observed in participants on a PI over time. Longitudinal studies on weight gain in individuals on PIs are warranted. Increased awareness and assessment of CV risk is needed.

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LIST OF ABBREVIATIONS

ART: Anti-retroviral therapy

ASCVD: Atherosclerotic cardiovascular disease

ASCVE: Atherosclerotic cardiovascular event

BMI: Body mass Index

CKD: Chronic kidney disease

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CT: Computed Tomography

CV: Cardiovascular

CVD: Cardiovascular disease

CYP450: Cytochrome P450

D:A:D: Data collection on Adverse effects of anti-retroviral Drugs study

DM: Diabetes mellitus

DTG: Dolutegravir

EFV: Efavirenz

ESC/EAS: European Society of Cardiology and European Society of Atherosclerosis

eGFR: Estimated glomerular filtration rate

HBA1c: Glycated haemoglobin A1c

HDLC: High-density lipoprotein cholesterol

HIV: Human immunodeficiency virus

II: Integrase Inhibitor

IIs: Integrase Inhibitors

INTREPID: HIV-infected patients and treatment with pitavastatin vs pravastatin for Dyslipidaemia

IQR: Interquartile range

LLT: Lipid lowering therapy

LDLC: Low-density lipoprotein cholesterol

LMIC: Low and middle-income countries

NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III

NNRTI: Non-nucleoside reverse transcriptase inhibitor

NNRTIs: Non-nucleoside reverse transcriptase inhibitors

NRTI: Nucleoside reverse transcriptase inhibitor

NRTIs: Nucleoside reverse transcriptase inhibitors

PI: Protease Inhibitor

PIs: Protease Inhibitors

PSCK 9: Proprotein convertase subtilisin/kexin type 9

PLWH: People living with HIV

SCORE: Systematic Coronary Risk Estimation System

SA: South Africa

TB: Tuberculosis

TC: Total cholesterol

TG: Triglycerides

VL: Viral load

WHO: World Health Organisation

CHAPTER 1: PROTOCOL WITH EXTENDED LITERATURE REVIEW

1. Extended literature review

1.1. Introduction

Human immunodeficiency virus (HIV) places a significant burden on the healthcare systems worldwide. At the end of 2019, there were over 38 million people living with HIV (PLWH). Africa accounts for approximately two thirds of this burden, with East and Southern Africa having close to 21 million PLWH (1). South Africa (SA) had approximately 7.5 million PLWH with an incidence of about 200,000 new infections in 2019 (2). New HIV infections is on the downward trend with the success and widespread availability of anti-retroviral therapy (ART), since the incidence rate topped in 1998 (1).

In SA, ART is easily obtainable by all PLWH with early initiation of ART advised (3). Approximately 5.2 million PLWH are receiving ART in SA (2). ART reduces HIV related infections and improves quality of life for PLWH (4). Nowadays, PLWH are at risk of non-communicable diseases in particular atherosclerotic cardiovascular disease (ASCVD). There is cumulative literature in developed countries that PLWH are more at risk of an atherosclerotic cardiovascular event (ASCVE) in contrast to HIV negative individuals once other ASCVD risk factors are accounted for (5). Many metabolic disorders that increase the risk for an ASCVE have been linked to HIV and certain classes of ART, however data from South African PLWH have not shown a greater risk of ASCVD compared to HIV negative individuals (6-8).

1.2. Current anti-retroviral therapy guidelines

The World Health Organization's (WHO) most recent ART policy recommends the use of two nucleoside reverse transcriptase inhibitors (NRTIs) and the integrase inhibitor (II) dolutegravir for first line ART in low and middle-income countries (LMICs) (9). The use of dolutegravir in first line ART has been applied to SA's latest adult ART guidelines (3). Until 2019, before dolutegravir became more easily accessible, the preferred first line ART regimen included two NRTIs with a non-nucleoside reverse transcriptase inhibitor (NNRTI), which was primarily efavirenz (10, 11). Advised second line ART has also subsequently changed. If dolutegravir was used then the use of a protease inhibitor (PI) together with a switch of NRTIs, is advised. The advised PIs in order of preference being atazanavir, lopinavir, or darunavir all of which are boosted with ritonavir. If an NNRTI was used in first line therapy then dolutegravir is advised with changing of the NRTI regimen (9). However, prior to 2019 and the widespread availability of dolutegravir, a PI was used in second line ART in SA (10, 11).

1.3. Metabolic complications associated with HIV

Dyslipidaemia is a well-recognised metabolic complication associated with HIV. It occurs in both individuals who have never been exposed to ART and those on ART (12).

Glucose intolerance is another complication associated with HIV itself but more so with ART.(13) In SA, glucose intolerance is noted to be more prevalent in those on PIs (14). Diabetes mellitus (DM) in PLWH is diagnosed most accurately using an oral glucose tolerance test and not fasting plasma glucose or glycosylated haemoglobin

(HBA1c) (15). A systematic review and metanalysis of insulin sensitivity on PIs revealed there were no changes in insulin sensitivity in PLWH taking the PIs darunavir and atazanavir but there was reduced insulin sensitivity observed in PLWH taking lopinavir (16).

Lipodystrophy is a cosmetically unappealing metabolic side effect of ART. It was seen more commonly in individuals who were prescribed older NRTI regimens, which included thymidine analogues, and especially individuals who started ART with lower pre-ART CD4 counts. Although, there is no exact clinical definition, it is a well-recognised entity that is comprised of a pattern of peripheral fat loss (lipoatrophy), central fat accumulation and areas of subcutaneous fat accumulation (lipomatosis) (17, 18). All PIs have been associated with causing lipodystrophy, but the older PIs have been implicated more than the PIs, atazanavir and darunavir, particularly when boosted with ritonavir. Lopinavir/ritonavir does not cause lipodystrophy to the same extent as efavirenz when used in combination with the NRTIs zidovudine and lamivudine. There is minimal data on lipodystrophy being caused by contemporary ART such as entry inhibitors or IIs (19).

Recently, substantial weight gain has been associated with the use of current ART, such as the NRTIs, tenofovir alafenamide and abacavir, and PIs, but more so with IIs, in particular dolutegravir (20-22). Weight gain has particularly been described in PLWH who are black, female and started ART with lower CD4 counts and higher viral loads (VL). PLWH that gained more weight than others had a correlation of initiating current ART with a normal BMI or pre-obese BMI, and not with a starting point of an obese BMI classification (20, 22, 23). A phase 3 open labelled randomized control trial in SA

conducted by Venter et al demonstrated that substantial weight gain has been associated with the use of dolutegravir. In particular, excessive weight gain occurred in PLWH using dolutegravir in combination with the NRTIs tenofovir alafenamide and abacavir (21, 24). Mechanisms for this increase in weight gain were previously thought to be a “return to normal’ phenomenon, which may have been the case in earlier years, but more recently it is suggested to be due to less gastro-intestinal side effects from a boosted PI and ART regimens that are more tolerable. More research on the exact mechanism of newer ART causing substantial weight gain, is needed (20, 22-24).

Radiological data from computed tomography (CT) angiograms has shown that PLWH have a higher frequency of plaques in the coronary vessels as compared to HIV negative individuals, with a similar frequency of calcified plaques in both groups and a much higher frequency of non-calcified plaques found in PLWH (5, 25). Non-calcified plaques have a unique structure; a thin fibrous cap surrounding a necrotic core. These features make these plaques more susceptible to rupture and can cause an ASCVE, specifically a myocardial infarction (5, 25). Ultrasound of the carotid arteries has also shown that PLWH have an increased carotid intima-media thickness. This is a valuable indicator of ASCVD and has been found more often to be increased in PLWH on PIs (26).

1.4. Definition of dyslipidaemia

Guidelines from the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) suggest that dyslipidaemia is defined as having any one or more of the following lipid derangements:

i. a total cholesterol (TC) value of greater than 5.2 mmol/L;

ii. a low-density lipoprotein cholesterol (LDLC) of greater than 2.5 mmol/L;
iii. a triglycerides (TG) value of greater than 1.7 mmol/L; or
iv. a high-density lipoprotein cholesterol (HDLC) value of less than 1.0 mmol/L (12).

1.5. Causes of dyslipidaemia in PLWH

Dyslipidaemia in PLWH is multi-factorial which involves the acute and chronic inflammatory viral states of HIV, ART, nutrition, genetics and now ART induced weight gain (4, 21).

In the acute or unsuppressed viral states, HIV viral replication can be associated with a reduction in TC and LDLC but is characterised predominantly by low HDLC and a raised TG value (27). The rise in TG is due to the acute inflammatory response that is associated with an increase in pro-inflammatory cytokines and reduced lipoprotein lipase activity (12, 28).

There is ongoing chronic inflammation in an individual who has achieved HIV VL suppression. This is due to persistent activation of T cells, inflammatory cytokines, monocytes, macrophages, an increase in coagulation markers, T regulation cell abnormalities as well as unique changes in lipid subclasses (29, 30). All of these and many other factors in both the innate and adaptive immune system play a role in increasing ASCVD despite HIV VL suppression (29).

In terms of ART, NRTIs and in particular thymidine analogues, such as stavudine and zidovudine, raise predominantly TG, and to lesser extent TC and LDLC (11, 31). Other

more commonly used NRTIs such as tenofovir, abacavir and lamivudine have a minor effect on lipids (31).

The NNRTI, efavirenz, raises TC and TGs (11). Efavirenz is the NNRTI of choice recommended by the WHO for alternative first line therapy in combination with two NRTIs in LMICs, when dolutegravir is not available. The previous recommended dose was 600 mg, but this has now been changed to 400 mg as it has been shown to have less severe adverse effects whilst still sustaining VL suppression (9). However, when the dose is reduced further to 200 mg, there is a statistically significant reduction in TC and LDLC, whilst still maintaining the primary aim of VL suppression (32).

The class of PIs are particularly responsible for lipid disorders (8). Many forms of mixed lipid disorders are seen in individuals that are on PIs (33). It is suggested that ritonavir, which is also a PI and is used to boost the effect of other PIs, causes dyslipidaemia (27).

It is well recognised that certain PIs cause endothelial dysfunction which leads to atherosclerosis via complex molecular mechanisms (34). This is usually caused by older PIs which includes lopinavir boosted with ritonavir (34, 35). The PIs darunavir and atazanavir have a less toxic effect on the endothelium. Atazanavir has a moderate effect whilst darunavir has a mild to almost no effect on endothelial function. The dose of ritonavir has also been shown to increase the degree of endothelial dysfunction when used in combination with other PIs. Darunavir had very little effect on the endothelium when used alone, however when used with ritonavir there was more

endothelia dysfunction noted, and subsequently the higher the dose of ritonavir the more endothelial dysfunction was shown (35).

In terms of PIs advised for use in second line ART, lopinavir boosted with ritonavir raises TC levels to a greater extent than when compared to alternative PIs, atazanavir or darunavir boosted with ritonavir (33). Lopinavir boosted with ritonavir raises TG levels, however this increase is never significant enough to cause complications such as pancreatitis, unless there is a co-existing genetic abnormality (36). Ritonavir boosted atazanavir is the PI combination of choice due to its improved gastrointestinal and lipid side effect profile. It is known to cause an unconjugated hyper-bilirubinaemia which is cosmetically unappealing. A challenge is that atazanavir cannot be used with rifampicin, which is needed to treat tuberculosis. Rifampicin, which is an important anti-tuberculosis drug, increases the action of cytochrome P450 (CYP450) 3A4 which is responsible for the metabolism of PIs, therefore increasing drug metabolism and reducing atazanavir levels, with a consequential decrease in VL suppression (37). Lopinavir boosted with ritonavir is double dosed when used with rifampicin to ensure adequate drug levels (11).

Darunavir is a recommended PI that has a better side effect profile and an improved lipid profile. The recommended dose of darunavir boosted with ritonavir is 800 mg and 100 mg respectively for second line ART. For third line ART, darunavir boosted with ritonavir can be used at this dose if there are no darunavir resistant associated mutations, otherwise darunavir 600 mg with ritonavir 100 mg twice daily is needed (38). However, the increased costs and greater drug interactions seen with rifampicin have limited its use in LMICs (11).

In terms of preferred PIs, observational studies have shown a link to ASCVD with the use of darunavir boosted with ritonavir, particularly with collective use over time (39, 40). An important observational study that demonstrated this is a large multi-centre observational study by Ryom et al, which utilised data from the Data collection on Adverse effects of anti-retroviral Drugs (D:A:D) study (40). There was a slow increase in ASCVD risk shown over time, it was present even with the removal of confounders, and it is postulated that the increased risk of ASCVD with darunavir may be dose related (39, 40). In contrast, there is no increase in ASCVD when atazanavir boosted with ritonavir is used over a collective period of time. This is due to the protective effects of raised unconjugated bilirubin. This demonstrates that dyslipidaemia is not a class adverse effect of all PIs. It must be noted that these are observational studies and randomised control trials are needed (39-41).

Genetics is a factor to consider as a cause of dyslipidaemia in some PLWH on PIs. There is evidence that certain genetic single nucleotide polymorphisms that are involved in cholesterol regulation have a role in raising TG, LDLC and HDLC in certain individuals receiving PIs (28).

Children and adolescents with vertical transmission of HIV have been and will continue to be exposed to long term PI use and mainly the PI, lopinavir/ritonavir. Despite their improved survival and quality of life, it is well described that these children and adolescents are exposed to the same metabolic problems that occur in adults living with HIV (42, 43).

In the WRHI 052 study conducted by Venter et al at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), it was demonstrated that low dose ritonavir (100 mg) boosted darunavir (400 mg) sustained VL suppression equivalently as the lopinavir/ritonavir combination used for second line ART in SA. The WRHI 052 study proved that the lower dose of darunavir/ritonavir at 400 mg/100 mg is as effective at maintaining VL suppression. The lower dose could be a more affordable option and become more widely available (44). This lower dose may also potentially be associated with reduced atherosclerosis (39).

A prospective open label trial in Italy by Ucceferri et al, showed a switch in ART regimen from ritonavir boosted lopinavir to ritonavir boosted darunavir maintained VL suppression and improved lipid profiles with a statistical significant decline in TC and LDLC (45). Lipid profiles, in particular TC and TC/HDLC ratios, improved significantly in children and adolescents living with HIV, who were previously receiving an older PI regimen which included ritonavir boosted lopinavir, when they were changed to a newer PI regimen either being atazanavir boosted with ritonavir or darunavir boosted with ritonavir (42).

Most of the studies analysing dyslipidaemia and cardiovascular (CV) risk of PLWH have been from higher income countries (12). The phenotype of PLWH in first world countries differs from that in the developing world. In SA, the majority of PLWH are women and are younger compared to higher income countries whereby majority are middle aged males. Data cannot be extrapolated to LMICs as variations in nutrition, culture and inherited genetics may further alter lipid profiles in comparison to higher income countries, therefore local research is necessary (12, 46, 47).

In 2016, a cross-sectional study done in SA by Dave et al, looked at the metabolic effects of ART. Dyslipidaemia occurred in 90% of PLWH who were ART naive and 85% of those on ART. Low HDLC was the most frequent lipid abnormality in the ART naive group. The ART group received an NNRTI-based regimen or a PI-based regimen and were found to have higher TC, TG, LDLC and HDLC than the ART naive group. In this study, severe dyslipidaemia requiring prompt management was a rare outcome (12). True hyper-triglyceridaemia in PLWH is not commonly reported in SA unless it is in combination with a genetic cause (7).

Despite the well described increase in atherogenic lipoproteins in PLWH on ART, the CV risk in these individuals within the South African context differs from the developed world, and therefore a CV risk assessment should be carried out (6, 7).

1.6. Cardiovascular risk assessment

A CV risk score is defined as the ten-year risk of having an ASCVE and is applied to determine which individuals require further management in terms of lipid lowering therapy (LLT). Ideally each population should have a population specific scoring system. This is not always readily available and the European Society of Cardiology and European Society of Atherosclerosis (ESC/EAS) recommend using the Systematic Coronary Risk Estimation system (SCORE) to estimate CV risk (48-50).

In SA, the management of dyslipidaemia is based on guidelines from the 2018 South African dyslipidaemia consensus statement. These principles are derived from the ESC/EAS 2016 guidelines (7). Since these guidelines, an update with lower

cholesterol targets for individuals at high or very high ASCVD risk has been published (51).

The Framingham CV risk score is the ten-year risk of having cardiovascular disease (CVD), such as heart failure, stroke, myocardial infarction or peripheral arterial disease. The Framingham risk score is calculated using the following variables: age, gender, systolic blood pressure, TC, HDLC and smoking status. Limitations of the Framingham score is that it was not specifically designed for the South African population, nor PLWH in SA, with few trials using this scoring system in PLWH in SA (7).

A large multicentre study in Germany proved that the Framingham score and SCORE for ASCVD risk assessments underestimated CV risk in correlation with ASCVEs that occurred in PLWH compared to HIV negative individuals. Therefore, more research is needed and a CV risk score for PLWH is needed (52). Despite these limitations, the Framingham risk score is recommended in SA, as this CV score originated from an ethnically varied cohort which may be similar to SA's ethnically diverse population (7, 49).

CV risk scores are categorised as low, moderate, high or very-high risk. High or very high risk do not need scoring. Conditions that contribute to categorising an individual as very high risk include established atherosclerosis, chronic kidney disease (CKD) with an estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, complicated Type 1 and Type 2 DM or genetic lipid abnormalities. High risk CV conditions include severe hypertension, uncomplicated Type 1 or 2 DM and CKD with an eGFR 30-59

ml/min/1.73m^2. The Framingham score is used as follows: very high risk is classified as a score of > 30, high risk as 15-30, moderate risk as 3-15 and low risk as a score of less than 3. Depending on the CV risk, the goals of therapy can then be determined (7).

1.7. Goals of cholesterol lowering therapy

LDLC is the most significant causative factor in the formation of atherosclerosis (53). Therefore, reducing LDLC has a significant impact on CV risk reduction (7). A meta-analysis of 52 randomised controlled trials by Wang et al, showed a direct linear relationship between LDLC lowering and relative risk reduction of ASCVE. This meta-analysis has proven that for each 1 mmol/L decrease in LDLC, a corresponding reduction is seen in all-cause mortality by 10%, death due to ASCVD by 20%, and major ASCVE by 24% (54). Furthermore, there is no lower threshold for LDLC lowering in achieving additional CV benefit (53, 54). The greater the percentage decrease in LDLC, the greater the decrease in not only CV risk but the magnitude of an ASCVE (53). Therefore, LLT is directed at achieving a target LDLC based on the calculated CV risk (49). This target is achieved with lifestyle change in combination with pharmacological therapy (7).

The most recent 2019 and updated 2021 ESC/EAS guidelines recommend new targets. In the very-high risk group, LDLC should be reduced by fifty percent of the initial value and be less than 1.4mmol/L. For high risk, moderate risk and low risk, target LDLC should be less than 1.8mmol/L, 2.6mmol/L and 3.0mmol/L respectively (48, 50). The South African dyslipidaemia guidelines have adopted these recommendations (51). Despite LDLC being the target of therapy, a full lipogram is

recommended for the diagnosis of dyslipidaemia, thereafter LDLC only are sufficient for follow up (7).

According to the 2017 Southern African ART clinical guidelines, routine lipid profile screening is not essential at baseline when starting ART, and is only recommended at 3 months after initiating a PI. This lipid screening includes TC and TG levels (11). However, in the 2018 South African dyslipidaemia consensus statement, full lipograms are suggested before initiating ART (7). The 2017 Adult ART guidelines, advises young adults living with HIV without any other ASCVD to initiate statin therapy only if TC>7.5mmol/l or LDLC >5.0mmol/l (11). The ESC/EAS guidelines have no specific reference value for starting statin therapy in PLWH, instead a CV risk assessment is to be performed in the same way as HIV negative individuals and values from the Adult Treatment Panel III as reference ranges for dyslipidaemia have been adopted (30, 49, 50).

Follow up testing is important to ensure LDLC targets are met. Proposed LDLC follow up from the South African dyslipidaemia guideline consensus statement is advised after 6 months if an individual has started lifestyle modification alone. If lifestyle modification is combined with pharmacotherapy, then follow up LDLC levels should be conducted after two monthly intervals until the target LDLC is met, and then subsequently six monthly (7).

1.8. Management of dyslipidaemia

Lifestyle modification remains the cornerstone of ASCVD risk reduction. This includes optimising weight, dietary changes with a decrease in refined sugar and carbohydrate

intake, and blood pressure control (53). Even in PLWH, the single most important modifiable risk factor to reduce ASCVD is cessation of smoking (18, 53).

The backbone of pharmacological therapy for dyslipidaemia including PLWH is statins (4). Statins are 3-hydroxy-3-methylgutaryl co-enzyme A reductase inhibitors. They are highly effective at reducing lipids, especially LDLC (53, 55). It has been noted that the effects of statins are similar in both PLWH and HIV negative individuals (4). Treating PLWH with statin therapy is beneficial in improving the immune system by reducing T cell activation, vascular inflammation as well as reducing atherosclerosis with its antioxidant properties (49, 56). The benefits of statin therapy accumulate with length of time of use (53).

However, most of the statins have drug interactions with PIs, especially ritonavir. This is due to their shared metabolism via the CYP450 system, either via the CYP3A4 pathway or as a substrate for transport mechanism in this pathway. Statins that do not have drug metabolism interactions with PIs, include fluvastatin, rosuvastatin, pitvastatin and pravastatin (55). Rosuvastatin's bioavailability is increased with any PI, therefore a low dose of 10 mg per day is not to be exceeded. Darunavir increases pravastatin concentrations (33). In the HIV-infected patients and treatment with pitvastatin vs pravastatin for dyslipidaemia (Intrepid) study conducted by Aberg et al, pitvastatin was more successful at reducing and maintaining LDLC reduction compared to pravastatin. However, there is no variation when comparing their effects on HDLC and TG levels. There is also no dose restriction when pitvastatin is used in combination with any PI (57). Rosuvastatin has been proven to still be the most

successful statin in reducing all lipid parameters, especially lowering LDLC levels and raising HDLC levels in both PLWH and HIV negative individuals (55, 58).

An important finding with the use of pravastatin, is the positive effect it has on endothelial dysfunction caused by PIs, almost to the extent of reversing this. More studies are needed to definitely conclude that statin therapy improves PI induced endothelial dysfunction (35).

The South African ART and the South African dyslipidaemia consensus statement only advise the use of pravastatin, fluvastatin, or low dose atorvastatin at 10 mg, which is considered the drug of choice. Simvastatin and losuvastatin are absolute contra-indications (11).

Changing ART, known as switch therapy, is an alternative option for reducing dyslipidaemia, but only if it does not interfere with VL suppression (4). Firstly, it is recommended that lopinavir be switched to atazanavir or secondly darunavir. Switch therapy is advised before statin therapy in the 2017 Adult ART guidelines (11).

In children and adolescents living with HIV, who have been exposed to long term PI use, the option of switching therapy to either atazanavir/ritonavir or darunavir/ritonavir has been shown to lower TC, TC/HDL ratio and TGs. This reduction in CVD risk is important in the PLWH who will potentially be exposed to ART for most of their lives (42).

Dyslipidaemia improved after a PI switch to atazanavir, however the endothelial dysfunction does not reverse, which implies PIs may have a toxic effect on the vasculature that increases ASCVD (59).

An alternative to PI switch therapy is changing the PI to a different ART drug class such an II, either raltegravir or the more widely available dolutegravir (3, 33). Importantly, literature has shown that treating with statin therapy such as rosuvastatin is more effective at reducing ASCVD than switching the PI to another PI or an alternative class (33).

Additional drugs are available if target LDLC is not achieved despite the use of a maximally tolerated statin dose. These include Ezetimibe and Proprotein Convertase Subtilisin/Kexin type 9 (PSCK9) inhibitors. Ezetimibe works as an inhibitor of intestinal absorption of bile acid and cholesterol and it is not metabolised by the CYP 450 system. From the few studies done on PLWH, it has minimal side effects and decreases LDLC successfully when used in combination with statin therapy at the highest possible dose (60, 61). PSCK9 inhibitors remain costly and long term trials on effectiveness and safety in PLWH are currently underway (62). The South African dyslipidaemia guidelines only recommend the use of fibrate therapy in individuals at high/very high risk for CVD and or pancreatitis with a TG > 2.3 mmol/L. Otherwise, unless TG > 10 mmol/L the preferred treatment is a statin (7).

An alternative option may be to change ritonavir to cobicistat. Cobicistat is a booster similar to ritonavir, that reduces the metabolism of PIs or IIs, by inhibiting CYP450 3A. In this way, PIs and IIs have increased concentrations. When PLWH who were

previously taking a darunavir boosted with ritonavir regimen are subsequently changed to darunavir combined with cobicistat, there is evidence of improved HDLC, LDLC and in particular TG levels (63). Darunavir combined with cobicistat is available as a fixed drug combination, which improves compliance and reduces pill burden (64).

Only a small fraction of PLWH that meet criteria for the treatment of dyslipidaemia are meeting target LDLC values (65). Mosepele et al, showed that health care screening, assessing CV risk, monitoring of metabolic parameters and achieving targets for lowering CV risk in PLWH in Botswana was suboptimal (66). A systemic review and metanalysis by Gili et al, highlighted that primary prevention with statin therapy is crucial in PLWH and those at risk of ASCVD. If statin therapy was prescribed as per the ASCVD primary prevention guidelines as it is for HIV negative individuals at risk of an ASCVE, then it is estimated that 20-26% of PLWH would be on statin therapy for primary prevention (58).

2. Rationale for proposed secondary analysis

In this secondary analysis of the WRHI 052 study, the metabolic profiles of individuals receiving the reduced darunavir/ritonavir (400 mg/100 mg) combination or the routine lopinavir/ritonavir (800 mg/200 mg) combination will be compared to assess if there are improved metabolic profiles on the darunavir/ritonavir combination. This analysis will provide insight into the metabolic profile of these two groups of participants with a potential solution for improving metabolic complications of individuals on PIs whilst still maintaining VL suppression. It will also provide data for recommendations on screening and monitoring.

Secondly, lipid management in PLWH will be assessed to determine if treatment protocols are being followed. In instances where protocols are followed, a further assessment will be performed looking at whether treatment is effective in meeting target LDLC levels in order to reduce CV atherosclerotic risk in the South African setting.

3. Study aims and objectives

3.1. Aims

The aim of this secondary analysis is to determine if there is a change in metabolic parameters of HIV positive individuals on two different PIs when one group is changed to low dose darunavir/ritonavir and the other group remains on routine lopinavir/ritonavir prescribed for second line ART in South Africa.

3.2. Primary objectives

To describe the lipid parameters in HIV positive individuals on two different PIs:

1. Describe the lipid parameters which includes TC, TG, HDLC and calculated LDLC at baseline, 24 weeks, 48 weeks and after two years of follow up

3.3. Secondary objectives

1. Describe fasting glucose at baseline and 48 weeks.
2. Describe body mass index at baseline, 24 weeks, 48 weeks and after 2 years of follow up
3. Describe the lipid disorders in those with abnormal lipograms;
4. Risk stratify individuals using the Framingham CV risk score;
5. Describe the management of dyslipidaemia in these participants including:
    a. The prescription of a statin;
    b. The type of statin prescribed;
    c. The dose of statin prescribed.
6. Determine if LDLC targets are being achieved based on CV risk classification;
7. Determine if regular LDLC monitoring is needed once targets are reached.

4. Study methods

This is a retrospective cross-sectional analysis of the metabolic profile of HIV positive individuals who participated in the WRHI 052 study, a randomised parallel-group open label non-inferiority phase 3 trial, at CMJAH from 30 June 2016 to 15 June 2017, and who remained at CMJAH for follow up after this study period.

4.1. Patient population

300 HIV positive individuals were included in this study. These HIV positive individuals were already taking lopinavir/ritonavir with two NRTIs and had a HIV RNA VL of less than 50 copies/ml. Patients were randomly assigned to 2 groups: to either remain on their current lopinavir/ritonavir regimen, or switched to low dose darunavir/ritonavir (400mg/ 100mg) together with their original NRTIs. The study was conducted over 48 weeks. Of these patients, 144 continued to completion in the darunavir/ritonavir arm and 149 continued to completion in the lopinavir/ritonavir arm. From this WRHI 052 study, it was concluded that a lower dose of darunavir at 400mg with ritonavir was noninferior to the routine lopinavir/ritonavir (800mg/ 200mg) at maintaining viral suppression.

The inclusion criteria for the study included HIV positive individuals:

i. over the age of 18 years old with a weight of more than 40kg;
ii. already on lopinavir/ritonavir for more than 6 months;
iii. with HIV RNA VL of less than 50 copies/ml; and
iv. having given voluntary informed consent.

The exclusion criteria for the study included:

i. Being on any other form of ART other than NRTIs and lopinavir/ritonavir;
ii. Being on rifampicin or any other therapy with CYP450 interaction within the last month prior to enrolment; or
iii. Females who were pregnant or planned to fall pregnant.

4.2. Study design and data capturing

In this retrospective cross-sectional analysis, the metabolic parameters on the two different PI arms in the WHRI 052 study will be compared. This will only be done for participants who completed the study and who are being followed within the clinical site where information is available.

Firstly, with permission from Ezintsha Wits RHI (see Appendix A), data from the original study will be collected from the WHRI 052 RedCap database, or the participants file when RedCap data are missing, and captured into a RedCap Data Capturing sheet. This document will be password protected and access will be strictly guarded. The information that will be captured in the RedCap Data Capturing sheet is included in Appendix B.

Secondly, for the post study period data, study participants resumed follow up at the CMJAH HIV clinic. Data from this follow up will be collected from participant records at the HIV clinic for the 2-year period after the end of the study. This data will also be captured into the same RedCap Database. The Redcap data capture sheet for the 2-year period after the study is included in Appendix C.

4.3. Data Analysis

The RedCap database of collected data will be exported into the relevant data analysis software platform, either R or SPSS. Descriptive statistics will be calculated for each data population to provide an overview of the data sets, including the median and interquartile range of the measured parameters at each measurement point. Graphical and tabular representation of the population distributions will also be presented.

Analysis of the data to test the primary outcomes of this study will require a two-step statistical testing process for each data parameter, ultimately testing the median of the populations against each other to confirm whether statistically significant differences are found between the participants treated with lopinavir or darunavir. The first test applied to the data sets will test each distribution to confirm normality of the data, using the Shapiro-Wilk test at a 5% significance level. If the p-value of the Shapiro-Wilk test is less than 0.05, signifying the distribution of the population is normally distributed, a Student t-test will be used, at a 5% significance level, to test for a statistically different mean between each parameter. If the distributions are not normal, the Mann-Whitney U test will be used to test for statistical difference.

For the secondary outcomes, the qualitative data parameters will be analysed using pie charts to represent the proportional breakdown of the data. From the data set, for the subset of the population that have been on statin treatment during the study and the follow up, a statistical test of the mean subset LDLC value between the study and the follow up will be performed.

Based on the data structure and the required data analysis, it is not envisaged that a statistical expert will be required to participate in the data analysis.

5. Ethics

Ethical approval will be sought from the University of the Witwatersrand Human Research Ethics Committee. (Appendix D)

Permission from the CEO of CMJAH will also be obtained for access to patient records and clinical data. (Appendix E)

Permission from Ezintsha has been obtained in order to obtain data from participant records.

6. Timeframe

A detailed timeline for that analysis is included below.

Proposed Mmed Schedule																	
	2019			2020												2021	
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Literature Review																	
Preparing Protocol																	
Protocol Assessment																	
Ethics Application																	
Collecting Data																	
Data Analysis																	
Writing up-paper																	

7. Funding/Expenses

Only printing expenses should in incurred for this analysis, and this will be minimal. Hence, no funding will be sort from any association.

8. Limitations

Potential limitations for the analysis that have been identified include:

1. The relatively small sample size of the study population;
2. Measurement bias: poor record keeping may be encountered when capturing data from hospital records for the 2-year follow up period.
3. Confounding bias: socio-demographic factors, lifestyle modification, adherence to therapy and patient related factors could have an impact on outcome measures.
4. Assessment of glycaemic control in HIV positive individuals is not best assessed using fasting plasma glucose and HBA1c, but rather using an oral glucose tolerance test.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Title: Lipid changes on Protease Inhibitors in South Africa

A retrospective cross-sectional analysis comparing metabolic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Johannesburg Academic Hospital

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Conflict of interest: None

Keywords: HIV, protease inhibitors, lipids, cardiovascular risk

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Abstract word count: 449 Total Word count: 5733

*Article has been prepared for submission to the Southern African Journal of HIV Medicine

ABSTRACT

Background

People living with HIV (PLWH) are at risk of cardiovascular (CV) disease, with few studies having assessed CV risk in Southern Africa. Protease inhibitors (PIs) are known to cause dyslipidaemia, in particular, lopinavir more so than darunavir. The WRHI 052 study by Venter et al, a randomised parallel-group open label non-inferiority phase 3 trial, at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), demonstrated that low dose darunavir/ritonavir (400 mg/ 100 mg) sustained viral load suppression equivalently to lopinavir/ritonavir (800 mg/ 200 mg). This could be a cost-effective option with improved metabolic effects.

Objective:

To assess CV risk screening in PLWH receiving two different PI based antiretroviral therapy (ART) regimens and exploring the difference in the metabolic profiles between the two PI groups.

Methods:

A retrospective sub-analysis of the WRHI 052 study. Between June 30 2016, and June 15 2017, 300 PLWH on a lopinavir/ritonavir regimen were randomized into two groups, 152 continued the lopinavir/ritonavir combination and 148 were switched to darunavir/ritonavir. The metabolic parameters of the two PI arms were compared at 24 and 48 weeks. A 2-year post study completion retrospective analysis was performed at the CMJAH HIV clinic where routine care was continued. An analysis of CV risk using the Framingham CV risk score, lipid management and achievement of low-density lipoprotein cholesterol (LDLC) goals was performed.

Results:

In the darunavir/ritonavir group, a significant decrease in total cholesterol (TC) of 7.7% at 48 weeks (95% CI, p<0.001) was observed, and the TC/HDLC ratio declined at 24 and 48 weeks by 1.7% and 3.9% respectively (95% CI, p=0.09; p=0.99). The TC increased by 0.4% at 48 weeks in the lopinavir/ritonavir group (95% CI, p=0.01) and the TC/HDLC ratio increased by 3.3% and 0.9% at 24 and 48 weeks (95%CI, p=0.04 and p=0.12). There was no statistical significance between the median differences and median percentage difference in TC/HDLC ratios between PI groups. LDLC decreased significantly in both PI groups at 24 and 48 weeks, however there was no statistical significance between the PI groups Weight and consequently body mass index (BMI) increased over time with a consistent trend in both groups and genders. Due to weight gain, the percentage of participants with a normal BMI declined from 41.5% at baseline to 33% and 35% at 24 and 48 weeks. Eighty-three percent of all participants had dyslipidaemia with 22% receiving lipid lowering therapy. Eighty-five percent of all participants had a low Framingham CV risk score with 52% achieving target LDLC.

Conclusion

TC and TC/HDLC ratio improved when switched to darunavir/ritonavir. Significant weight gain was observed in participants on a PI over time. Longitudinal studies on weight gain in individuals on PIs are warranted. Increased awareness and assessment of CV risk is needed.

Introduction

Human immunodeficiency virus (HIV) has placed a significant burden on healthcare systems worldwide (1). At the end of 2019, South Africa (SA) had approximately 7.5 million people living with HIV (PLWH) (2). In SA, anti-retroviral therapy (ART) is easily obtainable with early initiation of therapy advised (3). ART reduces the risk of HIV related infections (4). PLWH are also at risk of metabolic disorders that lead to atherosclerotic cardiovascular disease (ASCVD). There is cumulative literature in developed countries that PLWH are more at risk of atherosclerotic cardiovascular events (ASCVE) compared to HIV negative individuals (5). Many metabolic disorders that increase the risk of an ASCVE have been linked to HIV and certain classes of ART, however data from South African PLWH have not shown a greater risk of ASCVD (6-8).

Metabolic complications associated with HIV

Dyslipidaemia is a well-recognised metabolic complication associated with HIV. It occurs in both individuals who have never been exposed to ART and those on ART (9).

Glucose intolerance is another complication associated with HIV itself, but more so with ART (10). In SA, glucose intolerance is noted to be more prevalent in PLWH on protease inhibitors (PIs) (11).

Lipodystrophy, which is the pattern of peripheral fat loss, central fat accumulation and areas of subcutaneous fat accumulation, was more commonly seen with the use of

older nucleoside reverse transcriptase inhibitors (NRTIs), namely the thymidine analogues (12, 13).

Recently, substantial weight gain has been associated with the use of current ART, such as the NRTIs, tenofovir alafenamide and abacavir, and PIs, but more so with integrase inhibitors (IIs), and in particular, dolutegravir (14-16). Weight gain has particularly been described in PLWH who are black, female, started ART with lower CD4 counts, and higher viral loads (VL) (14-17). A phase 3 open labelled randomized control trial in SA conducted by Venter et al demonstrated that substantial weight gain was associated with the use of dolutegravir, in particular in combination with the NRTIs, tenofovir alafenamide and abacavir (15, 18).

Definition of dyslipidaemia

Guidelines from the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) suggest that dyslipidaemia is defined as having any one or more of the following lipid derangements:

v. a total cholesterol (TC) value of greater than 5.2mmol/L;
vi. a low-density lipoprotein cholesterol (LDLC) of greater than 2.5 mmol/L;
vii. a triglycerides (TG) value of greater than 1.7mmol/L; or
viii. a high-density lipoprotein cholesterol (HDLC) value of less than 1.0mmol/L (9).

Causes of dyslipidaemia in PLWH

Dyslipidaemia in PLWH is multi-factorial. Factors include the acute and chronic inflammatory viral states of HIV, ART, nutrition, genetics and now ART induced weight gain (4, 15).

In the acute or unsuppressed viral state, HIV viral replication can be associated with a reduction in TC and LDLC, but it is predominantly characterised by low HDLC and raised TG values (19).

In terms of ART, NRTIs and more specifically thymidine analogues raise TGs, and to a lesser extent contribute to raising TC and LDLC (20, 21). The non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz, which was previously recommended as first line ART, raise TC and TGs (3, 20). However, it is the class of PIs that are particularly responsible for lipid disorders (4, 19). Many forms of mixed lipid disorders occur in individuals taking PIs (22).

Prior to 2019 and the widespread availability of dolutegravir in SA, PIs were used in combination with two NRTIs for second line ART. The recommended PIs in order of preference were atazanavir, lopinavir or darunavir, all of which are boosted with the PI, ritonavir (20, 23). Atazanavir/ritonavir is the PI combination of choice due to its improved lipid and gastrointestinal side effects however, it cannot be used with rifampicin (20). Lopinavir/ritonavir raises TC to a greater extent when compared to the alternative PIs (4, 22). Darunavir has fewer side effects and a better lipid profile. The recommended dose of darunavir/ritonavir for second line ART is 800 mg/ 100 mg (24).

However, higher costs and greater drug interactions seen with rifampicin have limited its use in low and middle-income countries (LMICs) (20).

In the WRHI 052 study conducted by Venter et al at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), it was demonstrated that low dose darunavir (400 mg) boosted with ritonavir (100 mg) resulted in sustained VL suppression equivalently the lopinavir/ritonavir combination (800 mg/ 200 mg) used for second line ART in SA at that time. The WRHI 052 study proved that the lower dose of darunavir/ritonavir is as effective at maintaining VL suppression, and could be a more affordable option and become more widely available (25). This lower dose may also potentially be less lipogenic (26).

Studies from higher income countries in adults, and children and adolescents with vertical transmission have shown an improvement in TC, TC/HDLC ratios and LDLC when lopinavir/ritonavir was changed to darunavir/ritonavir (27, 28).

Most of the studies analysing dyslipidaemia and cardiovascular (CV) risk of PLWH have been from higher income countries (9). This data cannot be extrapolated to LMICs and local research is necessary (29).

In 2016, a cross-sectional study done in SA was conducted by Dave et al, that looked at the metabolic effects of ART. Dyslipidaemia occurred in 90% of PLWH who were ART naive and 85% of those on ART. Low HDLC was the most frequent lipid abnormality in the ART naive group. The ART group received an NNRTI-based regimen or a PI-based regimen and were found to have higher TC, TG, LDLC and

HDLC than the ART naive group. In this study, severe dyslipidaemia requiring prompt management was a rare outcome (9).

Despite the well described increase in atherogenic lipoproteins in PLWH on ART, the CV risk in these individuals within the South African context differs from the developed world, and thus a CV risk assessment should be carried out (6, 7).

Cardiovascular risk assessment

A CV risk score is defined as the ten-year risk of having an ASCVE and is applied to determine which individuals need further management in terms of lipid lowering therapy (LLT). Ideally each population should have a population specific scoring system (30, 31).

In SA, the management of dyslipidaemia is based on guidelines from the 2018 South African dyslipidaemia consensus statement. These principles are derived from the ESC/EAS 2016 guidelines (7). Since these guidelines were released, an update with lower cholesterol targets for individuals at high or very high ASCVD risk has been published (32). Limitations of the Framingham score are that it was not specifically designed for the South African population, nor PLWH in SA, with few trials using this scoring system in PLWH in SA (7). A large multicentre study in Germany proved that the Framingham risk score and the Systematic Coronary Risk Estimation system (SCORE) underestimated CV risk for ASCVEs that occurred in PLWH (33). Despite these limitations, the Framingham risk score is recommended in SA, as this CV score originated from an ethnically varied cohort which may be similar to SA's ethnically diverse population (7, 30).

CV risk scores are categorised as low, moderate, high or very-high risk. Conditions that do not need scoring include very high-risk conditions include established atherosclerosis, chronic kidney disease (CKD) with an estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, complicated Type 1 and Type 2 diabetes mellitus (DM) or genetic lipid abnormalities. High-risk conditions include severe hypertension, uncomplicated Type 1 or 2 DM and CKD with an eGFR 30-59 ml/min/1.73m^2. The Framingham score is used as follows: very high risk is classified as a score of > 30, high risk as 15-30, moderate risk as 3-15 and low risk is a score of less than 3. Depending on the CV risk, the goals of therapy can then be determined (7).

LDLC is the most significant causative factor in the formation of atherosclerosis (34). Therefore, reducing LDLC has a significant impact on CV risk reduction (7). There is no lower threshold for LDLC lowering in achieving additional CV benefit (34, 35). Therefore, LLT is directed at achieving a target LDLC based on the calculated CV risk (30).

Goals of cholesterol lowering therapy

The 2019 and updated 2021 ESC/EAS guidelines recommend new targets. In the very-high risk group, LDLC should be reduced by fifty percent of the initial value and be less than 1.4mmol/L. For high risk, moderate risk and low risk, target LDLC should be less than 1.8mmol/L, 2.6mmol/L and 3.0mmol/L respectively (31, 36). The South African dyslipidaemia guidelines have adopted these recommendations (32). Despite LDLC being the target of therapy, a full lipogram is recommended for the diagnosis of dyslipidaemia, thereafter LDLC only is sufficient for follow up (30, 32).

According to the 2017 Southern African ART clinical guidelines, routine lipid profile screening is not essential at baseline when starting ART, and is only recommended at 3 months after initiating a PI. This lipid screening includes TC and TG levels (20). However, in the 2018 South African dyslipidaemia consensus statement, full lipograms are suggested before initiating ART. Follow up testing is important to ensure LDLC targets are met (7).

Management of dyslipidaemia

Lifestyle modification remains the cornerstone of ASCVD risk reduction (34). The backbone of pharmacological therapy of dyslipidaemia including for PLWH is statins (4). They are highly effective at reducing lipids, especially LDLC (34, 37).

However, most of the statins have drug interactions with PIs, especially ritonavir. This is due to their shared metabolism via the cytochrome P450 (CYP450) system and the potent inhibition of CYP450 enzymes by ritonavir. Statins that do not have drug metabolism interactions with PIs, include fluvastatin, rosuvastatin, pitvastatin and pravastatin (37). Atorvastatin and simvastatin are the only statin available in the public sector. PIs raise atorvastatin levels; however, it is recommended at a dose of 10 mg with monitoring and simvastatin is absolutely contra-indicated (20).

Changing ART, known as switch therapy, is an alternative option for reducing dyslipidaemia, provided it does not interfere with HIV VL suppression (4). Firstly, it is recommended that lopinavir be switched to atazanavir or secondly darunavir. Switching ART is advised before statin therapy in the 2017 Adult ART guidelines (20). However, it must be noted that treating with statin therapy such as rosuvastatin

reduces TC and LDLC more effectively than switching the PI (22). The guidelines only recommend the use of fibrate therapy in individuals at high, very high risk for CVD and or pancreatitis with a TG > 2.3 mmol/L. Otherwise, unless TG > 10 mmol/L the preferred treatment is a statin (7).

Only a small fraction of PLWH that meet criteria for the treatment of dyslipidaemia are meeting target LDLC (38). Mosepele et al, showed that health care screening, assessing CV risk, monitoring of metabolic parameters and achieving targets for lowering CV risk in PLWH in Botswana was suboptimal (39). A metanalysis by Gili et al, highlighted that primary prevention with statin therapy is crucial in PLWH and those at risk of ASCVD. (40).

Rationale for this secondary analysis

This was a retrospective cross-sectional secondary analysis of the WRHI 052 study where the metabolic profiles of individuals receiving the reduced darunavir/ritonavir (400 mg/ 100 mg) combination or the routine lopinavir/ritonavir (800 mg/ 200 mg) combination were compared. CV risk assessment, lipid management and LDLC target achievement was also assessed. This analysis will provide insight into the metabolic profile of these two groups as well as further clarification for improving metabolic complications of individuals on PIs whilst still maintaining VL suppression. It will also provide data for recommendations on screening and monitoring to reduce CV atherosclerotic risk in the South African setting.

Methods

Study design and participants

A retrospective cross-sectional analysis of the metabolic profile of PLWH who participated in the WRHI 052 study, a randomised parallel-group open label non-inferiority phase 3 trial, conducted at the CMJAH HIV clinic, was performed.

The inclusion criteria for the WRHI 052 study included PLWH and:

I. ≥ 18 years old with a weight of more than 40 kg;
II. on lopinavir/ritonavir for more than 6 months;
III. HIV RNA VL of < 50 copies/ml within 60 days before enrolment; and
IV. having given voluntary informed consent

The exclusion criteria for the WRHI 052 study included:

i. being on any other form of ART other than NRTIs and lopinavir/ritonavir;
ii. being on rifampicin or any other therapy with CYP450 interaction within the last month prior to enrolment; or
iii. females who were pregnant, breastfeeding or planned to fall pregnant;
iv. sulphonamide allergy;
v. genotype PI resistance;
vi. alcohol misuse with potential adherence impediment.

Randomisation and masking

Three-hundred HIV positive individuals who were taking an ART regimen consisting of lopinavir/ritonavir (800 mg/ 200 mg) with two NRTIs and had a HIV RNA VL of < 50 copies/ml within the previous 60 days, were included in the WRHI 052 study.

Participants were randomly assigned to two groups: to either remain on their current lopinavir/ritonavir regimen, or switched to low dose darunavir/ritonavir (400 mg/ 100 mg) together with their original NRTIs. Enrolment occurred over a period from 30 June 2016 to 15 June 2017. The study was conducted over 48 weeks.

Procedure

The metabolic parameters of the two PI arms were compared. This included body mass index (BMI) and lipograms at baseline, 24 weeks and 48 weeks. Glucose was compared at baseline and 48 weeks. A CV risk assessment using the Framingham risk score was performed on all participants at baseline and an assessment of target LDLC achievement was conducted at 48 weeks. Information on LLT therapy and DM treatment was collected.

Secondly, a 2-year post study completion retrospective analysis was conducted at the CMJAH HIV clinic, where individuals were followed up for routine care. This included a 2-year post study BMI, LDLC, assessment of LDLC targets achievement and an analysis of LLT and DM treatment.

Statistical analysis

Data from the original study was collected from the WHRI 052 RedCap database, and captured into a RedCap Data capturing sheet. The 2-year follow up data was collected from participant records at the CMJAH HIV clinic. This data was captured into the RedCap Database.

The RedCap database was exported into a Microsoft Excel, and specific data fields extracted to GraphPad Prism version 9.2.0. for statistical analysis. Confidentiality of the study participants in the dataset was achieved by applying a unique patient number to each participant. The data was non-normally distributed, hence non-parametric descriptive statistics were applied to each treatment group to provide an overview of the data sets, using the median and interquartile range (IQR) for continuous variables and frequency counts and percentages for categorical variables. The Wilcoxon sign-rank test was used to compare the paired data within each PI arm. The Wilcoxon sign rank test (Mann-Whitney), was used to assess the median difference and median of percentage difference for all participants across different time points in order to compare changes between the PI arms. For the statistical analysis, statistical significance was set at 5%.

Ethical considerations

Ethical approval was obtained from the University of the Witwatersrand Human Research Ethics Committee, clearance certificate number M200829 (Appendix D)
Permission from Ezintsha WRHI was obtained in order to perform this retrospective secondary analysis. Permission from the CEO of CMJAH was obtained for access to patient records and clinical data.

Results

Three hundred participants were enrolled into the WRHI 052 study, 152 participants were randomly assigned to continue on the lopinavir/ritonavir regimen and 148 were switched to darunavir/ritonavir (400 mg/ 100 mg). Ninety-eight percent (n=149) of participants in the lopinavir/ritonavir group and 97% (n=144) of participants in the

darunavir/ritonavir group completed the 48-week study. At 2 years post study completion, 102 participants continued on their original study regimen for follow up at the CMJAH HIV clinic as part of standard of care (Figure B-1).

The baseline characteristics of the study population were well matched in the two groups as shown in Table A-1. The median age of participants was 42 years. 68% of participants were women and 99% of participants were of black ethnicity.

A comparison of the metabolic parameters of the two groups over time are demonstrated in Table A-2. Changes in TC in both groups are described in Figure B-2. The median TC in the lopinavir/ritonavir group increased from 4,7 mmol/L to 4.72 mmol/L from baseline to 24 weeks, and plateaued at 4.72 mmol/L until 48 weeks. (95% CI, p=0.02). In the darunavir/ritonavir group, the absolute TC significantly decreased from 4,96mmol/L to 4.56 mmol/L from baseline to 24 weeks and increased to 4.58 mmol/L at 48 weeks, with a percentage decrease of 8.1% and 7.7% at 24 and 48 weeks respectively (95% CI, p<0.001).

Table A-3 compares the median differences and median of percentage differences across PI groups at different time points. In the darunavir/ritonavir group, there was a statistically significant reduction in TC in the median difference from baseline to 24 weeks of 0.4 (95% CI, p<0.001) and from baseline to 48 weeks of 0.3 (95% CI, p=0.02) compared to the lopinavir/ritonavir group. The median percentage difference across the PI arms revealed a statically significant decline in the darunavir/ritonavir group at 24 weeks and 48 weeks (95% CL, p<0.001 and p=0.01) compared to the lopinavir/ritonavir arm.

There was a significant decline in HDLC in both PI groups, with a 4.2% and 5.6% reduction in the lopinavir ritonavir group at 24 and 48 weeks (95% CI, p=0.01 and p=0.03), and a 11.2% and 7.7% reduction in the darunavir/ritonavir group (95% CI, p<0.001 at 24 and 48 weeks). There was only a statistically significant decrease in the median difference and median of percentage differences at 24 weeks in the darunavir/ritonavir group compared to the lopinavir/ritonavir group (Table A-3).

In the lopinavir/ritonavir group the mean TC/HDLC ratio significantly increased from 3.32 to 3.43 from baseline to 24 weeks (95% CI, p=0.05) and then declined to 3.35 at 48 weeks (95% CI, p=0.12). In the darunavir/ritonavir group the median TC/HDLC ratio declined throughout the study, from an absolute TC/HDLC ratio of 3.59 to 3.53 and 3.45 from baseline to 24 and 48 weeks (95% CI, p=0.09; p=0.99). Comparison of the median difference and median of percentage differences of TC/HDLC across both PI arms at 24 and 48 weeks, revealed no statistical significance (Table A-3)

The median LDLC decreased significantly in both study groups at 24 and 48 weeks, with a 10.2% reduction in the lopinavir/ritonavir group from baseline to 2-years (95% CI, p=0.03) and a 23.1% reduction in the darunavir/ritonavir group at 2 years (95% CI, p=0.12). (Figure C-1). Comparison of the median differences and median of percentage differences of both PI arms at 24 and 48 weeks, revealed no statistical significance (Table A-3).

Eighty-three percent (n=249) of all participants had dyslipidaemia, which was defined according to the NCEP ATP III dyslipidaemia criteria, at the start of the WRHI 052

study. Of participants with dyslipidaemia, 44.2% had a mixed lipid disorder with a raised TC and LDLC, and 43.4% had a raised LDLC only. 2.4% (n=6) of participants with a background history of dyslipidaemia and receiving LLT, did not meet NCEP ATP III dyslipidaemia criteria for dyslipidaemia (Table C-1). 3% of participants developed dyslipidaemia during the study.

LLT was prescribed to 22.1% (n=55) of participants with dyslipidaemia, 15.3% (n=38) already receiving therapy prior to study enrolment. Seventeen participants (6.8%) were initiated on therapy at study enrolment, with 15 participants prescribed atorvastatin and 2 prescribed bezafibrate. Figure B-3 describes the dosage and combinations of LLT prescribed for all participants on LLT.

At 2- year follow up, 32 participants (32.4%) had documentation of LLT. This included 4 participants that had treatment stopped for unknown reasons and 7 participants had atorvastatin 20mg prescribed for the first time.

Both median weight and consequently median BMI increased from baseline to 48 weeks in both groups (Figure B-4). In the darunavir/ritonavir group a significant percentage increase in the median BMI of 4.6% and 7.2% at 24 and 48 weeks respectively was observed (95% CI; $p<0.001$; $p<0.001$) (Table A-2). A percentage increase in the median BMI was also seen in the lopinavir/ritonavir group of 4.5% and 4.9% respectively (95% CI; $p=0.43$; $p=0.02$) (Table A-2). There was no statistical significance in the median difference and the median of percentage differences in the change in BMI across both PI arms at 24 and 48 weeks (Table A-3).

At baseline, 41.5% (n=127) of all participants had a normal BMI. This percentage decreased during the study due to weight gain. At 24 and 48 weeks, 33%, and 35% and of all participants had a normal BMI. This was seen in both PI groups. In the darunavir/ritonavir group 42.6% of participants had a normal BMI classification at baseline and this declined to 32.9% at 48 weeks due to weight gain. In the lopinavir/ritonavir arm the percentage of participants with a normal BMI declined from 40.4 % at baseline to 36.7% at 48 weeks.

The percentage of participants with pre-obese, obesity class I BMI and obesity class II BMI classifications increased over time (Figure B-5). Of the participants (n=127) that had a normal BMI at the start of the study, BMI measurements during the study showed 58.9% continued to have a normal BMI, 19.4% changed to an obese BMI classification (either pre-obese or obese) and 21.7% had no weight measured after enrolment. (Figure B-6). Seventy- one participants had an initial BMI classification as pre-obese, 63.4% remained at a BMI as pre-obese, 19.7% changed to an obese BMI classification during the study and 16.9% had no weight documented after enrolment (Figure B-7). BMI classification changes in males and females in both groups were similar (Figure C-2 - Figure C-5). At enrolment, 1.4% (n=4) of participants, and 1.1% (n=2) at 48 weeks had a BMIs categorised as underweight.

According to the Framingham CV risk score, 85.7% of all participants had a low CV risk score, 9.6% a moderate risk, 2% a high-risk score, and 2.7% were stratified as very high risk of having an ASCVE within 10 years.

Forty-five percent (n=135) of participants did not achieve target LDLC according to their Framingham CV risk score, and 3% (n= 9) had missing data at 48 weeks. LDLC target achievements were similar in both PI groups. Twenty-three participants (41.8%) on LLT achieved target LDLC. At 2-year follow up, 56.9 % (n=58) of participants had an LDLC documented as part of routine care of whom 58.6% (n=34) achieved target LDLC. 37.5% (n=12) of participants on LLT at 2-year follow up achieved target LDLC.

In terms of glucose monitoring across the study, the median fasting glucose remained the same, 4.7 mmol/L at baseline and 48 weeks for all participants. The median fasting glucose in both groups was similar. 12 participants in the study had type 2 DM as a pre-existing co-morbidity. No detailed statistical analysis was conducted on this sub-population due to the small sample size. 8 of the diabetic participants had a very high CV risk score, 4 a high CV risk score and 1 achieved target LDLC.

Discussion

The WRHI 052 study conducted by Venter et al concluded that a low dose of darunavir (400 mg) boosted with ritonavir (100 mg) was non-inferior at maintaining VL suppression compared to ritonavir boosted lopinavir that is commonly used as second line ART (25). The WRHI 052 study was a good representation of the demographics of PLWH in SA. Firstly, in this analysis a reduction in TC and TC/HDLC ratio was found in participants that were switched from a ritonavir boosted lopinavir regimen to the ritonavir boosted darunavir. And secondly, the most prominent observation, of progressive weight gain in both groups over time.

In individuals on a PI, a switch from lopinavir/ritonavir to low dose darunavir/ritonavir was associated with a significant 7.7% decrease in TC over time (Table A2), and a significant decline in the median percentage difference in the darunavir/ritonavir group compared to the lopinavir/ritonavir group at 24 and 48 weeks (Table A3). TC in the lopinavir/ritonavir group was associated with a statistically significant increase of 0.2 mmol/L from baseline to 48 weeks (95% CI; p=0.02). However, it is unknown if this absolute increase in TC translates into a clinically significant increase in CV risk in PLWH who will be exposed to ART for many years. This is in keeping with prior literature that TC decreases in PLWH when switched from a ritonavir boosted lopinavir regimen to a ritonavir boosted darunavir regimen (19, 27, 28).

A significant decrease in HDLC was observed in both groups. However, an increase in the TC/HDL ratio was observed in the lopinavir/ritonavir group, whereas a consistent decrease was seen in the darunavir/ritonavir group throughout the study, which was not of statistical significance (table A2). The median difference in TC/HDLC and median percentage difference of the TC/HDLC ratio declined at 48 weeks in the darunavir/ritonavir, which may be due to the plateau of HDLC and decline in TC. The rise in the TC/HDLC ratio in the lopinavir/ritonavir group may be due to a continuing rise in TC and decrease in HDLC, which if it continues to less than 1mmol/L is defined as dyslipidaemia (9). The decline in the TC/HDLC ratio in the darunavir/ritonavir group is in keeping with prior studies that TC/HDLC ratio decreases when a ritonavir boosted lopinavir regimen is switched to a ritonavir boosted darunavir regimen (27, 28).

A significant decrease in the median LDLC was seen in in each study group, however, this was not statistically significant between the groups. The median LDLC remained

above 2.5 mmol/L throughout the study which is defined as dyslipidaemia according to the NCEP ATP III dyslipidaemia criteria (9).

The data from our sub-analysis of the WRHI 052 trial provides insight into the metabolic profiles of PLWH on two different PIs. A reduction in TC and TC/HDLC could potentially translate into lower CV risk in PLWH who will be exposed to ART for many years, and may prevent the need for LLT which would further add to pill burden (9, 41). However, CV outcome data and larger randomised trials will need to be conducted (7, 9, 42).

In this analysis, increase in weight was observed in individuals on a PI over time. Progressive weight gain and subsequently BMI increased in both groups. Analysis of the median difference and median percentage difference of BMI between groups indicated there was no statistical difference in weight gain between the two groups (table A3). It is noted at 24 weeks that the median percentage difference in the darunavir/ritonavir is trending towards statistical significance, however there is missing data at this point.

The observed increase in weight was associated with a consequent change in BMI classifications throughout the study. A consistent trend in the changes in BMI classifications were seen in both study groups as well as across genders. The changes in BMI classes were predominantly in participants with a normal or pre-obese BMI classification at the start of the study and lead to a consequent increase in numbers of participants with pre- obese and obese BMI classifications as shown in Figure B-5- Figure B-7 and Figure C-5.

This is in keeping with prior observational and randomised control trials whereby weight gain was associated more frequently in PLWH that started newer ART with a normal or pre-obese BMI classification (14, 16, 17). Prior studies observed significant weight gain predominantly in young black females, however in this analysis weight gain was observed across genders (14-16). The South African study by Venter et al, described considerable weight gain with the use of dolutegravir (18). The ART regimen in this analysis did not contain dolutegravir, but considerable weight gain has been observed in individuals on PIs (14, 15, 17). Mechanisms for substantial weight gain was previously thought to be a “return to normal’ phenomenon”, which may have been the case in earlier years, but more recently it is suggested to be due to fewer gastro-intestinal side effects from boosted PIs and ART regimens that are more tolerable. More research is needed regarding the exact mechanism by which newer ART causes substantial weight gain. Longer trials are also needed assess whether weight gain plateaus, and whether long term weight gain differs in lopinavir and darunavir based regimens. Further research is also needed compare weight gain across genders and ethnic groups in PLWH on ART (14, 17, 18).

In this analysis, the majority of participants (85.7%) had a low Framingham CV risk score. Even though the CV risk scores were low in the majority of participants, it must be kept in mind that as this population ages, the risk of CVD due to the metabolic side effects of HIV, ART and ART induced weight gain is likely to increase (4, 15). CV risk scores should be assessed regularly and a specific scoring system for PLWH in SA needs to be identified (33).

At the start of the study, 83% of all participants had dyslipidaemia according to meeting at least 1 NCEP ATP III criteria. This may have been due to the lopinavir/ritonavir regimen prescribed prior to enrolment. A variety of mixed lipid disorders were observed however, the majority were either a mixed lipid disorders with raised LDLC and TC, or secondly raised LDLC only. The raised TC and LDLC disorders observed are in keeping with literature from higher income countries and the South African study by Dave et al, that suggested that PIs impacts lipids by raising TC, LDLC, and TGs, and decrease HDLC (9, 19, 22). A limitation of the WRHI 052 study was that TGs were not recorded but a high TG would be expected to have been found on individuals on a PI (9). However, LDLC is the most significant causative factor in the formation of atherosclerosis (34). Therefore, reducing LDLC leads to CV risk reduction (7, 35).

Assessment of dyslipidaemia screening, management and LDLC target achievement according to Framingham risk stratification at 48 weeks and 2-year follow up, revealed that this was lacking for routine patient care. This was demonstrated by 83% of participants having dyslipidaemia, however 22.1% were prescribed LLT, and only 52% of all participants achieved LDLC targets at 48 weeks. Achievement of LDLC target were similar in the both groups. At 2-year follow up, documentation of LDLC monitoring, prescription of LLT and achievement of target LDLC was low.

This is as suggested by prior studies, screening, assessment of CV risk, prescription of statin therapy for primary prevention of ASCVD and achievement of target LDLC is lacking and attention needs to paid to these practices (38, 39). Possible reasons for this are lack of awareness of the need to screen, monitor and treat dyslipidaemia in

PLWH lack of compliance with guidelines; or facilities being over-burdened with high patient numbers and a main focus on ensuring VL suppression.

A systemic review and metanalysis by Gili et al demonstrated that primary prevention of ASCVD in PLWH is crucial as dyslipidaemia leads to ASCVE, which may occur more frequently in PLWH who are exposed to long term ART and who are overweight and aging (40).

In the WRHI 052 study, there were no significant changes in fasting plasma glucose noted from baseline to 48 weeks in both PI groups. Reasons for this may be that only two fasting plasma glucose tests were done throughout the study and no HbA1cs were performed on non-diabetic patients. A systematic review and metanalysis of insulin sensitivity on PIs revealed there were no changes in insulin sensitivity in PLWH taking the PIs darunavir and atazanavir but there was reduced insulin sensitivity observed in PLWH taking lopinavir (43). More frequent monitoring in long-term studies is needed to assess this in PLWH in SA.

The ESC/EAS recommends a CV risk assessment be performed in the same way as in HIV negative individuals and recommends follow up testing for LDLC to ensure LDLC targets are met (30, 36). Routine lipid screening is not essential when starting ART according to the 2017 Southern African ART clinical guidelines, and only 3 months after initiating a PI. This lipid screening includes a TC and TG level (20). Since LDLC is the causative factor in atherosclerosis, and CV risk reduction is aimed at LDLC reduction according to a CV risk stratification, it is beneficial that CV risk

assessments and LDLC screening and monitoring be performed as part of HIV management protocols for primary prevention of ASCVD in PLWH (35, 40).

Limitations

Baseline pre-ART initiation lipid panel and TGs were unavailable from the WRHI 052 study. Given the known association of raised triglycerides with ART, the difference in TG between the two groups would have been beneficial in monitoring the trend in PLWH on PIs.

Fasting plasma glucose was only measured at baseline and 48 weeks, and there were no HBA1c performed during the WRHI 052 study. More monitoring of these parameters is needed to assess the impact of PIs against the long-term risk on impaired insulin sensitivity.

Weight gain was poorly documented during the study at 48 weeks and at 2-year follow up, therefore limiting weight and BMI assessments to a small sample size. Limited data was available for analysis. In the lopinavir/ritonavir group 69.1%, 64.5% and 29.6% had weight documented at 24 weeks, 48 weeks and 2-year follow up respectively. In the darunavir/ritonavir group 60.1%, 55.4%, and 25.7% at 24 weeks, 48 weeks and 2-year follow up had documented weight for analysis. No statistical comparisons were done at 2-year follow up due to this limitation.

Truncal fat was not measured as part of the WRHI 052 study. This would have been beneficial to assess body fat distribution in PLWH on ART.

Limited data was available for the period post study completion due to inadequate record keeping and incomplete follow up. Many patients were lost to follow up, or stepped down to local clinics.

Conclusion

In this retrospective secondary analysis, improvement in lipid parameters was demonstrated, more specifically a reduction in TC and TC/HDLC ratios, when participants were switched from a lopinavir/ritonavir regimen to a darunavir/ritonavir regimen. Significant weight gain was observed in participants on a PIs over time, including both darunavir/ritonavir and lopinavir/ritonavir. Further studies in PLWH on PI's and II, should be directed at assessing changes in weight and metabolic parameters. Appropriate CV risk screening will guide therapeutic stages based on establishing an LDLC target upfront.

Recommendations

Further longitudinal trials and randomized control trials of weight gain in PLWH on PIs and IIs in SA is needed. HIV clinics should focus on improving the awareness of CV risk screening in all PLWH on PIs, as well as updating protocols to include routine monitoring of glucose and LDLC, with the goal of achieving target LDLC based on CV risk.

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APPENDIX A: LIST OF TABLES

Table A-1: Baseline characteristics of all participants and the distribution in each PI study arm

	All study participants	Lopinavir/ ritonavir arm	Darunavir/ ritonavir arm
Patient characteristics			
Age,years median,(IQR)	42 (20-75)	42 (20-69)	42 (26-75)
Sex, no. (%)			
Male	96 (32)	45 (30)	51 (34)
Female	204 (68)	107 (70)	97 (66)
Ethnicity, no. (%)			
Black	299	152 (100)	147 (99)
Coloured	1	0	1
CD4 count (cells/mm^3)-mean, (IQR)	601 (436-806)	596 (462-830)	604 (431-783)

Note IQR, interquartile range

Table A-2: Characteristic changes within the different PI study arms over time

	Baseline	24 weeks	48 weeks	2 years
Weight (Kg)				
Lopinavir/ritonavir				
Median	68.65	71.65	73.7	76.8
Interquartile range	(61.1-83.5)	(62.5-84.6)	(61.7-85.8)	(63.6-85.4)
N	152	106	98	45
Darunavir/ritonavir				
Median	71.85	73.1	74.65	76
Interquartile range	(59.7-82.2)	(62.1-84.0)	(65.5-83.3)	(67.0-83.3)
N	148	89	82	38
BMI				
Lopinavir/ritonavir				
Median	26.5	27.7	27.8	28.5
Interquartile range	(23.0-32.6)	(23.4-33.3)	(23.2-33.2)	(22.8-34.2)
N	151	105	98	45
Percentage difference (%)		4.5	4.9	7.5
P value		0.43	**0.02**	0.10
Darunavir/ritonavir				
Median	26.3	27.5	28.2	27.95
Interquartile range	(22.8-31.1)	(23.4-32.5)	(23.6-32.6)	(24.9-34.1)
N	148	89	82	38
Percentage difference		4.6	7.2	6.1
P value		**p<0.001**	**p<0.001**	**0.02**
Total Cholesterol (mmol/L)				
Lopinavir/ritonavir				
Median	4.7	4.72	4.72	
Interquartile range	(4.21-5.46)	(4.04-5.3)	(3.90-5.32)	
N	152	148	150	
Percentage difference (%)		0.4	0.4	
P value		0.41	**0.02**	
Darunavir/ritonavir				
Median	4.96	4.56	4.58	
Interquartile range	(4.40-5.58)	(3.98-5.1)	(3.9-5.13)	
Percentage difference (%)		-8.1	-7.7	
N	148	144	141	
P value		**p<0.001**	**p<0.001**	
HDLC (mmol/L)				
Lopinavir/ritonavir				
Median	1.42	1.36	1.34	

	Baseline	24 weeks	48 weeks	2 years
Interquartile range	(1.20-1.63)	(1.15-1.67)	(1.12-1.65)	
N	152	148	150	
Percentage difference (%)		-4.2	-5.6	
P value		**0.01**	**0.03**	
Darunavir/ritonavir				
Median	1.42	1.23	1.32	
Interquartile range	(1.19-1.67)	(1.01-1.50)	(1.09-1.49)	
N	148	144	141	
Percentage difference (%)		-11.2	-7.7	
P value		**p<0.001**	**p<0.001**	
TC/HDLC ratio				
Lopinavir/ritonavir				
Median	3.32	3.43	3.35	
Interquartile range	(2.75-4.07)	(2.75-4.20)	(2.82-4.16)	
N	152	148	150	
Percentage difference (%)		3.2	0.9	
P value		**0.05**	0.12	
Darunavir/ritonavir				
Median	3.59	3.53	3.45	
Interquartile range	(2.91-4.34)	(3.04-4.55)	(2.93-4.30)	
N	148	144	141	
Percentage difference (%)		-1.7	-3.9	
P value		0.09	0.99	
LDLC (mmol/L)				
Lopinavir/ritonavir				
Median	3.01	2.95	2.8	2.7
Interquartile range	(2.52-3.60)	(2.33-3.53)	(2.36-3.36)	(2.26-3.39)
N	152	148	150	31
Percentage difference (%)		-2	-7	10.3
P value		**0.01**	**p<0.001**	**0.03**
Darunavir/ritonavir				
Median	3.20	2.91	2.94	2.46
Interquartile range	(2.65-3.82)	(2.45-3.47)	(2.32-3.44)	(2.20-3.52)
N	148	144	141	27
Percentage difference (%)		-9	-8.1	23.2
P value		**p<0.001**	**p<0.001**	0.12
Fasting Glucose (mmol/L)				
Lopinavir/ritonavir				
Median	4.7		4.6	
Interquartile range	(4.4-5.0)		(4.4-5.0)	

	Baseline	**24 weeks**	**48 weeks**	**2 years**
N	150		149	
P value			0.75	
Darunavir/ritonavir				
Median	4.6		4.7	
Interquartile range	(4.3-4.9)		(4.4-5.0)	
N	147		143	
P value			**0.02**	

Note: All **bold** values in the table are p-values of statistical significance *P* <0.05. Percentage differences were calculated from the median of the metabolic parameters in this table.

BMI, body mass index; HDLC, high density lipoprotein cholesterol; TC/HDLC, total cholesterol/ high density lipoprotein cholesterol ratio; LDLC, low density lipoprotein cholesterol

Table A-3: Comparison of the median of differences and median of percentage differences of metabolic parameters across different time points between PI groups

	Lopinavir/ritonavir	Darunavir/ritonavir	P value
Total Cholesterol (mmol/L)			
Median difference baseline to 24 weeks	-0.1	-0.4	**p<0.001**
Median difference baseline to 48 weeks	-0.14	-0.3	**p=0.02**
Median percentage difference baseline to 24 weeks	-0.31	-7.9	**p<0.001**
Median percentage difference baseline to 48 weeks	-3.25	-7	**p=0.01**
HDLC (mmol/L)			
Median difference baseline to 24 weeks	-0.03	-0.14	**P<0.001**
Median difference baseline to 48 weeks	-0.07	-0.09	p=0.08
Median percentage difference baseline to 24 weeks	-2.38	-9.62	**P<0.001**
Median percentage difference baseline to 48 weeks	-5.13	-6.58	p=0.12
TC/HDLC ratio			
Median difference baseline to 24 weeks	+0.08	+0.08	p=0.88
Median difference baseline to 48 weeks	+0.1	-0.06	p=0.36
Median percentage difference baseline to 24 weeks	+1.81	+2.45	p=0.91
Median percentage difference baseline to 48 weeks	+2.92	-1.71	p=0.34
LDLC (mmol/L)			
Median difference baseline to 24 weeks	-0.1	-0.23	p=0.09
Median difference baseline to 48 weeks	-0.19	-0.15	p=0.89
Median percentage difference baseline to 24 weeks	-2.73	_-7.48	p=0.13
Median percentage difference baseline to 48 weeks	-7.49	_-4.56	p=0.92
BMI			
Median difference baseline to 24 weeks	+0.9	+0.39	p=0.11
Median difference baseline to 48 weeks	+0.19	+0.38	p=0.34
Median percentage difference baseline to 24 weeks	+0.38	+1.37	p=0.08
Median percentage difference baseline to 48 weeks	+0.6	+1.53	p=0.22

Note: All **bold** values in the table are p-values of statistical significance *P* <0.05. Median absolute difference and percentage differences between each PI group, of TC, HDLC, TC: HDLC, LDLC and BMI, was calculated based on the median difference of each participant at baseline, 24 and 48 weeks. BMI, body mass index; HDLC, high density lipoprotein cholesterol; TC/HDLC, total cholesterol/ high density lipoprotein cholesterol ratio; LDLC, low density lipoprotein cholesterol.

APPENDIX B: LIST OF FIGURES

Figure B-1: Trial profile from screening through to follow up 2-years post study completion.

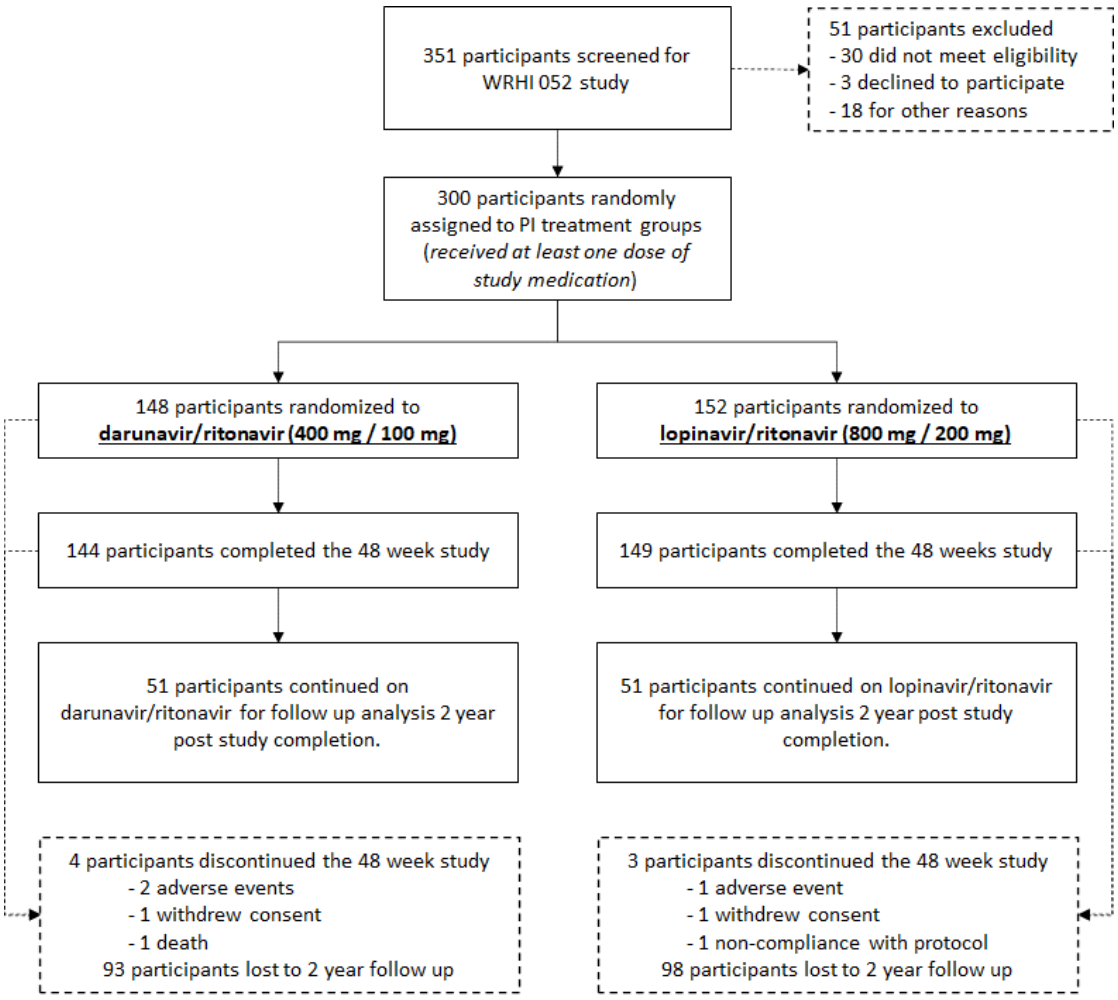
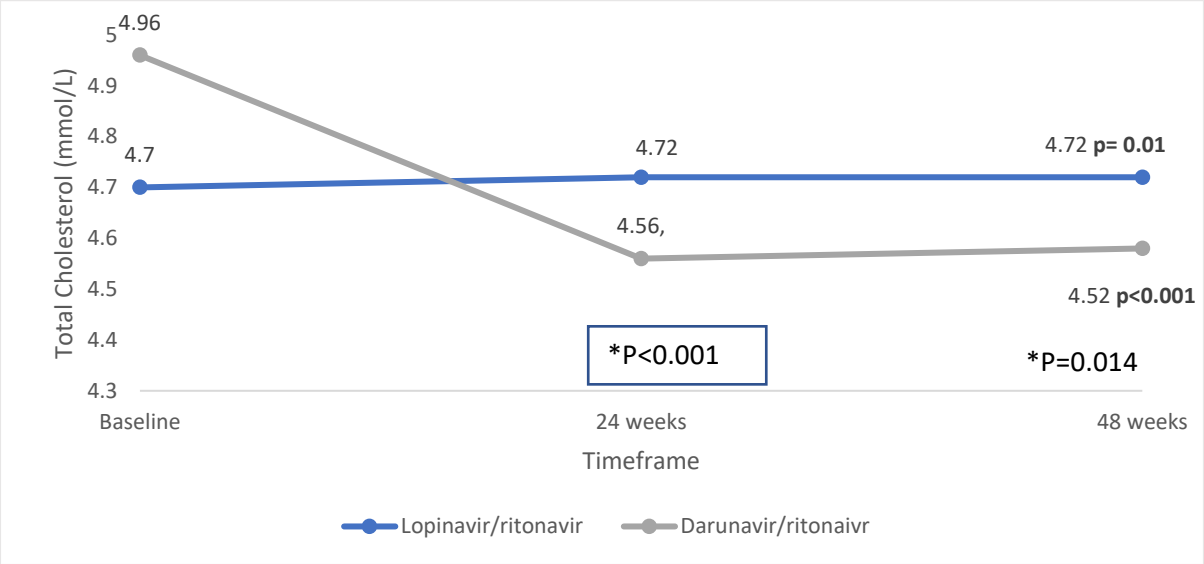


Figure B-2: Comparison of total cholesterol over time in both study groups



Note: All **bold** values in the table are p-values of statistical significance *P* <0.05

**P value comparing arms is the median of percentage differences comparing baseline to 24 weeks or 48 weeks using Wilcoxon sign-rank test.*

Figure B-3: Types of lipid-lowering therapy and the dosage of therapy prescribed

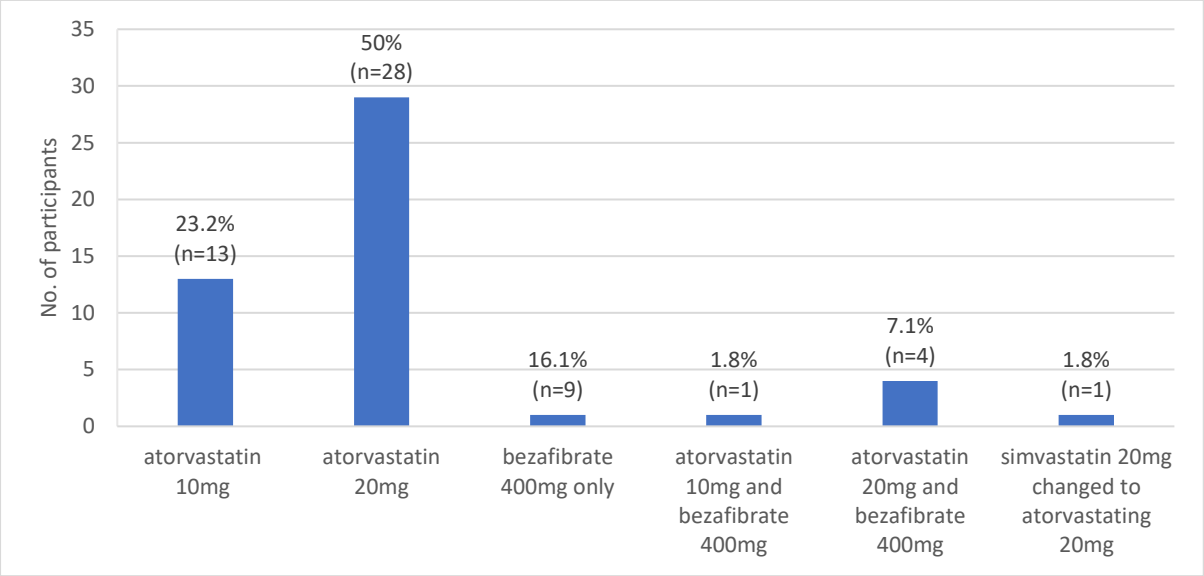
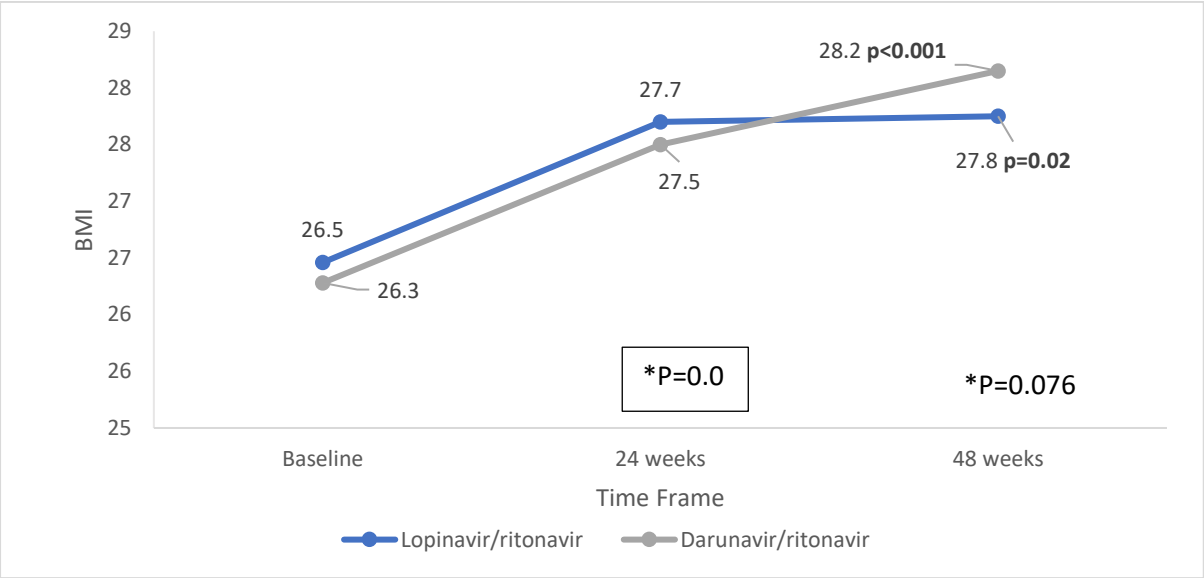


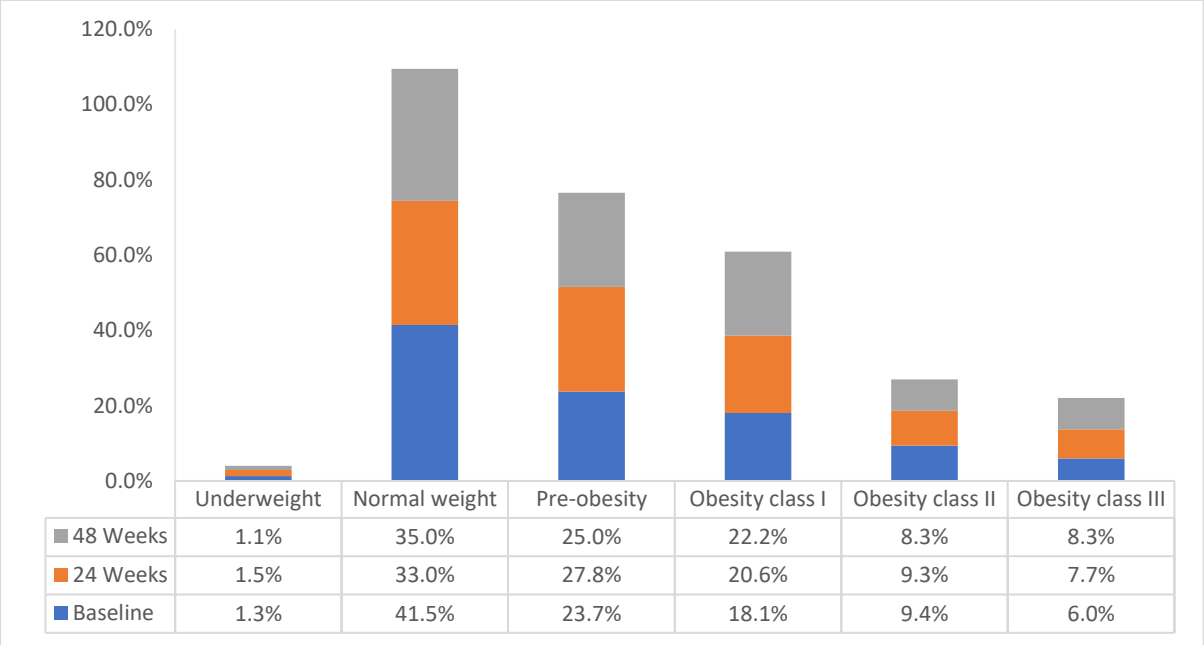
Figure B-4: Comparison of median BMI over time in both study groups



Note: All **bold** values in the table are p-values of statistical significance *P* <0.05

**P value comparing arms is the median of percentage differences comparing baseline to 24 weeks or 48 weeks using Wilcoxon sign-rank test*

Figure B-5: Changes in the percent of participants within each BMI classification over time



	Underweight	Normal weight	Pre-obesity	Obesity class I	Obesity class II	Obesity class III
48 Weeks	1.1%	35.0%	25.0%	22.2%	8.3%	8.3%
24 Weeks	1.5%	33.0%	27.8%	20.6%	9.3%	7.7%
Baseline	1.3%	41.5%	23.7%	18.1%	9.4%	6.0%

Figure B-6: The percentage change in BMI classification during the study for participants that started the with a normal BMI

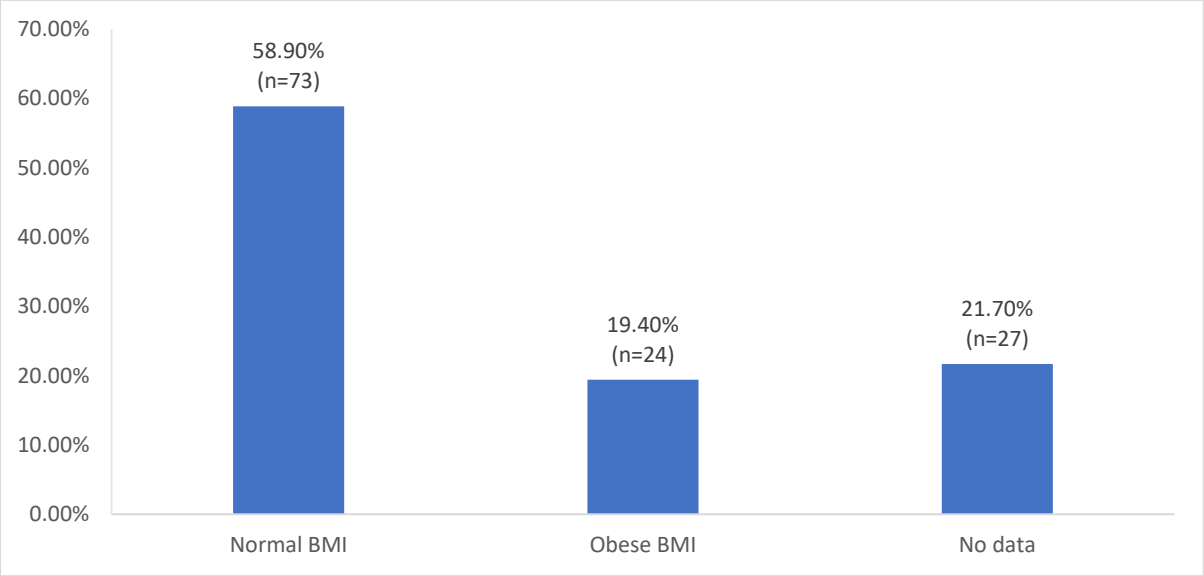
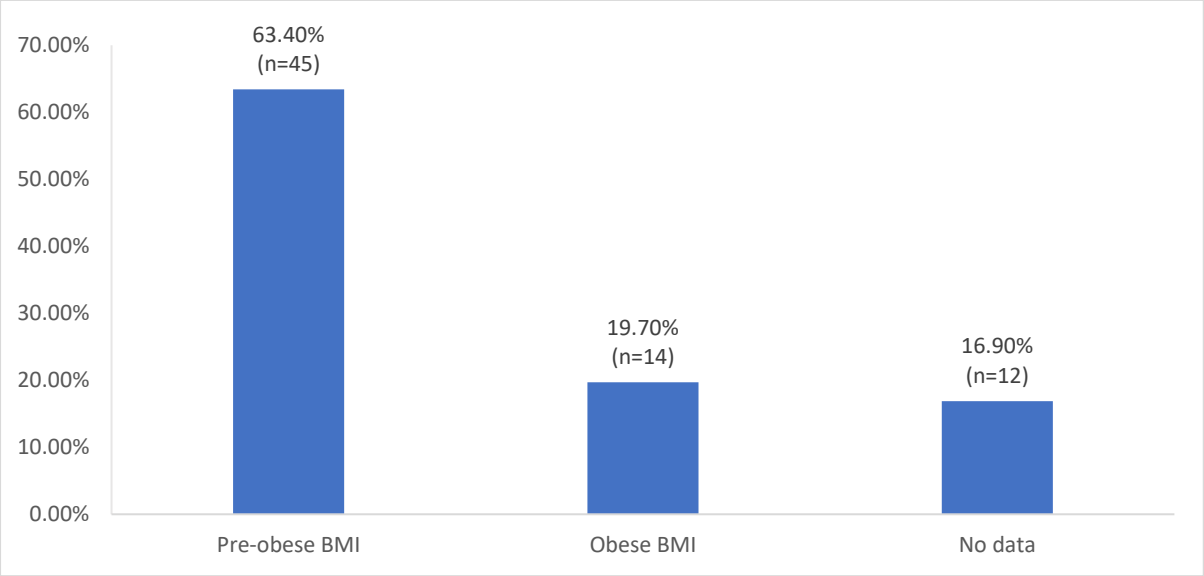
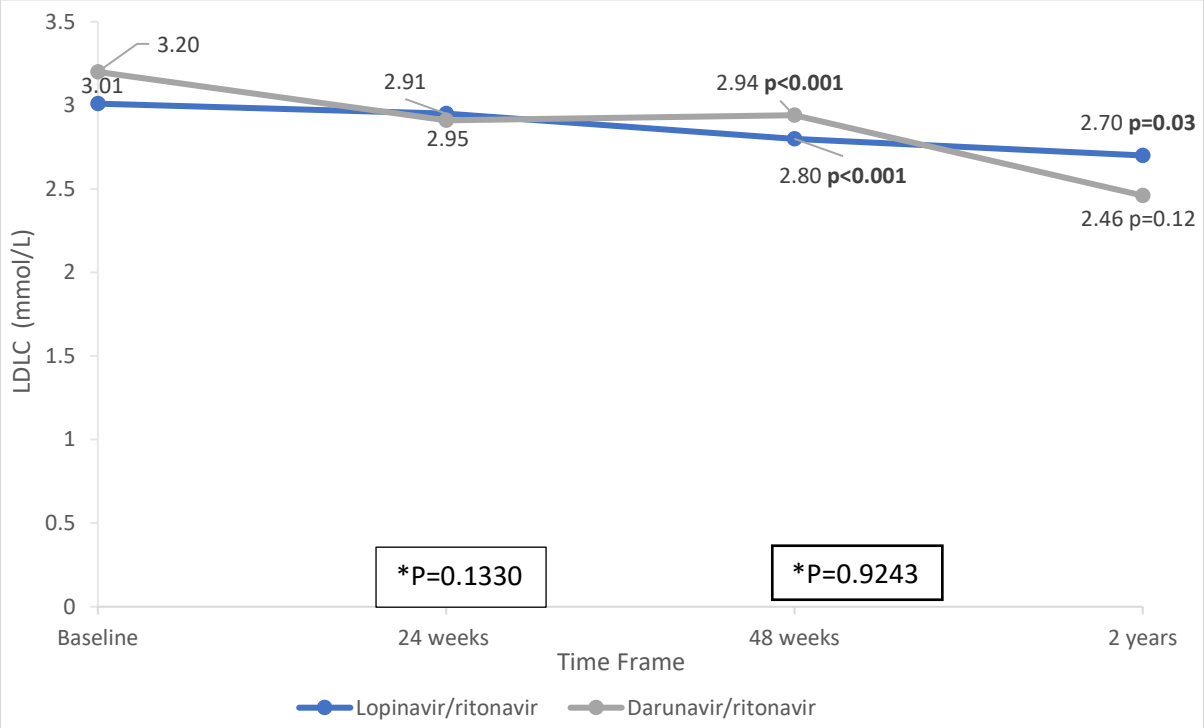


Figure B-7: The percentage change in BMI classification during the study for participants that started the with a pre-obese BMI



APPENDIX C: SUPPLEMENTARY APPENDIX

Figure C-1: Comparison of the median LDLC across both study groups over time

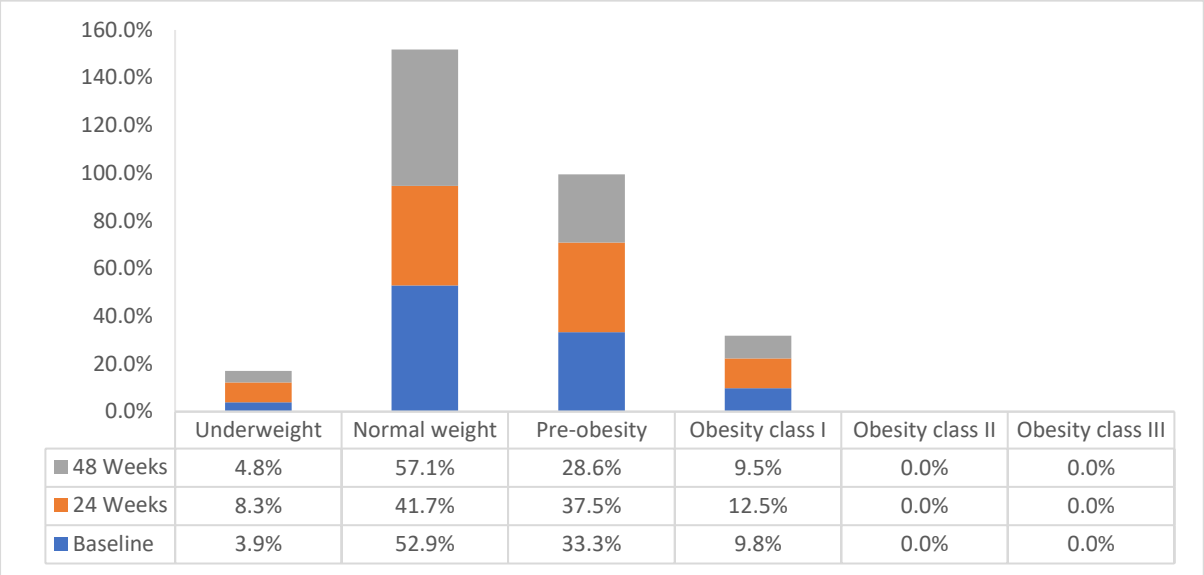


Note: All **bold** values in the table are p-values of statistical significance *P* <0.05

**P value comparing arms is the median of percentage differences comparing baseline to 24 weeks or 48 weeks using Wilcoxon sign-rank test.*

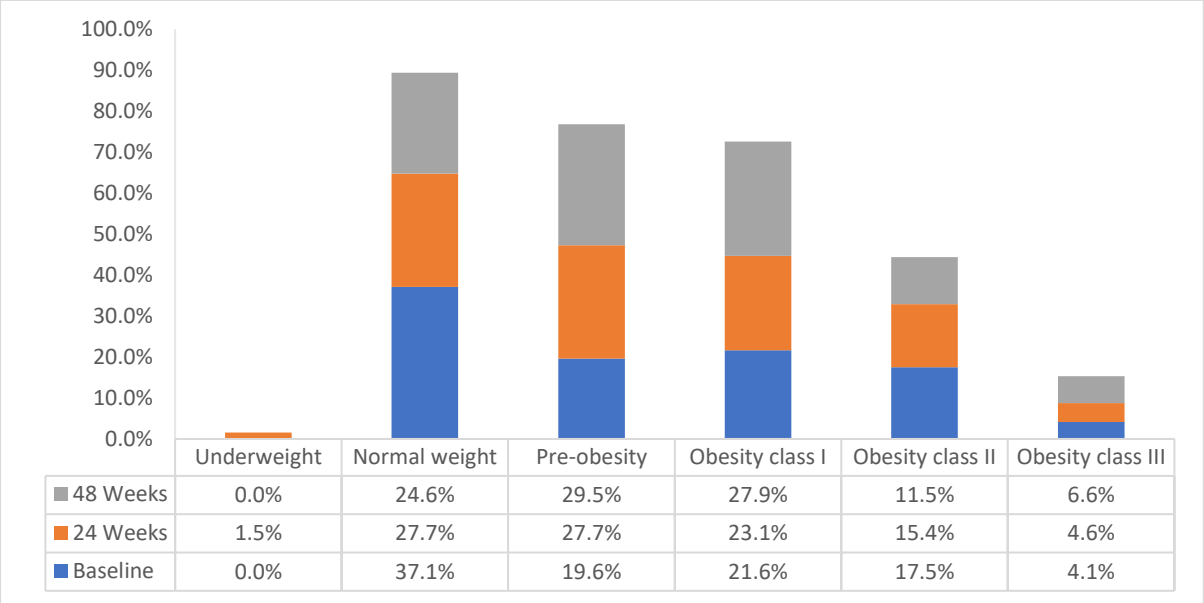
LDLC, low density lipoprotein cholesterol

Figure C-2: BMI classification and changes in male participants in the darunavir/ritonavir group.



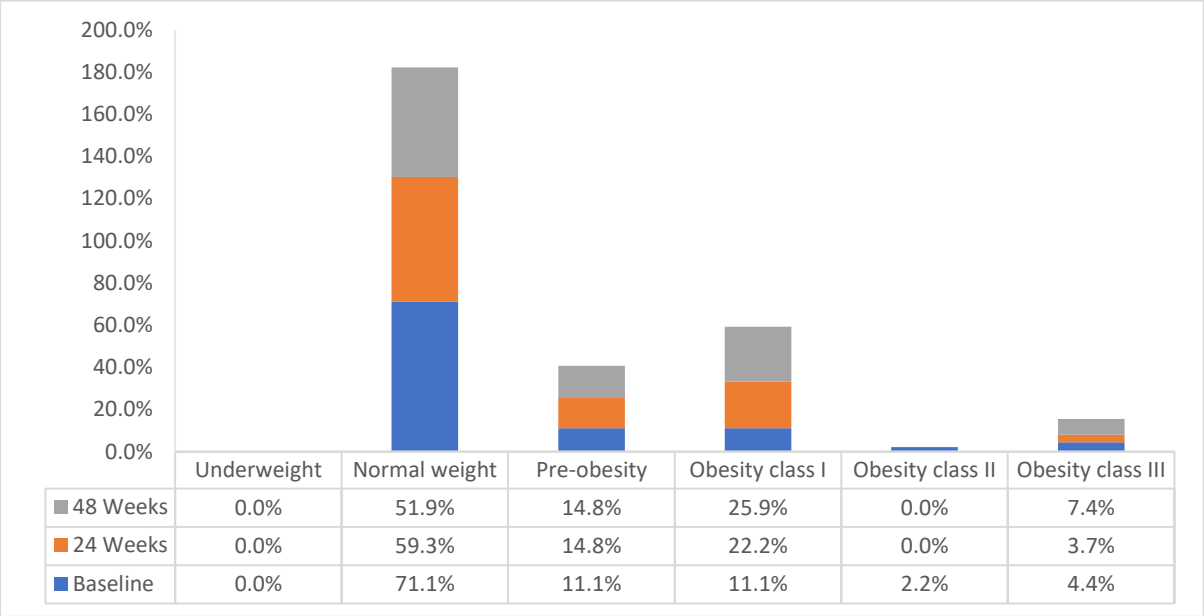
	Underweight	Normal weight	Pre-obesity	Obesity class I	Obesity class II	Obesity class III
48 Weeks	4.8%	57.1%	28.6%	9.5%	0.0%	0.0%
24 Weeks	8.3%	41.7%	37.5%	12.5%	0.0%	0.0%
Baseline	3.9%	52.9%	33.3%	9.8%	0.0%	0.0%

Figure C-3: BMI changes and classification changes in females in the darunavir/ritonavir group



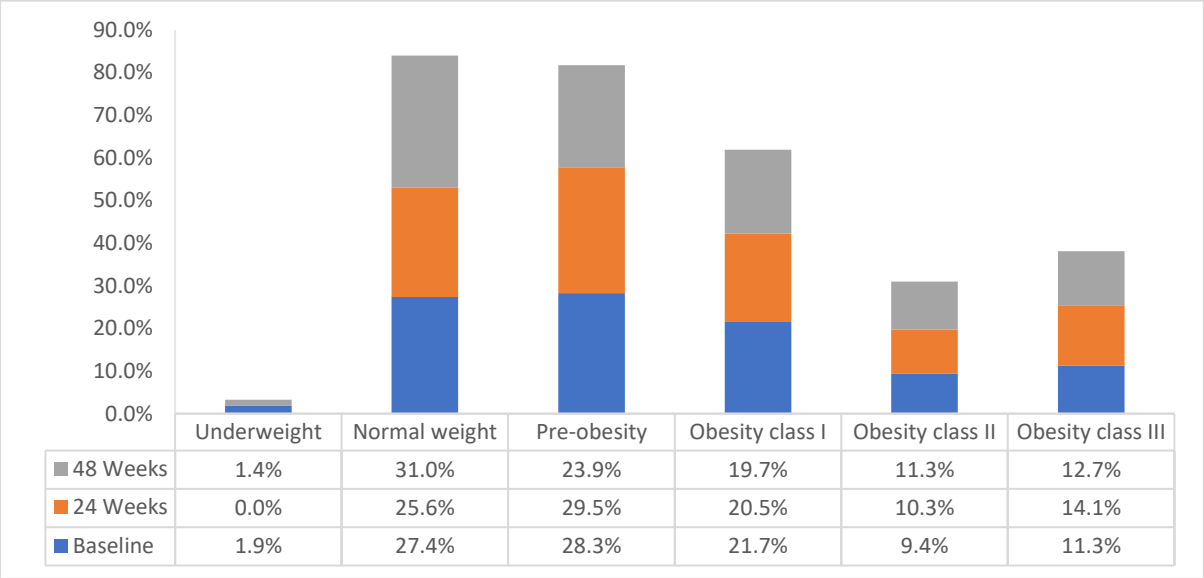
	Underweight	Normal weight	Pre-obesity	Obesity class I	Obesity class II	Obesity class III
48 Weeks	0.0%	24.6%	29.5%	27.9%	11.5%	6.6%
24 Weeks	1.5%	27.7%	27.7%	23.1%	15.4%	4.6%
Baseline	0.0%	37.1%	19.6%	21.6%	17.5%	4.1%

Figure C-4: BMI change and classification changes in males in the lopinavir/ritonavir group



	Underweight	Normal weight	Pre-obesity	Obesity class I	Obesity class II	Obesity class III
48 Weeks	0.0%	51.9%	14.8%	25.9%	0.0%	7.4%
24 Weeks	0.0%	59.3%	14.8%	22.2%	0.0%	3.7%
Baseline	0.0%	71.1%	11.1%	11.1%	2.2%	4.4%

Figure C-5: BMI changes and classifications in female participants in the lopinavir/ritonavir group



	Underweight	Normal weight	Pre-obesity	Obesity class I	Obesity class II	Obesity class III
48 Weeks	1.4%	31.0%	23.9%	19.7%	11.3%	12.7%
24 Weeks	0.0%	25.6%	29.5%	20.5%	10.3%	14.1%
Baseline	1.9%	27.4%	28.3%	21.7%	9.4%	11.3%

Table C-1: Types of lipid disorders in participants that had dyslipidaemia at baseline

High LDLC	43.37%
Low HDLC	2.81%
Mixed dyslipidaemia: high TC and high LDLC	44.18%
Mixed dyslipidaemia: high TC, high LDLC and low HDLC	2.81%
Mixed dyslipidaemia: high LDLC, low HDLC	4.02%
Mixed dyslipidaemia: high TC and low HDLC	0.40%
Baseline-normalised	2.41%

APPENDIX D: PERMISSION FROM WRHI EZINTSHA

4 May 2020

CONSENT TO ACCESS STUDY DATABASE

I, Prof WD Francois Venter, principal investigator of the WRHI 052 study (HREC ref no: 150705B) give permission to Dr Robyn Bruce to access the abovementioned study database for purposes of extracting the selected variables (of participants who completed WRHI 052) outlined in the study protocol *(Lipid changes on Protease Inhibitors in South Africa: A retrospective cross-sectional analysis comparing metabolic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Johannesburg Academic Hospital)* under review.

Should you have any questions please do not hesitate to contact me or Dr Lalla-Edward.

Professor WD Francois Venter PhD, FCP (SA)

Division Head: Ezintsha

Associate Professor, Department of Medicine

University of the Witwatersrand, Johannesburg, South Africa

fventer@wrhi.ac.za

Dr Samanta Lalla-Edward PhD
Head: Research Development
Ezintsha
slalla-edward@whri.ac.za
+27 82 6172490

APPENDIX E: REDCAP DATA CAPTURE SHEET 1

Data during the initial study period

Patient Information

Patients Name

Patient Number

Study Arm

BMI

Weight

Height

BMI

Baseline bloods

Total cholesterol

Baseline HDL

LDL

triglycerides

24 week Lipids

Total Cholesterol 24 weeks

LDL 24 weeks

HDL 24 weeks

Triglycerides 24 weeks

48 week Lipids

Total cholesterol 48 weeks

_____

LDL 48 weeks

_____

HDL 48 weeks

_____

Trigylcerides 48 weeks

_____

Glucose

Baseline fasting glucose

_____

Fasting glucose_48 weeks

_____

Dyslipidaemia treatment prior to entering study

Statin used

◯ Yes
◯ No

Statin type

_____

Statin dose

_____

Other cholesterol lowering medication

◯ Yes
◯ No

Which type?

_____

Was the patient dyslipidaemic at screening/enrollment

Is Statin initiated at screening or enrollment?

◯ Yes
◯ No

Statin used at screening/enrollment?

_____

statin dose used at screening?

_____

Other cholesterol lowering medication initiated at screening/enrollment?

_____

Dyslipidaemia during the study

Did the patient develop dyslipidaemia during the study?
◯ Yes
◯ No

Was therapy initiated?
◯ Yes
◯ No

What Statin was issued?
_____

What dose of statin was issued?
_____

Was anything else issued for cholesterol lowering
◯ Yes
◯ No

What was issued
_____

History of diabetes?

Is the patient diabetic?
◯ Yes
◯ No

What type if medication is the patient on?
_____

What is the HbA1c?
_____

Was a Framingham score calculated?
◯ Yes
◯ No

What was the scrore?
_____

Was target LDL reached
◯ Yes
◯ No

APPENDIX F: REDCAP DATA CAPTURE SHEET 2

2 year follow up

Patient Information

_____

Dyslipidaemia follow up

LDL at follow up

_____

Is the patient on medication for the dyslipidaemia?

◯ Yes
◯ No

What type of Statin is the patient on?

_____

What dose of Statin is the patient on?

_____

Is the patient on any other cholesterol lowering medication

◯ Yes
◯ No

What other medication is the patient on to lower cholesterol?

_____

Is target LDL reached?

◯ Yes
◯ No

Diabetes

Is the patient diabetic?

◯ Yes
◯ No

What is the HBA1c?

_____

What Diabetic medication is the patient on?

_____

APPENDIX G: ETHICS APPROVAL CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr R Bruce
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Medical School
University

E-mail: robynhbruce@gmail.com

CC: Supervisor: Drs F Mohamed & S Stacey; Professor F Venter
<farzahna.mohamed@yahoo.com>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/10/30

REF: *R14/49*

PROTOCOL NO: **M200829** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Lipid changes on protease inhibitors in South Africa: a retrospective cross-sectional analysis comparing metabolȹic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Johannesburg Academic Hospital*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr R Bruce

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200829

NAME: **(Principal Investigator)**	Dr R Bruce
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine Medical School University
PROJECT TITLE:	Lipid changes on protease inhibitors in South Africa: a retrospective cross-sectional analysis comparing metabolic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	2020/08/28
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Drs F Mohamed & S Stacey; Professor F Venter
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/10/30

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore reports and re-certification will be due early in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

APPENDIX H: PERMISSION FROM CMJAH CEO

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. X Dhlamini
Office of the Clinical Director
Email: xolisane.dhlamini@gauteng.gov.za
Tel: (011): 488-3710
08 June 2020

Dear Dr R Bruce

STUDY TITLE: Lipid changes on protease inhibitors in South Africa: A retrospective cross-sectional analysis comparing metabolic profiles and lipid management in patients living with HIV on different protease inhibitors at Charlotte Maxeke Academic hospital.

Permission to conduct the above-mentioned study is provisional approved. Your study can only commence once Ethics approval is obtained. Please forward a copy of your Ethics Clearance Certificate as soon as the study is approved by the Ethics Committee for the CEO's office to give you the final approval to conduct the study.

Supported / not supported

Dr. PN Africa
Acting Clinical Director
DATE:

Approved / not approved

Ms. G. Bogoshi
Chief Executive Officer
DATE:

APPENDIX I: TURNITIN REPORT

Submission date: 19-Oct-2022 05:47PM (UTC+0200)

Submission ID: 1929714337

Word count: 12142

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ORIGINALITY REPORT

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PRIMARY SOURCES

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APPENDIX J: WRHI 052 Study

Low-dose ritonavir-boosted darunavir once daily versus ritonavir-boosted lopinavir for participants with less than 50 HIV RNA copies per mL (WRHI 052): a randomised, open-label, phase 3, non-inferiority trial

Willem D F Venter, Michelle Moorhouse, Simiso Sokhela, Celicia Serenata, Godspower Akpomiemie, Ambar Qavi, Nonkululeko Mashabane, Natasha Arulappan, Joel W Sim, Phumla Z Sinxadi, Lubbe Wiesner, Ellisha Maharaj, Carole Wallis, Tom Boyles, David Ripin, Sarah Stacey, Gila Chitauri, Andrew Hill

Lancet HIV 2019; 6: e428–37

Published Online June 12, 2019 http://dx.doi.org/10.1016/S2352-3018(19)30081-5

See Comment page e412

Wits Reproductive Health and HIV Institute, Faculty of Health Sciences (Prof W D F Venter FCP, M Moorhouse FRSPH, S Sokhela MBChB, C Serenata MBA, G Akpomiemie MPH, N Mashabane BPharm, N Arulappan ND, E Maharaj BSc, T Boyles MD, S Stacey FCP), **and Division of Infectious Diseases, Charlotte Maxeke Johannesburg Academic Hospital** (S Stacey FCP, G Chitauri MBBCh), **University of the Witwatersrand, Johannesburg, South Africa; Faculty of Medicine, Imperial College London, London, UK** (A Qavi MPH, J Weibo Sim MPH); **Division of Clinical Pharmacology, Department of Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa** (P Z Sinxadi PhD, Prof L Wiesner PhD); **BARC, Lancet Laboratories, Johannesburg, South Africa** (C Wallis PhD); **Clinton Health Access Initiative, Boston, MA, USA** (D Ripin PhD); **and Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK** (Prof A Hill PhD)

Correspondence to: Prof William D F Venter, Wits Reproductive Health and HIV Institute, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
fventer@wrhi.ac.za

Summary

Background Pilot studies suggest that ritonavir-boosted darunavir could show high efficacy at doses below those currently approved. We investigated whether switch to 400 mg of darunavir boosted with 100 mg ritonavir once daily could show equivalent efficacy to continuation of ritonavir-boosted lopinavir (a protease inhibitor commonly used in low-income and middle-income countries) for individuals with HIV RNA suppression.

Methods In the WRHI 052 study, a randomised, parallel-group, open-label, non-inferiority phase 3 trial, adults who were HIV-1 positive were enrolled in Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa. Eligible participants were 18 years or older, who tolerated ritonavir-boosted lopinavir in combination with two nucleoside analogues (standard of care) for 6 months or more, and had plasma HIV-1 RNA of less than 50 copies per mL within 60 days of enrolment. We randomly assigned participants (1:1), using a computer-generated randomisation plan, to switch to darunavir (400 mg) boosted with ritonavir (100 mg) once daily or remain on ritonavir-boosted lopinavir (800 mg [plus 200 mg ritonavir]), with nucleoside analogues left unchanged. The primary endpoint was the proportion of patients with less than 50 HIV-1 RNA copies per mL at week 48 (US Food and Drug Administration snapshot algorithm; non-inferiority margin –4%). Primary and safety analyses included participants receiving at least one dose of darunavir boosted with ritonavir. This trial is registered with ClinicalTrials.gov, number NCT02671383.

Findings Between June 30, 2016, and June 15, 2017, 148 participants were assigned to ritonavir-boosted darunavir 400 mg and 152 continued on their lopinavir-containing regimen. Four (3%) patients in the darunavir group and three (2%) in the lopinavir group discontinued before week 48. At week 48, darunavir was non-inferior to lopinavir for the primary outcome (142 [96%] of 148 participants on darunavir had <50 HIV-1 RNA copies per mL *vs* 143 [94%] of 152 participants on lopinavir; difference 1·9% [95% CI –3·4 to 7·3]), with a predefined margin of –4%. More participants taking darunavir (30 [20%] participants) had drug-related adverse events than those on lopinavir (eight [5%]), but the adverse events were generally asymptomatic and resolved when switching back to lopinavir. Elevated liver transaminase in three (1%; one symptomatic) darunavir participants led to study withdrawal; all transaminase elevations resolved on restarting lopinavir.

Interpretation Low-dose ritonavir-boosted darunavir might be a safe and efficacious switch option to maintain HIV suppression for patients on lopinavir. However, an adequately powered and designed study in viraemic participants is needed.

Funding South African Medical Research Council, United States Agency for International Development, and US National Institute of Allergy and Infectious Diseases.

Introduction

Most low-income and middle-income countries (LMICs) use a protease inhibitor with two nucleoside reverse transcriptase inhibitors (NRTIs) to treat HIV after failure of first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy, in accordance with WHO antiretroviral guidelines.[1] This treatment strategy is effective for second-line treatment.[2,3] The second-line protease inhibitor most commonly used in LMICs is ritonavir-boosted lopinavir, which has been associated with increased lipid concentrations, gastrointestinal adverse events, and dosing of two pills twice daily. Ritonavir-boosted atazanavir, an alternative boosted protease inhibitor better tolerated than ritonavir-boosted lopinavir in terms of gastrointestinal and lipid profiles, and increasingly used in LMICs, has additional risks of scleral icterus and renal adverse events.[3]

Research in context

Evidence before this study

We searched PubMed and ClinicalTrials.gov between Sept 1, 2006, and Aug 9, 2018, for clinical trials and pharmacokinetic or cohort studies of darunavir boosted with ritonavir. We used combinations, abbreviations, and variations of the title or abstract search terms of "darunavir", "TMC114", and "dose optimization". We also used general internet searches to acquire relevant conference abstracts. Searches were limited to articles published in English. Our search yielded four articles summarising results of pharmacokinetics studies supporting the use of reduced-dose darunavir. One study showed that darunavir 800 mg resulted in a lower darunavir minimum concentration when boosted with cobicistat compared with ritonavir as a booster, with equal potency. This effect is further supported by two pharmacokinetic studies that did not show any significantly different drug exposures in both blood and seminal plasma, despite a reduction from 800 mg of darunavir boosted with 100 mg of ritonavir once daily to 400 mg darunavir (same ritonavir dose). Cerebrospinal fluid concentrations were also found to be similar between 800 mg and 600 mg darunavir (boosted with 100 mg of ritonavir) once daily in one study. The search also yielded three articles evaluating non-standard doses of darunavir (600 mg or 400 mg daily) as either a first-line treatment, or as a switch option in HIV-1 infected individuals who were virologically suppressed on the standard 800 mg once-daily dose with a dual nucleoside backbone, and all of which showed equivalent potency.

Added value of this study

Our findings show non-inferiority of switching from standard dose lopinavir-boosted ritonavir (frequently used in low-income and middle-income countries [LMICs]) to darunavir (400 mg) boosted with ritonavir (100 mg) once daily while maintaining the same dual nucleoside backbone in HIV-1 infected participants with less than 50 HIV-1 RNA copies per mL. To date, this study comprises the largest trial population in which the efficacy and safety of a reduced once-daily dose of 400 mg of darunavir has been evaluated. These results are consistent with pilot studies of low-dose ritonavir-boosted darunavir, which showed no difference in efficacy compared with currently recommended 800 mg once-daily dose for patients who are protease inhibitor-naive.

Implications of all the available evidence

Co-formulated ritonavir-boosted lopinavir is currently used in LMICs because it costs less than standard darunavir (800 mg) boosted with ritonavir (100 mg). The lower 400 mg once-daily dose of darunavir could allow widespread use of darunavir in LMICs, at an affordable price. These findings support further evaluation of darunavir, widely acknowledged in the medical community as the most potent and best tolerated protease inhibitor, at the lower dose of 400 mg once daily, improving safety and lowering long-term costs of mass treatment in LMICs. Ongoing studies are evaluating the use of alternative dosing for ritonavir-boosted darunavir with rifampicin, as dose-adjusted ritonavir-boosted lopinavir is currently the only recommended protease inhibitor with first-line tuberculosis therapy. Pregnancy might be associated with lower darunavir exposure, and further studies are needed before recommending the low-dose treatment to pregnant women. Future clinical trials should evaluate this new reduced dose of darunavir in protease inhibitor-naive participants after first-line treatment failure.

Darunavir boosted with ritonavir is the most widely recommended protease inhibitor treatment in high-income countries. The recommended dose is 800 mg of darunavir plus 100 mg of ritonavir once daily for individuals with no protease inhibitor resistance, and 600 mg of darunavir plus 100 mg of ritonavir twice daily for patients pretreated with a protease inhibitor but with known resistance to the therapy.[4] Results from studies on ritonavir-boosted darunavir have shown efficacy and safety benefits over ritonavir-boosted lopinavir and atazanavir.[5–9] However, darunavir has traditionally not been considered as a substitute for these protease inhibitor combinations in LMICs because of its higher cost.[3]

Darunavir is the only protease inhibitor with the optimal dose established in trials of individuals highly pretreated for HIV with antiretrovirals. In the POWER studies,[8–10] for the subset of participants harbouring virus phenotypically sensitive to darunavir, no difference in HIV RNA reductions was measured between the 400 mg once-daily dose and higher doses up to 600 mg twice daily. POWER 1 and 2 were randomised phase 3b studies done over 48 weeks, and had low numbers of patients (110 participants in the darunavir group and 120 in the non-darunavir protease inhibitor group), which, although favouring the darunavir group for both efficacy and safety, might have obscured efficacy differences within the group.

The pharmacokinetics of ritonavir-boosted darunavir is not proportional to dose. The dose–concentration relationship is non-linear with increasing doses of ritonavir-boosted darunavir, which might be attributed to the multidirectional interaction between darunavir and ritonavir. With the reduced ritonavir-boosted darunavir dose (400 mg of darunavir and 100 mg of ritonavir), the induction of ritonavir is also reduced, which explains the high ritonavir concentrations measured with this dose. In turn, this increase in ritonavir concentration increases its pharmacokinetic enhancing effect. In the original dose-ranging studies, the area under the curve (AUC) of the 400 mg darunavir and 100 mg ritonavir once-daily dose was only 27% lower than the AUC when the darunavir dose was 800 mg (once daily).[11] In the DARULIGHT study,[12] participants' treatment was switched from 800 mg darunavir to 400 mg darunavir once daily (both boosted

with 100 mg ritonavir). The AUC and maximum plasma concentration of darunavir did not change substantially despite the 50% reduction in dose.

For protease inhibitor-naive individuals, the association between darunavir pharmacokinetics and efficacy has not been established. In the ARTEMIS study,[13] treatment-naive individuals were given 800 mg of darunavir plus 100 mg of ritonavir once daily; participants with the highest darunavir AUC were significantly less likely to show HIV RNA suppression by week 48. In the ODIN study,[14,15] with the same dose, no significant association was found between darunavir AUC and efficacy.[14,15] Darunavir has been approved at the 800 mg once-daily dose boosted with cobicistat (150 mg once daily), which leads to significantly lower darunavir minimum concentrations than ritonavir-boosted darunavir.[4] In healthy volunteers, the minimum plasma concentration of darunavir 800 mg once daily was significantly lower when boosted with cobicistat (mean 1478 ng/mL) than when boosted with ritonavir (2015 ng/mL), with the authors suggesting that any reduction in the darunavir minimum concentration up to 50% should not adversely affect the efficacy profile of darunavir.[16]

Finally, three pilot studies have evaluated the efficacy of darunavir boosted with ritonavir (100 mg) at doses lower than 800 mg. The randomised GESIDA study[17] and an Italian study[18] evaluated 600 mg once daily, whereas the DARULIGHT study evaluated 400 mg once daily.[12] Results from these pilot studies suggested that ritonavir-boosted darunavir could achieve high rates of HIV RNA suppression at doses lower than 800 mg.

The Clinton Health Access Initiative has projected cost savings of low-dose darunavir boosted with ritonavir compared with ritonavir-boosted lopinavir, if substantial manufacturing volumes are attained. This strategy would mean that ritonavir-boosted darunavir could be slightly cheaper than conventional protease inhibitor formulations (US$170 per patient per year for darunavir 400 mg boosted with ritonavir 100 mg, and at scale as low as $120 per patient per year, instead of $159–165 for ritonavir-boosted atazanavir and $203 for ritonavir-boosted lopinavir).[19]

Reductions in the dose of darunavir could improve safety and lower costs of mass treatment in LMICs. The Wits Reproductive Health and HIV Institute (WRHI) 052 study was designed to evaluate whether switching to 400 mg of darunavir boosted with 100 mg ritonavir once daily could show equivalent efficacy to continuation of ritonavir-boosted lopinavir in individuals with HIV RNA suppression.

Methods

Study design and participants

WRHI 052 is a 48-week, phase 3, randomised, open-label, parallel-group, non-inferiority study done at Charlotte Maxeke Johannesburg Academic Hospital, a large teaching hospital in central Johannesburg, South Africa, and was approved by the drug regulator (the South African Health Products Regulatory Authority), local authority, and ethics committee, and overseen by an independent data safety monitoring board (DSMB). All participants provided written informed consent before the study.

Investigators enrolled HIV-1-positive adults (aged ≥18 years) on ritonavir-boosted lopinavir and two NRTIs (standard of care) for more than 6 months, weighing more than 40 kg, and with no history of protease inhibitor use other than ritonavir-boosted lopinavir. Participants had to be virologically suppressed (plasma HIV-1 RNA <50 copies per mL) within 60 days before enrolment. Exclusion criteria were being on any other antiretrovirals other than NRTIs and ritonavir-boosted lopinavir; being on rifampicin or any other therapy with major cytochrome P450 interactions; pregnancy, breastfeeding, or desiring pregnancy; sulfonamide allergy; any history of genotype-documented protease inhibitor resistance, history of drug or alcohol misuse that was deemed to be a potential impediment to adherence, and a likelihood to relocate to an area away from the study site. HIV-1 drug resistance testing is not standard of care in South Africa, unless the patient is not responding to second-line protease inhibitor-based regimen, so genotype results before randomisation were not anticipated.

Randomisation and masking

Randomisation was done with a computer-generated randomisation plan. Site pharmacists managed a system of sealed envelopes with all participants assigned a unique participant number based on the randomisation plan. Participants were randomly assigned (1:1) to switch to 400 mg of darunavir plus 100 mg of ritonavir without changing their dual-NRTI backbone, or to continue their current regimen containing lopinavir boosted with ritonavir for 48 weeks; allocation was open-label. Masking was not attempted, because the additional value to the study against cost was small and it would have added substantial complexity.

Procedures

The trial included a screening period of 60 days and a 48-week treatment period during which participants were switched to the study regimen or continued their current regimen (two fixed-doses of lopinavir [200 mg per tablet] and ritonavir [50 mg per tablet] tablets twice daily in combination with two NRTIs). The NRTIs were typically either tenofovir plus emtricitabine or zidovudine plus lamivudine.

The study regimen consisted of darunavir (one tablet of 400 mg) and ritonavir (one tablet of 100 mg), with the dual-NRTI backbone left unchanged. Study drugs were procured from Hetero Drugs (Hyderabad, India), with the other medication supplied via the South African Department of Health. Patients in the darunavir group were advised to take their medication with food.

Study visits were scheduled for baseline, and weeks 12, 24, 36, and 48, with unscheduled visits occurring between

these visits. Participants who prematurely discontinued or changed study treatment were encouraged to complete end-of-study assessments. Any participant who had an ongoing or serious adverse event at their last study visit had a 30-day follow-up visit unless consent had been withdrawn.

We monitored treatment adherence through participant self-report and pill count. HIV-1 RNA was quantified by the central laboratory using the Abbott RealTime HIV-1 viral load assay (Abbott, Chicago, IL, USA), according to manufacturer instructions.

For participants with more than 200 HIV-1 RNA copies per mL at any visit up until the end of week 48, HIV-1 resistance testing was done at a central laboratory with a laboratory-developed population-based assay that sequenced the full protease and reverse transcriptase regions of HIV. All participants with detectable viral loads received adherence counselling.

Safety was assessed through symptom screening with a directed physical examination along with laboratory analyses. Laboratory tests included haematology, clinical chemistry, renal and liver function, fasting lipid and glucose variables, and CD4 cell counts. All darunavir concentrations were measured after trial completion. Laboratory assay details are supplied in the appendix.

Any adverse events and clinically significant laboratory abnormalities were graded according to the Division of AIDS grading table scale.[20] If grade 3 or 4 laboratory abnormalities occurred, the study doctors and principal investigators were notified, and confirmatory tests were done. Investigators deemed adverse events as drug related when appropriate. Women were counselled to avoid pregnancy, which was a key feature of informed consent; the site has free and ready access to multiple forms of contraception. Pregnancy testing was done at all visits on women of childbearing potential. Pregnancy was not a reason for termination from the study, although the dose of darunavir was increased to 800 mg once daily for the duration of the pregnancy.

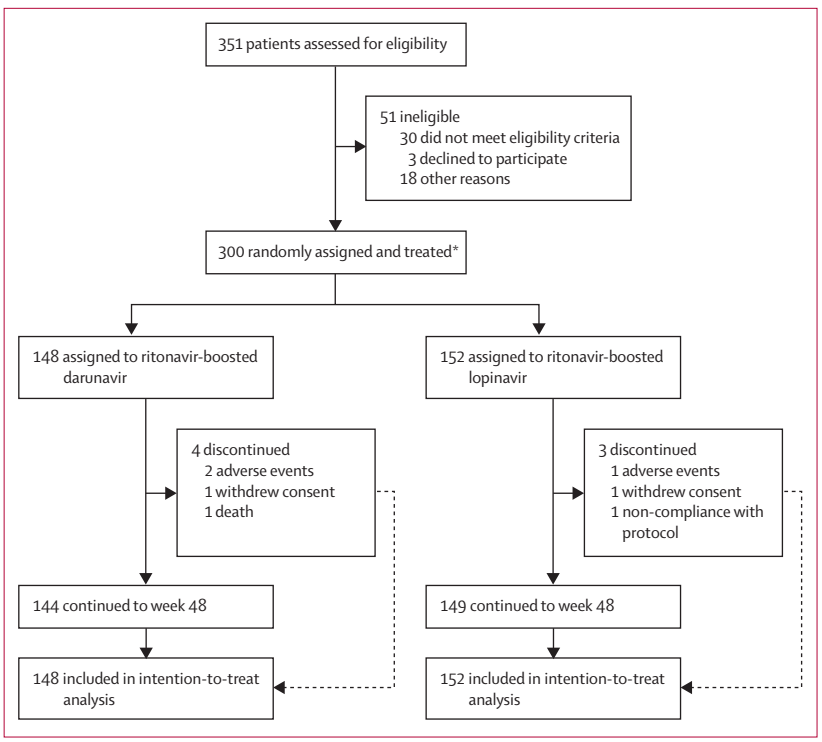

Figure 1: **Trial profile**
*Received at least one dose of study medication.

Outcomes

The primary efficacy endpoint was HIV RNA suppression to less than 50 copies per mL at the week 48 timepoint, by use of the US Food and Drug Administration (FDA) snapshot endpoint approach (intention-to-treat [ITT]). With this approach, a participant was classified as a treatment success if their HIV RNA was less than 50 copies per mL at week 48 (range 46–50); viral failure was classified as either HIV RNA of 50 or more copies per mL until the week 48 time window, missing HIV RNA data during this period, or earlier discontinuation of randomised treatment for adverse events or other reasons.[21] We also analysed the primary outcome with one modification to the FDA snapshot method: participants who had missing HIV RNA data within the 48-week time period, but HIV RNA suppression of less than 50 copies per mL at study visits before and after week 48, were classified as treatment successes (modified FDA snapshot).

See Online for appendix

Secondary endpoints were time to virological failure (defined as confirmed HIV-1 RNA concentrations of ≥200 copies per mL), change from baseline in plasma HIV-1 RNA and CD4 cell concentrations, and safety and tolerability measures, including the laboratory measures described. In addition, we assessed HIV-1 drug resistance mutations to NRTIs or protease inhibitors in those with persistently detectable viraemia of more than 200 copies per mL.[22]

Statistical analysis

WRHI 052 was designed to assess whether the efficacy of darunavir (400 mg) boosted with ritonavir (100 mg) once daily is non-inferior to ritonavir-boosted lopinavir at week 48. The original sample size calculation assumed an overall response rate of 80%, power of 80%, a two-sided 5% significance level, and non-inferiority margin of –12%.[23] Given these estimates, a sample size of 150 participants per group was required. During the course of the trial, the FDA changed their guidance for non-inferiority trials to specify a more stringent non-inferiority margin of –4% for switching studies.[21] After discussion with the trial DSMB statisticians, the statistical analysis plan was modified to include testing for non-inferiority with the –4% margin.

We used a Z test to compare the proportion of participants with less than 50 HIV RNA copies per mL

	Ritonavir-boosted darunavir (n=148)	Ritonavir-boosted lopinavir (n=152)
Patient characteristics		
Age, years	42 (26–75)	42 (20–69)
Sex		
Male	51 (34%)	45 (30%)
Female	97 (66%)	107 (70%)
Ethnicity		
Black	147 (99%)	152 (100%)
Mixed	1 (1%)	0
Weight, kg	72 (44–119)	70 (42–137)
Body-mass index (kg/m²)	26 (18–46)	27 (18–48)
Disease characteristics		
<50 HIV-1 RNA copies per mL	148 (100%)	149 (98%)
Mean CD4-positive cell count (SD), cells per µL	623 (270)	646 (275)
NRTIs		
Tenofovir plus emtricitabine	67 (45%)	50 (33%)
Tenofovir plus lamivudine	10 (7%)	14 (9%)
Lamivudine plus zidovudine	61 (41%)	79 (52%)
Other	10 (7%)	9 (6%)

Data are n (%) or median (IQR), unless otherwise stated. NRTI=nucleoside reverse transcriptase inhibitor.

Table 1: **Baseline characteristics**

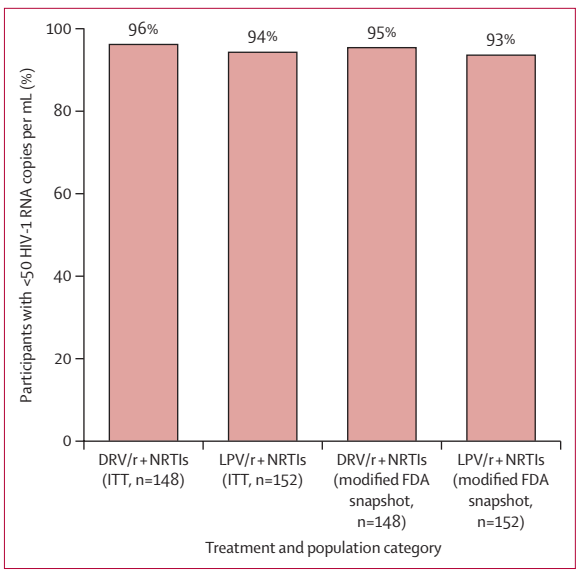

Figure 2: **Virological outcomes**
DRV/r=ritonavir-boosted darunavir. NRTI=nucleoside reverse transcriptase inhibitor. ITT=intention-to-treat. LPV/r=ritonavir-boosted lopinavir. FDA=Food and Drug Administration.

between the two treatment groups. 95% CIs were then calculated to provide an estimate of the difference between the two groups. All data, when applicable, were summarised by treatment group using descriptive statistics. Continuous variables are summarised by number of participants and the median. Categorical variables were summarised using frequency counts and percentages. The primary endpoint and safety analyses were done for the ITT population. We also did a sensitivity analysis in participants who had discontinued randomised treatment (excluding pregnant women); this analysis included all HIV RNA results at the week 48 timepoint, even if participants were then taking antiretrovirals they had not been originally randomly assigned.

All analyses were done with STATA version 13.1. This study is registered with ClinicalTrials.gov, number NCT02671383.

Role of the funding source

The funders (other than the National Institute of Allergy and Infectious Diseases) were involved in the study design and were given regular reports on the study conduct. The funders had no role in data collection, data analysis, data interpretation, or writing of the report (the United States Agency for International Development reviewed the manuscript). The corresponding author had full access to all the data and final responsibility for the decision to submit for publication.

Results

Between June 30, 2016, and June 15, 2017, 351 participants were assessed for eligibility. 300 participants previously stable on two NRTIs and ritonavir-boosted lopinavir with HIV RNA of less than 50 copies per mL were randomly assigned to ritonavir-boosted darunavir (n=148) or stay on ritonavir-boosted lopinavir (n=152; figure 1). Baseline study entry characteristics were similar between the two groups (table 1); almost all participants were black (299 [>99%] of 300 participants) and the majority (204 [68%] participants) were female, with high CD4 cell counts. Although most patients were South African, 87 (29%) patients (42 [28%] in the ritonavir-boosted darunavir group and 45 [30%] in the ritonavir-boosted lopinavir group) were from Zimbabwe and 16 (5%; six [4%] in the ritonavir-boosted darunavir group and ten [7%] in ritonavir-boosted lopinavir group) from other African countries.

11 women became pregnant during the study: four women in the ritonavir-boosted darunavir group and seven in the ritonavir-boosted lopinavir group. One woman in the lopinavir group was pregnant twice. Five pregnancies were carried to term: one in the ritonavir-boosted darunavir group and four in the ritonavir-boosted lopinavir group. All pregnant women received dose escalation of darunavir, according to protocol. Within this group of pregnancies, only one woman, in the ritonavir-boosted darunavir group, was viraemic at any point before or after dose escalation during the study (viral load of 116 copies per mL at week 48). Three miscarriages occurred, at pregnancy week 3 (darunavir group), 7 (lopinavir group), and 14 (lopinavir group). No other data on fetal outcomes are available.

Non-inferiority was confirmed in the primary efficacy analysis (ITT, using the original FDA snapshot approach): 142 (96%) of 148 participants in the ritonavir-boosted darunavir group had less than 50 HIV-1 RNA copies per mL versus 143 (94%) of 152 participants in the ritonavir-boosted lopinavir group (difference 1·9% [95% CI −3·4 to 7·3]; figures 2, 3); findings were similar using the modified FDA snapshot method (141 [95%] of 148 participants in the ritonavir-boosted darunavir group *vs* 142 [93%] of 152 participants in the ritonavir-boosted lopinavir group; difference 1·9% [95% CI −3·7 to 7·5]; figures 2, 3). Of the six participants in the ritonavir-boosted darunavir group and nine participants in the ritonavir-boosted lopinavir group without documented HIV RNA of less than 50 copies per mL by week 48, one patient and six patients, respectively, had low-concentration viraemia (50–199 copies per mL) and two in the darunavir group had transient high-concentration viraemia at week 48, which resolved after counselling on treatment adherence; seven participants in the ritonavir-boosted darunavir group and ten participants in the ritonavir-boosted lopinavir group were lost to follow-up and had missing data. At week 48, 140 (99%) of 141 participants in the ritonavir-boosted darunavir group versus 142 (97%) of 147 participants in the ritonavir-boosted lopinavir group had less than 50 HIV RNA copies per mL (modified FDA snapshot approach; figure 4).

Four (67%) of the six participants in the ritonavir-boosted lopinavir group who had persistently detectable viraemia had NRTI or NNRTI mutations during moments of transient viraemia versus one (25%) of four participants in the darunavir group (table 2). No major protease inhibitor mutations were observed in any participants. Samples from six participants did not amplify (two in the darunavir group, four in the lopinavir group).

Non-inferior efficacy was also shown when analysing only patients who had 50 copies of HIV-1 RNA per mL or more at week 48 after exclusion of pregnant women (sensitivity analysis; appendix p 1). The FDA snapshot modification allowed us to include data on an additional three participants, two in the ritonavir-boosted darunavir group and one in the ritonavir-boosted lopinavir group; however, even without these data, the non-inferiority finding held (figure 3). At week 48 in the ritonavir-boosted darunavir group, darunavir concentrations were lower than the limit of quantification of the assay in two (67%) of three participants with viral load of more than 40 copies per mL versus two (1%) of 140 participants with supressed viral load (p=0·002).

The new darunavir dose was well tolerated; investigator-assessed drug-related adverse events were almost all asymptomatic and confined to transient changes in hepatic function (table 3). No differences were observed in LDL or HDL, haemoglobin, or fasting glucose between groups (appendix). Minor differences were measured in total cholesterol, creatinine, aspartate transaminase, and alanine transaminase, with a mean decrease of 62·6 CD4 cells per µL (SD 167·2) from baseline to week 48 in the darunavir group (appendix p 2). Drug-related adverse events seen in the darunavir group were mostly due to the development of transaminitis. However, almost all laboratory abnormalities spontaneously resolved without intervention.

Notably, three participants in the darunavir group developed liver dysfunction (including one at the

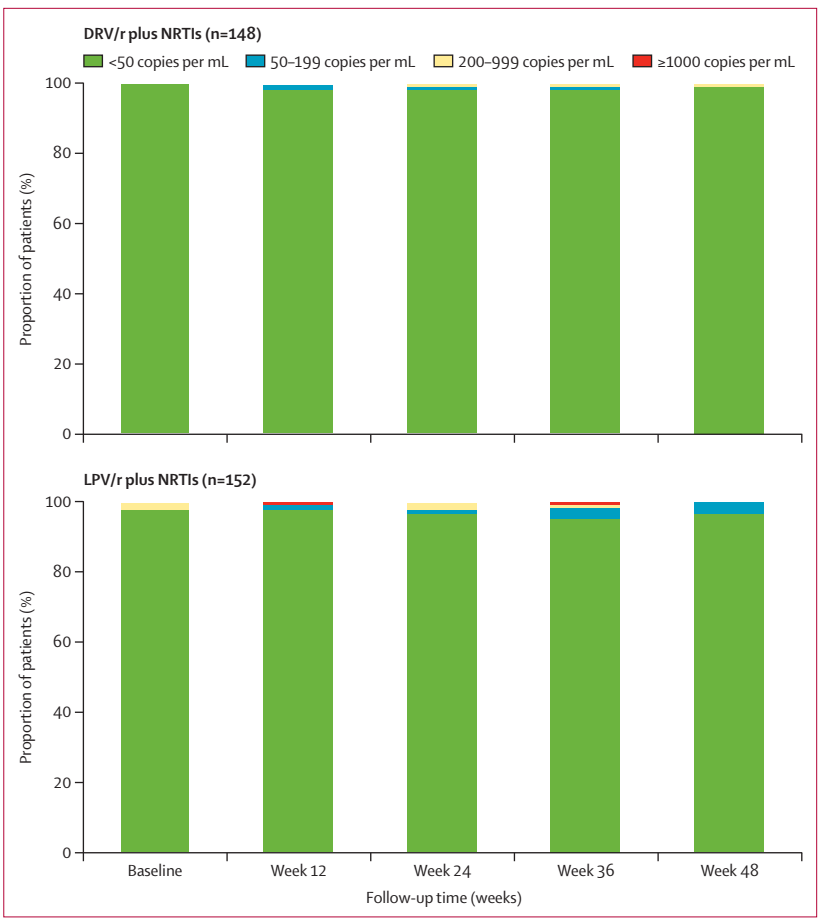

Figure 3: **Number of HIV RNA copies by treatment group (observed data)**
DRV/r=ritonavir-boosted darunavir. NRTI=nucleoside reverse transcriptase inhibitor. LPV/r=ritonavir-boosted lopinavir.

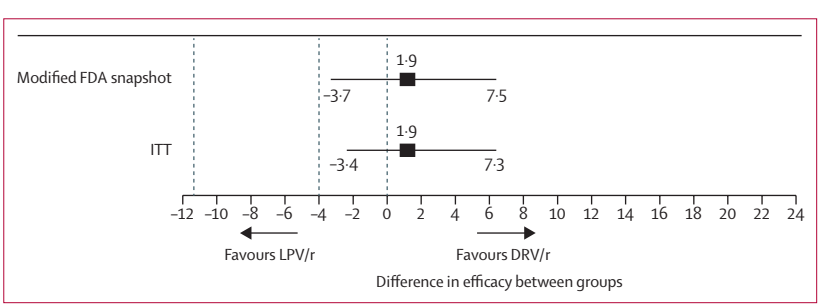

Figure 4: **Primary efficacy analysis at week 48**
Modified FDA snapshot (HIV RNA is less than 50 copies per mL at week 48; if unavailable, previous or subsequent viral load is used) and intention-to-treat (all patients assigned to treatment) methods. FDA=Food and Drug Administration. ITT=intention to treat. LPV/r=ritonavir-boosted lopinavir. DRV/r=ritonavir-boosted darunavir.

	DRV/r plus NRTIs (n=4)	LPV/r plus NRTIs (n=6)
No protease inhibitor or NRTI mutations	3 (75%)	2 (33%)
Protease inhibitor mutations	0	0
NRTI mutations	1 (25%)	4 (67%)
Met184Val	1 (25%)	3 (50%)
Lys219Glu	0	1 (17%)
Lys65Arg	0	1 (17%)
Tyr115Glu	0	1 (17%)
Lys70Arg	0	1 (17%)

Data are n (%). All samples had more than 200 HIV RNA copies per mL at any visit until week 48. DRV/r=ritonavir-boosted darunavir. LPV/r= ritonavir-boosted lopinavir. NRTI=nucleoside reverse transcriptase inhibitor.

Table 2: **Population-based HIV-1 drug resistance tests**

	DRV/r plus NRTIs (n=148)	LPV/r plus NRTIs (n=152)
Any adverse event	100 (68%)	106 (70%)
Most common adverse events (in ≥4% of participants in one group)		
Respiratory tract infection	31 (21%)	34 (22%)
Influenza	14 (9%)	13 (9%)
Rash	3 (2%)	11 (7%)
Elevated alanine transaminase	8 (5%)	5 (3%)
Headache	6 (4%)	5 (3%)
Backache	3 (2%)	8 (5%)
Hypertension	6 (4%)	3 (2%)
Transaminitis	7 (5%)	2 (1%)
Constipation	6 (4%)	2 (1%)
Grade 3–5 adverse events	7 (5%)	5 (3%)
Drug-related adverse events	30 (20%)	8 (5%)
Serious adverse events	6 (4%)*	3 (2%)
Drug-related serious adverse events	4 (3%)†	1 (1%)
Adverse events leading to withdrawal‡	2 (1%)	1 (1%)

Data are n (%). DRV/r=ritonavir-boosted darunavir. LPV/r=ritonavir-boosted lopinavir. NRTI=nucleoside reverse transcriptase inhibitor. *One patient died from myocardial infarction after week 12. †Includes one patient who developed anaemia after a miscarriage, and for whom zidovudine was substituted with abacavir. ‡DRV group: both hepatitis cases; LPV group: relocated after developing tuberculosis and LPV double-dosing.

Table 3: **Summary of adverse events**

end-of-study 48-week visit), all of which resolved on interruption of therapy followed by a switch to lopinavir, after a thorough medical history, ultrasound, and hepatitis screening showed no alternative explanation for the abnormal laboratory testing. In one participant (on atorvastatin), the liver dysfunction was accompanied by slow-onset abdominal pain. Darunavir treatment was interrupted for this participant until symptoms and hepatic function had returned to normal; however, reintroduction led to similar symptoms and laboratory findings, and the participant was successfully switched back to lopinavir. The darunavir concentration (time from last dose not recorded) was 4160 ng/mL. The other two liver dysfunction cases were asymptomatic, and similarly were resolved after reinitiating lopinavir treatment. One patient had a hepatotoxic event at week 12, with a 2810 ng/mL darunavir concentration measured 12 h after the last dose. The third patient had a hepatotoxic event at week 48 with a concentration of 1430 ng/mL measured 15 h after the previous dose, lower than the previous concentration of 2230 ng/mL measured 18 h after the last dose at week 36 (time of measurement for weeks 12 and 24 was not recorded, but concentrations were 2200 ng/mL and 2910 ng/mL, respectively). In the three patients with hepatotoxicity, darunavir concentrations were similar to those without hepatotoxicity (appendix p 11). A single death at home, from presumed myocardial infarction, occurred in the darunavir group, after the 12-week visit. One patient on an atazanavir plus lamivudine backbone developed anaemia after a miscarriage and the attending doctor elected to alter her entire regimen.

Discussion

In this randomised non-inferiority trial, the low dose of darunavir (400 mg) boosted with ritonavir (100 mg) was non-inferior to ritonavir-boosted lopinavir in terms of virological suppression and was well tolerated; the three cases of liver dysfunction (not reported in other studies) were resolved after switching back to ritonavir-boosted lopinavir, suggesting that clinical and laboratory monitoring might be required after switching. Consistent results were seen in the secondary analysis. We kept the entry criteria as realistic as possible, anticipating that any switch strategy in large public sector clinics might not assess treatment histories, but rather assess if the patient is stable on ritonavir-boosted lopinavir and virologically suppressed before switching treatments. No treatment-emergent drug resistance to protease inhibitors occurred in either group among the few participants who became viraemic during the trial. The results from this study are consistent with previous pilot studies, which also showed high numbers of HIV RNA suppression for participants treated with ritonavir-boosted darunavir (either 400 mg or 600 mg of darunavir once daily).

This study has several key limitations. Firstly, the sample size of 300 participants was originally set to establish non-inferior efficacy for darunavir boosted with ritonavir with a predicted response rate of 80% at week 48 and the non-inferiority margin of –12%.[23] During the course of the study, the FDA changed their guidelines for non-inferiority trials to specify a new smaller margin of –4% for treatment switch studies.[21] After consultation with the trial DSMB, the analysis plan was modified to test for non-inferiority at this new margin, because the overall response was higher than originally predicted. The final 48-week analysis showed non-inferiority within the 4% margin, but switching studies prospectively designed with this endpoint would typically be much larger (approximately 1300 participants).[21] The WRHI 052 trial showed non-inferior efficacy despite the relatively small sample size

because the response rate (>95% participants with <50 HIV RNA copies per mL at week 48) was substantially higher than predicted (80%).

Additionally, this study recruited participants who tolerated ritonavir-boosted lopinavir. Participants with serious adverse events from this treatment, such as gastrointestinal disturbance or elevations in lipids, would probably have already discontinued the treatment earlier and not entered the trial. This effect might have led to an underestimation of the difference in safety between ritonavir-boosted lopinavir and darunavir, which was clearly observed in first-line studies, such as ARTEMIS.[5] Switching patients who are stable and presumably tolerating a regimen can only lead to the same or worse outcomes; in our study, four times as many patients in the darunavir group had drug-related adverse events, albeit events that were minor and largely laboratory-based, and which were resolved. However, three patients had laboratory and clinical features that required substitution of ritonavir-boosted darunavir, meaning that patient education and clinical and laboratory monitoring might be required if this strategy is used. The WRHI 052 study is a switch trial in participants with HIV RNA suppression on protease inhibitor-based treatment. The most common use for second-line combinations of two NRTIs with ritonavir-boosted lopinavir would be in individuals who have not responded to a first-line treatment regimen consisting of two NRTIs plus an NNRTI. We do not have accurate data on the proportion of patients who were taking ritonavir-boosted lopinavir because of failure of a first-line regimen or because they were intolerant to NNRTIs and switched to ritonavir-boosted lopinavir, but clinical experience of the site suggests most patients were on lopinavir because of virological failure. A new randomised trial is required to compare ritonavir-boosted darunavir at the standard and lower doses for people who do not respond to a first-line regimen. Baseline differences existed between the two groups in the distribution of the NRTI backbone, but as potency remained strong across groups, we are unable to comment on whether backbone nucleoside potency affects response. The fact that the study was not blinded might have affected both patient and clinician reporting of adverse events. Generalisability of the study is enhanced by over a third of patients not being from South Africa; however, the site is well resourced, and applicability to less resourced sites should be assessed with care. Exclusion criteria, specifically around pregnant women and nucleoside backbone selection, might limit applicability to all patients who are HIV-positive. Finally, even if the 400 mg once-daily dose is proven to be efficacious in a second-line regimen, some individuals will have low darunavir drug concentrations, either from pregnancy or interactions with concomitant medications (such as rifampicin).[3,4,24] If this regimen is to be developed for widespread use, the efficacy of this dose needs to be documented in key populations. Any strategy that involves this lower dose switch in women requires attention to dose escalation in the event of pregnancy, until data suggest that concentrations remain sufficiently therapeutic with 400 mg throughout pregnancy. Ongoing studies are evaluating the efficacy of different doses of darunavir in the presence of rifampicin.[25]

Lowering drug dose represents an often untapped possibility for decreasing costs and toxicity. In high-income countries, manufacturing costs for originator companies comprised only a small part of the final price of the drug. Therefore, originator pharmaceutical companies have little incentive to study lower doses. With the rise of generic manufacturers, as well as increased licensing by originator companies to other pharmaceutical companies, costs of raw materials to manufacture the drugs (active product ingredient) have become a more substantial component of total production cost because prices have decreased. Any cost savings analysis would need to factor in the cost of monitoring, as well as potentially large cost drivers, such as liver disease, after switching doses.

Decreasing the dosages of commonly used antiretrovirals presents a major opportunity to reduce overall drug cost, with this success being realised with the ENCORE1 study,[26] which showed that treatment with a 400 mg dose of efavirenz was similar to the 600 mg dose. Similar results were obtained with the LASA study,[27] showing non-inferior efficacy for atazanavir (200 mg) boosted with ritonavir (100 mg) once daily, compared with the standard 300 mg of atazanavir and 100 mg of ritonavir, as a switch option in Thai participants with HIV RNA suppression.

Darunavir is now regarded as the most effective and least toxic antiretroviral in its class. Decreasing its price, while preserving potency and further reducing toxicity, might allow LMICs to access standard-of-care drugs within the small range of second-line antiretrovirals available to them. For most countries, individuals transition to a twice-daily atazanavir-containing regimen; this option currently is not a substantial gain in terms of cost or potency over the twice-a-day ritonavir-boosted lopinavir. However, in the future, darunavir could possibly be combined with daily dolutegravir, other integrase inhibitors, or other daily NRTIs, and studies are being designed to investigate whether this potentially efficacious, safe, and once-daily regimen will be an effective alternative to the current twice-daily second-line regimens. Nanoformulations of a lower dose of ritonavir-boosted darunavir could further reduce costs.[28]

Treatment with 400 mg of darunavir and 100 mg of ritonavir is appropriate as a switch strategy for individuals with suppressed HIV RNA on lopinavir boosted with ritonavir, although liver toxicity monitoring and possible pregnancy add complexity. A study looking at viral suppression of 400 mg darunavir for protease inhibitor-naive individuals who do not respond to NNRTI-based first-line therapy might be justified, although the move to integrase inhibitor-based therapies

in LMICs might make this approach less compelling. Further work is required to optimise the dose for pregnant women; currently, it seems prudent to maintain them on the 800 mg dose and escalate the dose for women who become pregnant, and for patients with tuberculosis, to whom a double-dosing strategy in the presence of rifampicin is being explored. Finally, exciting nanotechnology developments mean that darunavir doses as low as 200 mg (with equivalent exposure in patients to the 400 mg dose) are being explored, which might lead to further substantial cost and safety improvements.

Contributors

WDFV and AH led the protocol development and, with MM, wrote the initial manuscript drafts. WDFV provided overall oversight of the study with SS, NM, NA, GA, EM, and CS. GA, AQ, JW, AH, and CW assisted with data review. All authors reviewed the manuscript and approved its final version.

Declaration of interests

WDFV reports grants from the South African Medical Research Council (SAMRC) and United States Agency for International Development (USAID), during the conduct of the study; personal fees and non-financial support from ViiV Healthcare, Gilead Sciences, and Roche and personal fees from Mylan, Merk, Aspen, Adcock Ingram, and Virology Education, outside the submitted work. MM reports grants from the SAMRC and USAID, during the conduct and outside the study, and from Unitaid, outside the submitted work; personal fees and non-financial support from ViiV Healthcare and Gilead Sciences; and personal fees from Mylan, Janssen, Cipla, AbbVie, Aspen, and Merck, outside the submitted work. All other authors declare no competing interests.

Data sharing

De-identified data, with data labels, are available for further analysis with appropriate motivation and ethics committee authorisation, upon publication of the primary manuscript; all non-identifying data are available. The study protocol, statistical analysis plan, and informed consent form will be freely available from the corresponding author.

Acknowledgments

The study was funded by the SAMRC and Project OPTIMIZE, with substantial in-kind support from the South African Department of Health, and technical support from the South African Department of Science and Technology. Project OPTIMIZE (AID-OAA-A-15–00069) is funded by the US Agency for International Development under the US President's Emergency Plan for AIDS Relief. This assistance is from the American people. Research reported in this publication was also supported by National Institute of Allergy and Infectious Diseases of the National Institutes of Health (award numbers UM1 AI068634, UM1 AI068636, and UM1 AI106701). The views expressed in this Article are our own and not necessarily those of the US or South African Governments, or sponsors.

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