PROTOCOL

STUDY TITLE:

Effect of atorvastatin on subclinical atherosclerosis in virally-suppressed HIV-infected patients with CMV seropositivity: a randomized double-blind placebo-controlled trial

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LIST OF ABBREVIATIONS

ART	Antiretroviral Therapy
Co-PI	Co- Principal Investigator
GCP	Good Clinical Practice
ICF	Informed Consent Form
MPR	Medication Possession Ratio
PI	Principal Investigator

PROTOCOL SUMMARY

Full Title	Effect of atorvastatin on subclinical atherosclerosis in virally-supressed HIV-infected patients with CMV seropositivity: a randomized double-blind placebo-controlled trial
Protocol registration number	
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Background and rationale	Persistent immune activation and inflammation in virologically suppressed HIV-infection are linked to excess cardiovascular risk and other non-communicable diseases. Cytomegalovirus (CMV) seropositivity is common in Indonesian people, including HIV-infected patients. However, treatment of CMV infection is not accessible for most patients. Periodic asymptomatic CMV-reactive patients in HIV infected patients over a life time may contribute to non-AIDS defining morbidity, including cardiovascular disease, neurocognitive impairment, cancer and renal disease. Despite undetectable levels of HIV and CMV in plasma, these patients continue to have increased levels of biomarkers and immune activations. Statin administration is supposed to reduce subclinical atherosclerosis by decreasing LDL cholesterol levels, possibly via lipid-independent anti-inflammatory effect. Its pleiotropic properties also adding beneficial effect against CMV infection. We plan to study atorvastatin in virally- suppressed HIV-infected patients on stable ART with CMV seropositive and statin-naïve to evaluate the subclinical atherosclerosis changes assessed by carotid intima media thickness (CIMT)
Primary Study objectives	To compare the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on changes CIMT in virally supressed HIV patients with CMV seropositivity
Secondary Study Objectives	1) To evaluate the interaction between baseline CMV copy number and effect of atorvastatin on CIMT changes 2) To evaluate the effect of 48 weeks of 20 mg daily

	atorvastatin versus placebo on fasted lipid changes in virally supressed HIV patients with CMV seropositivity 3) To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on of brachial artery flow mediated vasodilatation (FMD) in virally supressed HIV patients with CMV seropositivity 4) To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on liver fibrosis and steatosis changes in virally supressed HIV patients with CMV seropositivity 5) To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on neurocognitive changes in virally supressed HIV patients with CMV seropositivity 6) To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on several immune activation biomarkers (sCD14, VCAM, ICAM, hsCRP, and **β**2M) changes in virally supressed HIV patients with CMV seropositivity 7) To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on oral periodontitis score changes in virally supressed HIV patients with CMV seropositivity 8) To evaluate the interaction between baseline CMV copy number and effect of atorvastatin on FMD, neurocognitive function, liver fibrosis and steatosis, lipid profile, and immune activation biomarkers changes in 48 weeks
Participant population	HIV-positive patients on ART will be recruited from the HIV Integrated Care Unit, Cipto Mangunkusumo Hospital, Jakarta. Each participant must meet eligibility criteria as defined below in order to be enrolled in the study. **Inclusion criteria:** 1. Ages between 20 to 45 years old 2. Using stable ART at least 1 year 3. Positive IgG CMV 4. Viral load HIV RNA <50 copies / ml **Exclusion criteria:** 1. Undergoing hepatitis C DAA therapy 2. Decompensated cirrhosis or acute liver failure 3. History of coronary artery disease 4. Diabetes mellitus 5. History of of brain infection, epilepsy, stroke 6. History of rhabdomyolysis or myopathy 7. Pregnant or breastfeeding 8. Severe depression 9. Using statin therapy in the past 6 weeks

	10. History of statin hypersensitivity 11. *Framingham Risk Score* above 10% within LDL ≥130 12. *Framingham Risk Score* under 10% within LDL ≥160 13. Out of Periodontitis Index (Upper right molars, top series, upper left molars, lower right molars, bottom series, lower left molars) **Drop out criteria:** 1. Becomes pregnant during this study 2. Stop using ART> 1 month 3. Stop or refuse to continue to use atorvastatin during this study 4. Not willing to continue as participant in this study
Participating sites	HIV Integrated Care Unit, Tertiary Hospital Cipto Mangunkusumo
Study Design	This study using double blind randomized, placebo-controlled clinical trial. Subject chosen by consecutive methods.
Study Scheme or Intervention	Eligible subjects will be randomized to receive an atorvastatin or placebo with a ratio of 1: 1. Atorvastatin 20 mg or identical placebo capsules will be given orally for 48 weeks.
Schedule of Procedures/ Evaluations	1. The participants who meet the inclusion and exclusion criterias (except viral load, anti-CMV, and Framingham score criterias) get an explanation of the purpose, method of the study, the rights to stop or refuse to take part during in the study. And each participant gives their sign to ICF. 2. Viral load, IgG anti-CMV, fasted lipid profile, fasting glucose and insuline to define Framingham score will be done as screening process. 3. Participants with CMV seropositivity, undetectable viral load, and Framingham risk score >10% LDL <130 or Framingham risk score <10% LDL <160 will continue the study process. 4. Participants will be asked to fast for 10 hours before taking 12 mL of venous blood: for examination of blood glucose, ALT, CMV DNA, and sCD14, VCAM, ICAM, hsCRP, β2M, and transient elastography with CAP. CIMT, FMD, neurocognitive examination, and Community Periodontal Index will be done within a week before taking study drugs. 5. Participants will be randomized to get atorvastatin therapy or placebo. While using study drugs, participants continue their ART and other standard of cares. 6. Study drugs will be dispensed monthly along with ART refill and the pharmacist records the remaining

	medication. 7. At week 24, blood collection procedures and neurocognitive examination procedures will be repeated. 8. At week 48, blood collection procedures, CIMT, FMD, transient elastography with CAP, neurocognitive examination, and community periodontal index procedures will be repeated.
Study Duration	The study will be conducted from April, 2019 to April, 2021. Predicted accrual period: 4 months Enrollment of the first study participant to enrollment of last participant schedule on four months in the first year.
Primary Endpoint(s)	The mean or median CIMT changes of virally supressed HIV patients with CMV seropositivity in atorvastatin group versus placebo group between baseline and week 48
Secondary endpoints	- Magnitude of change in effect of atorvastatin on CIMT associated with baseline CMV copy number - The mean or median changes of fasted lipid between baseline and week 24, week 24 and week 48 - The mean or median changes of brachial artery flow mediated vasodilatation (FMD) between baseline and week 48 - The mean or median changes of liver fibrosis and steatosis in atorvastatin and placebo group between baseline and week 48 - The mean or median changes of sCD14, VCAM, ICAM, CRP, and **β**2M in atorvastatin and placebo group lipid between baseline and week 24, week 24 and week 48 - The mean or median changes of neurocognitive function in atorvastatin and placebo group lipid between baseline and week 24, week 24 and week 48 - The mean or median changes of oral periodontitis score in atorvastatin and placebo group between baseline and week 48 - Magnitude of change in effect of atorvastatin on FMD, neurocognitive function, liver fibrosis and steatosis, lipid profile, and immune activation biomarkers associated with baseline CMV copy number

1. INTRODUCTION

1.1. Background

HIV continues to be a major public health issue. In Indonesia, from January to March 2017 there were 10,376 people infected with HIV (PLWH). The highest percentage(87.2%) was in the productive-aged grup (age 18 - 49 years).[1,2] Antiretroviral therapy (ART) has contributed to significant reduction in morbidity and mortality of HIV patients, as evidenced by reducing AIDS-related deaths from 2.3 million in 2005 to 1.6 million in 2016.[3] Free ART program conducted in Indonesia since 2004 had an impact on declining mortality of PLWH from 2.72% in 2013 to 1.22% at the end of 2014.[4] Decreased morbidity and mortality due to AIDS is accompanied by an increase of non-communicable diseases (NCD) in PLWH.

Based on 10 years observational research, 10% of deaths in PLWH caused by cardiovascular disease, even 3 out of 4 PLWHAs had a low risk of the disease in the beginning of the observation.[5] Increasing other NCD diseases, such as diabetes, neurocognitive disorder, chronic liver disease, chronic kidney disease, low bone mineral density, and other inflammatory related diseases, are associated with chronic immune and inflammatory activation that is still ongoing even in the state of viral suppression (undetectable viral load). Several antiretroviral drugs also increase to the worsening of these condition.

Cytomegalovirus (CMV) seropositivity is common in Indonesian people, including HIV-infected patients. In 2012, prevalence of CMV infection in pre-marital woman is 78,9%. In 2017, prevalence of CMV seropositivity is 98,23%.[6,7] However, treatment of CMV infection is not accessible for most patients. Periodic asymptomatic CMV-reactive patients in HIV infected patients over a life time may contribute to non-AIDS defining morbidity, including cardiovascular disease, neurocognitive impairment, cancer and renal disease.[8] Despite undetectable levels of HIV and CMV in plasma, these patients continue to have increased levels of biomarkers and immune activations.[9]

Atherosclerosis precedes cardiovascular events and has a prolonged asymptomatic phase during which the course of the disease can be modified by lifestyle modifications and treatment. The age of initiation and the rate of progression of atherosclerosis vary markedly among individuals and have been difficult to predict with traditional cardiovascular risk assessment models. Therefore, individuals with subclinical atherosclerosis should preferably be identified at an early stage, so that the primary prevention measures can be initiated. Early identification of subclinical atherosclerosis in individuals at low-to intermediate cardiovascular risk has been challenging.[8] There are several markers of subclinical atherosclerosis including Carotid Intima-Media Thickness (CIMT), Coronary Artery Calcium Score (CACS), Ankle-Brachial Index (ABI), and Flow-Mediated Dilatation (FMD).[10]

As a surrogate marker for subclinical atherosclerosis, CIMT evaluation is a non-invasive examination using B-mode ultrasonography which relatively reproducible, easy and safely performed to detect early stages of atherosclerosis and is accepted as one of the best methods for evaluation of arterial wall structure. There are several factors affecting changes on CIMT including traditional risk factors such as age, sex, dyslipidemia, diabetes mellitus, etc and also novel risk factors including hereditary, certain genotypic indices, anthropometric parameters, rheumatoid arthritis, immunologic and inflamatory cytokines, yet socioecomonic position (SEP) and job stress.

Recent years, systemic inflammation and immune activation have gained attention as an emerged factors of atherosclerosis. HIV is one of the common risk factor of atherosclerosis. The direct correlation between CIMT and atherosclerosis among HIV-

positive patients had been established in several studies. A prospective study of 307 patients (179 HIV positive and 128 HIV negative) found that despite HIV supression, lipid and hypertension control, HIV-positive patients have a disproportionately greater CIMT than HIV-negative comparators.[11] That facts parallel with one of our study which revealed that cIMT rose slightly in ART-naïve patients 12 months after commencing ART.[12] Cross-sectional study of 1183 HIV-infected patients compared with 297 HIV-uninfected patients from showed after multivariable adjustment for demographic characteristics and traditional cardiovascular risk factors, HIV infection was independently associated with subclinical atherosclerosis measured by cIMT.[13] Cohort study from 1563 HIV-infected patients compared to 584 HIV-uninfected Patients patient showed cIMT among middle-aged (30-49 years and older adult (50 – 75 years) that were similar to or lower than those in HIV-unifected participants. In contrast among those aged 6-29 years HIV infection was associated with higher cIMT.[14]

While HIV replication has been considered the major trigger of the immune system, persistent inflamation and immune activation have also found in patients receiving affective antiretroviral therapy (ART). Therefore, additional causes may elicit immune activation and might be involved in the accelerated course of atherosclerosis disease in virologically controlled HIV-infected patient. In HIV-infected patients, a relationship with subclinical atherosclerosis has been reported for CMV and herpes simplex virus type-2 (HSV-2). In HIV-infected patient, CMV antibody titers have been related to decreased artery distensibilty and carotid lesion in women, but no association was found with the CIMT measurement.[15]

Statins are inhibitors of *hydroxyl-3-methylglutaryl coenzyme A* which were first extracted from Pythim sp, Penicillium sp, and Aspergillus sp. Initially, the target of statin therapy was to reduce cholesterol levels. Recent studies shown that the use of statin also associated with decrease other atherogenic lipid particles, such as oxidized LDL and phospholipase associated with lipoprotein.[16] Statins remain key to primary and secondary CVD prevention; they improve endothelial function, slow the progression of atherosclerosis and stabilize atherosclerotic plaque. In the HIV population, decreased oxidized LDL is independently associated with a decrease in important markers of subclinical atherosclerosis, such as coronary plaque and carotid intima media thickness. The dual mechanism of statin use reduces LDL cholesterol, modifies inflammatory responses, antioxidant effects, antithrombic effects (clotting processes), and effects of removal of blood vessel plaques, thereby preventing cholesterol build-up and muscle proliferation.[17] Treating dyslipidemia with statin has been challenging in people with HIV due to potential drug interaction between statin and antiretroviral drug due to competing cytochrome P450. A study comparing comparing pitavastatin and pravastatin in patients using protease inhibitor shown higher virological failure in pravastatin group. Therefore, guideline recommend pitavastatin as a preferred drug in the treatment of dyslipidemia in PLWH.[18] However pitavastatin is expensive as it is not available in generic formulation. Atorvastatin, another moderate intensity statin is available in generic formulation and widely used in Indonesia. This drug has no interaction with all antiretroviral drugs use in Indonesia. Seropositivity to cytomegalovirus (CMV) may jointly predict increased mortality rate in patients with coronary heart disease (coronary heart disease). Statin therapy reduced lipid level and also has anti-inflamatory effect. Study showed statin reduce mortality rate among CMV-infected patients with CAD.[19] In Vitro study demonstrated Statins has a broad anti-cytomegalovirus activity. All statins dose-dependently reduced CMV titers in human aortic endothelial cells. These findings provide new insight into benefical effects of statins.[20]

1.2. Rationale

Non-communicable diseases that are rapidly developing among HIV-positive with stable ART, including cardiovascular disease. Atorvastatin administration supposed to have a positive effect on the reduction of subclinical atherosclerosis assessed by the thickness of the carotid artery intima media. The use of atorvastatin, a cheap and widely accessible drug in Indonesia, has not been specifically studied in term of improving cardiovascular disorder in HIV-infected patients under ART.

2. OBJECTIVES

2.1. Primary Objective

To compare the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on changes CIMT in virally supressed HIV patients with CMV seropositivity

2.2. Secondary Objective

1. To evaluate the interaction between baseline CMV copy number and effect of atorvastatin on CIMT changes
2. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on fasted lipid changes in virally supressed HIV patients with CMV seropositivity
3. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on of brachial artery flow mediated vasodilatation (FMD) in virally supressed HIV patients with CMV seropositivity
4. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on liver fibrosis and steatosis changes in virally supressed HIV patients with CMV seropositivity
5. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on neurocognitive changes in virally supressed HIV patients with CMV seropositivity
6. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on several immune activation biomarkers (sCD14, VCAM, ICAM, hsCRP, and **β**2M) changes in virally supressed HIV patients with CMV seropositivity
7. To evaluate the effect of 48 weeks of 20 mg daily atorvastatin versus placebo on oral periodontitis score changes in virally supressed HIV patients with CMV seropositivity
8. To evaluate the interaction between baseline CMV copy number and effect of atorvastatin on FMD, neurocognitive function, liver fibrosis and steatosis, lipid profile, and immune activation biomarkers changes in 48 weeks

3. STUDY ENDPOINTS

3.1. Primary endpoint

The mean or median CIMT changes of virally supressed HIV patients with CMV seropositivity in atorvastatin group versus placebo group between baseline and week 48

3.2. Secondary endpoints

1. Magnitude of change in effect of atorvastatin on CIMT associated with baseline CMV copy number
2. The mean or median changes of fasted lipid between baseline and week 24, week 24 and week 48

3. The mean or median changes of brachial artery flow mediated vasodilatation (FMD) between baseline and week 48
4. The mean or median changes of liver fibrosis and steatosis in atorvastatin and placebo group between baseline and week 48
5. The mean or median changes of sCD14, VCAM, ICAM, CRP, and **β**2M in atorvastatin and placebo group lipid between baseline and week 24, week 24 and week 48
6. The mean or median changes of neurocognitive function in atorvastatin and placebo group lipid between baseline and week 24, week 24 and week 48
7. The mean or median changes of oral periodontitis score in atorvastatin and placebo group between baseline and week 48
8. Magnitude of change in effect of atorvastatin on FMD, neurocognitive function, liver fibrosis and steatosis, lipid profile, and immune activation biomarkers associated with baseline CMV copy number

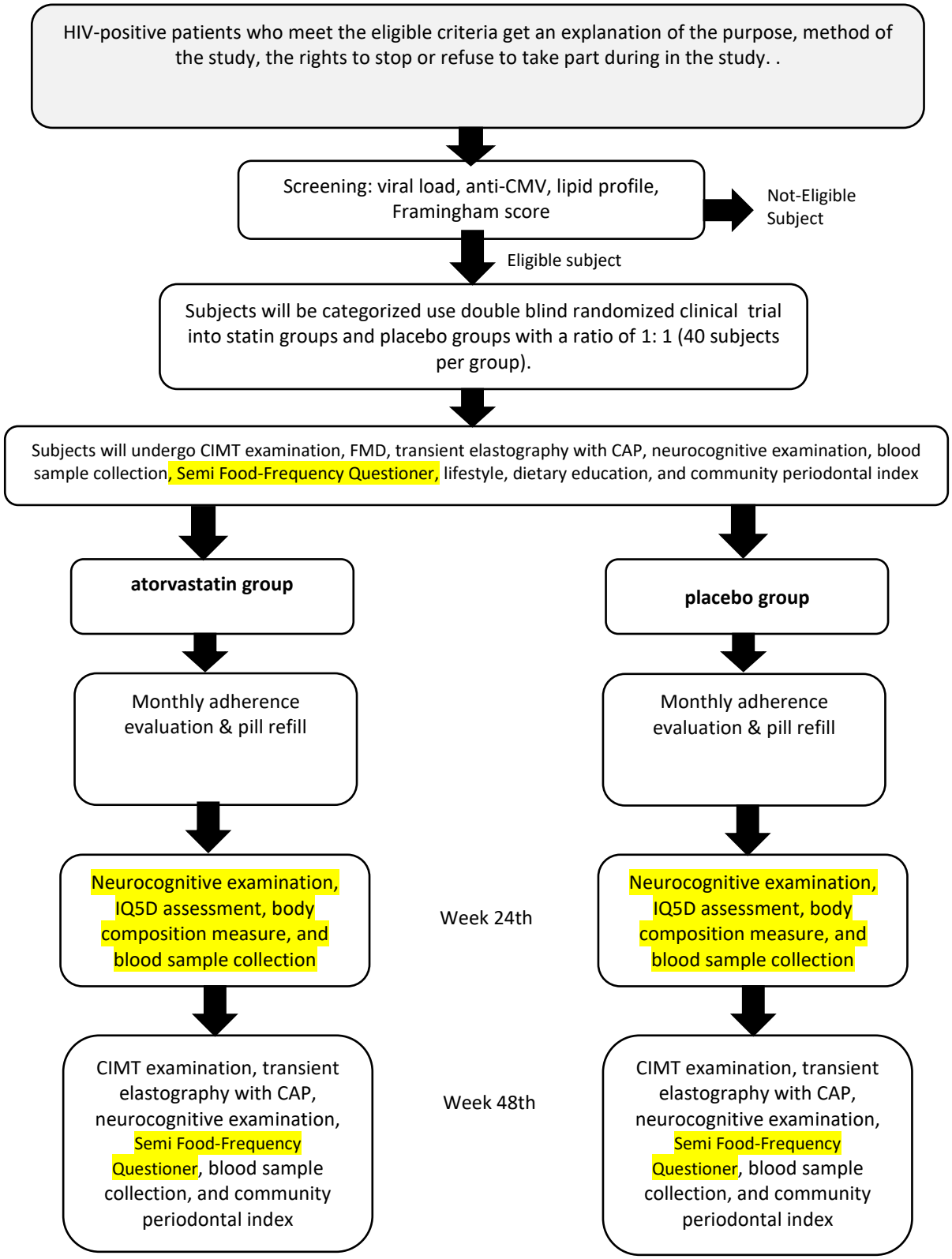
4. STUDY BENEFIT

4.1. Potential Benefit(s)

There are some potential benefits to those individuals who use atorvastatin such as lowering lipid level, but this will not be available for those who got placebo. However, society will gain general knowledge that will improve care of similar cases.

METHODS

4.2. Study Plan



4.3. Study Design

This study used a double-blind randomized placebo-controlled trial design. Participants who met the inclusion and exclusion criteria were randomized into atorvastatin groups and placebo groups with a ratio of 1: 1 (40 subjects per group). Randomization using a computer program based on randomization of permutation blocks with block 4 sizes carried out by statistical analysts. Then the code is given to pharmacists who involved in the study. Researchers, doctors, and participants did not know the results of randomization.

4.4. Study Sites and Duration

The study will conduct during 2019-2020 at HIV Integrated Care Unit and Integrated Cardiovascular Care Cipto Mangunkusumo Hospital, Jakarta.

4.5. Population and Sample Size

Target population is HIV-positive patient on stable ART routinely controlled in HIV Integrated Care Unit Cipto Mangunkusumo Hospital, Jakarta.

Assuming that the effect size is 0.1, standard deviation is 0.12, and the use of 2 sample t-test, the sample size needed for 90% power and 5% significant level is 32 per arm. With additional 20% drop, each arm (placebo and control group) will need 40 participants.

4.6. Eligibility Criteria

4.6.1. Inclusion Criteria

1. Age between 20 to 45 years old
2. Using stable ART at least 1 year
3. positive CMV IgG
4. Viral load HIV RNA <50 copies / ml

4.6.2. Exclusion Criteria

1. Undergoing for Hepatitis C DAA Therapy
2. Decompensated cirrhosis or acute liver failure
3. History of coronary artery disease
4. Diabetes mellitus
5. History of of brain infection, epilepsy, stroke
6. History of rhabdomyolysis or myopathy
7. Pregnant or breastfeeding
8. Severe depression
9. Using statin therapy in the past 6 weeks
10. History of statin hypersensitivity
11. *Framingham Risk Score* above 10% within LDL ≥130
12. *Framingham Risk Score* under 10% within LDL ≥160
13. Out of Periodontitis Index (Upper right molars, top series, upper left molars, lower right molars, bottom series, lower left molars)

4.6.3 Drop Out Criteria

1. Pregnant during this study
2. Stop using ART> 1 month
3. Stop or refuse to use atorvastatin during this study
4. Not willing to continue as participant in this study

4.7. Co-enrollment Criteria

HIV-positive patients who include into this study are still permitted to follow other observational studies as participants. However, patients who participate in this study are not permitted to take other interventional studies. Sticker affixed in participant's medical record as required by hospital policy will ensure that participant is not under other interventional study.

4.8. Study Instruments

Instruments used in this study included an ICF, demographic form contains medical record and essential information of each participant, CIMT examination form, blood collection and sample storage form, adherence form. A paper based forms that will be transferred to electronic format.

4.9. Strategies for Recruitment

Potential subjects as participants in this study are all adult patients who meet the eligible criteria and under antiretroviral therapy at HIV Integrated Care Unit Cipto Mangunkusumo Hospital. These patients regularly control once for each two or a month(s) but antiretroviral drugs be refilled monthly. A promotion poster will be placed in the clinic area during the enrollment period.

4.10. Participant Retention

To enhance participant retention, all participant will be monitored by research assistant using phone once every two weeks. In addition, a reminder of the next schedule visit will be sent a week before.

4.11. Study Intervention

4.11.1. Study drug and dosage:

- Atorvastatin 20 mg tablets in generic form
- The placebo tablets will be prepared by Cipto Mangunkusumo hospital pharmacist, were composed of starch and were similar to atorvastatin tablets in size, shape, and colour.

4.11.2. Treatment administration:

The administration of study drug is home-based participant self-administration. The participants will get the medication after randomization process (week – 0) at least 1 week after. The participants will get supply every month along with the refill of antiretroviral drugs. The drug and placebo tablets will be administered to patients by a staff member who are privy to the treatment. In the end of every month, each participant should return the unused pills every month. All prescriptions will use hospital Electronic Medical Record system.

4.11.3. Drug interaction:

During this study, each participant is not allowed to use cyclosporin, fenofibrate, and rice yeast herbal supplements.

4.12. Intervention Assignment Procedures

4.12.1. Randomization Procedures

Research assistant will do randomization for each participant using computerized statistic application.

4.12.2. Masking Procedures

Study pharmacist will make code (A and B) for atorvastatin and placebo, then save the code in safe place. Pharmacist will record each subject as participant received A or B intervention.

Patients, managing doctor, researcher, and those assessing outcomes would not know what type of drug given. The code will only be opened when a participant having adverse event that require identification of study drug, ie drug allergy and acute coronary syndrome.

4.13. Study Schedule and Procedures

Each participant will be followed for one year after start using study drug.

4.13.1. Screening

Patients meeting the initial screening criteria will be test for HIV RNA (viral load), anti-CMV and fasting lipid profile:

- Age between 20 to 45 years old
- Using ART at least 1 year
- Not under hepatitis C therapy
- No history of coronary artery disease, brain infection, epilepsy, stroke
- No diabetes mellitus
- Not pregnant or breastfeeding
- Not severe depression (using mini ICD10)
- Not using statin therapy

4.13.2. Baseline and Enrollment

Subjects with undetectable HIV-RNA, positive anti-CMV, having Framingham Risk Score above 10% with LDL < 130 or under 10% with LDL <160 will undergo thorough medical history and anthropometric examination as the initial data. Venous blood sample will be collected for fasting blood glucose, ALT, CMV DNA, hsCRP, sCD14, VCAM, ICAM, and β2M examinations. CIMT, transient elastography with CAP, neurocognitive function, and community periodontal index procedure will be done within a week before taking study drugs. CIMT and FMD evaluation will be done by trained cardiologist using B mode ultrasonography. Transient elastography with CAP will be done by trained cardiologist Fibroscan (Echosens) device. A trained neurologist will do neurocognitive evaluation using trail making test (TMT) A and TMT B (attention and executive function), digit symbol (processing speed), brief visuospatial memory test revised (visual and visuospatial memory), and California verbal making test (verbal memory).

4.13.3. Follow Up Visit(s)

Patient will be seen for regular clinical follow up as standard of care in the clinic (monthly/ bimonthly). Follow up visit will include clinical and laboratory procedure as presented in table study schedule (appendix).

At week 24, blood collection procedures and neurocognitive examination procedures will be repeated. At week 48, blood collection procedures, CIMT, FMD, transient elastography with CAP, neurocognitive examination, and community periodontal index procedures will be repeated.

4.13.4. Other visit

Other visit or investigations (or referral to other clinical specialists) may be requested as clinically indicated. These include side effect encountered and other treatment related issue.

4.13.5. End of Study Visit

In the end of study (week 48), a total drug consumption adherence will be assessed using Medication Possession Ratio (MPR) method.

5. SPECIMENS (REPOSITORY, USAGE, LOSS & DESTRUCTION)

5.1. Biohazard Containment

This study using a blood samples which will be analyzed by laboratory analysis. So, that will increase the risk of infection for laboratory analysis.

5.2. Specimen Preparation, Handling, Storage and Shipping

Before a blood sample collection, the subject is required to fasting minimum 8-10 hours. Blood glucose, lipid profile, IgG anti-CMV, ALT, and viral load will be checked at the time in hospital laboratory service. For other evaluation, the blood sample will be inserted into a tube with EDTA preservative and will be centrifuged to become plasma. Then, the plasma will be aliquoted into several tubes and will be saved in -80˚C refrigerator.

5.3. Total Blood Volume

Subjects who are willing to take this part in this study will take maximum 12 mL of venous blood sample in each visit (see appendix).

6. DATA MANAGEMENT, STATISTICAL CONSIDERATION & ANALYSIS PLAN

6.1. Data Management

Data management will be managed in HIV Integrated Care Unit, Cipto Mangunkusumo Hospital.

a. Specific Case Report Forms (CRF) will be created prior to study
b. A study-specific, relational electronic database will be created to house the anonymized study data by statistical analyst and saved by research team.
c. Prior to patient recruitment and data collection, all research team will receive training in study procedures.

All research team will have overall responsibility for maintaining the study CRFs. Corrections to the paper CRF documents must be made by striking through the incorrect entry with a single line (taking care not to obliterate or render the original entry illegible) and entering the correct information adjacent to the incorrect entry. Corrections to paper CRFs must be initialed and dated by the person making the correction. The researchers are responsible for maintaining accurate, complete and up-to-date records. These forms are to be completed on an on-going basis during the course of the study.

6.2. Statistical Consideration & Analysis Plan

Subjects will be analyzed in the group to which they were randomized, regardless of the treatment received.

6.2.1. Analysis of primary endpoints

T test will be used to compare the mean CIMT changes of virally supressed HIV patients with CMV seropositivity in atorvastatin group versus placebo group between baseline and week 48.

6.2.2. Analysis of secondary endpoints

- Linear regression with interaction will be used to assess magnitude of change in effect of atorvastatin on CIMT associated with baseline CMV copy number
- For other continuous endpoints, a 2 sample T-test

6.2.3 Missing data plan

We will compare subject characteristics in the drop out with remaining participants. If there is no different of the characteristic, we assume that the missing is at random.

7. RESEARCH MONITORING

Study will be monitored by Faculty of Medicine Universitas Indonesia CRO.

7.1. Record Retention

Research records for all study subjects including CRFs, laboratory and other investigations data/results are to be maintained by the investigator in secure storage for five (5) years. These records are to be maintained in compliance with all designated IRBs requirements.

It is the investigator's responsibility to retain copies of these study records until notified in that they can be destroyed.

6.2 Protocol Deviation

Protocol Deviation is defined as any change, divergence, or departure from the IRB approved study procedures in the research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. *Those that occur because a member of the research team deviates from the protocol.*
2. *Those that are identified before they occur, but cannot be prevented.*
3. *Those that are discovered after they occur*

Serious Protocol Deviation is a deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of participants or others.

7.2. Non-Compliance

Non-compliance is defined as the failure to comply with applicable IRB requirements, or regulatory requirements for the protection of human participants. Non-compliance is further characterized as

1. *Serious: Non-compliance that*
   - *Increases risks, or causes harm, to participants*
   - *Decreases potential benefits to participants*
   - *Compromises the integrity of the NIH-HRPP*
   - *Invalidates the study data*
2. *Continuing: Non-compliance that is recurring*
3. *Minor: Non-compliance that, is neither serious nor continuing.*

8. ASSESSMENT OF SAFETY

8.1. Adverse Events

Side effects that can occur in this study are the subjects feels pain at the site of injection for venous blood sample collection, and in some rarely case is a hematoma and dermatitis in the site of injection for venous blood sample.

Patients will be asked about signs and symptoms of atorvastatin toxicity. If any signs or symptoms are reported, patients will undergo clinical and laboratory evaluation to determine if the study drugs need to be stopped.

Stopping criterias:

- ALT level rise above 10-fold upper normal limit or persist in being above 5-fold elevated or are associated with symptoms of liver failure
- Creatine kinase rise 10-fold upper normal limit in patients with clinically rhabdomyolisis
- Severe drug allergic reactions, such as Stevens Johnson syndrome, toxic epidermal necrolysis

8.2. Serious Adverse Events

A serious adverse event (SAE) is defined as any adverse event that results in the following

- results in death;
- is life-threatening (places the participant at immediate risk of death from the event as it occurred);

- or based upon appropriate medical judgement, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

All SAE will be collected on a specific SAE form and reported to the Faculty of Medicine Universitas Indonesia IRB within a week of occurrence.
The management of AE will be according to best clinical practices at the judgment of the researcher at occurence.

8.3. Unanticipated Problem

An unanticipated problem (UP) is defined as any incident, experience, or outcome that is:

1. Unexpected in terms of nature, severity, or frequency in relation to:
   - the research risks that are described in the research protocol and informed consent document or other study documents; and
   - the characteristics of the participant population being studied; and
2. Related or possibly related to the participation in the research; and
3. Places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized or is an SAE.

8.4. IRB Reporting

8.5. Expedited Reporting to the NIHRD IRB

Unanticipated Problems, Serious Adverse Events, Serious Deviations, and Serious or Continuing Non-Compliance will be reported within 7 calendar days of investigator awareness to the IRB, regardless of expectedness.

8.6. Annual Reporting to the IRB

The following items will be reported to the IRB in summary at the time of Continuing Review:

- All deaths
- Summary of AEs, SAEs
- Serious and non-serious unanticipated problems
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

9. HUMAN SUBJECTS PROTECTIONS

9.1. Ethical Review

Prior to the protocol implementation, protocol, ICF, CRF, and any written information to be provided to participants for this study will be submitted for approval to the appropriate IRB. Any change to the protocol and ICF will only be implemented after approval from the IRB. Records of the IRB review and approval of all the documents pertaining to the study will be kept on file by the site investigator and will be made available for inspection at any time during the study. Continuing reviews, including a study progress report will be submitted according to the requirement of the appropriate IRB, but not less than once a year at a minimum.

9.2. Potential Risks and Benefit to the Participant

9.2.1. Potential Risks

Subjects can feel less comfortable and pain in blood sampling. Other complications which may arise are the occurrence of phlebitis (infection in the place of needle puncture) and hematoma (bruising at the place of needle puncture). To reduce discomfort in each subject, blood sampling will be carried out by experienced staff. Furthermore, researchers will guarantee the confidentiality of data from research subjects. The community periodontal index procedures might cause little bleeding.

The possibility of getting side effect related to atorvastatin can also make subject less comfortable. Common reactions include headache, difficulty sleeping, muscle aches, muscle tenderness, or muscle weakness, nausea, vomiting, diarrhea, bloating and constipation. Serious reactions might include severe liver toxicity, tendon rupture, severe allergic reaction and rhabdomyolisis.

9.2.2. Potential Benefit(s)

Subject will get various diagnostic tests which covered by this study. For those in atorvastatin arm, additional benefit such as decreasing cholesterol levels, might also possible. Information gained from this study may improve future treatment of similar patients.

9.3. Participant Compensation

The study will cover the costs of the tests used in this research, for those that are not free of charge to participants within the standard of care. Study participants will receive financial support for transportation to and from sites on visits days, as well as for time consented for the study procedures once per study-procedures from beginning to end per subject.

The management of AE will be using participant's national health insurance and will be based on best clinical practices. For this reason, researchers will provide treatment recommendations for each subject of research.

9.4. Vulnerable Participants or Special Population

No vulnerable participant will be included in this study among all special populations.

9.5. Informed Consent

A written informed consent to participation will be obtained from each participant.

The consent form will describe the purpose of the study, the study procedures and the risks and benefits of participation, in accordance with all applicable regulations. Consent will also include mention of the recording of all relevant clinical and para-clinical data obtained as part of participation in the study.

The consent form will be in the appropriate language(s) of the country. Literate patients will document their provision of informed consent by signing and dating the informed consent forms. Non-literate patients will be asked to mark their informed consent forms (with thumb print) in the presence of a literate third party witness who will also sign the consent as a witness.

Signed consents must remain in each study participant's study file. The informed consent procedure will be documented in the patient's medical records. A copy of the signed consent must be provided to the study participant.

9.6. Participant Privacy and Confidentiality

Private identity of each participant will be saved privately. Name, domain, and others private identity will not mention in publication, report, public media, and others. Researchers will replace the identity of each participant using codes. Each file only can be accessed by researchers, pharmacist, and analyst. This study is voluntary based. Each participant may decide at any time and for any reason withdraw from participation in the study. Any such decision will not affect the standard of care that patient in the hospital. Researcher may also at any time at discontinue subjects from receiving treatment or participating in the study, for their benefit and well-being. Information related to treatment or study follow up interruption will be documented in the study CRF.

8. RESEARCH TEAM

Name	Role	Institution & Contact Information
Evy Yunihastuti	PI	Allergic-Immunology Consultant, HIV Integrated Care Unit, Cipto Mangunkusumo Hospital
Chyntia Olivia Maurine Jasirwan	Co-PI	Hepatobiliary Consultant, Cipto Mangunkusumo Hospital
RR Dyah Purnamasari Sulistianingsih	Co-PI	Metabolic-Endocrine Consultant, Cipto Mangunkusumo Hospital
Riwanti Estiasari,	Co-PI	Neuro-Infection Consultant, Department of Neurology, Cipto Mangunkusumo Hospital
Lusiani Siregar, Internist, Cardiology Consultant	Co-PI	Cardiovascular Consultant, Department of Internal Medicine, Cipto Mangunkusumo Hospital
Sally Aman Nasution, Internist, Cardiology Consultant	Co-PI	Cardiovascular Consultant, Department of Internal Medicine, Cipto Mangunkusumo Hospital
Endah Ayu Tri Wulandari, Oral Medicine Specialist	Co-PI	Oral Medicine Specialist, Departemen of Dentistry, Cipto Mangunkusumo Hospital

12. REFERENCES

1. World Health Organization. Number of people living with HIV estimates by WHO region [Internet]. [updated 2016; cited 2018 Dec 20]. Available from: http://apps.who.int/gho/data/view.main.22100WHO?lang=en
2. Ministry of Health RI. The progress report of HIV AIDS and sexually transmitted infections (PIMS) in the first quarter of 2017 [Internet]. [updated 2017; cited 2018 Dec 20]. Available from: http://siha.depkes.go.id/portal/files_upload/Laporan_HIV_AIDS_tw_1_2017_rev.pdf
3. Marcolina S. Statin use and cognitive effects: not a brain drain [Internet]. [updated 2015; cited 2018 Dec 20]. Available from: http://reliasmedia.com/articles/135948-statin-use-n-cognitive-effects-note-a-brain-drain.
4. Disease Control and Environmental Health General Directorate. Action plan for disease control and environmental health programs in 2015-2019 [Internet]. [updated 2015; cited 2018 Dec 20]. Available from: http://depkes.go.id/resourcecs/download/RAP%20Unit20Utama%202015-2019/5.%20Ditjen%20P2P.pdf
5. Chastain DB, Kayl RS, Daniel MR. Evidence-based review of statin use in patients with HIV on antiretroviral theraphy. J Clin Transl Endocrinol. 2017;8:6-14.
6. Noviar G, Ritchie NK, Bela B, Soedarmono YSM. Prevalensi antibody IgG dan DNA cytomegalovirus pada darah donor di unit transfuse darah provinsi DKI Jakarta. JHECDs. 2017;3(5):28-35.
7. Gugun AM. Prevalensi seropositive IgM/IgG cytomegalovirus pada populasi wanita pra-nikah dengan riwayat konsumsi makan lesehan. Mutiara Medika. 2012;12(2):124-31.
8. Lederman MM, Funderburg NT, Sekaly RP. Residual immune dysregulation syndrome in treated HIV infection. Adv Immunol. 2013;119:51-83.
9. Barrett L, Fowke KR, Grant MD. Cytomegalovirus, aging, and HIV: a perfect storm. AIDS Rev. 2012;14(3):159-67.
10. Papageorgiou N, Briasoulis A, Androulakis E, Tousoulis E. Imaging subclinical atherosclerosis: where do we stand? Curr cardiol Rev. 2017;13(1):47-53.
11. Hsu R, Patton K, Liang J, Okabe R, Aberg J, Fineberg N. Independent Predictors of Carotid Intimal Thickness Differ Between HIV+ and HIV- Patients with Respect to Traditional Cardiac Risk Factors, Risk Calculators, Lipid Subfractions, and Inflammatory Markers. Paper presented at: 7th International AIDS Conference on HIV Pathogenesis, Treatment, and Prevention. Kuala Lumpur, Malaysia July 3, 2013)
12. Karim B, Wijaya IP, Rahmaniyah R, Ariyanto I, Waters S, Estiasari R, et al. Factors affecting affect cardiovascular health in Indonesian HIV patients beginning ART. AIDS Res Ther. 2017;14(52):1-7
13. Grunfel C, Delaney J, Wanke C, Currier J, Scherzer R, Biggs M, et al. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from FRAM study. Wolters Kluwer Health. 2009;23:1841–9.
14. Hanna D, Guo M, Buzkova P, Miller T, Post W, Stein J, et al. HIV infection and carotid artery intima—media thickness: pooled analysis across 5 cohorts of the NHLBI HIV-CVD collaborative. Oxf J Leg Stud. 2016;63(2):249-56.
15. Masia M, Robledano C, Tabla V, Antequera P, Lopez N, Gutierrez F. Increased carotid intima-media thickness associated with antibody responses to varicella-zoster virus and cytomegalovirus in hiv-infected patients. PLoS ONE. 2013;8(5):1-6.

16. Hemkens LG, Bucher HC. HIV infection and cardiovascular disease. Eur Heart J. 2014;35(21):1373-81.
17. Eckard AR, Grace AM. The role of statins in the setting of HIV infection. Curr HIV/AIDS Repp. 2015;12(3):305-12.
18. Aberg JA, Sponseller CA, Ward DJ, Kryzhanovski VA, Campbell SA, Thompson MA. Pitavastatin versus pravastatin in adults with HIV-1 infection and dyslipidaemia (INTREPID): 12 week and 52 week results of a phase 4, multicentre, randomised, double-blind, superiority trial. Lancet HIV. 2017;4(7): e284-94
19. Benjamin D, Muhleistein J, Carlquist J, Tami L, Madsen T, Hart N, et al. Seropositivity and high c-reactive protein in reducing mortality among patient with angiographically significant coronary disease. American Heart Asociation. 2003;107:258-263
20. Ponroy N, Traveira A, Mueller NJ, Milliard AL. Statins demonstrate a broad anti-cytomegalovirus activity in vitro ganciclovir-susceptible and resistant strains. J Med Virol. 2015;87(1):141-53.

APPENDIX A. SCHEDULE OF EVENTS

{Schedule of Events diagram or table, must match with section 4.12}

Study flowchart and schedule

	Screening	Randomization (visit 0)	Week 24 (visit 1)	Week 48 (visit 2)	Extra Visit
Clinical assessment					
Informed consent	V				
Review eligibility	V	V			V
Physical examination		V	V	V	V*
CIMT examination (puasa 4 jam), pemeriksaan 1-3 menit		V		V	
Transient elastography and CAP		v		v	
Neurocognitive examination		V	V	V	
Community Periodontal Index		V		V	
Lab assessment					
Viral load	V				
Lipid profile	V		V	V	
IgG Anti-CMV	V				
CBC		V			
ALT		V			V*
Creatinin		V			
CK					V*
Fasting blood glucose and insulin		V	V	V	
DPL	V*				
LED	V*				
Store sample					
Serum		V	V	V	
Other Assessment					
Depression assessment	v				

*According to patient's symptom.

Blood volume collection

	Type	Blood collected	screening	randomization	Week 24	Week 48	Extravisit
1 Viral load	Plasma	3 mL	3 mL				
Lipid profile	Serum	2 mL	2 mL		2 mL	2 mL	
Fasting blood glucose	Serum	1 mL	1 mL		1 mL	1 mL	
IgG Anti-CMV	Serum	1 mL	1 mL				
Complete blood count	Plasma EDTA	3 mL		3 mL			
ALT, Creatine	Plasma EDTA	1 mL		1 mL			1 mL
CK	Plasma EDTA	1 mL		1 mL			1 mL
Storage sample	plasma	6 mL	5 mL	6 mL	9 mL	9 mL	
TOTAL volume			**12 mL**	**12 mL**	**12 mL**	**12 mL**	**2 mL**

APPENDIX B. OPERATIONAL DEFINITION

{Operational definition table, please describe the main variables and how to measure them.}

Variable	Operational Definition	Measurement
CIMT changes	Numerical changes (in millimeter) between baseline and 48 weeks CIMT	Common Carotid Artery (CCA) intima media thickness measured by using B mode imaging system, equipped with a linear array transducer > 7 MHz with minimal compression (<10:1) and footprint of at least 3 cm (https://www.escardio.org/Journals/E-Journal-of-Cardiology-Practice/Volume-13/Intima-media-thickness-Appropriate-evaluation-and-proper-measurement-described)
FMD changes	Numerical changes (in percentage) between baseline and 48 weeks FMD	Using a high-resolution ultrasound linear array transducer, longitudinal images of the right brachial artery (mostly 3-15 cm above the elbow) were recorded at the baseline and for 3 minutes after cuff deflation following suprasystolic compression (50 mmHg over the systolic blood pressure) of the right forearm for 5 minutes. https://www.jstage.jst.go.jp/article/ihj/advpub/0/advpub_17-013/_pdf; EHJ 2010. 2854-61 Assesment of atherosclerosis : the rolw of FMD
Liver fibrosis change	Numerical changes (in KPa) between baseline and 48 weeks	The measurement of liver stiffness which uses the velocity of shear waves that travel through the liver using the Fibroscan (Echosens) device
Liver steatosis change	Numerical changes (in dB/m) between baseline and 48 weeks	The measurement of steatosis using the Fibroscan (Echosens) equipped with CAP software. dB/m

APPENDIX C. BUDGET

	Items	Cost
Personnel	Research assistant, pharmacist and laboratory assistant	184.000.000 (IDR)
Research cost	Study drug, laboratory, CIMT and TE examination, patient transportation	647.240.000 (IDR)
Direct cost	Ethical committee approval, stationary, communication, publication	81.000.000 (IDR)
TOTAL		912.000.000 (IDR)