STUDY PROTOCOL

PROTOCOL TITLE:
Study on the Effect of Atorvastatin Co-administered with Omeprazole on statin Lactone (SEACOL)

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1. BACKGROUND AND RATIONALE

Goh et al. 2023 found that the co-prescription of omeprazole is associated with increased atorvastatin lactone (ATVlac) levels and increased risk of MACE. Omeprazole's role in inducing UGT1A activity has been previously studied and UGT1A is involved in the metabolism of statin to statin lactone. Although statin lactone production may independently reduce the benefit of statin in about 30% of patients, to date, there has not been any published interventional study investigating the combined effect of statin and proton pump inhibitor (ppi) on statin lactone levels. Thus, this study aims to investigate the effect of Atorvastatin co-administered with Omeprazole versus Atorvastatin monotherapy on ATVlac level. We hypothesize that co-administration with Omeprazole results in a higher ATVlac level.

1.1. General Introduction

Both drugs are US FDA and Singapore HSA approved drugs.

(i) Atorvastatin is a synthetic lipid-lowering agent, which is an inhibitor of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.Atorvastatin is indicated as an adjunct to diet for reduction of elevated total-cholesterol (total-C), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (apo B) and triglycerides (TG) in adults, adolescents and children aged 10 years or older with primary hypercholesterolemia, heterozygous familial hypercholesterolemia or combined (mixed) hyperlipidaemia (Fredrickson Types IIa and IIb), elevated serum triglyceride (TG) levels (Fredrickson Type IV), and for patients with dysbetalipoproteinemia (Fredrickson Type III) when response to diet and other non-pharmacological measures is inadequate. Atorvastatin is also indicated to reduce total-C and LDL-C in adults with homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering treatments (e.g. LDL apheresis) or if such treatments are unavailable. Atorvastatin is also indicated to reduce the risk of myocardial infarction (MI) in adult hypertensive patients without clinically evident coronary heart disease (CHD), but with at least three additional risk factors for CHD such as age >55 years, male sex, smoking, left ventricular hypertrophy, other specified abnormalities on electrocardiogram (ECG), microalbuminuria or proteinuria, ratio of plasma total cholesterol (total-C) to HDL-cholesterol >6, or premature family history of CHD. Atorvastatin is indicated in adults with type 2 diabetes and without clinically evident CHD, but with multiple risk factors for CHD such as retinopathy, albuminuria, smoking or hypertension, as well as in adults with clinically evident CHD.

(ii) Omeprazole is a proton pump inhibitor which suppresses gastric basal and stimulated acid secretion by inhibiting the parietal cell H+/K+ ATP pump. Omeprazole is indicated for the treatment of gastroesophageal reflux disease (acute, healing or symptomatic), heartburn, helicobacter pylori eradication, peptic ulcer disease, duodenal or gastric ulcers and zollinger-ellison syndrome.

Rationale of the dose and schedule of administration: The proposed dosing used is 40mg/day for Atorvastatin and 20mg/day for Omeprazole, which are within approved therapeutic ranges (Atorvastatin 10 - 80mg, Omeprazole 20 - 360mg). The chosen doses are also the commonly prescribed doses for each drug based on clinical experience, with well-documented safety and efficacy profile. Since Atorvastatin is dosed once daily while Omeprazole can be dosed once or twice a day, for convenience and to improve compliance, we decided to schedule both drugs for once a day administration. Omeprazole should preferably be taken on an empty stomach, such as 30 - 60 mins before breakfast, while Atorvastatin can be administered without regard to food, hence, to standardize administration across all treatment arms, we will advise patients to take their assigned drugs 30 mins before breakfast.

Manufacturing, Packaging and labeling: The investigational product (IP) will be packaged and labelled by the manufacturer before shipping to the study site. All the participants will receive identical-looking Atorvastatin tablets while half of the participants will also receive Omeprazole capsules. Each drug will be packaged in a separate bottle with labels containing information on the test product including the IP code, expiry, allergen instructions, directions for use and its purpose i.e. “for clinical use only”. They will contain instructions for the participant on how to appropriately administer the drugs.

Receipt, handling, storage and disposal of drugs: The drugs will be received directly from the manufacturers by the Co-I, who is a registered pharmacist. Once received, the drugs will be stored under lock and key, whereby only the PI and Co-I will have access to the drugs and hold the keys to the cabinet. When a participant is enrolled in the study, the PI or Co-I will retrieve the necessary drugs from the cabinet and the Co-I will pass the drugs directly to the participant, along with an explanation on how and when to consume the drugs. Participants will be requested to return any unused drugs to the PI or Co-I at the end of the study period. These unused drugs will be returned to the manufacturer for proper disposal.

IP randomization: The pharmacist will dispense the IP once during the study period. Each patient is supplied either 30 tablets in 1 bottle (control arm) or 60 tablets / capsules in 2 separate bottles (intervention arm). 30 Atorvastatin tablets will be packaged in 1 bottle and the other 30 Omeprazole capsules will be packaged in another bottle. An additional 5 surplus tablets / capsules of each drug will also be supplied in separate bottles to replace any damaged or missing tablets / capsules. We will provide the pharmacist with a code list. The pharmacist will dispense the drugs to the participants based on the unique randomisation code for that participant. Only trial pharmacy staff at the study site will be notified of the treatment allocation while all other site staff and research coordinators will be blinded. Delegated pharmacists will have access to an unbinding code list which they will use to cross reference the randomisation code in the email with the treatment allocations on the code list. To promote compliance to the study protocol, participants will be sent reminder texts before each visit. Compliance will be measured both by tablet / capsule counting (participants will be asked to return untaken tablets / capsules at the second visit) and a self-reported compliance questionnaire.

1.2. Rationale and justification for the Study

a. Rationale for the Study Purpose

A higher level of Atorvastatin lactone has been found to be associated with an increased risk of Major Adverse Cardiovascular Events (MACE) and the co-prescription of Omeprazole was found to be associated with a higher concentration of Atorvastatin lactone in the same study. However, the above study was conducted retrospectively and we think that it would be clinically important to conduct a prospective interventional trial to assess the effect of co-administration of Omeprazole with Atorvastatin on the concentration of Atorvastatin lactone. If it is indeed found that patients on both Omeprazole and Atorvastatin have a higher Atorvastatin lactone level, then an individualized risk-benefit assessment should be conducted to weigh the higher risk of MACE with co-prescription of Omeprazole against the benefit of Omeprazole for its prescribed indication.

b. Rationale for Doses Selected

The proposed dosing used is 40mg/day for Atorvastatin and 20mg/day for Omeprazole, which are within approved therapeutic ranges (Atorvastatin 10 - 80mg, Omeprazole 20 - 360mg). The chosen doses are also the commonly prescribed doses for each drug based on clinical experience, with well-documented safety and efficacy profile. Since Atorvastatin is dosed once daily while Omeprazole can be dosed once or twice a day, for convenience and to improve compliance, we decided to schedule both drugs for once a day administration. Omeprazole should preferably be taken on an empty stomach, such as 30 - 60 mins before breakfast, while Atorvastatin can be administered without regard to food, hence, to standardize administration across all treatment arms, we will advise patients to take their assigned drugs 30 mins before breakfast.

c. Rationale for Study Population

Healthy volunteers will be selected for this study as we would like to prospectively study the effect of co-administration of Atorvastatin and Omeprazole on Atorvastatin lactone, so we would like to recruit subjects who are not currently taking a statin or ppi. Nonetheless, it may be difficult to identify such patients within the outpatient or inpatient setting, hence we feel that healthy volunteers may will be the most suitable.

d. Rationale for Study Design

A 2-arm trial was selected to allow us to compare the Atorvastatin lactone metabolite level across the participants in this study. The study duration of 30 days was selected in view of the study's secondary endpoint to measure c-reactive protein (CRP) level. Atorvastatin induces a significant and extensive decrease in CRP level within the first 4 weeks of therapy but with no further decrease after 3 months [5]. This means that at the 30-days timepoint, we should be able to capture any significant changes in CRP.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

Co-administration of Omeprazole results in higher atorvastatin lactone level

2.2. Primary Objectives

To investigate the effect of co-administration of Omeprazole with Atorvastatin versus Atorvastatin monotherapy on atorvastatin lactone level.

Primary endpoint: Higher statin lactone level in intervention arm (Atorvastatin + Omeprazole) after 30 days

2.3 Secondary Objectives

Secondary endpoints:

- Higher hs-CRP in intervention arm

- Lipid panel
- Incidence of adverse drug side effects (e.g. muscle-related or elevated LFTs)

For safety endpoint, if the principal investigator, study team member, or the patient's primary treating physician notices anything with potential medical significance in any of the tests (blood test and vital signs), the physician will assess the causality and seriousness of the adverse event (AE) / serious adverse event (SAE). If found, all AE/SAE will be immediately notified to the Sponsor, Research Ethics Committee (DSRB), HSA authority, and the drug manufacturer, and full report will be submitted within the time frame specified. Given the relatively low risk associated with Atorvastatin and Omeprazole and short study duration, the study does not have an independent data monitoring committee. In the unlikely event of a AE/SAE, medical treatment will be allocated to ensure patient safety

2.4 Potential Risks and benefits:

We consider potentially minimal risk to the study participants. This study will involve (1) A demographics + medical + medication history survey, (2) Blood sampling (18mL per sample per subject, performed by a trained phlebotomist), and (3) A self-reported compliance questionnaire.

Potential benefits:

There is no direct individual benefits from participating in this study. However, the knowledge gained from this study may contribute to the medical field regarding the possible negative implications of co-prescription of drugs.

Potential risks:

i) Breach of confidentiality may happen during the study, such as after collection of personal information

ii) Subjects may primarily experience pain during the venipuncture blood draw when the needle goes through the skin. In less than 10% of cases, a small amount of bleeding under the skin will produce a bruise (minute hematoma). The puncture site may be visible and sore to the touch for a short period of time after the collection. Dizziness or light-headedness (syncope). The risk of local infection is less than 1 in 1,000.

iii) There is a possibility that subjects may suffer from an allergic reaction to Atorvastatin and / or Omeprazole or even suffer from adverse effects to Atorvastatin. The risk of elevated serum transaminases with Atorvastatin 40mg is 0.6%. The risk of rhabdomyolysis, a severe form of muscle injury, resulting from Atorvastatin therapy is about 0.5 – 1.0% over 5 years.

a. End Points - Efficacy

Results from the metabolic panel and biomarkers obtained from blood sample.

b. End Points - Safety

Although both drugs are approved by regulatory authorities such as Singapore Health Sciences Authority (HSA) as well as the United States (US) Food and Drug Administration (FDA) and are also widely prescribed, there is still a possibility that you may experience an allergic reaction to Atorvastatin and / or Omeprazole or even suffer from the side effects of Atorvastatin. There

is a possibility that Atorvastatin may result in liver injury or muscle-related side-effects. The risk of getting a severe form of muscle injury is about 0.5 – 1.0% over 5 years.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

We aim to assess the effect size of a minimum clinically significant difference in Atorvastatin lactone concentration of 1.68μg/L for the Atorvastatin + Omeprazole group compared to the Atorvastatin monotherapy group. Goh et al. 2023 reported a 1.4 times higher ATVlac in patients who were on ATV + OMP and literature on the pharmacokinetics of ATV reported the Cmax of ATVlac to be 4.2 ± 2.4 μg/L following oral administration of ATV 40mg. By inference, we expect a ATVlac concentration of 4.2 x 1.4 = 5.88μg/L in the ATV + OMP arm and this equates to a difference of 1.68μg/L in ATVlac concentration compared to the ATV monotherapy arm. Using a 2-sample t-test at a power (1-beta) of 85% and one-sided significance level (α) of 95%, we will need 30 subjects per treatment arm. To account for 30% drop-out after randomization, we will need to recruit at least 39 subjects per treatment arm and a total of 78 subjects for the study.

3.2. Criteria for Recruitment

3.3. Inclusion Criteria

1) Males between 21 and 75 years old or females between 50 and 75 years old.
2) No contraindications to the use of Atorvastatin amp; Omeprazole.
3) Ability to comply with study requirements eg administer Atorvastatin and Omeprazole once daily and return for follow-up 1 month later.
4) Capable of understanding the study requirements and provide informed written consent to participate.
5) Have not taken any statins or proton pump inhibitors in the past 30 days.

3.4. Exclusion Criteria

1) Women of child-bearing age (<50 years old)
2) Pregnancy / Planning to conceive or breast-feeding
3) Current or recent history of liver disease / renal impairment / myopathy / rhabdomyolysis
4) Recent history of alcohol or drug abuse
5) Concurrent use of other drugs that may interact with Atorvastatin, Omeprazole eg clopidogrel
6) Acute infection or illness
7) Allergy to any statins or proton pump inhibitors
8) Medical condition(s) that might compromise safety or successful completion of study

3.5. Withdrawal Criteria

Participation in the study is fully voluntary. Study participants can withdraw from the study at any time without comment or penalty. The decision to participate will in no way impact upon their present or future care. In addition, study participants may be withdrawn from the study

solely at the discretion of the Investigator or nominee. The investigator can withdraw a subject from the study for (1) they decide that it is in the patient's best interests due to urgent medical reasons or (2) if a protocol violation occurs or (3) if the patient requests withdrawal. Withdrawn patients will not be replaced in this study.

The reasons for discontinuation/withdrawal will be recorded in the study participant's Case Report Form (CRF). Any study participant(s) withdrawn from the study prior to their completion for any reason will have data compiled to the point of discontinuation to be used for analysis.

3.6. Subject Replacement

There is no need for subject replacement.

4. TRIAL SCHEDULE

Table 1 shows the trial plan and procedures to be conducted on each study visit.

5. STUDY DESIGN

5.1. Summary of Study Design

This is a 2-arm, prospective, randomized, parallel-group, open-label, longitudinal study that lasts 30 days for each study participant. Assessment of primary and secondary outcomes have been planned in an exploratory setting.
Fig. 1 shows the study protocol.
Table 1 shows trial plan and procedure to be conducted on each study visit.

2. Description of study visits:
a) Pre-screening, consenting process, and screening procedures
Potential subjects will be identified through (i) direct advertisement such as posters to be put up around NUS / NUH, (ii) indirect – through word or mouth or snowballing. Participants who are interested will be interviewed over the phone by the study's Clinical Research Coordinator (CRC). During the telephone interview, participants will be given a brief description of the study design and procedures to be followed. If the participant is eligible, with their verbal agreement, arrangement will be made for them to visit NUHS IMU (located in NUS MD6, level 7) for a screening and enrolment visit.

b) Informed consent
Informed consent will be taken by the PI. The study will be explained to the participant in detail. This will be performed in the privacy of a closed-door room. During the consenting process, the study will first be explained to the participants and ample time will be provided for patients to read through the informed consent form. They will also be (i) given opportunity to clarify any doubts before acknowledging the consent form; (ii) allowed to return home to discuss their participation with their family prior to consenting. Non-English speakers will have the English informed consent form with a short consent form in their language, so that they can understand the purpose of this study and what is required of them. If the participant agrees to participate, the informed consent form will be signed by

the PI and the participant, in the presence of a prescribed witness. In the event that the participant is illiterate, or a short consent form will be used, an impartial witness will be present instead. A copy of the informed consent will be kept by both the PI and the participant. Enrolled subjects will be updated in the Screening and Enrolment Log. Unselected subjects will be documented in the Pre-screening Log.

(c) Screening and baseline data collection
Participants who have signed the informed consent form will be assessed for eligibility to participate, based on the following assessments: (i) Demographic data; (ii) Medical and medication history; (iii) history of adverse drug reactions (if any). Patients will be interviewed directly to provide the above health information; medical records will not be assessed. Based on the collected data, approval for participation will be finalised by either the PI or Co-PI. Participants who are eligible for the study will be informed by the CRC and enrolled as a subject for the study.

(d) Blood draw
Post-fasting (8 hours) whole blood will be drawn twice. 18mL will be collected by ventipuncture each time, once at the time of recruitment (before intervention) and a second time on the second visit (T2) 30 days after the start of the intervention period. This amounts to a total of 36mL of blood collected throughout the study period. Blood will be drawn into EDTA vacutainers. All samples will be transferred from NUHS IMU to the PI's research laboratory (located in CDrum lab, NUS MD6, level 8). All the blood will be spun down to plasma. With an estimated plasma extraction efficiency of 45% via centrifugation, we expect to obtain about 8.1mL of plasma from 18mL of blood. The plasma will be aliquoted and stored in a -80 degrees Celsius freezer until analysis. For atorvastatin lactone metabolite profiling, a 1mL aliquot will be subjected to protein precipitation and used for analysis. Another 1mL aliquot will be used for high sensitivity c-reactive protein (hs-CRP) assays using the cobas c-111 machine. Another 3mL of plasma will be used for lipid panel profiling using cobas c-111 machine. The remaining plasma samples will be stored at -80 degrees Celsius for repeated analysis, if necessary.

3. Randomization and treatment allocation
The study will be open-label since our primary endpoint is an objective pharmacokinetic parameter.

Treatment allocation between the Atorvastatin + Omeprazole and Atorvastatin only arms will be generated by a random allocation sequence using real-time web-based randomization system (IVRS) in a 1:1 ratio. Our desired allocation ratio (1:1) can be achieved using permuted block randomization. Block randomization ensures balance between the 2 groups, in which the order of allocation is chosen at random at the beginning of the block. Block size will be determined by the study team member.
Intervention group 1: Atorvastatin 40mg/day + Omeprazole 20mg/day
Control group: Atorvastatin 40mg/day
We target to recruit 78 patients, of whom 39 patients will be assigned to each of the 2 treatment arms. The pharmacist will dispense the IP to the study participants. Each participant will be supplied 30 tablets of Atorvastatin and those in the intervention arm will receive an additional 30 capsules of Omeprazole during the trial. A total of 60 tablets / capsules will be dispensed to each participant in the intervention arm and 30 tablets to each participant in the control arm. An additional 5 surplus tablets / capsules of each drug will also be supplied to replace any damaged or missing tablets / capsules. Atorvastatin and Omeprazole will all be packaged and coded by the manufacturer. The pharmacist will be provided with the code list and will dispense the IP to each participant based on the unique subject ID for that participant. A CRC will provide a prescription on the day of the study visit and ensure that it is kept at the appropriate storage condition prior to administration.

Possible IFs:

a) Adverse events following co-administration of atorvastatin and omeprazole in healthy participants.
b) Participant found to have an elevated C-reactive protein (CRP) level (>10mg/L), which may indicate that the participant has an underlying inflammation or infectious condition.
c) Participant found to have elevated low-density lipoprotein (LDL) and/or triglycerides (TG) (LDL ?4.9 mmol/L, TG ?4.5 mmol/L), which may indicate that the participant may have hyperlipidemia and/or hypertriglyceridemia.

4. Incidental Finding (IF) management plan:
(a) In the event that the researchers believe that the IF may affect the health or well-being of the study participant, the researchers will base their determination of the IF on criterias including how accurate the test that identified the IF was, and the researchers will have the test repeated and validated in a clinical laboratory, should a valid test be available to confirm the IF. Otherwise, justification will be submitted to the IRB as to why clinical verification is not ethically appropriate or practicably possible, and therefore, the results will not be returned to the participant. The PI is responsible for reporting IFs, in accordance with evidence-based best practice's guideline for managing IFs. The PI, who is a qualified medical professional, is responsible for assessing the clinical significance of the IF, and managing the IF to ensure the safety of all the study participants. When an IF has an "immediate clinical relevance", in the absence of clear evidence or when faced with unanticipated IFs of potential concern, researchers will (i) consult qualified clinical experts to assess the clinical significance of the finding and plan a response; (ii) consult with colleagues to determine the standards within the discipline, and combine with the practical wisdom of a consultant medical specialist; or (iii) consult DSRB ethics board about possible disclosure and be ethically responsible or required to disclose such findings to study participants. Prior to disclosure, the results will be verified by an accredited diagnostic laboratory / expert, whether it is practicably possible. Please refer to IF Management Plan on the types of IFs that may arise from this study. Considering (i) the preferences of participants in the decision-making regarding IF disclosure; (ii) the potential benefits vs harms of disclosure, the study PI or another qualified member of the research team will communicate the IF to the participant, either directly or indirectly by the participant's primary treating physician. The IFs will be communicated verbally, in a timely mannger, and documented in writing, and the process of communicating IFs should be completely transparent. The informed consent process will include IFs (including information about follow-up and the cost of managing the finding). During the consent process, research participants should be informed of these practices.

(b) The decision of whether to return IFs will typically be the responsibility of the study team members, i.e., Co-I, who is a GCP qualified and registered medical practitioner under the Singapore Medical Council (SMC) respectively. This is because IFs are typically more of a clinical issue rather than a research issue. For medical reasons, it is desirable that the information concerning the IFs be returned by a person who has a clinical relationship with the participant. If the researcher is not a physician or does not have a clinical relationship with the participant, someone else, such as a medical counsellor, may be more suitable for returning the IF to the participant. Only verified results will be returned and the primary treating physician responsible for the participant's care should always be notified. Physicians who can judge the potential health implications of a finding will advise the research participant, and fully inform the participant of the likelihood of IFs and the threat the IFs might pose.

(c) The mode of communication to be used and the limits of effort of re-contacting in case an IF is identified during the course of study: physician will notify the participant if they choose to be notified as indicated in their consent form. In such an event, the study PI or delegated study team member will attempt to contact the participant by (i) mailing a general letter; (ii) by email and (iii) phone call at the requested time using their preferred number. Extended efforts with follow-up and phone call strategies

(varying calling times, calling multiple times) until contact is successfully made with the study participant. The researcher will communicate the IF to the participant in a way that is sensitive to the issues raised. If it is anticipated that there will be a high likelihood of finding grave IFs in the study, researchers may ask the participant during the consent process if they consent to disclosure of medically significant IFs to their primary care physician, with whom they already have a relationship. Researchers disclosing an IF to the participant may consider communicating the IF directly to the participant's attending physician, so that the attending physician can discuss and address the IF promptly with the participant. If the participant has no physician, the researchers should offer to suggest one. Depending on the specific IFs revealed, the participant may ask the researchers to suggest a specialist, such as a cardiologist. The researchers should respond to such requests with recommendations or referral. The study team may provide a reasonable standard of care and give feedback on health information that is likely to result in avoidance of significant harm to the participant, whichever appropriate.

6. METHODS AND ASSESSMENTS

6.1. Randomisation and Blinding

The study will be open-label since our primary endpoint is an objective pharmacokinetic parameter.

Treatment allocation between the Atorvastatin + Omeprazole and Atorvastatin only arms will be generated by a random allocation sequence using real-time web-based randomization system (IVRS) in a 1:1 ratio. Our desired allocation ratio (1:1) can be achieved using permuted block randomization. Block randomization ensures balance between the 2 groups, in which the order of allocation is chosen at random at the beginning of the block. Block size will be determined by the study team member.
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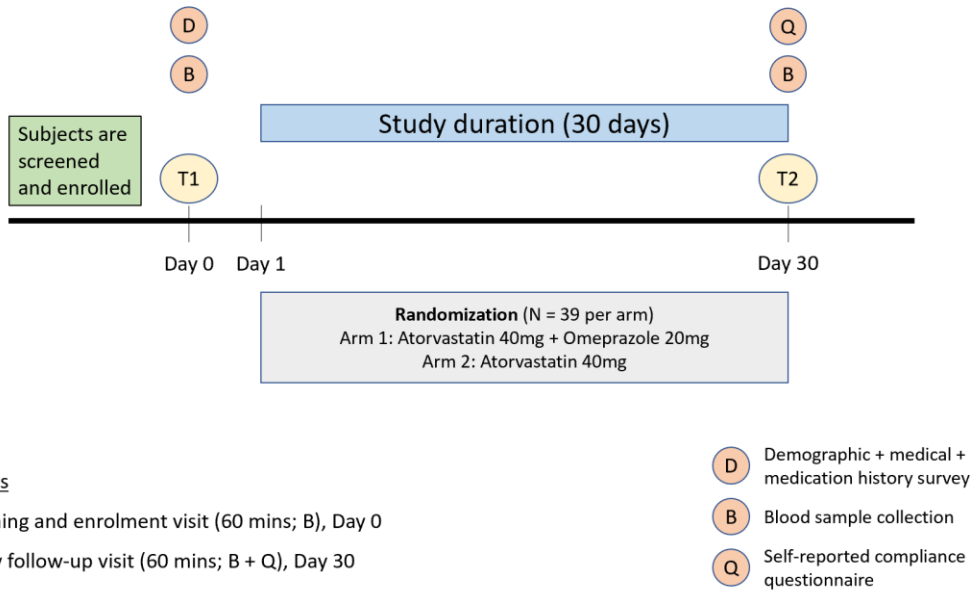
6.2. Study Visits and Procedures

The total study duration is 31 days and consists of 2 study visits (Fig. 1). Blood samples will be collected at each timepoint (T1 and T2) for metabolic panel and biomarker measurements. All blood sampling (B) are study activities and not routine medical care.

Study Visit T1 (Day 0)
This will be the screening and enrolment visit. Estimated duration is 90 mins. The following procedures will be performed during this visit:
i) Confirming eligibility and Informed consent taking (30 minutes)
ii) Completion of demographic + medical + medication history survey (10 minutes)
iii) Blood sample collection (10 minutes)
iv) Post-collection health monitoring (20 minutes)
v) Randomization, distribution of drugs and explain how to take the assigned medications (20 minutes)

Study Visit T2 (Day 30)
T2 will be follow-up visit 30 days after intervention. Estimated duration is 60 mins. The following procedures will be performed during this visit:
i) Self-reported compliance questionnaire and return of unused drugs if any (20 minutes)
ii) Blood sample collection (10 minutes)
iii) Post-collection health monitoring (20 minutes)
iv) Reimbursement (10 minutes)

Figure 1 SEACOL Study protocol



a. Post Study Follow up and Procedures

There will not be post study follow-up or procedures.

b. Discontinuation Visit and Procedures

The last study visit (T2) should be done if withdrawal occurs. Participants may withdraw voluntarily from the study at any time and for any reason without penalty. This will not affect their routine medical care. If voluntary withdrawal occurs, the participant should be asked to continue scheduled evaluations, complete an end of study evaluation and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or until the participant's condition stabilizes. The PI may also withdraw the patient's participation in the study at any time if they decide that it is in the patient's best interests or if they have other medical problems.

The reasons for withdrawal or discontinuation will be documented in the participant's Case Report Form. In the case of any premature withdrawal from the study, blood samples and study data that have been collected up to the time of withdrawal or discontinuation will be kept and analysed as intention-to-treat analysis.

c. Measurements

1) Demographic + medical + medication history survey on the first visit. Information to be collected include: age, gender, race, history of liver or kidney disease, whether a statin or proton pump inhibitor has been taken in the past 30 days, any diagnosed medical condition and regular medications, as well as whether the participant is taking any supplements, traditional or herbal products.
2) Blood sample collection (18mL each time x 2 times, on enrolment and after 30 days of intervention).
3) Consumption of investigational drugs (Atorvastatin or Atorvastatin + Omeprazole)
4) Self-reported compliance questionnaire at the second visit timepoint. Questions include: (i) Have you missed taking the medications on any day(s) during the 30 days study period? (Yes / No. If yes, please state how many days: ___); (ii) How many tablets / capsules have you been taking each day? (___)
5) LC-MS measurement: Blood samples will be processed to extract plasma. Plasma samples, calibrators and quality control samples will be transferred into a deep well 96-well plate and standard curve will be used for quantification of Atorvastatin lactone levels in the plasma samples.
6) hs-CRP and lipid panel assays on participant's plasma samples using cobas c-111 machine

7. TRIAL MATERIALS

7.1. Trial Product (s)

Both drugs are US FDA and Singapore HSA approved drugs.

(i) Atorvastatin is a synthetic lipid-lowering agent, which is an inhibitor of HMG-CoA reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis.Atorvastatin is indicated as an adjunct to diet for reduction of elevated total-cholesterol (total-C), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (apo B) and triglycerides (TG) in adults, adolescents and children aged 10 years or older with primary hypercholesterolemia, heterozygous familial hypercholesterolemia or

combined (mixed) hyperlipidaemia (Fredrickson Types IIa and IIb), elevated serum triglyceride (TG) levels (Fredrickson Type IV), and for patients with dysbetalipoproteinemia (Fredrickson Type III) when response to diet and other non-pharmacological measures is inadequate. Atorvastatin is also indicated to reduce total-C and LDL-C in adults with homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering treatments (e.g. LDL apheresis) or if such treatments are unavailable. Atorvastatin is also indicated to reduce the risk of myocardial infarction (MI) in adult hypertensive patients without clinically evident coronary heart disease (CHD), but with at least three additional risk factors for CHD such as age ? 55 years, male sex, smoking, left ventricular hypertrophy, other specified abnormalities on electrocardiogram (ECG), microalbuminuria or proteinuria, ratio of plasma total cholesterol (total-C) to HDL-cholesterol ? 6, or premature family history of CHD. Atorvastatin is indicated in adults with type 2 diabetes and without clinically evident CHD, but with multiple risk factors for CHD such as retinopathy, albuminuria, smoking or hypertension, as well as in adults with clinically evident CHD.

(ii) Omeprazole is a proton pump inhibitor which suppresses gastric basal and stimulated acid secretion by inhibiting the parietal cell H+/K+ ATP pump. Omeprazole is indicated for the treatment of gastroesophageal reflux disease (acute, healing or symptomatic), heartburn, helicobacter pylori eradication, peptic ulcer disease, duodenal or gastric ulcers and zollinger-ellison syndrome.

There is a possibility that subjects may suffer from an allergic reaction to Atorvastatin and / or Omeprazole or even suffer from adverse effects to Atorvastatin. The risk of elevated serum transaminases with Atorvastatin 40mg is 0.6%. The risk of rhabdomyolysis, a severe form of muscle injury, resulting from Atorvastatin therapy is about 0.5 – 1.0% over 5 years.

7.2. Storage and Drug Accountability

Both drugs have similar storage requirements, with reference to their individual product information leaflet. The drugs need to be store in a cool and dry place, away from direct sunlight, at or below 30°C. Specifically for Atorvastatin, it should also be stored in its original aluminium blister to protect from moisture. Hence, if we are packaging Atorvastatin tablets in bottles for the subjects, a moisture absorber should be included in the bottle, such as a packet of silica gel, and subjects should be reminded not to remove that moisture absorber as well as to minimize exposure of the medication to moisture.

8. TREATMENT

8.1. Rationale for Selection of Dose

The proposed dosing used is 40mg/day for Atorvastatin and 20mg/day for Omeprazole, which are within approved therapeutic ranges (Atorvastatin 10 - 80mg, Omeprazole 20 - 360mg). The chosen doses are also the commonly prescribed doses for each drug based on clinical experience, with well-documented safety and efficacy profile.

8.2. Study Drug Formulations

Atorvastatin is formulated as oral tablets while Omeprazole is formulated as oral capsules.

8.3. Study Drug Administration

Since Atorvastatin is dosed once daily while Omeprazole can be dosed once or twice a day, for convenience and to improve compliance, we decided to schedule both drugs for once a day administration. Omeprazole should preferably be taken on an empty stomach, such as 30 - 60 mins before breakfast, while Atorvastatin can be administered without regard to food, hence, to standardize administration across all treatment arms, we will advise patients to take their assigned drugs 30 mins before breakfast.

8.4. Specific Restrictions / Requirements

The risk of myopathy during treatment with Atorvastatin (HMG-CoA reductase inhibitor) is increased with concurrent administration of cyclosporine, fibric acid derivatives, lipid-modifying doses of niacin or CYP 3A4/transporter inhibitors (e.g., erythromycin and azole antifungals). These medications should be avoided during the study period or spaced at least 2 – 4 hours apart from Atorvastatin if not possible to omit.

The Omeprazole and Clopidogrel combination is a well-known drug interaction whereby Omeprazole may diminish the antiplatelet effects of Clopidogrel due to Omeprazole-mediated inhibition of CYP2C19, an enzyme responsible for clopidogrel metabolism to its active metabolite. Nonetheless, clinical evidence has suggested limited impact on clinical efficacy for using this drug combination and it is still a popularly prescribed pair of drugs, but for the purpose of this study, we will advise against initiating Clopidogrel during the study period.

8.5. Blinding

The study will be open-label.

8.6. Concomitant therapy

All medications (prescription and over the counter), vitamin and mineral supplements, and / or traditional or herbal products taken by the participant should be documented.

9. SAFETY MEASUREMENTS

9.1 Collecting, Recording and Reporting of "Unanticipated Problems Involving Risk to Subjects or Others" – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. **Unexpected**
   In terms of nature, severity or frequency of the problem as described in the study documentation (eg: Protocol, Consent documents etc).

2. **Related or possibly related to participation in the research**
   Possibly related means there is a reasonable possibility that the problem may have been

caused by the procedures involved in the research; and

3. **Risk of harm**
   Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB.

1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.

2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

9.2 Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

1. **For Principal Investigator initiated Trials**
   All SAEs that are unexpected and related to the study drug must be reported to HSA.

"A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious."

All SAEs that are unexpected and related to the study drug will be reported. The investigator is responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

9.3 Safety Monitoring Plan

Safety assessments during the study include monitoring the wellbeing of each study participant regarding any adverse events he/she may experience. All study participants receiving treatment will be attended by the site PI if any medical issues arise.

Regular reports on conduct and progress of the project will be provided by the study team to the Domain Specific Review Board (DSRB) ethics committees and to the team of investigators. The team of investigators will conduct random inspections of the research procedures, data and consent documentation, which includes the checking of the completeness of the Investigator File and verifying data collection forms. In addition, the Investigators will ensure the integrity of the data through the following strategies:

- Regular project meetings (monthly) with project progress (written and verbal) and dedicated time for discussion and intellectual exchange (monthly)
- Rotation of meetings among different sites to enable easy attendance
- Use of secured shared IT procedures to allow investigators and collaborators from the different organizations and sites to access shared databases.

9.4 Complaint Handling –

All complains or feedback about this research study will either be dealt with by the Principal Investigator and/or the NHG Domain Specific Review Board Secretariat. Participants will be notified in the patient information consent form to contact the PI if they have any questions, complaints or concerns. In addition, they can also contact the NHG Domain Specific Review Board Secretariat at 6471-3266 if they want an independent opinion to discuss problems and questions, obtain information and offer inputs on their rights as a research subject.

10 DATA ANALYSIS

10.1 Data Quality Assurance

LC-MS metabolite quantification as well as hs-CRP and lipid panel assays will be done in duplicates or triplicates to ensure accurate and reproducible results.

10.2 Data Entry and Storage

Data collected and entered in the Case Report Forms (CRF) and questionnaires will be stored in a secure location at the main study site (NUS CVRI). A patient coded electronic database containing data extracted from the Case Report Forms will also be securely stored. Only the research team will have access to the Case Report CRF or any medical data. In the event of any publication regarding this study, the data will be de-identified so that the identity of the patient remains confidential.

11 SAMPLE SIZE AND STATISTICAL METHODS

11.1 Determination of Sample Size

We aim to assess the effect size of a minimum clinically significant difference in Atorvastatin lactone concentration of 1.68μg/L for the Atorvastatin + Omeprazole group compared to the Atorvastatin monotherapy group. Goh et al. 2023 reported a 1.4 times higher ATVlac in patients who were on ATV + OMP and literature on the pharmacokinetics of ATV reported the Cmax of ATVlac to be 4.2 ± 2.4 μg/L following oral administration of ATV 40mg. By inference, we expect a ATVlac concentration of 4.2 x 1.4 = 5.88μg/L in the ATV + OMP arm

and this equates to a difference of 1.68μg/L in ATVlac concentration compared to the ATV monotherapy arm. Using a 2-sample t-test at a power (1-beta) of 85% and one-sided significance level (α) of 95%, we will need 30 subjects per treatment arm. To account for 30% drop-out after randomization, we will need to recruit at least 39 subjects per treatment arm and a total of 78 subjects for the study.

11.2 Statistical and Analytical Plans

To avoid potential bias due to exclusion of patients (type 1 error), the effect of intervention will be assessed according to the Intention-to-Treat (ITT) principle, such that every patient randomized in the study will enter the primary analysis. Since we expect dropout of some patients on follow-up assessments, all randomized patients will be included in the intention-to-treat analysis, where missing data will be imputed by a multiple imputation method.

We will compare the atorvastatin lactone level between the 2 treatment arms at the second visit timepoint. A one-sided t-test will be performed using R 4.3.1 software.

No interim analyses will be carried out since the study has a short study duration.

12 ETHICAL CONSIDERATIONS

12.1 Informed Consent

Informed consent will be obtained by the PI who is a qualified practitioner. Co-I or CRC will only assist with the informed consent process. The study will be explained to patients in detail. This will be performed in the privacy of a closed-door room within NUHS IMU. During the consenting process, the study will first be explained to participants, and they will be given ample time to read through the informed consent form. Participants will be (1) given opportunity to clarify any doubts before acknowledging the consent form and (2) allowed to return home to discuss their participation with their family prior to consenting. Non-English speaking participants will have the English informed consent form with a short consent form translated in their preferred language. Hence, the informed consent process gives participants sufficient time to consider their participation in the study by having all their questions answered and allowing them to consult others, such as their physician, as well as give them the option of further discussing with their family members before making a decision. Participants would not be approached in a situation where they may feel compromised, thus preventing coercion and undue influence. If the participant agrees to participate, the informed consent form will be signed by the PI and the patient, in the presence of a prescribed witness. If a short consent form is used or if the participant is illiterate, an impartial witness will be present instead. A copy of the informed consent will be kept by both the PI and the participant.

12.2 IRB review

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by the NHG DSRB.

12.3 Confidentiality of Data and Patient Records

The study team would store all research data within the institution. For hardcopy data, they will be stored in designated locked cabinet(s) or room(s) that are accessible to authorized study personnel only. For electronic data, they will be stored in a secured computer that is password protected. The databases will not contain subject identifiers and the data linking subject identifiers and the subject identification codes will be stored separately. When portable media (e.g. CD, USB drives etc.) are used to store the data, subject identifiers are stored separately. Records for all participants, including all source documentations (containing evidence to study eligibility, history, billing information and physical findings, laboratory data, etc.) as well as IRB records and other regulatory documentation will be retained by the PI in a secure storage facility at the institution. All information will be coded, stored in a secure area and treated confidentially. Only the PI, co-I, study administrators and research coordinators will have access to the research data. All data files will be password protected and the study team will have access to the research data. All research data and biological materials (blood samples) will be collected prospectively and kept at the PI's lab at NUS-MD6 Centre for Translational Medicine for up to 6 years, for future research. Collected data and blood samples will be de-identified / coded. The PI and / or delegated CRCs will maintain the source code.

13 PUBLICATIONS

In the event of any publication regarding this study, the data will be de-identified so that the identity of the patient remains confidential. In addition, no identifying information or names will be included in any transcripts, conference presentations, research / industry reports or publications.

14 RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation should be retained by the PI as original hard copy in a secure storage facility and data stored in computer databases will be kept in secure network drives during and after the project, for up to 6 years. The records should be accessible for inspection and copying by authorized authorities.

List of Attachments

Appendix 1 Study Schedule

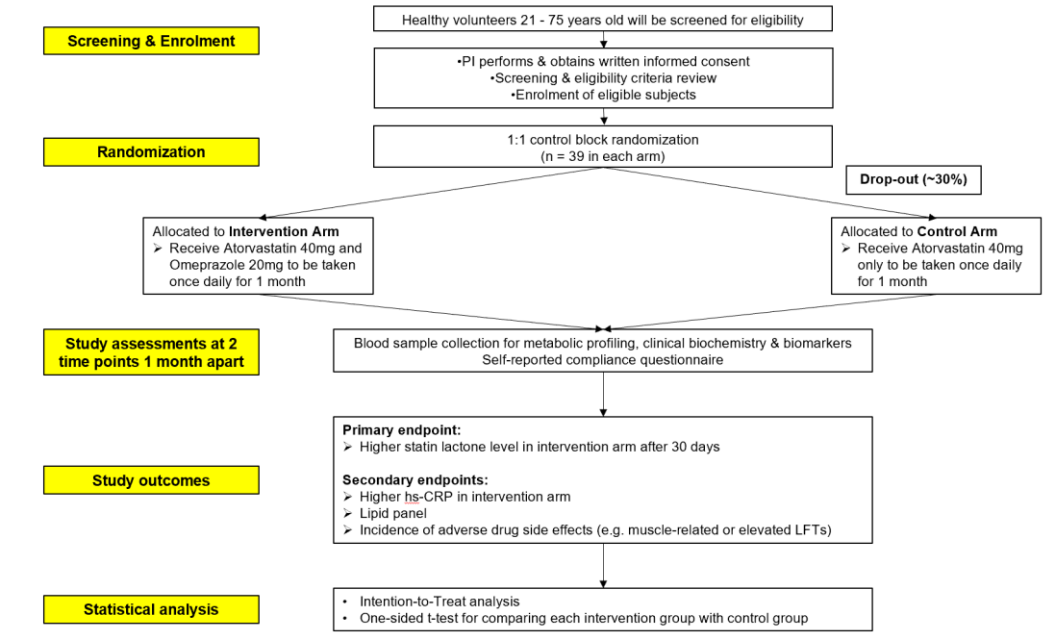
Procedure	Screening & enrolment (visit T1)	30-days follow-up (visit T2)
Subject information & Informed consent signature	X	[redacted]
Verification of inclusion and exclusion criteria	X	
Demographics, medical & medication history survey (D)	X	
Randomisation	X	
Peripheral intravenous blood collection (B)	X	X
Self-reported compliance questionnaire (Q)	[redacted]	X
Oral administration of drugs Atorvastatin or Atorvastatin + Omeprazole	<==========➔	
Serious/Adverse events (AE/SAE)	<==========➔	

X = experimental procedure; ==> = Intervention.
Timepoint: T1 = Screening & enrolment; T2 = Follow-up after 30 days of intervention

Appendix 2 Study design flow chart

Study flow chart

Figure 2. Study on the Effect of Atorvastatin Co-administered with Omeprazole on statin Lactone (SEACOL)



Appendix 3 Demographic + Medical + Medication history survey

Subject ID:
Date & Time:

---

1) Age (as of January 2024): _____

2) Gender: ☐ Male ☐ Female

3) Race: ☐ Chinese ☐ Malay ☐ Tamil

☐ Others (Please specify:

_____)

4) Have you ever been diagnosed with the following? (please tick if applicable)

☐ Liver disease

☐ Kidney disease

5) Do you have any other diagnosed medication conditions? (E.g., High blood pressure, high cholesterol, diabetes, heart conditions etc.)

Please specify:

_____

6) Have you taken any of the following medications in the past 30 days? (please tick if applicable)

☐ Statin (E.g., Atorvastatin, Rosuvastatin, Simvastatin, Pravastatin)

☐ Omeprazole / Esomeprazole / Pantoprazole

7) Are you taking any medications on a regular basis e.g., every day?

   Please specify the name of the medication + dose (e.g., 5mg) + how often you are taking it (e.g., two times a day):

   _____

   _____

   _____

8) Are you taking any supplements, herbal or traditional products?

Please specify: _____

Appendix 4 Self-reported compliance questionnaire

Subject ID:
Date & Time:
Study intervention period (e.g., DD/MM/YY to DD/MM/YY):

1) Have you missed taking your assigned medication(s) on any day(s)?

   ☐ Yes

☐ No

2) If your answer to Question 1 above is 'Yes', please state how many day(s) you have missed:

_____

You may also circle the dates you have missed taking medication(s) on the calendar below if you remember the dates:
(A monthly calendar will be attached here based on the subject's study intervention period, example shown below)

May 2024						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

3) How many tablet(s) / capsule(s) of each of these medications have you been taking each day? (Indicate 'NA' for the medication you did not receive)

_____

References

Studies on Atorvastatin + Omeprazole on Atorvastatin lactone:
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2) Turner, R. M., Fontana, V., FitzGerald, R., Morris, A. P., & Pirmohamed, M. (2020). Investigating the clinical factors and comedications associated with circulating levels of atorvastatin and its major metabolites in secondary prevention. British Journal of Clinical Pharmacology, 86(1), 62–74. doi:10.1111/bcp.14133

Studies related to giving Atorvastatin to healthy volunteers:
1) Cilla, D. D., Whitfield, L. R., Gibson, D. M., Sedman, A. J., & Posvar, E. L. (1996). Multiple-dose pharmacokinetics, pharmacodynamics, and safety of Atorvastatin, an inhibitor of HMG-COA reductase, in healthy subjects. Clinical Pharmacology & Therapeutics, 60(6), 687–695. doi:10.1016/s0009-9236(96)90218-0
2) Abdelkawy, K. S., Abdelaziz, R. M., Abdelmageed, A. M., Donia, A. M., & El-Khodary, N. M. (2020). Effects of green tea extract on atorvastatin pharmacokinetics in healthy volunteers. European Journal of Drug Metabolism and Pharmacokinetics, 45(3), 351–360. doi:10.1007/s13318-020-00608-6

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1) Baldwin, R. M., Ohlsson, S., Pedersen, R. S., Mwinyi, J., Ingelman-Sundberg, M., Eliasson, E., & Bertilsson, L. (2008). Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. British Journal of Clinical Pharmacology, 65(5), 767–774. doi:10.1111/j.1365-2125.2008.03104.x

For sample size calculation:
1) Lennernäs, H. (2003a). Clinical pharmacokinetics of atorvastatin. Clinical Pharmacokinetics, 42(13), 1141–1160. doi:10.2165/00003088-200342130-00005