CLINICAL STUDY PROTOCOL

A randomized, open, parallel design study to evaluate the effect on liver fat, adipose tissue and metabolic parameters when switching a protease inhibitor or efavirenz to once daily raltegravir in HIV-infected patients with body mass index over 25 kg/m^2 and with at least one metabolic syndrome component.

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Organization of the research:

This study is an investigator initiated study in collaboration with Infectious Diseases Department, HUH and with Obesity Research Unit, University of Helsinki.
The research project is coordinated by PI Jussi Sutinen and co-investigator Anna Hanttu.
The recruiting and clinical follow-up of study subjects will take place in Infectious Diseases Clinic, Helsinki. There will be two study visits in the Obesity Research Unit, where the metabolic procedures will be done. Additionally, liver fat and abdominal fat distribution will be measured by MRI/MRS and liver fibrosis by transient elastography, both at the Helsinki University Hospital.

Funding of this project comes from Merck Sharp & Dohme Corp. With this funding, we anticipate to organize the study visits of the study subjects in Obesity Research Unit and all the metabolic and imaging studies planned for this study. In the future, we will apply funding for stool sample analyzes (microbiome studies) and for the gene expression studies of the fat biopsies.

1. Background and rationale of the study

The prevalences of overweight (body-mass-index, BMI 25-30 kg/m^2) and obesity (BMI>30 kg/m^2) are steadily increasing among HIV-infected patients globally. In our HIV outpatient clinic in Helsinki, approximately half of the patients are overweight or obese (unpublished data). Obesity is often associated with metabolic syndrome, which manifests with dyslipidemia (with high triglycerides and low high-density lipoprotein cholesterol (HDL) levels), impaired fasting glucose (≥ 5.6 mmol/l), high blood pressure (BP≥ 130/≥ 85 mmHg) and increased visceral adipose tissue mass. Inflammation markers, such as hsCRP and IL6 are often increased in serum and there are various alterations in bowel microbiota supporting ongoing low-grade inflammation. Similar changes in inflammation markers and in metabolism are seen in HIV-infected patients even with normal body weight.

As the prevalences of obesity and metabolic disorders among HIV patients gradually increase, the risk of non-alcoholic fatty liver disease (NAFLD) increases parallel, as shown by the ECHAM (European Cohort on HIV, Ageing and Metabolic liver disease) Study (1). NAFLD is characterized by excessive hepatic fat accumulation and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a weight fraction >5.6% assessed by proton magnetic resonance spectroscopy (1HMRS) or proton density fat fraction – a quantitative chemical shift-based water and fat separation -magnetic resonance imaging (MRI), which are the golden standards in diagnostics (2). The diagnosis of NAFLD requires the exclusion of secondary causes, such as excessive daily alcohol consumption (3). NAFLD, however, is a spectrum of pathologically distinct liver diseases, where simple steatosis is considered to be a non-progressive form of NAFLD, and non-alcoholic steatohepatitis (NASH) is considered to be the progressive form of NAFLD. NASH manifests with inflammation and fibrosis and can lead to cirrhosis and hepatocellular carcinoma (HCC) (2). Clinically alarming are the data which suggest that HIV infected individuals have higher rates of progressive form of NAFLD than non-HIV infected age, gender and BMI matched controls (4).

Antiretroviral therapy (ART) for HIV-infected patients may promote unfavourable changes in metabolism and in trunk fat redistribution. Antiretroviral drugs may increase biosynthesis and reduce hepatic clearance of serum cholesterol. In a systematic review regarding ART-associated changes in metabolism, association between ART exposure and mean serum total cholesterol level and triglycerides was significantly stronger with the use of protease inhibitors (PI), compared with the use of other ART regimens (5). Yet the clinical effect of individual agents within the PI class varies. Other antiretroviral medications may contribute to dyslipidemia as well. Of the NNRTI-group (non-nucleoside reverse transcriptase inhibitor), efavirenz has been associated with the worst lipid profile, causing elevated triglycerides and total cholesterol and unfavourable body fat redistribution (5).

According to several studies, raltegravir (RAL) has been shown to have neutral metabolic effects in the management of HIV. In (ACTG) A5257 study, when treatment-naive adult subjects were randomized either to ritonavir-boosted protease inhibitor atazanavir (ATV/r) or darunavir (DRV/r), or RAL-based ART, RAL produced the most favorable lipid profile (6). In SPIRAL-trial, ART-treated, virologically suppressed HIV-infected patients switching from a PI/r containing ART to RAL-based ART showed a favourable impact on lipids (lowering of triglycerides, total and LDL cholesterol) as compared to those continuing with the PI/r based ART. (7). In the same study, insulin and some inflammatory biomarkers decreased in the RAL-arm (hsCRP, IL-6, TNF-α). In a study conducted by Nguyen et al (SWITCH-ER study), favourable changes in total cholesterol, triglycerides and LDL cholesterol were seen in the RAL-arm compared to the control arm continuing their current efavirenz-containing ART (8).

Although raltegravir has been demonstrated to have beneficial impact on some metabolic parameters compared to the PI class or to efavirenz, very little is known whether raltegravir could improve hepatic steatosis in HIV-associated metabolic syndrome. Recent studies presented in CROI (Conference on Retroviruses and Opportunistic Infections) 2017 showed that serum

biomarker Chi3L1 (hepatic steatosis/fibrosis marker) decreased in HIV-infected women with lipohypertrophy when switching from NNRTI- or PI-based regimens to raltegravir. In the same study adiponectin concentration decreased, which is surprising knowing the inverse correlation between serum/plasma adiponectin concentration and liver fat content (9, 10). STERAL study presented also in CROI 2017, showed that switching from efavirenz to raltegravir decreased hepatic steatosis, as measured by controlled attenuation parameter (CAP) at 48 weeks, compared with those continuing with efavirenz in HIV/HCV co-infected patients (11).

There are confounding data on the effect of raltegravir on body weight and fat tissue. In a study with thirty-nine obese women, a switch to raltegravir from a PI or a NNRTI did not reduce visceral fat as measured by computed tomography (12). In SPIRAL-LIP substudy there were no significant changes in total body fat between maintaining a PI-based regimen or switching to a raltegravir–based regimen as measured by DEXA and CT. However, maintaining a PI-based regimen was associated with a significant increase in visceral adipose tissue and total adipose tissue (13). In a retrospective analysis of the ACTG A5257 study, where ART-naïve HIV-infected individuals were randomized to one of 3 regimens: atazanavir/ritonavir (ATV/r), darunavir/ritonavir (DRV/r), or raltegravir (RAL) each in combination with tenofovir/emtricitabine, the odds of severe weight gain were significantly higher for RAL than for ATV/r and for DRV/r (14).

In summary, raltegravir seems to have a favourable impact on distinct metabolic components compared to PI class or efavirenz. Raltegravir also seems to decrease hepatic steatosis measured by CAP in HIV-infected subjects co-infected with HCV. These intriguing results set up a hypothesis whether raltegravir could improve liver fat content in HIV-infected population without HCV, who are overweight or obese and have other metabolic syndrome components and who currently use a PI or efavirenz in their ART. To test this hypothesis, we plan to run a prospective, randomized, open, parallel design study to compare the liver fat content in study subjects either continuing their current ART regimens (which include a PI or efavirenz) or switching to raltegravir based ART. For the quantification of liver fat content, we aim to use the proton magnetic resonance spectroscopy – the golden standard method -, which has not been used previously in PI/ efavirenz/ raltegravir-switch studies.

As there are confounding data on body fat composition changes with raltegravir in studies using single slice CT scan and DEXA methods, the plan is to characterize the adipose tissue changes in more detail with MRI, using 16 slices described in more detail below in methods. To acquire more knowledge on metabolic effects in adipose tissue level, we also intend to take subcutaneous adipose tissue biopsies together with blood, saliva and feces samples. With these study methods we aim to characterize in more detail the metabolic effects of protease inhibitors, efavirenz and raltegravir in HIV-infected overweight or obese patients with the presence of at least one metabolic syndrome component.

2. List of study objectives

Primary objective is:

1. Baseline to 24-week Change in Liver Fat (measured by proton magnetic resonance spectroscopy, Δ %)

Secondary objectives are:

1. Baseline to 24-week Change in Body Composition including subcutaneous and visceral adipose tissue volume (measured by magnetic resonance imaging), total body fat and lean tissue mass (measured by Bioelectrical Impedance Analysis)

2. Baseline to 24-week Change in Liver Stiffness measured by transient elastography (Fibroscan ®)

3. Baseline to 24-week Change in Glucose Metabolism including fasting glucose, insulin, 2-h oral glucose tolerance test

4. Baseline to 24-week Change in Lipid Profile (LDL and HDL cholesterol, Triglyceride)

5. Baseline to 24-week Change in Metabolic and Inflammatory Biomarkers (e.g. high-sensitivity C-reactive protein (hsCRP), adiponectin, etc)

6. To collect subcutaneous adipose tissue samples at baseline and 24 weeks for future gene expression studies

7. To collect stool and saliva samples at baseline and 24 weeks for future microbiome studies

8. To collect safety and tolerability data related to the switch to raltegravir

9. Determination of the fatty liver adipose allele (B-PNPLA3)

10. Determination of basal metabolic rate by indirect calorimetry at screening and at the end of study

3. Study subjects

Actual subject recruitment will start after ethics committee (EC), competent authority (CA) and clinic approvals have been granted. The study subjects will be recruited and screened in a single center (Aurora hospital, Infectious diseases clinic, HUH, Helsinki). All the eligible study subject candidates will be contacted during regular visits and informed of the study by study investigators verbally and with a written information hand out. After adequate time and opportunity to inquire about details of the study and to decide whether or not to participate in the study, the candidates will sign and personally date an informed consent. After the study has finished, all the study subjects will continue their regular follow up with a chance to continue their current ART or to switch to another regimen.

The total number of study subjects included in the study is 45. Study subjects will be randomized 1:1 to continue current ART regimens or to switch their PI or efavirenz to once daily raltegravir

plus continue current NRTIs. The randomization will be stratified by age, sex and antiretroviral regimens (PI or efavirenz).

The inclusion criteria are:

- Written informed consent (IC) obtained.
- HIV-positive adult (age over 18) subjects currently on stable ART, with no changes in the ART regimens during the past 6 months.
- Current ART includes either a protease inhibitor or efavirenz.
- No documented or suspected resistance to integrase inhibitors or to NRTIs.
- No prior history of virologic failure. Failure is defined as a confirmed plasma viral load > 200 cop/ml measured no less than six months after initiation or modification of therapy.
- Virological blips accepted only if a single viral load measurement has been between 50-200 cop/ml followed by VL < 50 cop/ml without the need to initiate a change in ART and no blip within 12 month window period prior to screening.
- Documented evidence of at least two HIV VL < 50 cop/ml measurements during the past 12 months prior to inclusion: one within 6 months prior to screening.
- HIV VL < 50 cop/ml at screening.
- BMI>25 kg/m^2 and one metabolic syndrome condition, which are
    - BP ≥ 130/≥ 85 mmHg or hypertension medication currently in use or
    - fasting glucose ≥ 5.6 mmol/l or B-HbA1C > 42 mmol/mol or diabetes medication currently in use or
    - HDL < 1.0 mmol/l in men and < 1.3 mmol/l in women or triglycerides ≥ 1.7 mmol/l or a cholesterol-lowering regimen currently in use or
    - waist circumference > 94 cm in men and >80 cm in women (or respective cut off values for non-European ethnic groups as defined by International Diabetes Federation).

      OR

    - ultrasound or biopsy proven hepatosteatosis.

Exclusion criteria are:

- Within 12 month window period prior to screening, HIV VL measurement of ≥50 cop/ml.
- More than one consecutive HIV VL measurements of ≥ 50 cop/ml in the treatment history after initial viral suppression with ART.
- Chronic hepatitis B or C.
- Daily alcohol consumption ≥ 30 g for men and ≥ 20 g for women.
- Pregnancy or planned pregnancy during the study period.

- Lipid or glucose lowering regimen or hormonal supplement started within 3 months before the planned study start.

- Psychiatric disorder, which prevents a study subject to understand the study protocol.

- Other serious disease, which prevents a study subject to participate in the study.

- For MRI/spectroscopy imaging: metal objects in the body or claustrophobia.

4. Study procedures and assessments

This study is an investigator initiated, single-center, prospective, randomized, open-label, parallel design study.

Study flow chart consists of

1. Screening visit, signing of the informed consent and randomization
2. Baseline study visit in the Obesity Research Unit with study measurements detailed below in section “Study procedures in Obesity Research Unit”.
3. Baseline visit in Meilahti hospital, where MR imaging and spectroscopy, and transient elastography will be done.
4. Visit at the Infectious Diseases Clinic, where the current ART will be continued or modified according to the randomization. Patient follow up will take place according to the standard of care with two safety laboratory tests at weeks 2 and 8.
5. 24 week study visit in the Obesity Research Unit with the same study measurements as detailed at the baseline (see section below).
6. 24 week study visit in Meilahti hospital, where MR imaging and spectroscopy, and transient elastography will be repeated.
7. End of study visit (24 weeks after study start) at the Infectious Diseases Clinic, where current ART can be continued or modified according to the standard of care.

4.1. Screening visit

A screening visit will be performed prior to first study visit in Obesity Research Unit. The screening visit consists of the following procedures:

- A physical examination will be performed and demographic data will be recorded including subject’s gender, date of birth, ethnicity, substance use, recent and current alcohol use (AUDIT questionnaire), smoking status, weight, height, BMI, and waist and hip circumferences.
- Medical history and current medical conditions and concomitant treatments will be inquired and recorded.
- Blood pressure and heart rate will be measured.

The subject may enter into the study if all the inclusion criteria and none of the exclusion criteria are met and will be randomized to one of the treatment arms after signing the informed consent (IC). Recording of adverse events (AEs) starts from the time that a study subject signs the IC form. The study subjects will be given a study diary, where they can fill in AEs and possible concomitant treatments during the study. They will be asked to fill a dietary and exercise diary for three days at the study start and at the end of study.

4.2. Clinical follow up visits in Infectious Diseases Clinic (Aurora hospital)

Table 1 lists all study procedures in Aurora hospital, Helsinki.

Clinical follow up	Lab measurements	Other
first visit, day 1	HIV viral load, complete blood count, Creatinine, ALT, AST, CD4 count	According to the randomization, switching of a PI or efavirenz to raltegravir, or continuing with the previous ART.
remote contact by phone 1, week 2	compete blood count, CRP, ALT, Glucose, Creatinine	AEs inquired and reported
remote contact by phone 2, week 8	complete blood count, ALT, AST, Creatinine, HIV viral load	AEs inquired and reported
last visit, week 24	HIV viral load, complete blood count, Creatinine, ALT, AST, CD4 count	AEs inquired and reported

4.3. Study procedures in the Obesity Research Unit and in the Radiology Department

The study protocol includes two visits in the Obesity Research Unit. The first visit will take place after randomization and before the switch of the ART. The second visit will take place after 24 weeks of follow up.

Study subjects are advised to fast 10-12 hours before each visit in the Obesity Research Unit (water is allowed during fasting). Alcohol intake is prohibited from 48 h before each study visit. Smoking is prohibited during the visits. Apart from these restrictions the subjects are advised to keep to their usual exercise and lifestyle habits for the duration of the study.

The visits consist of the following study measurements: Oral glucose tolerance test (OGTT), measurements of liver fat by 1H-spectroscopy, body composition by MRI, hepatic stiffness by transient elastography and calorimetry for measurement of basal metabolic rate. Samples of blood, urine, stool and saliva are collected, ECG and subcutaneous adipose tissue biopsies taken, and health questionnaire filled in.

Body composition studies

Body composition (total lean and fat mass) will be analyzed by a non-invasive BioElectrical Impedance Analyzer System.

Abdominal subcutaneous and visceral fat (MRI)

A series of T1-weighted trans-axial images for the determination of visceral and subcutaneous fat will be acquired from a region extending from 8 cm above to 8 cm below the 4th and 5th lumbar intervertebral discs (16 slices, field of view 375 x 500 mm2, slice thickness 10 mm, breath-hold TR/TE 91 ms/5.24 ms). Water signal is suppressed using a frequency selective fat excitation. Visceral and subcutaneous fat volumes will be measured using an image analysis program (SliceOmatic, http://www.tomovision.com/products/sliceomatic.html).

Intrahepatic fat content (1H Magnetic resonance spectroscopy, MRS)

Single voxel (25x25x25 mm3) proton spectra from the liver will be acquired using 16 exitations, a body matrix surface coil and a 1.5-tesla magnetic resonance device (Avanto^{fit};

Siemens, Erlangen, Germany). A Point resolved spectroscopy (PRESS) single voxel technique will be applied, with an echo time of 30 ms. Respiratory motion will be triggered using a navigator belt so that repetition time will be kept > 3000 ms. Intensities of water signal (S_{water}) ppm and methylene signal (S_{fat}) representing intracellular triglyceride in the liver, resonating at 4.8 and 1.4 ppm, respectively, will be determined. Effects of T2 relaxation will be corrected for water and fat intensities and hepatic fat percentage will be calculated by dividing S_{fat} by the sum of S_{fat} and S_{water}. Signal ratios will be further converted to mass fractions as previously described (15). This measurement of percent hepatic fat by proton spectroscopy has been validated against the lipid content of liver biopsies in humans (16). MRI and MRS methods involve no ionizing radiation exposure.

Transient elastography (Fibroscan®)

Transient elastography examinations will be carried out by a single gastroenterologist (PA) with Fibroscan® (Echosens, France). The examinations will be performed through one intercostal space that provides an optimal unobstructed view to the right lobe of the liver. The results will be based on a median of ten validated measurements. Transient elastography results with an interquartile range (IQR) below 30% of the median value and a success rate of at least 60% will be accepted, according to manufacturer's recommendations.

Indirect calorimetry

Indirect calorimetry (Datex Metabolic Monitor, Datex, Helsinki, Finland) will be used to estimate basal metabolic rate from measurements of oxygen (O2) consumption and carbon dioxide (CO2) production. Patient is lying supine in bed, breathing calmly and regularly in the canopy with a constant air flow (to be adjusted to give O2 and CO2 concentrations within workable range). O2 (VO2) consumption, CO2 (VCO2) production, a urinary nitrogen excretion during the study will be measured and on the assumption that all the oxygen is used to oxidize degradable fuels and all the CO2 thereby evolved is recovered, it is possible to estimate the amounts of glucose and lipids oxidized by the body and calculate the basal energy expenditure.

Fat biopsy

A subcutaneous fat biopsy (appr. 2-3 g) will be taken under local anesthesia from abdominal area. Biopsy will be taken using mini-liposuction technique. Part of the sample will be immediately frozen and stored in liquid nitrogen or -80°C until used for transcriptional, protein or other biochemical analyses. Another part will be used for measurement of adipocyte size under a light microscope.

Fasting blood samples

Routine laboratory tests, including HbA1c, total, LDL and HDL cholesterol, and triglycerides, thyroid function (TSH, T4v) and liver enzymes (AST, ALT, GGT, AFOS) will be collected and analyzed in HUSLAB. B-PNPLA3 sample will be collected and send to Yhtyneet Medix laboratory where the sample will be analyzed for the fatty liver adipose allele. In addition, extra samples of plasma and serum will be stored for further analysis, e.g. for metabolomics, lipidomics, proteomics, hormones, adipokines.

Oral Glucose Tolerance Test (OGTT)

OGTT (oral glucose tolerance test) will be performed for all patients after an overnight (10-12 h) fast. A fasting blood sample will be collected after which patients will take a 75 g oral glucose dose in about 5 minutes. Zero time is the beginning of the drink and blood samples are collected at time points 0, 30, 60 and 120 minutes for measurement of plasma glucose, insulin and c-peptide. Extra samples will be collected at each time point for the above mentioned purposes.

Saliva

Saliva will be collected after stimulation by chewing parafilm. Saliva samples will be stored for later purposes and can be used e.g. for microbiota, cortisol, and metabolomics analyses.

Feces

Feces will be collected either at home or at the research center. Collection specimen and instructions will be given during the first visit. Feces samples can be used later to analyze the fecal metabolites, including fatty acid composition and the microbiota.

Urine

Urine samples will be collected on a study morning to perform a pregnancy test for females at childbearing age. Additionally urine samples can be stored for later analyzes, e.g. metabolomics.

5. Safety assessments and ethical considerations

5.1. Study sites and study procedures

The investigator is responsible for ensuring adequate medical expertise and facilities to conduct the study and to handle possible emergency situations during the study. All procedures and possible hazards, risks and discomforts are explained to the subjects both verbally and in writing (see informed consent).

For the blood sampling, a forearm vein will be cannulated. In this study, the maximum volume of blood drawn per subject during the visits in Obesity Research Unit will be approximately 300 ml.

There is no ionizing radiation in MRI/MRS/ transient elastography studies.

The subcutaneous abdominal fat biopsies will be taken under local anesthesia with sterile conditions. The needle used for local anesthesia can cause slight discomfort but the biopsies themselves are painless. A fat biopsy will be taken using a mini-liposuction technique. The incision is closed with skin tape and covered by pressure bandages. The subjects will be adviced to avoid strenuous exercise for two days after the procedure. The biopsy site might have a dull ache for 24-48 h for which the subjects are provided pain-killers. However, medications such as aspirin, or ibuprofen are not recommended since they may contribute to bleeding and/or bruising during the post-biopsy period.

Study subjects may experience moderate discomfort from the blood samples and biopsies.

The subjects will have to reserve two days for the study visits in Obesity Research Unit besides the normal follow up in Aurora hospital. Travel costs incurred as a result of a study visit will be reimbursed on the basis of supporting documents according to actual costs.

Study personnel will conduct all procedures according to GCP (good clinical practice) guidelines. In the case of a needle stick injury, personnel is advised to contact immediately the study investigator or the infectious diseases specialist on duty in the Infectious Diseases Clinic for the evaluation of the need for HIV post exposure prophylaxis.

5.2 Raltegravir

Raltegravir (trade name Isentress®) is an HIV medicine that is used in combination with other HIV medicines to treat adults and children who are infected with human immunodeficiency virus (HIV-1). It is developed and manufactured by Merck Sharp & Dohme Corp (MSD). The European Commission granted a conditional marketing authorisation valid throughout the European Union for Isentress on 20th December 2007. This was switched to a full marketing authorisation on 14th July 2009. A new formulation of Isentress 600mg coated tablets was granted a marketing authorisation by European Comission on 18th July 2017. The recommended daily dose for the new formulation is 1200 mg (two 600 mg tablets) once daily. In this study, we plan to use the once daily formulation of raltegravir. This study drug is provided by Merck Sharp & Dohme Corp.

Raltegravir is one of the best tolerated HIV-medicines. The most common reported adverse effects with Isentress (seen in between 1 and 10 patients in 100) are abnormal dreams, nightmares, insomnia, depression, abnormal behavior, dizziness, headache, psychomotor hyperactivity, vertigo, decreased appetite, abdominal distension, abdominal pain, diarrhea, flatulence, nausea, vomiting, dyspepsia, rash, asthenia, fatigue, pyrexia, atypical lymphocytes, and increased blood levels of some enzymes (alanine aminotransferase, aspartate aminotransferase, lipase and pancreatic amylase) and triglycerides. For more details of the study drug, see SmPC of Isentress attached.

5.3. Adverse events assessment

An AE is any untoward medical occurrence in a study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not related to the IMP. The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Thus, an AE may be an appearance or worsening of any undesirable sign or symptom, any worsening of the current medical conditions or onset of a new disease, compared with the previous observations or a clinically significant adverse change in a laboratory variable or other diagnostic finding.

A serious adverse event (SAE) is any untoward medical occurence that

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/ birth defect, or
- is an important medical event jeopardizing the patient or requiring intervention to prevent serious outcome (examples of such are intensive treatment in an emergency room, convulsions that do not result in hospitalization, etc.)

Any case of pregnancy during a clinical study will be reported by the investigator in the same way as an SAE.

Other significant AEs are AEs (other than those meeting the definition of serious) that are of clinical importance and lead to:

- a diagnostic or therapeutic intervention
- discontinuation of the IMP

- significant additional concomitant treatment, or
- marked hematological and other laboratory abnormalities

Suspected Unexpected Serious Adverse Reaction or "**SUSAR**" shall mean any Serious Adverse Event, the nature, severity or frequency of which is not consistent with information in the most current Summary of Product Characteristics (SmPC) or Package Insert.

All AEs must be elicited, documented and reported by the investigator. The investigator will assess and record the causality and severity of the AEs due to IMP (see criteria for causality and severity below). SAEs and other significant AEs should be followed up until resolved or until the event is considered a chronic or stable outcome, or both.

The investigator is responsible for expediting all suspected unexpected serious adverse reactions (SUSARS) as well as other safety issues requiring expedited reporting to the relevant authorities within applicable timelines.

Principal Investigator shall forward to MSD's Global Safety ("MSD GS") group, any SAE and SUSAR or Incident information, including, but not limited to, all initial and follow-up information involving any study subject in the study. Notification shall be in the form of a completed CIOMS I/MedWatch (or other mutually agreed upon format) within two (2) business days of learning of the information.

Causality criteria:

Related to the IMP: The temporal relationship of the AE/SAE onset to the administration of the IMP makes a causal relationship possible, and other drugs, therapeutic intervention or underlying conditions do not provide a sufficient explanation for the AE/SAE.

Not related to the IMP: The temporal relationship of the AE/SAE onset to the administration of the IMP makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the AE/SAE.

Severity criteria:

Mild: Discomfort noticed, but it does not affect normal activity.

Moderate: Discomfort sufficient to reduce or affect normal daily activity.

Severe: Incapacitating with inability to perform normal daily activity.

Clinical safety assessments are detailed in section "4.2. Clinical follow up visits in Aurora hospital." Additionally pregnancy test for females of child-bearing potential is done during the first visit in Obesity Research Unit.

Possible unexpected adverse events due to study regimens are covered by the **Pharmaceutical Injury Insurance (Lääkevahinkovakuutus).**

All subjects are insured by the **patient insurance of Helsinki University Hospital**.

6. Data analysis

The data will be analyzed by the investigators and the study group.

All variables of the data will be analyzed after the last subject's last visit, when the data have been declared complete.

The data will be analyzed using IBM SPSS Statistics v. 22. The differences between the groups will be analyzed by using chi-square, Fisher's exact, Mann-Whitney U test and others, as appropriate.

As there are no earlier ART-switch studies using 1H-Magnetic resonance spectroscopy for the quantification of liver fat, the calculation of the study power is an estimate. In the STERAL study evaluating the effect of switching from efavirenz (EFV) to RAL vs. continuing EFV-based ART, a statistically significant difference in the liver fat content using CAP (controlled attenuated parameter) was shown with a study sample of 19 +18 subjects after 48 weeks (7,3% decrease in the RAL vs. 11,0% increase in the EFV arm, p = 0,019) (11).

The power of this study is calculated with the assumption of liver fat being 4.4-5.0% in this study group at baseline. This estimation is based on our previous results published in 2002, when the median liver fat was 8% with lipodystrophic patients and 3% with non-lipodystrophic patients (17). Hadigan et al reported liver fat content being >5% in 42% of HIV positive subjects (18). As the liver fat content correlates with visceral fat mass, studying overweight or obese patients with at least one metabolic syndrome component includes patients at high risk for elevated liver fat content.

In the power calculation, we have used a coefficient of variation (CV) of 1H-Magnetic resonance spectroscopy being 11% (17). To detect a 9% difference between study arms with 80% power, a minimum sample size of 25 + 25 subjects with a 10% drop-out rate is needed.

Publication plan

We anticipate to submit one main manuscript with the results of the primary objective (liver fat change) and secondary objectives (1-3: body composition change as well as metabolic markers). Additional manuscripts will be submitted after further analysis of subcutaneous fat samples (for transcriptional, protein and other biochemical analyses), fecal samples (microbiota) and blood samples (inflammatory markers and metabolomics studies).

7. Study budget

Study budget is detailed in a separate sheet "Study Budget" attached.

8. References

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9. List of attachments:

1. Synopsis
2. CVs of Principal Investigator and Head of the Obesity Research Unit
3. SmPC of Isentress
4. Study budget
5. Patient registry report
6. Summary of the current state of the report to the national competent authority (Fimea)