SUPPLEMENTARY MATERIAL
AVERTAS TRIAL

Changes in weight, body composition and metabolic parameters after switch to dolutegravir and lamivudine compared to continued treatment with dolutegravir, abacavir and lamivudine for virologically suppressed HIV infection:

A randomized open-label superiority trial - The AVERTAS trial

Registration

Protocol version: Awaiting amendment approval, protocol version 9.0 April 04, 2023

Ethics Committee of the Capital Region, Denmark (H-20011433)

Danish Medicines Agency (EudraCT no. 2019-004999-19)

Regional Data Protection Centre (P-2020-207)

clinicaltrials.gov (NCT04904406)

TABLE OF CONTENT

APPENDIX 1

Radiological studies

Computed Tomography (CT) scan

All CT imaging will be performed using a 320-multidetector scanner (Toshiba Medical Systems, Otawara-shi, Tochigi-ken, Japan). CT scans will encompass a low-dose chest CT, an unenhanced coronary artery calcium score (CACS), and an unenhanced scan of the upper abdomen to determine liver steatosis and an abdominal single slice acquisition for measurements of visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT). No contrast will be used in the scan protocol. The CT scans will be optional. All CT-analyses will be conducted by trained physicians blinded to randomization, clinical and biochemical details of the study participants.

Chest CT

The chest examination will use a low-dose protocol with images captured at full inspiration using automatic exposure control at 120 kV with an SD of 15. AIDR will be used for image reconstruction with both 1/1 mm and 3/3 mm slices. A lung kernel (FC52) will be used for thin-slice dataset reconstruction to evaluate lung parenchyma and airways, while a soft tissue kernel will be used for thicker slices to evaluate mediastinum and pleurae. A soft tissue kernel (FC08) and filtered back projection will be used to reconstruct an additional dataset with 1/1 mm slices for quantitative emphysema measurements using a dedicated lung density program (Vitrea Vital Images, Minnetonka, MN, U.S.).

Coronary Artery Calcium Score (CACS)

120kV (BMI< 28) or 135 kV (BMI>28), automatic exposure control with an SD 55 (min 30 mA and max 300 mA). ECG-triggering with exposure at 75% of the RR-interval will be used, and reconstructions will be performed with a soft tissue kernel (FC12) and 3/3 mm slice thickness/increment.

Unenhanced CT scan of the upper abdomen

A single 16 cm volume scan extending from the left hemidiaphragm and downwards using 40 mA (fixed) and 120 kV. reconstructions will be performed with 1/1 mm and a soft tissue kernel (FC12) using an iterative reconstruction technique (Adaptive Iterative Dose Reduction, AIDR).

Liver attenuation will be measured using Vitrea 3.1 imaging software (Vital Images Inc., Minnetonka, MN, USA). Two regions of interest (ROI) with an area of 1500 mm2 (+/- 100 mm2) will be placed in Coinaud liver segments 5 and 6, and the average liver attenuation calculated in Hounsfield Units (HU). Moderate-to-severe hepatic steatosis will be defined as CT liver attenuation <48 HU with a specificity of 100%, sensitivity of 53.8%, positive predictive value of 100%, and negative predictive value of 93.9%.

Visceral and subcutaneous adipose tissue scan

A single 8 mm slice at the level of lumbar vertebra 4 (L4) will be performed using 120kV and 210 mA. Reconstructions will be performed with filtered back projection (FBP) and soft tissue kernel (FC08). Trained

personnel will use commercially available CT software (Fat Measurement, Aquilion ONE; Canon, Japan) to measure the cross-sectional area of adipose tissue defined as voxels with attenuation values in the range of −150 to − 70 Hounsfield units. From within a manually adjusted region of interest delineated by the muscular compartments, VAT area will be calculated automatically. SAT will be defined as adipose tissue superficial to the abdominal and paraspinal muscles. Intraintestinal and intramuscular adipose tissues will be manually excluded. Mean density for VAT and SAT, respectively, will be calculated and reported, using four regions of interest within each fat depot.

Transient liver elastography (Fibro scan)

Transient elastography will be performed by trained personnel using Fibro scan (EchosensTM, Paris, France) to assess liver stiffness and quantify liver fat. With the fasting participant in supine position, the transducer will be placed on the skin in an intercostal space in the right midaxillary line at the level of the right liver lobe. The liver stiffness and controlled attenuation parameter (CAP) is measured using a M- or XL probe. Liver stiffness will be expressed in kilopascal (kPa) and CAP in dB/m. The physiologic stiffness of the liver parenchyma is 5.5 ±1.6 kPa by transient elastography (1). Liver stiffness is positively correlated with liver fibrosis, yielding higher LSM with higher amounts of liver fibrosis. In this study we define significant liver fibrosis as LSM ≥7.6 kPa (2). CAP quantifies liver fat by applying a proprietary algorithm to evaluate the decrease in amplitude of ultrasound waves propagating through the (3). The cut-off for fatty liver will be set at 285 dB/m. Patients must be fasting for a minimum of two hours prior to the procedure.

Carotid Intima-Media Thickness (cIMT)

External carotid artery ultrasound will be performed to determine intimal thickness as a measure of arteriosclerosis. cIMT will be measured bilaterally at the far wall of the distal common carotid artery caudally of the sinus caroticus covering 10 mm using a Sonosite M-Turbo (Sonosite Inc., Bothell, WA, USA) with a 13.6 MHz linear transducer and automated software (SonoCalc IMT 5.0; Sonosite Inc.). The measurement will be performed in one projection in a longitudinal view with both the near and far wall visible. An increased cIMT will be defined as an average thickness of > 900 µm. Average measurements > 900 µm or visible plaques will result in referral to the Department of Clinical Physiology for further evaluation (4).

Dual Energy X-ray Absorptiometry (DEXA)

To estimate the amount of fat in the trunk and the extremities whole-body DEXA scanning [Hologic QDR-2000 W (Bedford, MA, USA) in single beam mode; in vivo coefficient of variation (CV) 1.6 for total and 3.2 for regional fat mass (10 duplicate measurements)] will be performed. The trunk will be defined as the region including the chest, abdomen, and pelvis. The upper limit of the leg region will be placed through the hip joints at an angle of approximately 45°, and the upper limit of the arm region will be placed vertically through the shoulder joints. Peripheral or limb fat mass will be defined as the sum of arm and leg fat masses. The percentage of limb

fat will be calculated as (limb fat mass/ total fat mass) x 100% (5). Patient's weight will be estimated by DEXA. Patients must be fasting for 6 hours prior to scanning. DEXA will be performed at baseline, week 24 and 48.

Cardiac Magnetic Resonance Imaging (Cardiac-MRI) SUB STUDY

The cardiac MRI protocol includes functional and structural MRI measurements and will be optional. MRI scans will be performed at a MAGNETOM Prisma 3 Tesla scan, SIEMENS Healthineers (Erlangen Germany) at Hvidovre hospital. A SSFP MRI imaging sequence will be applied to determine the volume and function of the atria and ventricles using a 2D cine imaging protocol. Biventricular and bi-atrial function are evaluated from trans axial (7mm, no gap, temporal resolution 25-45 msec) and from short axis slices (double oblique) which also entails the atria. In addition, 2,3, and 4 chamber sequences are obtained of the left ventricle. The blood flow will be determined in the pulmonary artery and aorta and the flow velocity and compliance of aorta will be evaluated using phase contrast flow sequences. Structural myocardial MRI will involve T1 measurements using a Modified Look-Locker Imaging (MOLLI) sequence performed during one breath-hold per slice (6). Patients with an eGFR larger than 45 ml/min will subsequently undergo additional T1 mapping after intravenous injection of gadolinium contrast (gadobutrol (gadovist) in low concentration (0.1 mmol/kg) to detect local and diffuse myocardial fibrosis. The MR images are subsequently transferred to a dedicated workstation where the imaging analysis will be performed.

To calculate extracellular volume (ECV) erythrocyte volume will be needed. This plasma analysis is already performed in the main study.

Holter Monitor ECG Recording (R-test)

Heart rhythm will be monitored by Holter monitor ECG recording (R-test) for 24 hours. A Cortrium C3+ Holter Monitor will be applied at to the chest with three pads. The 24-hour ECG data will be read and analyzed in a dedicated software system. Any event of arrhythmia will be registered during the R-test and evaluated.

APPENDIX 2

Informed Consent

Informed consent form for participation in a health research project.

Does Switching from 3-drug to 2-drug Therapy in People Living with HIV affect weight, metabolism and the heart? – The AVERTAS-1 Trial.

AVERTAS-1, H-20011433; EUDRA-CT NO.: 2019-004999-19, Protocol v. 9.0

Translation of the Danish Informed consent Form.

Participant's statement:

I have received written and oral information about the study, and I am sufficiently informed about the aim, methods, risks and benefits to agree to participate.

I know that participation is voluntary, and that I can withdraw my consent at any time without losing my current or future rights to treatment.

I give consent to participate in the research project and to the withdrawal and storage of my biological material in a research biobank. I have gotten a copy of this consent form and a copy of the participant information for own use.

Name of the participant: _____

Date: _____ Signature: _____

If any new essential health information about you should appear during the study, you will be informed.
If you do not want this essential health information, you should mark here: _____ (tick x)

Do you wish to receive information on the finale results of the study as well as any possible personal consequence? Yes _____ (tick) No _____ (tick x)

Statement from the informant:

I declare that the participant has received oral and written information about the research project.
In my belief sufficient information is provided for the subject to make the decision about participating in the study.

Name of the informant: _____

Date: _____ Signature: _____

APPENDIX 3

Biobank

Project Specific Biobank

At day 0, 24 and 48 3 ml will be collected for the purpose of detecting plasma interleukin 1β and 6, soluble P-selectin, soluble glycoprotein VI, vascular cell adhesion molecule 1, intercellular adhesion molecule 1. A project-specific research biobank will be established for freezer storage in a -80 °C freezer located at the Copenhagen University Hospital – Amager and Hvidovre. Plasma will be stored pseudo anonymized marked with study-IDs. The identification key will be kept separately. The project specific biobank will be terminated immediately after collection of the last patients' blood samples. Samples will be analyzed collectively. Any excess blood will be stored in a biobank for future research.

Biobank for Future Research

A biobank for future research will be established. Besides excess blood material from the project specific biobank, additional blood samples are drawn (3 ml) at visit 1, 24 and 48 and stored in a -80 °C freezer. Patients must give separate written consent to allow storage of their biological material for future research outside this study. Permission on biobank for future research will be applied from The Danish Data Protection Agency. The biological material will be handled and stored according to the agency's guidelines.

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