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Changes in weight, body composition and metabolic parameters after switch to dolutegravir/lamivudine compared to continued treatment with dolutegravir/abacavir/lamivudine for virologically suppressed HIV infection: A randomized open-label superiority trial - The AVERTAS trial

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TITLE

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

A randomized open-label superiority trial - The AVERTAS trial

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Registration

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

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

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Roles and Responsibility

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Role of Sponsor and Funding

The study is a Sponsor-Investigator trial. The sponsor, Thomas Benfield has no conflicts of interest or commercial interest in the study. This work was supported by the Simonsen's Foundation and from Amager and Hvidovre Hospital's Research Foundation. Sponsor and investigators are independent of economic or competing interests. The grant is held in a foundation account managed by sponsor. Participants will not be financially compensated. Study results will be utilized only for scientific and public purpose and do not hold any commercial significance.

Word Count

2887

Key Words

Randomized controlled trail, RCT, HIV, antiretroviral therapy, weight, obesity, cardiovascular disease, Cardiac Magnetic Resonance, CMRI

Abstract

Introduction

With longer life expectancy in people living with HIV (PLWH) on antiretroviral therapy, cardiovascular disease (CVD) has become a common cause of mortality. Abacavir has been associated with an increased risk of myocardial infarction, but the mechanism is unknown. Additionally, abacavir may obesogenic which could mediate an additional risk factor of CVD. We aim to investigate if discontinuation of abacavir will have a favorable impact on body weight and cardiac parameters in PLWH.

Methods and Analysis

Randomized, controlled, superiority trial of virologically suppressed PLWH on dolutegravir, abacavir and lamivudine (DTG/ABC/3TC) for ≥ 6 months. In total, 84 PLWH will be randomized 1:2 to either continue DTG/ABC/3TC or to switch to dolutegravir and lamivudine (DTG/3TC) providing the power of 90% at alpha 5% to detect a mean difference in weight change of 2 kg (Δ) given a variance of 2.7 kg. Follow-up will be 48 weeks. Data will be collected at baseline and week 48. Primary outcome will be change in bodyweight from baseline to week 48 measured as difference between means in the two study arms. Secondary outcomes will be changes in cardiac-, inflammatory- and metabolic parameters, fat distribution, coagulation, endothelial, platelet function, quality of life and virological control from baseline to week 48. Measurements include computed tomography (CT) of thorax and abdomen, external carotid artery ultrasound, liver elastography and dual energy x-ray absorptiometry (DEXA) and blood analysis. Plasma HIV RNA will be measured at baseline, week 4, 24 and 48. Forty participants (20 from each arm) will be included in a sub-study involving cardiac magnetic resonance imaging at baseline and week 48. Twenty non-HIV-infected controls will be included with a single scan to compare with baseline scan data.

Ethics and Dissemination

Result from this study will lead to a better understanding of the association between antiretroviral therapy and the impact on weight and risk of CVD. Findings will be useful for both clinicians and PLWH in the guidance of a more individualized HIV treatment. Results from the main study and the sub study will be submitted for publication into a peer-review journal(s). The AVERTAS study is approved by the Ethics Committee of the Capital Region, Denmark (H-20011433), Danish Medicines Agency (EudraCT no. 2019-004999-19) and Regional Data Protection Centre (P-2020-207). The study is registered at clinicaltrials.gov (NCT04904406). Trial registration:

ClinicalTrials.gov Identifier: NCT04904406, registered the 27th of May 2021.

ARTICLE SUMMARY

Strengths and Limitations

- Study design is randomized controlled multicenter trial which limits confounding
- The study is limited by the unblinded setting which enables bias
- The study is carried out in outpatient clinics, where participants receive their usual care. This might enhance adherence to study visits

INTRODUCTION

Background and Rationale

The introduction of antiretroviral treatment (ART) with ≥3 drugs in 1996 changed the prognosis of HIV infection dramatically. Today, people living with HIV (PLWH) treated with ART has a life expectancy close to that of the HIV-uninfected population (1). However, this longer life expectancy has led to higher rates of serious non-AIDS events (SNAE) such as cardiovascular disease (CVD) (2–4). Today, CVD is the main cause of mortality in PLWH (5). The mechanism is thought to be a multifactorial interplay between traditional CVD risk factors, HIV specific factors and ART. Studies have shown a correlation between the use of the nucleoside reverse transcriptase inhibitor (NRTI) abacavir and myocardial infarction (6–10). The underlying mechanism remains largely unknown but may include endothelial dysfunction, increased inflammation, platelet activation and platelet/collagen interactions, all of which can modify CVD risk (11).

Initiating ART per se is associated with weight gain (12–14). Some of this weight gain is thought to be related to a “return-to-health” phenomenon where initiation of ART causes a return to baseline weight after HIV induced wasting. This mechanism is not fully understood and seems to be only partly responsible for the observed weight gain. A meta-analysis of 8 RCTs reported that weight increased more in ART-naïve PLWH initiating treatment with an integrase strand transfer inhibitor

(INSTI)-based regimen as compared to a non-NRTI or a protease inhibitor based regimen (15). Among NRTIs, abacavir and tenofovir alafenamide (TAF), were associated with more weight gain than tenofovir disoproxil fumarate (TDF) or zidovudine. Weight gain after ART switch is modest and it remains uncertain whether this is due to the loss of a weight suppressive effect of prior regimens with older agent such as TDF or efavirenz or a weight gain effect of the newer regimens especially TAF and/or INSTI, or both (16). Overweight and obesity are associated with an increased risk of metabolic syndrome, diabetes, hypertension and dyslipidaemia that converge as risk factors for CVD including myocardial infarction (17). Thus, it is conceivable that there could be synergistic effects and a higher overall risk of CVD using abacavir and dolutegravir in combination. We want to investigate the effect on weight change and cardiac function after discontinuation of abacavir in PLWH treated with abacavir, lamivudine and dolutegravir.

Objectives

The aim of this study is to investigate if discontinuing abacavir by switching from a 3-drug (3DR) regimen with dolutegravir, abacavir and lamivudine (DTG/ABC/3TC) to a 2-drug (2DR) regimen with dolutegravir and lamivudine (DTG/3TC) will decrease weight and improve metabolic and cardiac parameters in PLWH.

Trial Design

This study is a randomized, controlled, parallel, open-label, phase 4, interventional trial.

METHODS: PARTICIPANT, INTERVENTIONS, AND OUTCOMES

Study Design

Participants will be recruited from outpatient clinics at the departments of infectious diseases at two Danish hospitals: Copenhagen University Hospital - Amager and Hvidovre; and Copenhagen

University Hospital, Rigshospitalet. Both located in the Capital Region of Denmark. Study visits and data collection will be performed at Copenhagen University Hospital - Amager and Hvidovre.

Eligibility Criteria

Inclusion and exclusion criteria are listed in Table 1.

Table 1 Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
• Age ≥ 18 years • HIV infection • ongoing treatment with DTG/ABC/3TC ≥ 6 months • Plasma viral load (HIV-RNA) < 50 copies/ml at inclusion For women with childbearing potential • Willingness to use contraceptive during study period	• Pre-existing viral resistance to lamivudine or dolutegravir* • Presence of hepatitis B antigen (HBsAg) or HBV DNA • Cancer within the past five years • Pregnancy or breastfeeding (for women) • Unstable cardiovascular disease, diabetes (assessed by the treating physician)

Participants will be eligible for MRI sub study participation, if they comply to standard MRI safety guidelines.

*Existing genotypic resistance test results will be screened prior to inclusion.

INTERVENTIONS

At week 1 patients will be randomized to either continued therapy with co-formulated dolutegravir 50 mg, abacavir 600 mg, and lamivudine 300 mg (Triumeq, control arm) or switch to co-formulated dolutegravir 50 mg and lamivudine 300 mg (Dovato, intervention arm) for 48 weeks. See Figure 1 for participant timeline.

Administration

The Investigational Medicinal Products (IMP) will be self-administered in original pharmaceutical packaging by participants once daily. Double controlled administration of the IMP and IMP-log registration will be performed by two designated and GCP-trained study personnel. The study IMP will be distributed at week 1 and week 24 in original wrapping, with a study specific label. All IMPs are authorized, registered, and marketed by the Danish Medicines Agency and the European Medicines Agency (EMA). Dosage and administration frequency are assigned according to treatment guidelines.

Withdrawal from study

Participants will be withdrawn in case of viral rebound or in any other case of compromised participants safety assessed by the investigator. If patients drop out or are withdrawn, an immediate follow up meeting will be arranged to ensure return to their usual treatment and the treating clinician will be informed. Data will be collected at time of withdrawal with the patient's acceptance and used in the intention to treat analyses.

Adherence

Adherence will be monitored by measurements of plasma HIV-RNA viral load at baseline, weeks 4 (2DR group only), 24 and 48. Virological failure defined as two consecutive viral loads > 50 copies/ml with an interval of 14 to 30 days will lead to immediate withdrawal from the study.

OUTCOMES

Primary outcome

- Change in bodyweight from baseline to week 48 measured as difference between means in the two study arms

Secondary outcomes

- Development of metabolic syndrome at week 48 (18)
- Development of type 2 diabetes at week 48 (19)
- Impaired insulin resistance and/or β-cell function determined by changes in HOMA-IR at week 48 (20)
- Virological control at week 48 as defined by a plasma HIV-RNA <50 copies/ml

Changes from baseline to week 48 in:

- Self-rated health evaluated by 12-item Short Form Survey (SF-12)
- Framingham Risk Score (21,22)
- DAD CVD risk score (23)
- Blood HbA1c
- Total plasma cholesterol
- Plasma High Density lipoprotein cholesterol (HDL-cholesterol)
- Plasma Low Density Lipoprotein cholesterol (LDL-cholesterol)
- Plasma Very Low-Density Lipoprotein cholesterol (VLDL-cholesterol)
- Plasma Triglycerides
- Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) ratio (abdominal CT)

- Fat distribution in trunk, limb, and extremities measured by Dual-Energy X-ray Absorption (DEXA)
- Fatty infiltration of the liver evaluated as:
    - Development of steatosis or increase in existing steatosis from baseline (CT liver and Controlled Attenuated Parameter (CAP) (Fibroscan)
- Blood pressure
- Cardiac magnetic resonance imaging (MRI)
- Carotid artery intima-media thickness (cIMT) measured by ultrasound
- Coronary artery calcium score (CACS)
- Plasma N-terminal pro-B-type natriuretic peptide (Pro-BNP)
- Plasma Troponin T (TnT)
- Inflammation:
    - Plasma High-sensitive C-reactive protein
    - Plasma Interleukin 1β
    - Plasma Interleukin 6
- Endothelial function:
    - Plasma Vascular cell adhesion molecule 1
    - Plasma Intercellular adhesion molecule 1
- Platelet function:
    - Plasma Soluble P-selectin
    - Plasma Soluble glycoprotein VI.
- Coagulation:
- Plasma D-dimer

- Plasma coagulations factor 2, 7 and 10 (extrinsic pathway)
- Plasma Fibrinogen
- Blood hemoglobin, leucocyte count, and platelet count
- Plasma creatinine, urea, sodium, potassium, bilirubin, and alanine aminotransferase.

OUTCOMES, MR-SUB STUDY

Primary outcome

In the CMR sub-study the outcome is a composite endpoint of

- Decrease in extracellular myocardial volume (ECV) from baseline to week 48
- Decrease in left atrial volume from baseline to week 48
- Improvement in diastolic function from baseline to week 48
- Reduction in myocardial mass from baseline to week 48

Secondary outcomes

Changes in

- Left ventricular ejection fraction
- Myocardial perfusion
- Myocardial edema/inflammation
- Myocardial fibrosis
- Myocardial lipid-water profile

PARTICIPANT TIMELINE

Participant timeline is illustrated in Figure 1

SAMPLE SIZE

Anticipated annual weight change in the two groups are: DTG/3TC + 0 to -1 kg and DTG/ABC/3TC +2 kg. Sample size is estimated by Student's unpaired t-test. Patients will be randomized 2:1 to intervention or control.

Assuming a 2:1 randomization ratio for the intervention and control arm, a significance level (α) of 5%, a power (β) of 90%, mean difference in weight between the two arms of 2 kg (Δ) and a variance of 2.7 kg the estimated sample size required will be 60 randomized individuals in the intervention group and 30 randomized individuals in the control group. To account for a 5% withdrawal or dropout rate, sample size will be 95 patients in total

With a power of 80% the estimated sample size required will be 44 randomized individuals in the intervention group and 22 randomized individuals in the control group, plus 4 additional allowing a 5% dropout rate. Sample size will be 70 in total.

In this study we intend to include between 70-95 subject to ensure sufficient power.

RECRUITMENT

Eligible patients will be identified by treating physicians at outpatient clinics at the involved sites. Information on eligible patients will be disclosed to the responsible investigator for the purpose of recruitment. Eligible patients will receive verbal and written study information, and subsequently be offered participation. All participants must provide written informed consent.

METHODS: ASSIGNMENT OF INTERVENTIONS

Allocation

Eligible participants will be randomized in two parallel arms with an allocation ratio of 2:1 to switch to 2DR therapy with DTG/3TC or to usual 3DR treatment with DTG/ABC/3TC (control). Key variables will be entered into a secure web-based program (REDCap) to randomize patients into two parallel arms upon inclusion. The randomization list will be centrally generated in REDCap using random blocks stratified by study center and sex. Additionally, 40 participants (20 control and 20 intervention) will be invited to participate in the cardiac MRI sub-study. The study will be open label, both participants and the study personnel will be un-blinded to randomization.

METHODS: DATA COLLECTION, MANAGEMENT, AND ANALYSIS

Data Collection Methods

Study visits and data collection will be performed at Copenhagen University Hospital - Amager and Hvidovre and participants will be examined by trained clinicians or study nurses. At the inclusion visit (week 1) baseline data will be collected, and participants will be randomized. Subsequently a safety blood test will be performed at week 4 (for the 2DR arm), and follow-up data will be collected at study visits at week 24 (range 20-28) and finally at week 48 (range 46-52). An overview data collection is listed in Table 2 and 3. Blood testing is listed in Table 4. Detailed description on radiological tests are included in Appendix 2.

Participants must be fasting at least 6 hours prior to visits at week 1, 24 and 48, to ensure fasting measurements of weight, blood tests, transient liver elastography and Dual-Energy X-Ray Absorptiometry.

Table 2 Data Collection

	Week 1	Week 4	Week 24	Week 48
Informed consent	x			
Randomization	x			
Demographics[1]	x			
Framingham risk score	x		x	x
SF-12[2]	x		x	x
Body weight	x		x	x
Waist circumference	x		x	x
Vital signs	x		x	x
Blood tests[4]	x		x	x
HIV safety blood tests[5]	x	x	x	x
Transient elastography / Controlled Attenuated Parameter (CAP)	x		x	x
CT[6]	(x)		(x)	(x)
DEXA[7]	x		x	x
cIMT[8]	x		x	x
MR SUB STUDY				
Cardiac MRI[9]	x			x
24-hours ECG Holter monitoring	x			x

[1]Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living.

[2] 12-item short-form health survey (SF-12)

[3]Blood **pressure**, heart rate, respiration rate, peripheral oxygen saturation, temperature.

[4]**HIV safety blood tests:** Plasma HIV-RNA and CD4 count.

[5]**Blood tests:** leucocytes, platelets, hemoglobin, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, lactate dehydrogenase, erythrocyte fraction. **Metabolism:** Fasting p-glucose, insulin, glycated hemoglobin (HbA1c), total cholesterol, HDL, LDL, VLDL, triglyceride. **Inflammation:** High-sensitive c-reactive protein, interleukin 1 and 6. **Coagulation:** D-dimer, factor 2, 7 and 10, fibrinogen**. Platelet function:** soluble P-selectin, soluble glycoprotein VI**. Endothelial function:** Vascular cell adhesion molecule 1, intercellular adhesion molecule 1, Cardiac**:** N-terminal pro-B-type natriuretic peptide (Pro-BNP), troponin T (TnT).

[6]Low dose computed tomography of thorax and abdomen to determine coronary artery calcium score (CACs), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and liver steatosis.

[7]Dual energy x-ray absorptiometry, optional

[8]Carotid intima-media thickness (cIMT) determined by ultrasound.

[9]Cardiac magnetic resonance imaging sub study (optional).

Table 3 Clinical Measurements and Methods

Measurements	Description
Body weight	Measured after minimum 6 hours of fasting and without cloth Scale: Seca 701 7021099
Blood tests (fasting)	Peripheral venous blood
Transient liver elastography / Controlled Attenuated Parameter (CAP)	Fibro scan, M-XL probe, (Echosens, Paris, France)
Carotid intima-media thickness (cIMT)	Sonosite M-Turbo (Sonosite Inc., Bothell, WA, USA), analysed with a 13.6 MHz linear transducer and automated software (SonoCalc IMT 5.0; Sonosite Inc.)
CT scans	CT chest, CT upper abdomen and Coronary Calcium Scan Aquillion One scanner, Toshiba Medical Systems, (Otawara-shi, Tochigi-ken, Japan)
DEXA scan	Dual energy X-ray absorptiometry (DEXA) Whole-body DEXA scanning Hologic QDR-2000 W (Bedford, MA, USA)
Cardiac MRI	MAGNETOM Prisma 3 Tesla scan, SIEMENS Healthineers (Erlangen Germany)
Metabolic syndrome[1]	Central obesity: waist circumference ≥94 cm for males and ≥80 cm for females (Europids), or BMI>30 kg/m², plus any two of the following four factors: • Raised triglycerides: >1,7 mmol/L • Reduced HDL cholesterol: ≤1,03 mmol/L in males, <1,29 mmol/L in females

	• Raised blood pressure (BP): Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension • Raised fasting plasma glucose: ≥5,6 mmol/L or previously diagnosed type2-diabetes
Insulin resistance and β-cell function	Homeostatic Model Assessment of Insulin Resistance (HOMA-IR): $HOMA - IR = \frac{FPG\ (mmol/L) \cdot FPI\ (mU/L)}{22.5}$ $HOMA - \%B = \frac{20 \cdot FPI\ (mU/L)}{FPG\ (mmol/L) - 3{,}5}$
Type 2 diabetes[2]	• Fasting plasma glucose concentration ≥ 7.0 mmol/L or • a random venous plasma glucose concentration ≥ 11.1 mmol/L or • HbA1c > 48 mmol/mol
Cardiovascular disease risk scores Framingham Risk Score D:A:D risk score	Online calculation tool, CHIP – Centre of Excellence for Health & Framingham Heart Study
Survey	• 12-Item Short term Survey (SF-12) • Questionnaire on dietary and activity patterns

[1] Metabolic syndrome defined in accordance with The International Diabetes Federations definition

[2] WHO definition

Table 4 Blood and Plasma Analysis

Category	Blood and Plasma analysis
Hematology	Leucocyte count and differential count, hemoglobin, hematocrit
Electrolytes	Sodium, Potassium
Renal	Creatinine, urea, albumin
Liver	Alanine aminotransferase, aspartate transaminase, bilirubin, lactate dehydrogenase
HIV-safety	HIV-RNA*, CD4 cell count*
Metabolism	Glucose, insulin, blood glycated hemoglobin, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, LDL-cholesterol (calculated), triglycerides
Inflammation	High sensitivity C-reactive protein, interleukin 1β and 6*
Coagulation	D-dimer, factor II+VII+X, fibrinogen, platelet count

Platelet function	Soluble P-selectin*, Soluble glycoprotein VI*
Endothelial function	Vascular cell adhesion molecule 1*, intercellular adhesion, molecule 1*
Cardiac	N-terminal pro-B-type natriuretic peptide, Troponin T

Blood tests are performed at visits in week1, week24 and week48. Participants will be fasting minimum 6 hours prior to blood tests. Non-HIV-infected controls will have blood tests prior to- or at the day of CMRI and will not be fasting.

Blood analysis with * will be performed only in PLWH.

CARDIAC MRI SUB STUDY

Forty participants (randomized 1:1) from the main study will be included in the CMRI sub study. Cardiac MRI and ECG-monitoring will be performed on the sub-study population at baseline and week 48. Further, 20 non-HIV-infected controls will be recruited for a single CMRI to compare baseline data. CMRI technical details and scan protocol are elaborated in Appendix 2.

The following clinical information will be obtained from healthy controls or from their patient records: Medicine use, known medical conditions, cardiac risk (smoking status, alcohol consumption, family CVD history), height and weight. Blood pressure and heart rate will be measured at the day of CMRI. Blood tests will be performed prior to- or at the day of CMRI. See Table 4 for blood and plasma testing details.

DATA MANAGEMENT

The participant data including demographics, medical history, laboratory- and investigational results will be recorded in digital eCRFs in Research Electronic Data Capture (REDCap), a secure web

application for administration of databases in non-commercial clinical research. Investigators or appointed research nurses will manually enter the data. Only authorized personnel such as the sponsor, investigator, sub-investigator, or study nurses will have encoded access via personal user ID and password. All data will be handled confidentially, in accordance with "The Danish Act on Processing of Personal Data and General Data Protection Regulation (GDPR). Study data will be published in pseudonymous form only after being extracted by the primary investigator at study termination.

STATISTICAL METHODS

All participants will be included in the intention to treat analysis. Only patients who have completed the last follow up will be included in the per protocol analysis.

Descriptive Statistics

Baseline characteristics will be presented in Table 1 grouped by treatment arm. Continuous variables will be presented as median [IQR] or mean±SD and compared by unpaired t-test or Mann-Whitney U test depending on distribution of data. Categorical variables will be presented as number and percentage, groups will be compared with chi-square test or Fisher's exact test. Carciac Magnetic Resonance Sub Study data will be presented similarly.

Analysis Set

Data from randomized subjects who have completed their baseline visit and week 48 follow up, adherent to treatment as per protocol, will be included in the per protocol analysis in evaluation of primary outcome. Absolute change in mean fasting weight from baseline to week 48 will be compared between the intervention and the control group by two-tailed unpaired t-test. Continuous secondary endpoints will be analyzed and presented similarly to the primary analysis of weight

change. Categorical variables will be compared with chi-square test or Fisher's exact test. No interim analysis is planned.

Missing Data

Missing data will be analyzed with the assumption that data are "missing at random" (MAR) and analyzed using multiple imputation. At study termination an analysis to check for pattern of missing data and association between missing and observed data will be performed to ensure missing data is MAR.

METHODS: MONITORING

Data Monitoring

External data monitoring will be performed according to ICH-GCP by the public "Danish GCP Units, Copenhagen". Data collection and handling will be conducted according to a monitoring plan and written standard procedures (SOP) and in accordance with GCP regulations and requirements. There are no planned interim analyses in the study.

Harms

Serious adverse events (SAE) and unexpected serious adverse reactions will be evaluated and documented by the primary investigator and reported to the sponsor in accordance with GCP and Danish medicine Agency regulatory. A yearly report including all emerged SARs and SUSARs and comments on general safety in the study will be sent to the Danish Medicines Agency. In case of death or life-threatening disease, the sponsor will report the SUSAR to Danish Medicines Agency and the regional Research Health Ethics Committee within 7 days of the sponsor's knowledge of the event. A final report of registered events will be generated at study terminations and reported to the Danish Medicines Agency and Health Research Ethics committee of the Capital Region of Denmark.

ETHICS AND DISSEMINATION

Protocol Amendments

Any possible protocol modification will be reported in a protocol amendment to relevant public authorities.

Consent or Assent

All participants will be informed of the study by the means of oral and written information, per usual ICH standards, with full details of the study, including risk and benefits, before enrollment. Participants will be informed of the right to obtain an assessor. Only the principal investigator or co-investigators will be allowed to obtain informed consent from participants. Appendix 1 shows the informed consent form.

An additional consent will be retrieved for a project specific plasma biobank for subsequent analyses (project specific biobank) and a biobank for future research. Biobank details are listed in Appendix 3.

Confidentiality

Sponsor and investigators are obliged to handle all data on trial participants confidentially in accordance with the Act on Processing of Personal Data. At the end of the study, the primary investigator will extract data from the electronic database REDCap to perform the planned analyses on primary and secondary outcomes. Data will be processed and analyzed in the free statistic software R Studio. Study data will subsequently be published only in anonymous form. Data will be handled based on Danish law of data protection and Danish data protection regulation.

Declaration of Interest

There are no conflicts of interest to declare.

Data Access

The study is registered at ClinicalTrials.gov Identifier (NCT04904406, registered the 27th of May 2021). Access to final data will be limited to sponsor, primary investigator and personnel involved in the analysis of data, co-investigator, and statisticians. The data that support the findings of this study are available upon reasonable request. The data are not publicly available due to Danish legislation regarding General Data Protection Regulation.

Ancillary and post-trial care

All areas of the Danish health care system are covered by a publicly funded compensation scheme. The scheme covers if a participant is injured in connection with treatment at a public hospital. The scheme covers medicinal product injuries. This also applies for patients involved in research. At inclusion, the participants will be informed of the compensation and complaint avenues in case a drug injury occurs, which is in adherence to Danish law.

Dissemination policy: trial results

Upon the completion of the trial, the data collected from all participating sites will be pooled and analyzed together. Researchers involved in the trial will not be permitted to publish data until after the main study publication is released. The results of the primary study will be featured in a peer-reviewed journal, with the primary investigator as the first author, the sponsor as the senior author, and the participating investigators as co-authors based on their work and involvement in the study. All findings, whether positive, negative, or inconclusive, will be published. The cardiac MRI sub study data will be reported separately.

Patient and Public Involvement statement

Patients/public were not involved until initiation of inclusion in the trial. The research question was developed in the clinic by clinicians treating the involved patient group. The patients/public were

not involved in the design, conduct or recruitment of the study, but the study was designed to make the participation as accessible and effortless as possible for patients. Participants will be informed about the study results by letter at study termination unless the opt this out in the consent form.

Author Statement

Roles of corresponding author and co-authors:

Roles and Responsibility

Karen Brorup Heje Pedersen, corresponding author: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Project administration

Thomas Benfield, Sponsor: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization, Writing - Review & Editing, Project administration, Supervision, Funding acquisition

Andreas Knudsen: Conceptualization, Methodology, Writing - Review & Editing, Supervision

Søren Møller, Professor: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision,

Hartwig Roman Siebner: Conceptualization, Methodology, Resources , Writing - Review & Editing, Supervision

Jens Dahlgaard Hove: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - Review & Editing, Supervision,

Jan Gerstoft: Conceptualization, Methodology, Writing - Review & Editing, Supervision

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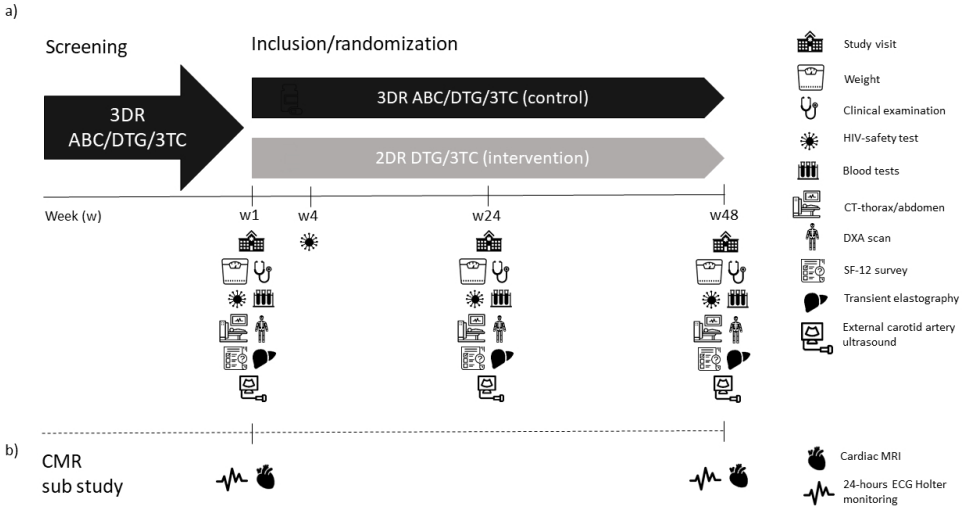
FIGURE LEGENDS

Figure 1: Participants Timeline and Data Collection

a) Eligible participants on abacavir/dolutegravir/lamivudine (ABC/DTG/3TC) for HIV infection for more than 6 months will be enrolled. Inclusion, randomization, and data collection will be performed at baseline visit in week 1 (w1). Participants will be randomized to either continuation of a three-drug regimen with ABC/DTG/3TC (control) or a two-drug regimen 2DR DTG/3TC (intervention). Data will be collected at follow up visits at week 4 (w4), week 24 (w24) and week 48 (w48). At study visits w1, w24, and w48 the following tests will be performed: Physical examination by clinician; blood tests including HIV-RNA and CD4 cell count; computed tomography scan (CT) of thorax and the upper abdomen, Dual-Energy x-ray absorptiometry (DXA) scan; 12-Item Short Form Survey (SF-12); liver elastography; External carotid artery ultrasound. Participants in the intervention arm will have plasma HIV RNA determined at week 4 for safety reasons. b) Participants enrolled in the cardiac MRI (CMR) sub study will additionally receive CMR and 24-hours Holter ECG monitoring at visits w1 and w48.



a) Eligible participants on abacavir/dolutegravir/lamivudine (ABC/DTG/3TC) for HIV infection for more than 6 months will be enrolled. Inclusion, randomization, and data collection will be performed at baseline visit in week 1 (w1). Participants will be randomized to either continuation of a three-drug regimen with ABC/DTG/3TC (control) or a two-drug regimen 2DR DTG/3TC (intervention). Data will be collected at follow up visits at week 4 (w4), week 24 (w24) and week 48 (w48). At study visits w1, w24, and w48 the following tests will be performed: Physical examination by clinician; blood tests including HIV-RNA and CD4 cell count; computed tomography scan (CT) of thorax and the upper abdomen, Dual-Energy x-ray absorptiometry (DXA) scan; 12-Item Short Form Survey (SF-12); liver elastography; External carotid artery ultrasound. Participants in the intervention arm will have plasma HIV RNA determined at week 4 for safety reasons. b) Participants enrolled in the cardiac MRI (CMR) sub study will additionally receive CMR and 24-hours Holter ECG monitoring at visits w1 and w48.

338x190mm (96 x 96 DPI)

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

Biobank

Project Specific Biobank

At week 1, 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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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	3
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,2

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	3
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	5
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	5
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8,9
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9,10

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found,	10

		if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	7
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	12

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	12
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication,	13

		reporting in results databases, or other data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	13
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Appendix 2, appendix 3

Notes:

- 33: Appendix 2, appendix 3 The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 08. May 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Protocol: Changes in weight, body composition and metabolic parameters after switch to dolutegravir/lamivudine compared to continued treatment with dolutegravir/abacavir/lamivudine for virologically suppressed HIV infection (The AVERTAS trial): A randomized open-label superiority trial in Copenhagen, Denmark

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	HIV/AIDS
Keywords:	Obesity, INFECTIOUS DISEASES, VIROLOGY, HIV & AIDS < INFECTIOUS DISEASES, Other metabolic, e.g. iron, porphyria < DIABETES & ENDOCRINOLOGY

TITLE

Protocol: Changes in weight, body composition and metabolic parameters after switch to dolutegravir/lamivudine compared to continued treatment with dolutegravir/abacavir/lamivudine for virologically suppressed HIV infection (The AVERTAS trial): A randomized open-label superiority trial in Copenhagen, Denmark.

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Registration

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

Regional Data Protection Centre (P-2020-207)

clinicaltrials.gov (NCT04904406)

Roles and Responsibility: Sponsor Contact Information

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Roles and responsibilities: Sponsor and Funder

The study is a Sponsor-Investigator trial. The sponsor, Thomas Benfield has no conflicts of interest or commercial interest in the study. Sponsor and investigators are independent of economic or competing interests. The grant is held in a foundation account managed by sponsor. Participants will

not be financially compensated. Study results will be utilized only for scientific and public purpose and do not hold any commercial significance.

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3828

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Randomized controlled trail, RCT, HIV, antiretroviral therapy, weight, obesity, cardiovascular disease, Cardiac Magnetic Resonance, CMRI

Abstract

Introduction

With longer life expectancy in people living with HIV (PLWH) on antiretroviral therapy, cardiovascular disease (CVD) has become a common cause of mortality. Abacavir has been associated with an increased risk of myocardial infarction, but the mechanism is unknown. Additionally, abacavir may be obesogenic which could mediate an additional risk factor of CVD. We aim to investigate if discontinuation of abacavir will have a favorable impact on body weight and cardiac parameters in PLWH.

Methods and Analysis

Randomized, controlled, superiority trial of virologically suppressed PLWH on dolutegravir, abacavir and lamivudine (DTG/ABC/3TC) for ≥ 6 months. In total, 70 PLWH will be randomized 1:2 to either continue DTG/ABC/3TC or to switch to dolutegravir and lamivudine (DTG/3TC) providing the power of 80% at alpha 5% to detect a mean difference in weight change of 2 kg (Δ) given a standard deviation of 2.7 kg. Follow-up will be 48 weeks. Data will be collected at baseline and week 48. Primary outcome will be change in mean bodyweight from baseline to week 24 and 48 evaluated in a linear mixed model. Secondary outcomes will be changes in cardiac-, inflammatory- and metabolic

parameters, fat distribution, coagulation, endothelial, platelet function, quality of life and virological control from baseline to week 48. Measurements include computed tomography (CT) of thorax and abdomen, external carotid artery ultrasound, liver elastography and dual energy x-ray absorptiometry (DEXA) and blood analysis. Plasma HIV RNA will be measured at baseline, week 4, 24 and 48. Forty participants (20 from each arm) will be included in a sub-study involving cardiac magnetic resonance imaging at baseline and week 48. Twenty non-HIV-infected controls will be included with a single scan to compare with baseline scan data.

Ethics and Dissemination

Result from this study will lead to a better understanding of the association between antiretroviral therapy and the impact on weight and risk of CVD. Findings will be useful for both clinicians and PLWH in the guidance of a more individualized HIV treatment. Results from the main study and the substudies will be submitted for publication in a peer-review journal(s). The AVERTAS study is approved by the Ethics Committee of the Capital Region, Denmark (H-20011433), Danish Medicines Agency (EudraCT no. 2019-004999-19) and Regional Data Protection Centre (P-2020-207). The study is registered at clinicaltrials.gov (NCT04904406). Trial registration:

ClinicalTrials.gov Identifier: NCT04904406, registered the 27th of May 2021.

ARTICLE SUMMARY

Strengths and Limitations

- Study design is randomized controlled multicenter trial which limits confounding
- The study is limited by the unblinded setting which enables bias
- The study is carried out in outpatient clinics, where participants receive their usual care. This might enhance adherence to study visits

INTRODUCTION

Background and Rationale

The introduction of antiretroviral treatment (ART) with ≥3 drugs in 1996 changed the prognosis of HIV infection dramatically. Today, people living with HIV (PLWH) treated with ART has a life expectancy close to that of the HIV-uninfected population (1). However, this longer life expectancy has led to higher rates of serious non-AIDS events (SNAE) such as cardiovascular disease (CVD) (2–4). Today, CVD is the main cause of mortality in PLWH (5). The mechanism is thought to be a multifactorial interplay between traditional CVD risk factors, HIV specific factors and ART. Studies have shown a correlation between the use of the nucleoside reverse transcriptase inhibitor (NRTI) abacavir and myocardial infarction (6–10). The underlying mechanism remains largely unknown but may include endothelial dysfunction, increased inflammation, platelet activation and platelet/collagen interactions, all of which can modify CVD risk (11).

In recent years, dual therapy as an alternative to traditional 3-drug-regimen (3DR) has emerged as a HIV treatment option. The combination of XTC/DTG is one of several 2-drug regimens now recommended in the EACS guidelines (v11.1) and IAS-USA for either initiating ART in ART-naïve adults with plasma HIV RNA < 500,000 copies/mL and without HBV infection or as a switch option for individuals with viral suppression (plasma HIVRNA < 50 copies/mL for the past 6 months) (12,13). These recommendations was supported by data from randomized controlled trials reporting non-inferiority in achieving or maintaining viral suppression (plasma HIV-RNA 50 copies/mL) in ART naïve and virally suppressed PLWH, respectively (14–17).

Initiating ART per se is associated with weight gain (18–20). Some of this weight gain is thought to be related to a “return-to-health” phenomenon where initiation of ART causes a return to baseline weight after HIV induced wasting. This mechanism is not fully understood and seems to be only

partly responsible for the observed weight gain. A meta-analysis of 8 RCTs reported that weight increased more in ART-naïve PLWH initiating treatment with an integrase strand transfer inhibitor (INSTI)-based regimen as compared to a non-NRTI or a protease inhibitor-based regimen. Among NRTIs, abacavir and tenofovir alafenamide (TAF), were associated with more weight gain than tenofovir disoproxil fumarate (TDF) or zidovudine (21). Weight gain after ART switch is modest and it remains uncertain whether this is due to the loss of a weight suppressive effect of prior regimens with older agent such as TDF or efavirenz or a weight gain effect of the newer regimens especially TAF and/or INSTI, or both (22). Overweight and obesity are associated with an increased risk of metabolic syndrome, diabetes, hypertension and dyslipidaemia that converge as risk factors for CVD including myocardial infarction (23). Thus, it is conceivable that there could be synergistic effects and a higher overall risk of CVD using abacavir and dolutegravir in combination. We want to investigate the effect on weight change and cardiac function after discontinuation of abacavir in PLWH treated with abacavir, lamivudine and dolutegravir.

Objectives

The aim of this study is to investigate if discontinuing abacavir by switching from a 3-drug (3DR) regimen with dolutegravir, abacavir and lamivudine (DTG/ABC/3TC) to a 2-drug (2DR) regimen with dolutegravir and lamivudine (DTG/3TC) will decrease weight and improve metabolic and cardiac parameters in PLWH.

Trial Design

This study is a randomized, controlled, parallel, open-label, phase 4, interventional trial.

METHODS: PARTICIPANT, INTERVENTIONS, AND OUTCOMES

Study Design

Participants will be recruited from outpatient clinics at the departments of infectious diseases at two hospitals: Copenhagen University Hospital - Amager and Hvidovre; and Copenhagen University Hospital - Rigshospitalet. Both located in the Capital Region of Denmark. Baseline and follow up study visits and data collection will be performed at Copenhagen University Hospital - Amager and Hvidovre.

Eligibility Criteria

Inclusion and exclusion criteria are listed in Table 1.

Table 1 Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
• Age ≥ 18 years • HIV infection • ongoing treatment with DTG/ABC/3TC ≥ 6 months • Plasma viral load (HIV-RNA) < 50 copies/ml at inclusion For women with childbearing potential • Willingness to use contraceptive during study period	• Pre-existing viral resistance to lamivudine or dolutegravir* • Presence of hepatitis B antigen (HBsAg) or HBV DNA • Cancer within the past five years • Pregnancy or breastfeeding (for women) • Unstable cardiovascular disease, diabetes (assessed by the treating physician)

Participants will be eligible for a magnetic resonance imaging (MRI) sub-study participation, if they comply to standard MRI safety guidelines.

*Existing genotypic resistance test results will be screened prior to inclusion.

Interventions

At day 0 patients will be randomized to either continued therapy with co-formulated dolutegravir 50 mg, abacavir 600 mg, and lamivudine 300 mg (Triumeq, control arm) or switch to co-formulated dolutegravir 50 mg and lamivudine 300 mg (Dovato, intervention arm) for 48 weeks. See Figure 1 for participant timeline.

Administration

The Investigational Medicinal Products (IMP) will be self-administered in original pharmaceutical packaging by participants once daily. Double controlled administration of the IMP and IMP-log registration will be performed by two designated and GCP-trained study personnel. The study IMP will be distributed at /day 0 and week 24 in original wrapping, with a study specific label. All IMPs are authorized, registered, and marketed by the Danish Medicines Agency and the European Medicines Agency (EMA). Dosage and administration frequency are assigned according to treatment guidelines.

Withdrawal from study

Participants will be withdrawn from the assigned study treatment in case of viral rebound or if any serious adverse reaction (SAE) or suspected unexpected serious adverse reaction (SUSAR) considered to compromise participants safety as assessed by the investigator. Any case of withdrawal will result in an immediate visit, where the study participant will be offered resistance test, and be assigned to a new suppressive HIV regimen. This will be managed by the study team and the participants primary healthcare provider, who will be involved in the decision and further

monitoring of viral load and participants. Participants withdrawn from the study will be included in the intention to treat analysis.

Adherence

Adherence will be monitored by measurements of plasma HIV-RNA viral load at baseline, weeks 4 (2DR group only), 24 and 48. Virological failure defined as two consecutive viral loads > 50 copies/ml with an interval of 14 to 30 days will lead to immediate withdrawal from the study.

If participants drop out or are withdrawn, an immediate follow up meeting will be arranged to make sure patients return to their usual treatment. A resistance test will be performed, and their regular physician will be informed. Data from withdrawn participants or dropouts will contribute to the intention to treat analysis. with the patient's acceptance.

Outcomes

Primary outcome

- Change in bodyweight from baseline to week 48 measured as difference between means in the two study arms

Secondary outcomes

- Development of metabolic syndrome at week 48 (24)
- Development of type 2 diabetes at week 48 (25)
- Impaired insulin resistance and/or β-cell function determined by changes in HOMA-IR at week 48 (26)
- Virological control at week 48 as defined by a plasma HIV-RNA <50 copies/ml

Changes from baseline to week 48 in:

- Self-rated health evaluated by 12-item Short Form Survey (SF-12)
- Framingham Risk Score (27,28)

- DAD CVD risk score (29)
- Blood HbA1c
- Total plasma cholesterol
- Plasma High Density lipoprotein cholesterol (HDL-cholesterol)
- Plasma Low Density Lipoprotein cholesterol (LDL-cholesterol)
- Plasma Very Low-Density Lipoprotein cholesterol (VLDL-cholesterol)
- Plasma Triglycerides
- Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) ratio (abdominal CT)
- Fat distribution in trunk, limb, and extremities measured by Dual-Energy X-ray Absorption (DEXA)
- Development of liver fibrosis or progression of existing liver fibrosis. Significant liver fibrosis as LSM ≥7.6 kPa.
- Fatty infiltration of the liver evaluated as:
    - Development of steatosis or increase in existing steatosis from baseline (CT liver and Controlled Attenuated Parameter (CAP) (Fibroscan)
- Blood pressure
- Cardiac magnetic resonance imaging (MRI)
- Carotid artery intima-media thickness (cIMT) measured by ultrasound
- Coronary artery calcium score (CACS)
- Plasma N-terminal pro-B-type natriuretic peptide (Pro-BNP)
- Plasma Troponin T (TnT)
- Inflammation:
    - Plasma High-sensitive C-reactive protein

- Plasma Interleukin 1β
- Plasma Interleukin 6

- Endothelial function:
  - Plasma Vascular cell adhesion molecule 1
  - Plasma Intercellular adhesion molecule 1
- Platelet function:
  - Plasma Soluble P-selectin
  - Plasma Soluble glycoprotein VI.
- Coagulation:
- Plasma D-dimer
- Plasma coagulations factor 2, 7 and 10 (extrinsic pathway)
- Plasma Fibrinogen
- Blood hemoglobin, leucocyte count, and platelet count
- Plasma creatinine, urea, sodium, potassium, bilirubin, and alanine aminotransferase.

Outcomes MRI Sub-study

Primary outcome

In the Cardiac MRI (CMRI) sub-study the outcome is a composite endpoint consisting of any abnormalities in

- Extracellular myocardial volume (ECV) from baseline to week 48
- Left atrial volume from baseline to week 48
- Diastolic function from baseline to week 48
- Myocardial mass from baseline to week 48

Secondary outcomes

Changes in

- Left ventricular ejection fraction
- Myocardial perfusion
- Myocardial edema/inflammation
- Myocardial fibrosis
- Myocardial lipid-water profile

Participant Timeline

Participant timeline is illustrated in Figure 1

Sample Size

The hypothesis on weight change in this study relies on a meta-analysis with pooled weight data from 8 RCTs of treatment naïve PLWH. In the meta-analysis mean weight gain with abacavir (ABC) was 3.08 kg (95% CI, 2.36–3.81) in 96 weeks. In the same meta-analysis dolutegravir lead to a mean weight gain on DTG, 4.07 kg [95% CI, 3.51–4.62] (21).

Since dolutegravir treatment continues in both study arms, we hypothesize the possible contribution from dolutegravir to weight gain will be equal in the groups. We speculate that that the absence of abacavir in the intervention (2DR) group can lead to a small weight loss in. Anticipated annual weight change in the two groups are: DTG/3TC + 0 to -1 kg and DTG/ABC/3TC +2 kg. Sample size is estimated by Student's unpaired t-test. Patients will be randomized 2:1 to intervention or control.

Assuming a 2:1 randomization ratio for the intervention and control arm, a significance level (α) of 5%, a power (β) of 80%, mean difference in weight between the two arms of 2 kg (Δ) and a standard deviation of 2.7 kg the estimated sample size required will be 44 randomized individuals in the

intervention group and 22 randomized individuals in the control group. To account for a 5% withdrawal or dropout rate, the sample size will be 70 patients in total.

Recruitment

Eligible patients will be identified by treating physicians at outpatient clinics at the involved sites. Information on eligible patients will be disclosed to the responsible investigator for the purpose of recruitment. Eligible patients will receive verbal and written study information, and subsequently be offered participation. All participants must provide written informed consent.

Study Status

The first participant was included the 20th of October 2020. Recruitment is expected to be completed by November 2023.

METHODS: ASSIGNMENT OF INTERVENTIONS

Allocation

Eligible participants will be randomized in two parallel arms with an allocation ratio of 2:1 to switch to 2DR therapy with DTG/3TC or to continue 3DR treatment with DTG/ABC/3TC (control). Key variables will be entered into a secure web-based program (REDCap) to randomize patients into two parallel arms upon inclusion. The randomization list will be centrally generated in REDCap using random blocks stratified by study center and sex. Additionally, 40 participants (20 control and 20 intervention) will be invited to participate in the cardiac MRI sub-study. The study will be open label, both participants and the study personnel will be un-blinded to randomization.

METHODS: DATA COLLECTION, MANAGEMENT, AND ANALYSIS

Data Collection Methods

Study visits and data collection will be performed at Copenhagen University Hospital - Amager and Hvidovre and participants will be examined by trained clinicians or study nurses. At the inclusion visit (day 0) baseline data will be collected, and participants will be randomized. Subsequently a safety blood test will be performed at week 4 (for the 2DR arm), and follow-up data will be collected at study visits at week 24 (range 20-28) and finally at week 48 (range 46-52). An overview data collection is listed in Table 2 and 3. Blood testing is listed in Table 4. Detailed description on radiological tests are included in Appendix 1.

Participants must be fasting at least 6 hours prior to visits at day 0, 24 and 48, to ensure fasting measurements of weight, blood tests, transient liver elastography and Dual-Energy X-Ray Absorptiometry.

Table 2 Data Collection

	Day 0	Week 4	Week 24	Week 48
Informed consent	x			
Randomization	x			
Demographics[1]	x			
Framingham risk score	x		x	x
SF-12[2]	x		x	x
Body weight	x		x	x
Waist circumference	x		x	x
Vital signs	x		x	x
Blood tests[4]	x		x	x
HIV safety blood tests[5]	x	x	x	x

Transient elastography / Controlled Attenuated Parameter (CAP)	x		x	x
CT[6]	(x)		(x)	(x)
DEXA[7]	x		x	x
cIMT[8]	x		x	x
MRI SUB-STUDY				
Cardiac MRI[9]	x			x
24-hours ECG Holter monitoring	x			x

[1]Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living.

[2] 12-item short-form health survey (SF-12)

[3]Blood **pressure**, heart rate, respiration rate, peripheral oxygen saturation, temperature.

[4]**HIV safety blood tests:** Plasma HIV-RNA and CD4 count.

[5]**Blood tests:** leucocytes, platelets, hemoglobin, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, lactate dehydrogenase, erythrocyte fraction. **Metabolism:** Fasting p-glucose, insulin, glycated hemoglobin (HbA1c), total cholesterol, HDL, LDL, VLDL, triglyceride. **Inflammation:** High-sensitive c-reactive protein, interleukin 1 and 6. **Coagulation:** D-dimer, factor 2, 7 and 10, fibrinogen**. Platelet function:** soluble P-selectin, soluble glycoprotein VI**. Endothelial function:** Vascular cell adhesion molecule 1, intercellular adhesion molecule 1, Cardiac**:** N-terminal pro-B-type natriuretic peptide (Pro-BNP), troponin T (TnT).

[6]Low dose computed tomography of thorax and abdomen to determine coronary artery calcium score (CACs), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and liver steatosis.

[7]Dual energy x-ray absorptiometry, optional

[8]Carotid intima-media thickness (cIMT) determined by ultrasound.

[9]Cardiac magnetic resonance imaging sub-study (optional)

Table 3 Clinical Measurements and Methods

Measurements	Description
Body weight	Measured after minimum 6 hours of fasting and without cloth Scale: Seca 701 7021099
Blood tests (fasting)	Peripheral venous blood
Transient liver elastography / Controlled Attenuated Parameter (CAP)	Fibro scan, M-XL probe, (Echosens, Paris, France)
Carotid intima-media thickness (cIMT)	Sonosite M-Turbo (Sonosite Inc., Bothell, WA, USA), analysed with a 13.6 MHz linear transducer and automated software (SonoCalc IMT 5.0; Sonosite Inc.)
CT scans	CT chest, CT upper abdomen and Coronary Calcium Scan Aquillion One scanner, Toshiba Medical Systems, (Otawara-shi, Tochigi-ken, Japan)
DEXA scan	Dual energy X-ray absorptiometry (DEXA) Whole-body DEXA scanning Hologic QDR-2000 W (Bedford, MA, USA)
Cardiac MRI	MAGNETOM Prisma 3 Tesla scan, SIEMENS Healthineers (Erlangen Germany)
Metabolic syndrome[1]	Central obesity: waist circumference ≥94 cm for males and ≥80 cm for females (Europids), or BMI>30 kg/m², plus any two of the following four factors: • Raised triglycerides: >1,7 mmol/L • Reduced HDL cholesterol: ≤1,03 mmol/L in males, <1,29 mmol/L in females • Raised blood pressure (BP): Systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or treatment of previously diagnosed hypertension • Raised fasting plasma glucose: ≥5,6 mmol/L or previously diagnosed type2-diabetes

Insulin resistance and β-cell function	Homeostatic Model Assessment of Insulin Resistance (HOMA-IR): $HOMA - IR = \frac{FPG\ (mmol/L) \cdot FPI\ (mU/L)}{22.5}$ $HOMA - \%B = \frac{20 \cdot FPI\ (mU/L)}{FPG\ (mmol/L) - 3{,}5}$
Type 2 diabetes[2]	• Fasting plasma glucose concentration ≥ 7.0 mmol/L or • a random venous plasma glucose concentration ≥ 11.1 mmol/L or • HbA1c > 48 mmol/mol
Cardiovascular disease risk scores Framingham Risk Score D:A:D risk score	Online calculation tool, CHIP – Centre of Excellence for Health & Framingham Heart Study
Survey	• 12-Item Short term Survey (SF-12) • Questionnaire on dietary and activity patterns

[1] Metabolic syndrome defined in accordance with The International Diabetes Federations definition

[2] WHO definition

Table 4 Blood and Plasma Analysis

Category	Blood and Plasma analysis
Hematology	Leucocyte count and differential count, hemoglobin, hematocrit
Electrolytes	Sodium, Potassium
Renal	Creatinine, urea, albumin
Liver	Alanine aminotransferase, aspartate transaminase, bilirubin, lactate dehydrogenase
HIV-safety	HIV-RNA*, CD4 cell count*
Metabolism	Glucose, insulin, blood glycated hemoglobin (HbA1c), total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol (calculated)
Inflammation	High sensitivity C-reactive protein, interleukin 1β and 6*
Coagulation	D-dimer, factor II+VII+X, fibrinogen, platelet count
Platelet function	Soluble P-selectin*, Soluble glycoprotein VI*
Endothelial function	Vascular cell adhesion molecule 1*, intercellular adhesion, molecule 1*
Cardiac	N-terminal pro-B-type natriuretic peptide, Troponin T

Blood tests will be performed at visits day 0 week 24 and week 48. Participants will be fasting minimum 6 hours prior to blood tests. Non-HIV-infected controls will have blood tests prior to- or at the day of CMRI and will not be fasting. Blood analysis with * will be performed only in PLWH.

Cardiac MRI Sub-study

Forty participants from the main study, 20 from the 2DR-group and 20 from the 3DR-group, will be included in the CMRI sub-study. Cardiac MRI and ECG-monitoring will be performed on the sub-study population at baseline and week 48. Further, 20 non-HIV-infected controls will be recruited for a single CMRI to compare baseline data. CMRI technical details and scan protocol are elaborated in Appendix 1.

The following clinical information will be obtained from healthy controls or from their patient records: Medicine use, known medical conditions, cardiac risk (smoking status, alcohol consumption, family CVD history), height and weight. Blood pressure and heart rate will be measured at the day of CMRI. Blood tests will be performed prior to- or at the day of CMRI. See Table 4 for blood and plasma testing details.

Data Management

The participant data including demographics, medical history, laboratory- and investigational results will be recorded in digital eCRFs in Research Electronic Data Capture (REDCap), a secure web application for administration of databases in non-commercial clinical research. Investigators or appointed research nurses will manually enter the data. Only authorized personnel such as the sponsor, investigator, sub-investigator, or study nurses will have encoded access via personal user ID and password. All data will be handled confidentially, in accordance with “The Danish Act on Processing of Personal Data and General Data Protection Regulation” (GDPR). Study data will be published in pseudonymous form only after being extracted by the primary investigator at study termination.

Statistical Methods

Intention-to-treat (ITT) analysis

The intention-to-treat analysis will include all participants who were randomized, regardless of their adherence to the assigned treatment or whether they discontinued the treatment. This analysis will also include participants who were lost to follow-up.

Per-protocol analysis (PP)

The per-protocol analysis will only include randomized participants who completed the entire study and the last follow-up. If participants did not adhere to the assigned treatment but adhered to one of the two treatment arms, they will be included in the per-protocol analysis with their actual treatment. However, if participants discontinued the treatment or started a treatment regimen outside the study protocol, they will only be included in the intention-to-treat analysis.

Descriptive Statistics

Baseline characteristics will be presented in Table 1 grouped by treatment arm. Continuous variables will be presented as median [IQR] or mean and standard deviation (SD) and compared by unpaired t-test or Mann-Whitney U test depending on distribution of data. Categorical variables will be presented as number and percentage, groups will be compared with chi-square test or Fisher's exact test. Cardiac Magnetic Resonance Sub-study data will be presented similarly.

Analysis Set

Absolute change in mean fasting weight from baseline to week 24 and from baseline to week 48 will included in a linear mixed model for analysis of the primary outcome. Continuous secondary endpoints will be analyzed and presented similarly to the primary analysis of weight change. Categorical variables will be compared with chi-square test or Fisher's exact test. No interim analysis is planned.

Missing Data

Missing data will be analyzed with the assumption that data are “missing at random” (MAR) and analyzed using multiple imputation. At study termination an analysis will be performed to check for pattern of missing data and association between missing and observed data will be performed to ensure missing data is MAR.

Loss to follow up

Participant who are lost to follow-up including dropouts withdrawing their consent or unreachable participant will be included in the intention to treat analysis. The participant's primary healthcare provider will be informed to ensure that regular monitoring and treatment will be resumed.

METHODS: MONITORING

Data Monitoring

External data monitoring will be performed according to ICH-GCP by the public “Danish GCP Units, Copenhagen”. Data collection and handling will be conducted according to a monitoring plan and written standard procedures (SOP) and in accordance with GCP regulations and requirements. There are no planned interim analyses in the study.

Harms

SAEs and SUSARs will be evaluated and documented by the primary investigator and reported to the sponsor in accordance with GCP and Danish Medicines Agency regulatory. A yearly report including all emerged SAEs) and SUSARs and comments on general safety in the study will be reported to the Danish Medicines Agency. In case of death or life-threatening disease, the sponsor will report the SUSAR to Danish Medicines Agency and the regional Research Health Ethics Committee within 7 days of the sponsor’s knowledge of the event. A final report of registered events

will be generated at study terminations and reported to the Danish Medicines Agency and Health Research Ethics committee of the Capital Region of Denmark.

Patient and Public Involvement statement

Patients/public were not involved until initiation of inclusion in the trial. The research question was developed in the clinic by clinicians treating the involved patient group. The patients/public were not involved in the design, conduct or recruitment of the study, but the study was designed to make the participation as accessible and effortless as possible for patients. Participants will be informed about the study results by letter at study termination unless the opt this out in the consent form.

ETHICS AND DISSEMINATION

Protocol Amendments

Any possible protocol modification will be reported in a protocol amendment to relevant public authorities.

Consent or Assent

All participants will be informed of the study by the means of oral and written information, per usual ICH standards, with full details of the study, including risk and benefits, before enrollment. Participants will be informed of the right to obtain an assessor. Only the principal investigator or co-investigators will be allowed to obtain informed consent from participants. Appendix 2 shows the informed consent form.

An additional consent will be retrieved for a project specific plasma biobank for subsequent analyses (project specific biobank) and a biobank for future research. Biobank details are listed in Appendix 3.

Confidentiality

Sponsor and investigators are obliged to handle all data on trial participants confidentially in accordance with the Act on Processing of Personal Data. At the end of the study, the primary investigator will extract data from the electronic database REDCap to perform the planned analyses on primary and secondary outcomes. Data will be processed and analyzed in the free statistic software R Studio. Study data will subsequently be published only in anonymous form. Data will be handled based on Danish law of data protection and Danish data protection regulation.

Data Access

The study is registered at ClinicalTrials.gov Identifier (NCT04904406, registered the 27th of May 2021). Access to final data will be limited to sponsor, primary investigator and personnel involved in the analysis of data, co-investigator, and statisticians. The data that support the findings of this study are available upon reasonable request. The data are not publicly available due to Danish legislation regarding General Data Protection Regulation.

Ancillary and post-trial care

All areas of the Danish health care system are covered by a publicly funded compensation scheme. The scheme covers if a participant is injured in connection with treatment at a public hospital. The scheme covers medicinal product injuries. This also applies for patients involved in research. At inclusion, the participants will be informed of the compensation and complaint avenues in case a drug injury occurs, which is in adherence to Danish law.

Dissemination policy: trial results

Upon the completion of the trial, the data collected from all participating sites will be pooled and analyzed together. Researchers involved in the trial will not be permitted to publish data until after the main study publication is released. The results of the primary study will be featured in a peer-

reviewed journal, with the primary investigator as the first author, the sponsor as the senior author, and the participating investigators as co-authors based on their work and involvement in the study. All findings, whether positive, negative, or inconclusive, will be published. The cardiac MRI sub-study data will be reported separately.

LIMITATIONS

The main limitation in this study is non-blinding of the intervention which introduces both observer - and performer bias. We try to minimize the performance bias by not informing the participants what to expect in terms of weight change with their assigned treatment. Further we limit this bias with a 48 week follow up as a long term study intervention may decrease the probability to adhere to other weight interventions such as diets or excessive training.

We expect high adherence to the study and to the randomization. We only include virally suppressed PLWH. PLWH are used to adhere to prescribed medication, and they are all treated with the drugs used at study entry (they either continue three drug and switch to a regimen consisting of two of the three drugs), thus we expect very few adverse events. The study setup an expected low AE rate is thought to limit drop out. The randomized controlled design of the study will contribute to even distribution of bias, non-adherence, and loss to follow up in the groups.

COMPETING INTERESTS

None to declare

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contribute to the design of the study, the decision to publish the findings, or the preparation of the manuscript.

Author Contributions Statement

The authors listed have all made contributions to this paper in accordance with the recommendations of the International Committee of Medical Journal Editors (ICMJE). Thomas Benfield (TB), MD, professor, DMSc; Jan Gerstoft (JG), MD, professor DMSC; Andreas Knudsen (AK), MD, DMSc and Karen Brorup Heje Pedersen (KBHP), MD contributed to the study design. Jens Dahlgaard Hove (JDH), MD, PhD, MSc, Associate Professor; Hartwig Roman Siebner (HRS), MD, professor, DMSc and Søren Møller (SM), MD, professor, DMSc contributed specifically to the radiological aspects of the protocol, including technical details, setup and access to all radiological examinations. All authors will be involved in the analysis and interpretation of data upon study termination. The primary drafting work was conducted and organized by the corresponding author, KBHP, and the study sponsor, TB. JDH and JDH drafted the CMRI-scan protocol. All authors participated in reviewing the manuscript and providing intellectual input.

The authors listed have approved the final version of the paper and are willing to participate in any future revisions. All authors (KBHP, AK, SM, HRS, JDH, JG, and TB) take responsibility for the accuracy and integrity of the work and are committed to addressing any concerns that may arise.

Artificial intelligence (AI)-Assisted Technology, ChatGBT from OpenAI (https://openai.com/blog/chatgpt), was used solely for grammatical and linguistic proofreading of the manuscript. No AI was involved in the study design, data analysis, interpretation, or substantial writing and reviewing of the manuscript.

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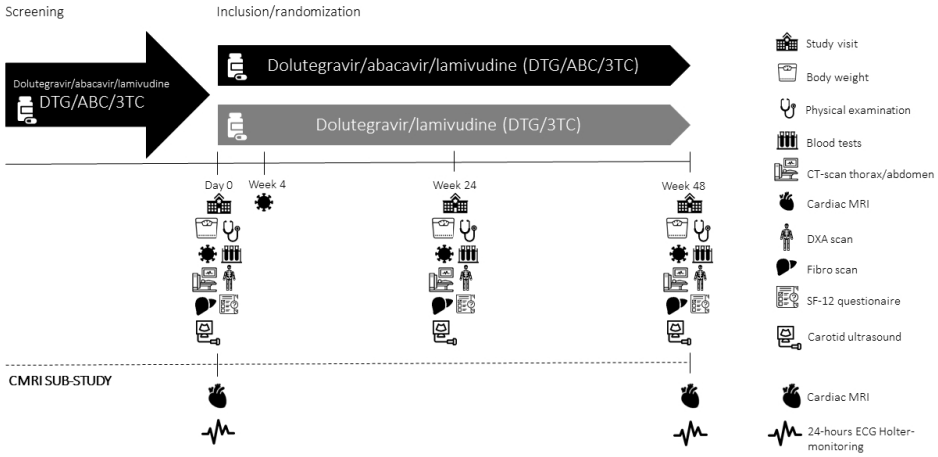
FIGURE LEGENDS

Figure 1: Participants Timeline and Data Collection

a) Eligible participants on abacavir/dolutegravir/lamivudine (ABC/DTG/3TC) for HIV infection for more than 6 months will be enrolled. Inclusion, randomization, and data collection will be performed at a baseline visit (Day 0) . Participants will be randomized to either continuation of a three-drug regimen with ABC/DTG/3TC (control) or a two-drug regimen with DTG/3TC (intervention). Data will be collected at follow up visits at week 4 (w4), week 24 (w24) and week 48 (w48). At study visits w1, w24, and w48 the following tests will be performed: Physical examination by clinician; blood tests including HIV-RNA and CD4 cell count; computed tomography scan (CT) of thorax and the upper abdomen, Dual-Energy x-ray absorptiometry (DXA) scan; 12-Item Short Form Survey (SF-12); liver elastography; External carotid artery ultrasound. Participants in the intervention arm will have plasma HIV RNA determined at week 4 for safety reasons. b) Participants enrolled in the cardiac MRI (CMRI) sub-study will additionally receive CMRI and 24-hours Holter ECG monitoring at visits w1 and w48.



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338x190mm (96 x 96 DPI)

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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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16, 17

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	2
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	1
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8-9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection,			

management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	6
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure;	14

		statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	16
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 2
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Appendix 3, Supplementary

Notes:

- 33: Appendix 3, Supplementary The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 07. July 2023 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai