Statistical Analysis Plan for Clinical Trials

Title: Switch from stable cART containing ABC/3TC or TAF/FTC plus dolutegravir or bictegravir to TDF/3TC/doravirine in people living with HIV: Impact on lipids, body composition, insulin sensitivity, neuroendocrine function and inflammation markers

A randomised, open label, two arm, phase II, clinical trial

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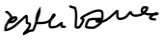
Signature  Date 18/08/2023

Table of Contents

List of abbreviations and definitions of terms

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
cART	Combination antiretroviral therapy
(e)CRF	(Electronic) Case Report Form
DOR	Doravirine
DSMB	Data Safety Monitoring Board
DTG	Dolutegravir
EuroQoL	European Quality of Life
FTC	Emtricitabine
GCP	Good Clinical Practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH-GCP	International conference of harmonization good clinical practice
INSTI	Integrase Strand Transfer Inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitors
PI	Principal Investigator
SmPC	Summary of Product Characteristics
PIS	Participant Information Sheet
PSQI	Pittsburgh Sleep Quality Index
PLWH	Person/People living with HIV
SOC	Standard Of Care
TAF	Tenofovir alafenamide
TDF	Tenofovir disoproxil
WOCBP	Women of Childbearing Potential

1 Introduction

This Statistical Analysis Plan (SAP) describes the planned efficacy of switching from stable cART containing ABC/3TC or TAF/FTC plus dolutegravir or bictegravir to Delstrigo (TDF/3TC/doravirine) in people living with HIV.

It consists of two components:

1. The main part which provides a more statistical and medical description of the statistical analyses
2. The specifications of derived data which are part of the body text

The analysis plan is based on the following documents:

- **MetaD Trial Protocol v4.5**

The SAP contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary variables and other data.

This version of the SAP is the final description of how the analysis should be performed. All analyses discussed in this document will be included in the report and the format and content of the tables and appendices will be as defined in the templates.

All deviations fromthe analysis as planned in the protocol will be explained and justified in the report. This document has been written in accordance with the recommendations outlined in the International Conference on Harmonisation (ICH) E9 Guideline entitled ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials.

2 Trial Design and Objectives

2.1 Trial Objectives

Primary

To quantify the effect on lipid profile (change from baseline in total fasting cholesterol to week 24) of switching from suppressive, stable cART containing ABC/3TC or TAF/FTC plus dolutegravir or bictegravir to Delstrigo (TDF/3TC/DOR) in HIV positive patients.

Secondary

To evaluate up to 48 weeks: To investigate the effect of switch on:

- Body composition changes when measured by Total Body DXA and by waist circumference at week 24 and week 48
- Safety and tolerability of switch from stable cART to Delstrigo (TDF/3TC/DOR)
- Change in insulin sensitivity from baseline to week 24 and week 48 by HOMA-IR (glucose & insulin levels)
- PBMC cholesterol and cholesterol levels
- Adipocytokines by assessing adiponectin, leptin
- Pituitary hormones (TSH, LH, FSH, IGF-1, Testosterone)
- Estimated cardiovascular risk (QRISK3 and D:A:D equations)
- Hepatic steatosis and fibrosis by transient elastography-CAP
- Dietary, Quality of Life, and Sleep quality Questionnaires
- Renal safety by uPCR, eGFR

Potential Exploratory Objectives

Platelet aggregation & endothelial markers, metabolomics

2.2 Trial Registration

EudraCT No: 2021-006507-15

IRAS Number: 1004705

Clinical Trial gov: NCT05289986

2.3 Trial Design

Primary Endpoint

Lipid biochemistry in the blood of participants will be analysed at the baseline and compared to week 24 after switching to Delstrigo without a change in detectable HIV RNA levels. Should a change in HIV RNA levels be detected and confirmed, then the trial will stop for that participant.

Exploratory Endpoints

Changes in platelet aggregation & endothelial markers, and changes in metabolism as measured using metabolomics

Metabolomics: Investigation of the metabolic changes associated with study treatments at week 0, 48: Metabolomics can be used to profile the maximum number of metabolites found within an organism tissue, cell or biofluids, enabling detailed mapping of perturbed pathways involved in cART drug response. This approach has the potential to unravel on- and off-target effects of cART and further our understanding of mechanisms of action, metabolism and toxicities. With new safer antiretrovirals becoming available, it is important to understand what the drug effect could be on certain metabolic pathways, especially in view of people living with HIV ageing and needing access to such safer drugs. We therefore aim to carry out metabolomic analysis on plasma and urine samples from the patients enrolled in the study before drug switch and after to investigate the metabolic changes associated with the study treatments.

These studies will not be powered for drug/metabolomics changes associations but will enable us to build a data base on drug/metabolomics changes. Prospective studies would need to be planned based on these preliminary data.

2.4 Randomisation

Eligible individuals will be randomly assigned in a 1:1 ratio to start TDF/3TC/DOR immediately or to delay the switch to week 24. Randomisation will be performed using sealed envelopes, and in blocks of patients, allowing interim analysis of results in appropriate steps during the study. A participant identification number will be provided by the system to the person entering the data.

2.5 Sample Size Justification

It is anticipated that data from 60 participants are adequate to meet the trial objectives (30 in the immediate switch arm and 30 in the delayed switch arm). The study will be powered based on lipid fractions: TC, non-HDL cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and TC/HDL cholesterol ratio at week 24, using total cholesterol as the primary endpoint (Reference: Venter WF e*t al.* ADVANCE Study; IAS 2019. Abstr WEAB0405LB).

We expect total cholesterol decrease of approximately 12% we varied this between 70-95% based on other available estimates. Using the most conservative inputs, to detect total cholesterol decrease with 80% power and a 5% two-sided significance level, requires recruitment of 60 participants. This figure

allows for a 20% non-response rate (ROCKET study; Moyle G, *et al.*, doi:10.1371/journal.pone.0116297).

Additional sample size verification:

Table 1 Changes in primary (change in limb fat) and secondary endpoints in each treatment group over 48 weeks and difference between the two treatment rms.

Total cholesterol (mmol/l) **Mean (SD) change over 48 weeks**	Tenofovir DF	Abacavir
	-0.45 (1.06)	0.21 (1.13)

Reference
DOI: 10.1097/01.aids.0000247574.33998.03 Reference
A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy

Table 1 is from another study (UK based with a different treatment design at 48 weeks (i.e. not a two-arm switch/cross over study over 48 weeks); SDs for each group is 1.06 and 1.13 at 48 weeks. (The study Moyle G, *et al.*, doi:10.1371/journal.pone.0116297 was about week 12).

Sample size estimation will depend on SD, and if SD for Meta-D study is 1 for each group, 30 participants per group would be enough to detect total cholesterol change with 80% power and a 5% two-sided significance level (based on the study Moyle G, *et al.*, doi:10.1371/journal.pone.0116297).

In case with SD 1.01 and 1.1 (approximation of the study Table 2 of the paper), 33 participants will be required for each group.

3 General Analysis Definitions

Unless otherwise stated the 'statistical unit' in the statistical analyses is the 'subject'. No patient will be enrolled more than once. For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation, median, minimum and maximum. Minimum and maximum are presented to the same number of decimal places (dps) as the raw data, mean and median show one more decimal place (dp) than raw data and the standard deviation shows one/ two dps more than the raw data. Additionally, for selected variables the standard error of the mean (SEM) and the coefficient of variation (CV) and standard errors show one/ two dps more than the raw data.

Frequencies and percentages will be displayed for categorical data. Percentages by categories will be based on the number of subjects of the appropriate analysis population.
In case no data is available for the variable to be listed, tabulated or displayed graphically, e.g. no serious adverse event (SAE) within the trial, a planned output must still be created putting a comment on the output "There is no data to display for this table/listing/graph."

The repeated measures analysis of variance (ANOVA) or analysis of covariance (ANCOVA) will be conducted accordingly.

All statistical comparisons will be made using two sided tests at the α=0.05 significance level unless specifically stated otherwise. All null hypotheses will be of no (treatment) difference. P-values will be displayed to 2 or 3 decimal places and in the case that a p-value is <0.001 it will be displayed as "<0.001".

3.1 Trial period and definition

This is an open label, randomised, two-arm switch study over 48 weeks in which virally suppressed participants on a stable combined ART regimen will be randomised (1:1) to an immediate switch to TDF/3TC/DOR (immediate switch arm, N=30) for the duration of the 48-week study, or to maintaining their current cART followed by a switch to TDF/3TC/DOR from week 24 to week 48 (delayed switch arm, N=30). Participants will be monitored for the length of the study (48 weeks) plus a 30-day follow-up period.

After screening (≤ 28 days prior to Baseline Visit), trial participants will spend 48 weeks from baseline to final visit. There is a 30-day follow-up assessment scheduled.

3.2 Handling of Missing Data, Outliers, and Visit windows

3.3.1 Handling of Missing Data

If not specified, missing values will not be imputed. (If it is required, Multiple Imputation using multivariate normal distribution can be additionally conducted.)

No assessment or replacement of outliers will be performed.

3.3.2 Visit Windows

The trial protocol gives the overall trial schedule and the permissible intervals for these visits expressed as the number of days relative to Baseline. Analyses will not exclude patient data due to the patient's failure to comply with the visit schedule.

The time windows applied for the weekly averages of endpoints (e.g. from diary) will be as follows:
Baseline: day 1
Health check (immediate at week 4, or delayed at week 28): week 4 or week 28
FU visit: week 12, week 36
Mid-point visit: week 24
End of Study, or ETV: week 48

All visits are to be scheduled relative to the Baseline visit date, and with +/- 7 days visit window; and will occur at Weeks 12, 24, 36 and 48.

3.3 Definition of Subgroups, Interactions, Covariates

No adjustment of the significance level alpha is implemented, and inflation of type I error due to multiple testing may not be corrected.
Subgroup analyses will be performed for the full-analysis set (FAS) only.

4 Analysis sets

In accordance with ICH recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses: total set, safety set (SAF), full analysis set (FAS), valid cases set (VCS). These analysis populations must be identified by the Medical Responsible,the Co-ordinating Trial Manager and the Trial Statistician. The reason(s) for exclusion of patients from any analysis set must be documented, signed and filed in the Trial Master File(TMF) and reported in the review meeting (DRM) minutes.

4.1 Total set

This dataset will include all patients enrolled in the trial, regardless whether the patient discontinued prior to randomization (e.g. screening failures) or whether the patient received any IMP after randomization. Patients who satisfied inclusion criteria will be analyzed according to the treatment assigned at randomization. If the same patient has been enrolled – only the first admission will be analyzed.

4.2 Full Analysis set

The Full Analysis Set (FAS) consists of all randomized subjects who received a fixed dose combination tablet, containing combined ART regimen to an immediate switch to TDF/3TC/DOR (immediate switch arm, N=30) for the duration of the 48-week study, or to maintaining their current cART followed by a switch to TDF/3TC/DOR from week 24 to week 48 (delayed switch arm, N=30).

The intention-to-treat (ITT) analysis is based on the FAS and will be conducted for the primary analysis of the primary endpoint. The ITT analysis will also be used for robustnessand sensitivity analyses performed to support the primary analysis of the primary endpointand it will be applied for all secondary efficacy endpoints. The FAS/ITT will be used for summaries of selected demographic and baseline characteristics.

Subjects will be assessed and analysed as a member of the treatment group to which they were randomised (‘as randomised’ analysis). In case patients were randomised and treated more than once by mistake, only the first randomisation (and treatment) of the patient will be included in the FAS.

4.3 Valid Case Set

This analysis set is only foreseen to be specified for the initial approach, excluding data beyond the initial study period from re-enrolled subjects. The valid case set (VCS) consists therefore of all patients of the initial approach FAS without any major protocol deviations. In this clinical trial, protocol deviations that would lead to exclusion from the VCS include but are not limited to exclusion criteria E1 to E5 (criteria affecting read-out parameters). Patients discontinuing prematurely will be included in the VCS provided that none of these major protocol deviations had occurred before discontinuation.

The per-protocol (PP) analysis is based on the VCS and will only be conducted as a supportive analysis of the primary endpoint. Also, the VCS/PP will be used for summaries of selected demographic and baseline characteristics.

Subjects will be assessed and analyzed as a member of the treatment group to which theywere randomized (‘as randomized’ analysis). In the VCS this is identical to the treatment group ‘as treated’ and reflects the first/initial and only the first/initial randomized treatment.

4.3.1 Major protocol violations

According to the trial protocol, the following violations are major protocol violations:

- Violation of inclusion/randomisation criteria as described in the trial protocol.

Final decisions on other major protocol violations will be made during the DSMC and should include any of the following violations:

- Entered the trial even though they did not satisfy the entry criteria
- Developed withdrawal criteria during the trial but were not withdrawn
- Incorrect treatment allocation
- Treatment compliance with GCP and regulatory agency guidelines during the treatment period
- Administration of forbidden concomitant medication as described in the trial protocol

Further major protocol violations might be identified during the treatment review.
In summaries, a patient with major protocol violations will be defined as a protocol violator (PV), whereas those who did not have any major protocol violations will be termed protocolcorrect (PC).

4.3.2 Treatment allocations and mis-randomisations

For the decision whether a patient was randomized or not the entry on the Randomization criteria check on Day 1 from the CRF at the question “Was the subject randomized?” (YES/NO) is decisive. If this question was not answered despite querying the investigator, the provided randomization number on the CRF and last the drug accountabilitydata are considered. More precisely, a provided randomization number or an IMP being dispensed according to drug accountability CRF, will be considered to indicate a patient was randomized. In case a patient’s data on treatment according to randomization and dispensed IMP differ, the IMP that is captured in the CRF will be considered as the IMP the patient received (as treated).

4.4 Safety Analysis Set

The safety analysis set (SAF) consists of all randomized subjects who received at least onedose of IMP. Subjects in the safety set for whom data is not reported after first dose of IMP are still considered as part of the safety set but are not included in the statistical summary (i.e., the missing records for these subjects would reduce the size of the population for the corresponding data evaluation, so that e.g. percentages are calculated based on the safetyset subjects with available safety records.)

The SAF will be used by default for all summaries of safety and tolerability related variables.The SAF should however be used for selected summaries of demographic and baseline characteristics only if corresponding FAS evaluation does not provide sufficient insight into the data (e.g. when total size of SAF and FAS differ by more than 3%). All adverse events will be listed and flagged as either treatment emergent (TE) or pre-treatment (P).

Comments regarding initial and extended approach FAS also apply for SAF. Subjects will beassigned to and analyzed in the treatment group that they actually received ('as treated' analysis). Multiple treated patients will be included for all treatments received.

4.5 Other analysis sets

Patients participating in measurements of arterial stiffness are those patients meeting all of the inclusion criteria and none of the exclusion criteria and agreeing to participate in the measurements of arterial stiffness. Patients who signed a separate PI and IC for the measurements of arterial stiffness will be included in this specific analysis set in the sense ofa subpopulation to the FAS. As part of the efficacy assessment, treatment allocation principles follow those described above.

5 Disposition of Subjects

Screening is defined as the post-consent assessment of whether patient data match with theclinical trials inclusion and exclusion criteria. A case report form (CRF) is required and should be completed for each patient having signed the IC and attending Day 1. All patients who attend screening must be listed on the respective paper form provided by the sponsor's representative.

For those patients who fail screening, the Eligibility Form must be completed with appropriate reason for discontinuation in the CRF. Discontinuations, apart from consent withdrawal, that occur prior to randomization are to be considered as "screening failures".

The following subject data will be summarized based on the total set and presented for/byeach treatment group (if not explicitly stated otherwise):

- Number and percentage of subjects screened, randomized, not randomized, their treatment assignment by site status overall (and not by treatment group);
- Number and percentage of subjects in each analysis set, additionally showing overall numbers;
- Number and percentage of subjects prematurely discontinued (with reasons) respectively completed, additionally showing overall numbers;

- Number and percentage of subjects who prematurely discontinued (with reasons) respectively completed, by trial periods, based on the FAS, additionally showing overall numbers

A listing will be provided (by enrolment season/run) showing all subjects' randomization and disposition data as well as their status (and related data).

6 Protocol Deviations

The trial is conducted in compliance with the protocol, GCP and the applicable regulatory requirements. All deviations from the statistical analysis plan will be recorded during the conduct of the interim and final analysis. If there is a decision for major modifications to the statistical analysis while the clinical trial is in progress, this will be part of a protocol amendment.

7 Trial population

7.1 Demographics and baseline characteristics

Demographic and baseline characteristics are those variables measured on or prior to Day 1 such as age, sex, race, body mass index (BMI), and lipids.
They will be analyzed using summary statistics, but no statistical testing will be performed. More precisely:
Descriptive statistics (n, mean, SD, median, min, max) for continuous variables will be presented by treatment group and overall:

- Age [years]
- Vital Signs such as Systolic/diastolic pressure at baseline/ changes
- CD4 and CD4:8 ratio from baseline to week 24 and week 48
- Lipids & estimated cardiovascular risk changes
- Waist circumference and/or Weight/ BMI changes

In addition,

- Changes (baseline to week 24 and week 48) in adipocytokines
- Changes in pituitary hormones
- Changes in estimated cardiovascular risk
- Changes in hepatic steatosis and fibrosis
- Changes in dietary, Quality of Life, and Sleep quality
- Changes in renal safety

Frequencies and percentages will be presented by category for categorical variables bytreatment group and overall:

- Gender [female / male]
- BMI [< 18.5/ 18.5-<25/ 25-<30/ 30-<35/ > 35]
- Ethnicity

All the above variables are either recorded on the eCRF, or else derived.

7.2 Medical history, concomitant medication and physical examination

The following evaluations must be performed within 42 days prior to study randomisation at baseline:

- Assessment of subject eligibility according to the inclusion and exclusion criteria
- Medical and social history (past and current), including HIV-associated conditions
- Full antiretroviral history (including resistance history i.e. record all resistance history by major mutations in NRTI, NNRTI, PI and II regions).
- Review of non-antiretroviral medication (any medication taken within the last 30 days)
- Concomitant medication check
- Physical examination
- Height, weight, waist circumference
- Vital signs (Temperature, Blood Pressure, Heart Rate and Respiratory Rate)
- HBV & HCV testing
- Whole blood cells count
- Creatinine, AST, ALT, urine protein
- HIV RNA viral load
- Urine or serum pregnancy test for WOCBP
- CD4 & CD8 count / % and ratio
- Urinalysis

8 Treatment compliance

Prior to the enrolment of participants, approval will be obtained from the NHS Research Ethics Committee (REC) and CAs for the conduct of the study at named sites, the protocol, the PIS and ICF, any other written information that will be provided to individuals before or when they are participants, and of any advertisements that will be used. The study will be conducted in compliance with this protocol, GCP, and all applicable regulatory requirements.

9 Efficacy

9.1 General considerations

A DSMB will be appointed to monitor this study and draw up their own charter and decide when they will meet based on the protocol. TSC will request that there is meeting when 50% of participants have reached 24 weeks to look at efficacy and safety, DSMB may also want to meet before or after that.
Decisions on stopping the study will be based on a combined evaluation of the efficacy and safety results.
The primary population for analysis will be Intent to Treat exposed, including all randomised patients who have received at least one dose of study medication. The analyses of safety will also be conducted on the Intent to Treat exposed population.

9.2 Efficacy variable(s)

The primary efficacy analysis will be repeated for the Per Protocol population, excluding patients with serious protocol violations which could interfere with the reliability of the efficacy outcome. Examples would include taking other antiretroviral treatment within the first 48 weeks of treatment, incorrect randomisation or early discontinuation from the study for reasons unconnected to efficacy or safety. This analysis will be conducted to check the reliability of the results from the primary efficacy analysis.

10 Safety

10.1 General considerations

Any significant worsening noted during interim or final physical examinations, or any other potential safety assessment required or not required by protocol should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

10.2 Adverse events

A serious adverse event is any untoward medical occurrence that at any dose:

i) Results in death
ii) Is life-threatening
iii) Requires in-patient hospitalisation or prolongation of existing hospitalisation
iv) Results in persistent or significant disability/ incapacity
v) Is a congenital anomaly/ birth defect

OR

vi) Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject, or may require intervention [e.g. medical, surgical] to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

Although pregnancy is not always serious by regulatory definition, for the purposes of this trial pregnancy must be handled as SAEs.
Any component of a study endpoint that is considered related to study therapy (e.g. death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) should be reported as SAE.

10.3 Clinical laboratory variables, Haematology, Pregnancy, CT and ECG

Blood samples for all biochemistry, haematology (including immunology) and virology (primary and secondary endpoints) assessments should be collected in accordance with routine clinical practice, according to local laboratory requirements.

10.4 Clinical observation / examination

Processing of samples:

The purpose of this section is to describe the procedures involved in the study for the collection, processing, storage, and shipment of the study's biological samples.

The samples include blood, plasma and urine.
Samples for **exploratory endpoints**:

Processing of samples:
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Blood samples for all biochemistry, haematology (including immunology) and virology (primary and secondary endpoints) assessments should be collected in accordance with routine clinical practice, according to local laboratory requirements.

11 Interim analyses

An interim analysis may be done at week 24 (mid-point visit) and/or week 36. Furthermore, a DSMB will be established and will review the data regularly during the study. Additional issues could include treatment-emergent drug resistance, serious adverse events, or high rates of discontinuation for adverse events.

12 Deviations from planned analysis in protocol

To be filed.

13 Planned Tables, Figures and Listings

This section lists tables, and/or figures and listings to be produced for inclusion in the Clinical Trial Report (CTR).

	Arm 1	**Arm 2**
Demographics		
Male (%)		
Age (years, SD)		
Race		
CD4 & CD8 (count / % and ratio)		
⋮		
Primary Outcomes		
(Changes from baseline to week 24 and week 48)		
Total fasting cholesterol		
Secondary Outcomes		
(Changes from baseline at week 24 and week 48)		
Body composition		
Insulin sensitivity		
PBMC cholesterol and cholesterol levels		
Adipocytokines by assessing adiponectin and leptin		
Pituitary hormones		
Estimated cardiovascular risk		
Hepatic steatosis and fibrosis		

Dietary consumption
Quality of Life
Sleep quality
Renal safety

*Some additional analyses could be conducted if it is needed. The planned sample is maybe underpowered. However, a different way of calculation (or something similar) will be need to get a robust outcome.

14 Appendices

14.1 Statistical monitoring plan(s)

Prior (1 or 2 weeks earlier) to the Primary and Secondary endpoint analyses:

- Standard deviation (SD) of measures and Outlier(s) across visits for each subject for vital signs and key laboratory parameters of the study will be conducted.

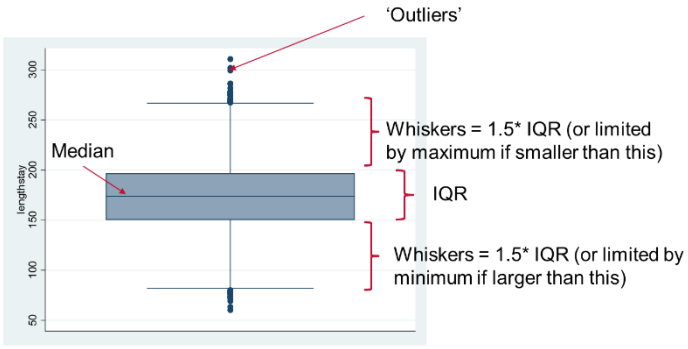

Figure 1 Box plot for continuous laboratory parameter

- Subject-, site-, and sub-group level correlations will be conducted between blood pressure measurements (SBP, DBP), and lipid profiles (e.g. total fasting cholesterol, total body DXC, waist circumference). For instance, a scatter plot of subject-level correlations between lipid profiles (for different types of sites) can be reported in terms of sensitivity and specificity.

- Additional algorithm(s) for detecting fake data per subject- and site-level can be created (if it is needed).