Chelsea and Westminster Hospital
NHS Foundation Trust

CLINICAL TRIAL PROTOCOL

Protocol Title:	**The effect of dolutegravir on whole-body insulin sensitivity, lipid and endocrine profile in healthy volunteers**
Protocol Number:	**CRF003**
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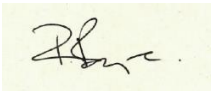
Sponsor:	Chelsea and Westminster Hospital NHS Foundation Trust 369 Fulham Road SW10 9NH London

Protocol Approval: 

Dr Ruth Byrne

20 Jan 2023
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GCP Compliance Statement:
This trial will be conducted in compliance with the protocol, the principles that have their origin in the Declaration of Helsinki and all applicable regulatory requirements

Synopsis

Name of Investigational Product:	Dolutegravir
Name of Active Ingredients:	Dolutegravir
Study Title:	The effect of dolutegravir on whole-body insulin sensitivity, lipid and endocrine profile in healthy volunteers
Phase of Study:	Phase I
Objectives:	The objectives of this study are:

Primary

To quantify the difference in change in insulin sensitivity (determined by peripheral glucose uptake using a euglycaemic clamp) with the administration of dolutegravir (DTG) compared to no DTG for 28 days in HIV seronegative healthy volunteers.

Secondary

To investigate the effect of the dolutegravir on:

1. Adipocytokines by assessing adiponectin and leptin levels
2. Ghrelin
3. Pituitary hormones
4. Lipid profile including lipid fractions
5. Changes in indirect calorimetry
6. Changes in food intake by food diaries
7. Change in sleep parameters by sleep questionnaires
8. Safety and tolerability of dolutegravir

Study Design	Randomised, crossover
Indication:	HIV negative volunteers
Methodology:	Insulin sensitivity determined by glucose disposal during steady state of euglycaemic clamp.
Planned Sample Size:	16
Summary of Eligibility Criteria:	• HIV negative subjects • Clinically well • BMI between 18 and 30kg/m^2 • Free from diabetes mellitus and metabolic syndrome • Not receiving any metabolically active medication or drugs likely to interact with antiretroviral therapy • Able and willing to provide informed consent
Number of Study Centres	One
Duration of Treatment:	28 days per sequence with washout between sequences
Dose and Route of Administration:	Orally administered dolutegravir 50 mg once daily
Criteria for Evaluation:	Subjects will undergo three euglycaemic clamp procedures in order to determine the extent of glucose disposal. The first clamp will be performed prior to the commencement of the first study drug administration/no treatment, the second one following four weeks of study drug/no treatment, the third after the crossover phase to study drug/no treatment.
Primary Endpoint:	Change from baseline in total body glucose disposal by euglycaemic clamp method following 28 days treatment

Secondary Endpoints:

- Change from baseline in serum levels of fasting cholesterol fractions and triglycerides
- Changes from baseline in serum adipocytokines, ghrelin and pituitary hormones
- Changes from baseline in indirect calorimetry
- Caloric intake by food diaries
- Quality of sleep by sleep questionnaires
- Safety data including the number and severity of adverse events, as assessed by the medical dictionary for regulatory activities (MedDRA), from baseline to day 100

General Study Information

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List of abbreviations and definitions of terms

ACTG	AIDS Clinical Trial Group
AE	Adverse event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body Mass Index
CM	Concomitant Medication
CRF	Case Report Form
DAIDS	Division of AIDS
ECG	Electrocardiogram
FBC	Full blood count
GCP	Good Clinical Practice
GP	General Practitioner
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
ICH-GCP	International conference of harmonization good clinical practice
IMP	Investigational Medicinal Product
LFT	Liver function test
MHRA	Medicines and Healthcare products Regulatory Agency
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
OD	Once daily
PI	Protease inhibitors

PLWH	People living with HIV
SAE	Serious Adverse Event
SmPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOPS	The over-volunteering prevention scheme
UK	United Kingdom
WOCBP	Women of Child Bearing Potential

TABLE OF CONTENTS

1. Background Information

1.1 Introduction & background

Metabolic changes and weight increase commonly occur early in ART. Weight gain observed in many patients shortly after ART initiation is thought to be due in part to a reduction in basal metabolic rate following suppression of plasma viremia, improved appetite, lower inflammatory cytokine effects and a reduction in the rate of protein turnover [1, 2].

Multiple studies in healthy volunteers have demonstrated acute onset of reversible insulin resistance, as assessed by euglycaemic hyperinsulinaemic clamp, inhibition of glucose transporters, in particular GLUT4, with protease inhibitors (PIs) and difference in effects on insulin resistance and other metabolic parameters is seen as a key differentiator of agents within the PI class. Other antiretroviral drug classes have also been extensively tested. Clinical cohort data and data from clinical trials have reported an association between some nucleoside reverse transcriptase inhibitors (NRTIs) (stavudine, zidovudine, didanosine) and insulin resistance or diabetes mellitus. As mitochondrial dysfunction has been proposed as playing a role in the pathogenesis of diabetes mellitus, the association with drugs which may impact mitochondrial DNA and diabetes is not surprising. Tenofovir DF use does not cause insulin resistance when investigated with euglycemic clamp methodology [3].

A recent case report of acute onset diabetes mellitus after a switch to an Integrase Strand Transfer Inhibitor (INSTI)-containing regimen (raltegravir/abacavir/lamivudine) postulated effects on bioavailable magnesium could alter insulin signalling characteristics [4].

The association between insulin resistance and body shape changes and weight gain during therapy has not been established. Insulin resistance triggered by some ARV drugs is apparently reversible, following switching to an agent not associated with insulin resistance. However, reduction in central adiposity has only been observed in highly selected subject, [5] weight gain in the setting of HIV may not be exclusively related to glucose and/ or fat handling, but may also relate to alterations in endocrine function, therefore we proposed study to investigate changes in insulin resistance, lipid metabolism and complete endocrine profile in subject exposed to dolutegravir to investigate the role all these different factors may potentially have in weight gain recently reported in clinical cohorts.

1.2 Rationale

In a retrospective analysis in the NA-ACCORD, 22% of those with normal BMI at the start of anti-retroviral therapy (ART) became overweight after 3 years of ART, and 18% of those overweight at baseline becoming obese [1]. And a recent retrospective analysis by Menard et al [6] on patients stopping dolutegravir (DTG) due to adverse effects in the clinical setting, found that 7% had done so due to weight gain. Norwood and colleagues [7] has reported significantly increased weight gain in those switching to an INSTI-based regimen from an efavirenz-based (Atripla) based regimen. This weight gain was greatest among patients switching to DTG/ABC/3TC [7]. The ethology of weight gain in patients with undetectable plasma HIV RNA who change to another regimen and remain suppressed is unclear. An abrupt reduction in insulin sensitivity could

promote storage of excess circulating glucose and lipids in adipose tissue, but this is was speculative until now, therefor we propose the study of insulin sensitivity to address the underlining reasons for weight change in patients receiving dolutegravir based therapy.

Other studies have used a range of ART for 28 days in healthy volunteers looking at similar metabolic, coagulation and inflammatory markers and pharmacokinetic [8-11]. Given DTG half-life we wished to establish steady state to adequately examine the effect of a drug on weight gain, lipids and neuroendocrine markers. 28 days of DTG is Centre for Disease Control (CDC) recommended post exposure prophylaxis (PEP), therefore this duration of dosing is wildly used and recommended in HIV-negative individuals [12].

HYPOTHESIS: There is no difference in change in insulin resistance and multiple lipid parameters in healthy volunteers when treated with dolutegravir compared with baseline values.

2. Trial Objectives

2.1 Primary Objective

To quantify the difference in change in insulin sensitivity (determined by peripheral glucose uptake using a euglycaemic clamp) with the administration of dolutegravir (DTG) compared to no DTG for 28 days in HIV-seronegative healthy volunteers.

2.2 Secondary Objectives

To investigate the effect of the dolutegravir on:

1. Adipocytokines by assessing fasting adiponectin and leptin levels
2. Fasting Ghrelin
3. Pituitary hormones
4. Lipid profile including lipid fractions
5. Changes in indirect calorimetry
6. Changes in food intake by food diaries
7. Change in sleep parameters by sleep questionnaires
8. Safety and tolerability of dolutegravir

3. Trial Design

3.1 Endpoints

Primary Endpoint

- Change from baseline in total body glucose disposal by euglycaemic clamp method with 28 days dolutegravir treatment.

Secondary Endpoints

- Change from baseline in serum levels of fasting cholesterol and triglycerides
- Changes in adipocytokines, ghrelin and pituitary hormones
- Changes in indirect calorimetry
- Changes in food intake by food questionnaires
- Changes in sleep quality by sleep questionnaires
- Safety data including the number and severity of adverse events, as assessed by the medical dictionary for regulatory activities (MedDRA), from baseline to day 100

3.2 Study design

This is a 72-day (excluding screening period and follow up phone call) randomized study with two arms. The study will utilize a crossover design as described below.

Subjects will be randomised to start on dolutegravir 50 mg once daily OR no treatment for the first 28 day dosing phase of the study, then will cross over to the alternative for the second dosing phase, following a 2 week washout period (equivalent to 5+ dolutegravir elimination half-lives). Subjects will be contacted approximately seven days after the final euglycaemic clamp visit for a telephone follow up.

- **Day -28 to 0:** Screening
- **Day 1 (am):** Euglycaemic clamp 1
- **Day 1 (pm) to 28:** Dolutegravir (n=8)/Control (n=8)
- **Day 28 (am):** Euglycaemic clamp 2
- **Day 29 to 43:** Wash out (14 days)
- **Day 44 to 72:** Control (n=8)/Dolutegravir (n=8)
- **Day 72 (am):** Euglycaemic clamp 3
- **Day 100** Post study telephone contact

3.3 Randomisation

After screening, eligible subjects satisfying the inclusion and exclusion criteria will be randomised 1:1 to one of two arms:

Group 1:

- Dolutegravir 50 mg once daily for the first 28 days of the study.
- No treatment for the last 44 days of the study.

Group 2:

- No treatment for the first 43 days of the study.
- Dolutegravir 50 mg once daily for the last 28 days of the study (day 44-72).

3.4 Benefit: Risk Assessment

- Key identified and potential risks of DTG and the clamp procedure are summarized in Appendix 4. More detailed information about the known and expected risks and reasonably expected adverse events (AEs) of DTG may be found in the SmPC for Tivicay. (Appendix 4)

4. Subject Population

4.1 Number of subjects and subject selection

Subjects will be HIV-1 negative volunteers aged between 18 to 60 years inclusive. The total number of subjects recruited will be 16 (8 per group)

4.1.1 Inclusion Criteria

- Willing and able to provide informed consent
- Cis-Male and Cis-Female healthy subjects without underlying conditions
- Subjects must have documented negative HIV serology by ELISA and P24 antigen and not receiving anti-HIV pre-exposure prophylaxis (PreP)
- Subjects must be clinically well volunteers aged between 18 to 60 years with BMI <30 kg/m^2 but >18 kg/m2
- Healthy, as determined by the investigator or medically qualified designee based on a medical evaluation, including medical history, physical examination, laboratory tests, and cardiac evaluation (including ECG)
- Non-fasting blood glucose, total cholesterol and triglycerides within normal limits
- Subjects should have complete blood count (FBC) with normal differential and platelet count (detail below of specified normal range, table 1)

Table 1 – Compete FBC with normal differential & platelets count ranges		
Test	Male Normal Range	Female Normal Range
Haemoglobin (g/L)	130-168	114-150
White blood cell count (x10^9/L)	4.2-10.6	4.2-11.2
Neutrophil count (x10^9/L)	2.0 - 7.1	
Lymphocyte count (x10^9/L)	1.1 - 3.6	
Monocyte count (x10^9/L)	0.2 - 0.9	
Eosinophil count (x10^9/L)	0.0 - 0.5	
Basophil count (x10^9/L)	0.0 - 0.2	

- A female, may be eligible to enter and participate in the study if she:
    - is of non-child-bearing potential defined as either post-menopausal (12 months of spontaneous amenorrhea and ≥45 years of age) or physically incapable of becoming pregnant with documented tubal ligation, hysterectomy or bilateral oophorectomy or,
    - is of child-bearing potential with a negative pregnancy test at both Screening and Day 1 and agrees to use one of the following methods of contraception to avoid pregnancy:
    - Complete abstinence from penile-vaginal intercourse. Abstinence is acceptable only as true abstinence when this is in line with the preferred and usual lifestyle of the participant;
    - Any intrauterine device with published data showing that the expected failure rate is <1% per year (not all intrauterine devices meet this criterion, see Appendix 6] for an example listing of approved intrauterine devices);
    - Male partner sterilization confirmed prior to the female subject's entry into the study, and this male is the sole partner for that subject;
    - Approved hormonal contraception (see Appendix 6] for a listing of examples of approved hormonal contraception)*;
    - Any other method with published data showing that the expected failure rate is <1% per year
- Men who have partners who are women of childbearing potential (WOCBP – definition in Appendix 6) must be using an adequate method of contraception to avoid pregnancy in their partner throughout the study and for a period of at least 4 weeks after the study;
    - Complete abstinence from penile-vaginal intercourse. Abstinence is acceptable only as true abstinence when this is in line with the preferred and usual lifestyle of the patient;
    - Double barrier method (male condom/spermicide, male condom/diaphragm, diaphragm/spermicide);

- Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year (not all IUDs meet this criterion, see Appendix 4 for an example listing of approved IUDs) plus male condom;
- Sterilisation confirmed prior to the subject's entry into the study
- Approved hormonal contraception used by female partner (see protocol appendix 4 for a listing of examples of approved hormonal contraception) plus male condom;
- Any other method with published data showing that the expected failure rate is <1% per year and not containing hormones plus male condom.
- Any contraception method must be used consistently, in accordance with the approved product label and for at least four weeks after discontinuation of IMP (Appendix 6).

Any contraception method must be used consistently, in accordance with the approved product label and for at least 28 days prior to the first dose of study medication and 4 weeks after discontinuing the study medication.

4.1.2 Exclusion Criteria

- Subjects with a waist hip ratio > 0.97 or BMI > 30kg/m^2 and BMI <18 kg/m^2 will be excluded
- Acute or chronic hepatitis B infection (determined by positive hepatitis B surface antigen result at the screening visit)
- Acute or chronic hepatitis C infection (determined by positive hepatitis C antibody result at the screening visit)
- Diabetes mellitus, other metabolic syndrome or disease process in the opinion of the investigator likely to cause marked disturbance in glucose and lipid homeostasis including hypertension. Subject with HbA1c >42 mmol/mol will be excluded.
- History or presence of allergy to the dolutegravir
- ALT or AST greater than or equal to 1.5 x Upper Limit of Normal (ULN) and total bilirubin greater than or equal to 1.5 x ULN excluded;
- Pregnancy and breastfeeding women
- Alcohol consumption >10 units/week
- Clinically relevant drug use (positive urine drug screen) or history of alcohol or drug use considered by the Investigator to be sufficient to hinder compliance with treatment, follow-up procedures or evaluation of adverse events. Smoking is permitted, but tobacco intake should remain consistent throughout the study.
- Unable to refrain from the use of prescription (e.g., dofetilide) or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's wort) within 7

days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives prior to the baseline visit and throughout the study until the follow-up period, unless in the opinion of the Investigator the medication will not interfere with the study procedures or compromise participant safety.

- This includes on-going therapy with any of the following

  - Metabolically active medications
  - Any lipid-lowering medication
  -Any testosterones treatments or supplements - Glucocorticoids including inhaled steroids except for 'as necessary' use
  - Beta-blockers
  - Thiazide diuretics and indapamide
  - Thyroid preparations
  - Psychotropic agents
  - Anabolic steroids
  - Megestrol acetate
  - Dofetilide (or pilsicainide)

4.2 Duration of Involvement

Total duration of involvement in the study is up to 72 days, plus a screening visit within 28 days prior to day 1 and follow up telephone call approximately 28 days after the last euglycaemic clamp.

4.3 Withdrawal of Subjects and Discontinuation Criteria

Subjects have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw subjects from the study in the event of intercurrent illness, adverse events, protocol violations, administrative reasons or other reasons. An excessive rate of withdrawals can render the study impossible to interpret. Therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

The investigator should contact the subject either by telephone or through a personal visit or a responsible relative must be contacted to determine as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an adverse event or an abnormal laboratory test result, the principal specific event or test will be recorded on the Case Report Form.

A subject will be considered to have withdrawn from the study if they do not complete all euglycaemic clamp procedures (day 1, 28 and day 72).

Subjects may be replaced at the discretion of the investigator.

5. Treatment of subjects

5.1. Study Treatment Regimen

Group 1

Subjects will receive dolutegravir 50 mg once daily from day 1 for 28 days.

On the evening after the second clamp (day 28) they will cease treatment and will continue study activities for following 44 days.

Group 2

Subjects will receive no treatment from day 1 for 43 days on the day 44 they will initiate dolutegravir 50 mg once daily for 28 days (until day 72).

Dolutegravir will be dispensed by the Chelsea and Westminster NHS Trust pharmacy labelled as clinical trial supply.

5.2 Treatment Compliance

Adherence to investigational product will be evaluated using pill counts of returned / unused investigational product. This assessment will be documented in the drug accountability record.

6. Visit schedule & Procedures

The schedule of assessments is summarised in Appendix 1.

Screening Visit

The screening visit should take place within 28 days prior to study day 1.

Subjects will be provided with written information about the study in the form of a subject information sheet and will be allowed adequate time for questions and to consider the study before agreeing to participate. It will be the responsibility of the investigator or co-investigator to obtain written informed consent prior to undertaking any procedures detailed in the protocol.

The investigator or designee must provide adequate explanation of the aims, methods, objectives and potential hazards of the study. It must also be explained to the subject that they are free to refuse or withdraw from the study for any reason without detriment to their future care or treatment.

The following information and procedures will be recorded and performed as part of the screening assessments:

- Written Informed Consent
- Medical and medication history, including smoking and substance history
- Demographics; gender, ethnic origin, age, height, weight, and body mass index (BMI)
- Vital signs: temperature, blood pressure, heart rate, respiration rate, oxygen saturation
- Physical examination including measurement of waist and hip size

- Haematology complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Pregnancy testing and last menstrual period (LMP) date in WOCBP subjects
- Biochemistry safety labs assessment for standard clinical chemistry (glucose, creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin, CRP), including HbA1c
- HIV antibody testing, hepatitis C (antibody) and B (surface antigen) screening
- ECG
- Urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) and urine drug screen

Baseline

Subjects who meet all the inclusion and exclusion criteria and wish to take part in the study will be admitted to the unit on the morning of day 1. Subjects will remain in the unit for approximately four to six hours.

The following study assessments will be performed (subjects must have fasted for at least eight hours):

- Adverse events
- Concomitant medication
- Vital signs (during the clamp procedure vital signs will occur every 30 minutes)
- Height/ weight/ BMI
- Measurement of waist and hip size
- Haematology: complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Biochemistry safety labs assessment for standard clinical chemistry (fasting glucose, creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), including thyroid hormones (TSH, ft4), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) CRP and Hb1aC
- Urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) and urine drug screen
- Pregnancy testing and LMP date in WOCBP subjects
- Adiponectin, leptin, ghrelin, testosterone
- Pituitary hormone function tests

- Dietary Questionnaire
- Sleep Questionnaires (PSQI)
- Euglycaemic clamp 1
- Insulin will be titrated 3 times during euglycaemic clamp procedure according to approved and verified formula sheet.
- ECG monitoring at 30 minute intervals during the clamp procedure
- Indirect calorimetry (all participants)

Prior to baseline visit subjects will be randomly assigned to Group 1 or Group 2:

Group 1:

- Dolutegravir 50 mg once daily for the first 28 days of the study.
- No treatment for the last 44 days of the study.

Subject will be dispensed study medication (Dolutegravir 50 mg) and instructed to self-administer the study drugs at home from the morning of day 2 until the morning prior to the next euglycaemic clamp day (Day 27).

Group 2:

- No treatment for the first 43 days of the study.
- Dolutegravir 50 mg once daily for the last 28 days of the study (day 44-72).

Subject will be dispensed study medication (Dolutegravir 50 mg) and instructed to self-administer the study drugs at home from the morning of day 45 until the morning prior to the next euglycaemic clamp day (Day 71).

Day 28

Subjects will be admitted to the unit in the morning and remain on the unit for approximately four to six hours. Group 1 subjects will take their last dose of study medication in the unit in the morning of the visit.

The following study assessments will be performed (subjects will have fasted for at least eight hours):

- Adverse events
- Concomitant medication
- Vital signs (during the clamp procedure vital signs will occur every 30 minutes)
- Height/ weight/ BMI
- Measurement of waist and hip size

- Haematology: complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Biochemistry safety labs assessment for standard clinical chemistry (fasting glucose,creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), including thyroid hormones (TSH, ft4), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) CRP and HbA1c
- Urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) and urine drug screen
- Pregnancy testing and LMP date in WOCBP subjects
- Adiponectin, leptin, ghrelin, testosterone
- Pituitary hormone function tests
- Dietary Questionnaire
- Sleep Questionnaires (PSQI)
- Euglycaemic clamp 2
- Insulin will be titrated 3 times during euglycaemic clamp procedure according to approved and verified formula sheet.
- ECG monitoring at 30 minute intervals during the clamp procedure
- Indirect calorimetry (Group 1)

Day 29 - Day 43

Wash out Period

Day 44

Subjects will be admitted to the unit in the morning and remain on the unit for approximately one to two hours. Subjects randomized to Group 2 will take their first dose of study medication in the unit in the morning of the visit.

The following study assessments will be performed (subjects must have fasted for at least 8 hours):

- Adverse events
- Concomitant medication
- Vital signs: temperature, blood pressure, heart rate, respiration rate, oxygen saturation
- Height/ weight/ BMI

- Measurement of waist and hip size
- Haematology: complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Biochemistry safety labs assessment for standard clinical chemistry (fasting glucose, creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), including thyroid hormones (TSH, ft4), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) CRP and HbA1c
- Urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) and urine drug screen
- Pregnancy testing and LMP date in WOCBP subjects
- Adiponectin, leptin, ghrelin, testosterone
- Pituitary hormone function tests
- Dietary Questionnaire
- Sleep Questionnaires (PSQI)

Day 72

Subjects will be admitted to the unit in the morning and remain on the unit for approximately four to six hours. Subjects will take their last dose of study medication (Group 2) in the unit in the morning of the visit.

The following study assessments will be performed (subjects must have fasted for at least 8 hours):

- Adverse events
- Concomitant medication
- Height/ weight/ BMI
- Vital signs (during the clamp procedure vital signs will occur every 30 minutes)
- Measurement of waist and hip size
- Haematology: complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Biochemistry safety labs assessment for standard clinical chemistry (fasting glucose, creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), including thyroid hormones (TSH, ft4), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) CRP and Hb1aC

- Urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) and urine drug screen
- Pregnancy testing and LMP date in WOCBP subjects
- Adiponectin, leptin, ghrelin, testosteron
- Pituitary hormone function tests
- Dietary Questionnaire
- Sleep Questionnaires (PSQI)
- Euglycaemic clamp 3
- Insulin will be titrated 3 times during euglycaemic clamp procedure according to approved and verified formula sheet.
- ECG monitoring at 30 minute intervals during the clamp procedure
- Indirect calorimetry (Group 2)

Follow up (Day 100)

The subject will be contacted by telephone approximately 28 days after the final euglycaemic clamp day to check whether they have experienced any adverse events. This follow up phone call should be documented in the subject's notes. WOCBP should take a home pregnancy test.

Early termination visit

If a subject withdraws or is discontinued from the study the subject should attend if at all possible for a discontinuation visit and undergo the following procedures:

- Haematology: complete blood count (FBC) with differential and platelet count (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
- Biochemistry safety labs assessment for standard clinical chemistry (fasting glucose,creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), including thyroid hormones (TSH, ft4), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) CRP and Hb1aC
- Symptom-directed physical examination (if any adverse event experienced)

6.1 Euglycaemic Clamp

Following completion of the procedures mentioned above on days 1, 28 and 72 a euglycaemic clamp study will be performed to determine the extent of total and non-oxidative glucose disposal. After an overnight fast of at least 8 hours, the subject will be admitted to the Clinical Research Facility. This procedure will take approximately 3 hours. A primed/ continuous intravenous infusion of insulin, through an intravenous cannula, will commence in the morning at

an infusion rate dictated by the verified formula sheet, maintaining a mid-physiological level of hyperinsulinaemia for 180 minutes . Whole blood glucose will be assayed from a separate intraveneous cannula every 5 minutes. A negative feedback formula will be used to adjust the rate of infusion of 20 % dextrose in order to maintain euglycaemia at 4.5 mmol.L^{-1}. Whole blood potassium and haematocrit is measured at 30 minute intervals during the clamp procedure with electrocardiographic monitoring half hourly in order to monitor for changes towards hypokalaemia.

At 180 min, the insulin and glucose infusion is stopped immediately, and the subject fed. A final blood sugar, potassium and haematocrit level, and vital signs check will be performed before the subject is discharged from the unit.

The visit window for the euglycaemic clamp is up to plus or minus two days. All other visits should be adjusted accordingly.

6.2 Indirect Calorimetry

Indirect calorimetry by ventilated hood expires gas analysis will be used to determine energy expenditure during the course of the visit. Indirect calorimetry is the method by which the type and rate of substrate utilization, and energy metabolism are estimated in vivo starting from gas exchange measurements (carbon dioxide production and oxygen consumption during rest and steady-state exercise). Indirect calorimetry with hood canopy and dilution pump will be performed. Duration of indirect calorimetry is 30-40 minutes.

7 Statistical Analysis

Statistical analysis will be carried out after all subjects complete the study. All patients who have at least one dose of the trial medication, whether withdrawn prematurely or not, will be included in the safety analysis.
All safety data and adverse events will be summarised.

7.1 Sample size

No formal power calculations have been performed however, based on a treatment study in HIV-negative healthy volunteers over 4 weeks therapy [2, 9-11]; a power calculation can be determined by using the change in insulin-mediated glucose uptake after four weeks of therapy as the primary end point.

The study with eight [5] subjects per sequence will achieve 80% power to show equivalency in insulin-mediated glucose disposal under DTG treatment between Baseline and Week 4, assuming a standard deviation of difference between baseline and week 4 is 1.4 mg/kg x min per microUnit/mL insulin [1], the insulin-mediated glucose disposal equivalence boundary will range between −1.1 mg/kg × min per microUnit/mL to 1.1 mg/kg × min per microUnit/mL.

The primary endpoint of this study is the change from baseline in insulin sensitivity by euglycaemic clamp method with 28 days treatment.

The secondary endpoints include:

- Change from baseline in serum levels of fasting cholesterol, triglycerides
- Changes from baseline in serum adipocytokines, ghrelin and pituitary hormones
- Changes from baseline in indirect calorimetry

7.2 Criteria for evaluation

All subjects who complete day 1, 28, 44 and 72 assessments (including all euglycaemic clamps) will form part of the evaluable cohort.

7.3 Statistical Methods

Baseline demographic characteristics will be summarized using the descriptive statistics by treatment arms. Where quantitative data are hyper geometrically distributed median with inter-quartile ranges will be presented while Gaussian normal data will be summarized using the mean and standard deviation while qualitative data will be summarized using number of subjects with percentages.

The primary efficacy endpoint will be evaluated using analysis of variance (ANOVA) or Analysis of covariance (ANCOVA) fitting models for the crossover experiment, including treatment, period, and sequence as fixed effects, and subject within sequence as a random effect in the model. Least square mean difference between dolutegravir and no treatment period will be obtained from the ANOVA, or ANCOVA adjusting for potential confounding factors and the associated two-sided 95% confidence intervals will be constructed. It will be concluded that dolutegravir has equivalent impact as no treatment on insulin-mediated glucose disposal if the 90% confidence interval for the mean difference between the two treatments is within −1.1 mg/kg × min per microunit/mL to 1.1 mg/kg × min per microunit/mL boundary.

All data analyses will be performed using STATA statistical software (version 17). All p-values presented will be two tailed.

All patients who attend the baseline visit whether withdrawn prematurely or not will be included in the safety analysis. All safety data and adverse events will be summarised. Adverse events will be mapped using medical dictionary for regulatory activities (MedDRA) (the latest available version). The incidence of adverse event will be tabulated by system organ class, preferred term, and treatment. Additional summaries of adverse events will be displayed by highest grade, investigator's assessment of relationship to study drug, and effect on study drug discontinuation.

Statistical methods and power calculations for the study have been previously published by multiple different groups [3, 14-16].

Exploratory analyses will look at the effects of age, baseline BMI, sex and race on treatment effects.

7.3.1 Efficacy parameters

There will be no assessment for efficacy over the course of the study as the subjects are HIV-1 seronegative volunteers.

7.3.1 Safety parameters

All patients who have at least one dose of the trial medication, whether withdrawn prematurely or not, will be included in the safety analysis.

All safety data and adverse events will be summarised.

7.4 Criteria for termination of the trial

The Sponsor or Investigator may terminate either part of, or the entire study for safety or administrative reasons. A written statement fully documenting the reasons for such a termination will be provided to the Ethics Committee and the Regulatory Authority as appropriate.

7.5 Toxicity Management

In the event of a discontinuation of a DTG for suspected drug induced liver injury, other clinically significant liver chemistry elevations, severe skin reaction or hypersensitivity reaction, subjects should not be re-challenged with a DTG-containing product due to the risk of a recurrent reaction. These subjects should be withdrawn from study.

- **Allergic reaction**

Subjects with Grade >1 or higher allergic reactions that are considered to be possibly or probably related to the investigational product should permanently discontinue the investigational product and the subject should be withdrawn from the study. Subjects should be treated as clinically appropriate and followed until resolution of the adverse event.

- **Rash**

Participants should be instructed to contact the Investigator as soon as possible if they develop a rash while on study. Mild to moderate rash is an expected adverse reaction for DTG-containing antiretroviral therapy.

Subjects should permanently discontinue investigational product (and all other concurrent medication(s) suspected in the Investigators causality assessment) for a rash Grade 1 or higher, and the subject should be withdrawn from the study. Subjects should be treated as clinically appropriate and followed until resolution of the adverse event.

The rash and any associated symptoms should be reported as adverse events and appropriate toxicity ratings will be used to grade the events (based on DAIDS [Division of AIDS] toxicity grading).

- **Liver Chemistry Stopping and Follow up Criteria** Summarized in Appendix 5.

- **Suicidal Risk Monitoring**

Subjects will be monitored appropriately and observed closely for suicidal ideation and behaviour, or any other unusual changes in behaviour. Mental health consultation will be considered or referral for subjects who experience signs of suicidal ideation or behaviour.

Subjects with evidence of suicidal ideation or behaviour changes should permanently discontinue the investigational product and the subject should be withdrawn from the study.

If any subject experiences a possible suicidality-related adverse event (PSRAE) while participating in this study that will be considered to meet ICH E2A (ICH E2A, 1994) definitions for seriousness, we will collect information using a PSRAE case report form in addition to reporting the event on a serious adverse event case report form. A PSRAE may include, but is not limited to, an event that involves suicidal ideation, a preparatory act toward imminent suicidal behaviour, a suicide attempt, or a completed suicide. The investigator will exercise his or her medical and scientific judgment in deciding whether an event is possibly suicide-related. PSRAE forms should be completed and reported to Chelsea and Westminster NHS Trust R&D within one week of the investigator diagnosing a possible suicidality-related serious adverse event.

7.6 Avoidance and Management of Pregnancy

Due to the potential risk from neural tube defect associated with DTG treatment during the first trimester of pregnancy, study staff must review the following information with all female participants of reproductive potential:

- Participants should be informed that in an early analysis of one observational study, women who were taking DTG when they became pregnant had an increased risk of having babies with serious brain and spine defects. These defects happen early in pregnancy, before many women even know they are pregnant.

- Women of reproductive potential should be counselled on the importance of avoiding pregnancy, safer sexual practices and the proper use of their chosen contraceptive methods in accordance with the applicable contraceptive product label, or, for non-product methods, as determined by the investigator.

- The need for the participant's chosen contraceptive method to be used for an adequate time period before dosing with the DTG is initiated.

- The need to continue the use of contraception throughout the treatment- and post-treatment- periods with DTG, until it is predicted that a clinically insignificant amount of DTG is present in the participant (i.e. 30 days or one month after stopping DTG).

- Females of reproductive potential should be reminded about the importance of pregnancy avoidance and of adherence to contraception requirements at every study visit.

Additionally, site staff should document the participant's chosen contraception methods in her medical records and study records/eCRF.

Any subject who becomes pregnant during the study should be immediately withdrawn from DTG, to eliminate further exposure to the embryo/foetus, and withdrawn from the study. Any pregnancy that occurs during study participation must be reported using the Pregnancy CRF (Appendix 6). To ensure subject safety, each pregnancy must be reported to the PI and the nominated person at the sponsor Chelsea and Westminster NHS Trust research and development (R&D) office (as set by the sponsor standard operating procedures (SOP)), within 24 hours of learning of its occurrence. The pregnancy should also be reported to ViiV Healthcare in accordance with the terms of the contract. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child, which must also be reported to the PI and the nominated person at the sponsor R&D office, using a the Pregnancy Follow-up CRF (Appendix 6). The pregnancy outcome should also be reported to ViiV Healthcare within one week of awareness in accordance with the contract. Pregnancy complications and elective terminations for medical reasons must be reported as an adverse event or serious adverse event. Spontaneous abortions must be reported as a serious adverse event.

Any serious adverse event occurring in association with a pregnancy brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to DTG, must be promptly reported to the PI and the nominated person at the sponsor R&D office and ViiV Healthcare.

8 Adverse Events

8.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the study

treatments. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

Adverse events observed by the investigator, or reported by the subject, and any remedial action taken, will be recorded in the subject's CRF and should be verifiable in the subject's notes throughout the study. The nature of each event, time of onset after drug administration, duration and severity will be documented together with the Investigator's opinion of the causal relationship to the treatment (unrelated, unlikely, possible, probable, and definite).

All subjects experiencing adverse events, whether considered associated with the use of the study medication or not, must be monitored until the symptoms subside and any clinically relevant changes in laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed.

Procedures such as surgery should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the adverse event and the resulting appendectomy noted on the CRF.

Planned procedures such as surgery planned prior to the subject's enrolment into the study need not be reported as adverse events if these are documented as planned at the screening visit.

Clinically significant changes in physical examination and blood safety profiles should also be recorded as adverse events.

8.1.1 Assessment of severity

Severity should be recorded and graded according to the current version of the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events (Appendix 3).

Note: There is a distinction between the gravity and the intensity of an adverse event. Severe is a measure of intensity; thus, a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity but would not be classified as serious unless it met one of the criteria for serious events.

8.1.2 Assessment of Causality

The relationship to study drug of each adverse event will be assessed using the following definitions:

DEFINITE: Distinct temporal relationship with drug treatment. Known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot be explained by subject's clinical state or other factors.

PROBABLE: Reasonable temporal relationship with drug treatment. Likely to be known reaction to agent or chemical group, or predicted by known pharmacology. Event cannot easily be explained by subject's clinical state or other factors.

POSSIBLE: Reasonable temporal relationship with drug treatment. Event could be explained by subject's clinical state or other factors.

UNLIKELY: Poor temporal relationship with drug treatment. Event easily explained by subject's clinical state or other factors.

UNRELATED: This is an event which occurs before dosing. Event or intercurrent illness is due wholly to factors other than drug treatment.

8.2 Collection and follow up of adverse events

All adverse events, however minor, will be documented in the CRF whether or not the investigator concludes the event to be related to drug treatment.

The adverse event reporting period will be from the time that the subject gives informed consent until the subjects final study visit. In addition, any untoward event that may occur subsequent to the reporting period that the Investigator assesses as possibly, probably or definitely related to the study drug medication should also be reported as an adverse event (AE).

Adverse events may be directly observed, reported spontaneously by the subject or by questioning the subject at each study visit.

All AE should be followed up until they are resolved or the subject's participation in the study ends (i.e. until the final CRF is completed for that subject). In addition, all serious and non-serious adverse events assessed by the Investigator as possibly related to the investigational medication should continue to be followed even after the subject's participation in the study is over. Such events should be followed until resolution, or until no further change can reasonably be expected. Deaths occurring more than 30 days after the final dose, which are considered to be unrelated to the study medication, should not be reported as SAE.

8.3 Serious Adverse Events (SAE)

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.

vi) Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may

jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

vii) All events of possible drug-induced liver injury with hyperbilirubinemia defined as ALT ≥3xULN and bilirubin ≥2xULN (>35% direct).

NOTE: bilirubin fractionation is performed if testing is available. If unavailable, the presence of detectable urinary bilirubin on a dipstick (indicating direct bilirubin elevations and suggesting liver injury) should be recorded. If testing is unavailable and a subject meets the criterion of total bilirubin ≥2xULN, then the event is still reported as a serious adverse event. If INR is obtained, include values on the serious adverse event form. INR elevations >1.5 suggest severe liver injury.

The SAE should be reported immediately to the Principal Investigator (within 24 hours of a member of the study team becoming aware of the event). SAEs, and Suspected Unexpected Serious Adverse Reactions (SUSARS) and pregnancies must be reported to the nominated person at the sponsor research and development office as set by the sponsor standard operating procedures (SOPs) within 24 hours of awareness of the event. All SAEs, SUSARs and pregnancies should also be notified to ViiV Healthcare, regardless of causality or expectedness, per the terms of the contract. The SAE should be reported to VIIV Healthcare within 24 hours of a member of the study team becoming aware of the event. A SAE form should be completed and an assessment of whether the SAE is a Suspected Unexpected Serious Adverse Reaction (SUSAR) conducted by the Principal Investigator.

Section 4.8 in the Summary of Product Characteristics (SmPC) represent the Reference Safety Information to determine expectedness of serious adverse events for expedited reporting.

The Principal Investigator is responsible for determining whether the SAE is a Suspected Unexpected Serious Adverse Reaction (SUSAR) and for reporting this in accordance with Good Clinical Practice (GCP) and applicable regulatory requirements.

8.4 Liver Events and possible suicide related events

Additional reporting for Liver Events and Possible Suicide Related Events are detailed in Appendix 5 and Section 7.5, respectfully.

9 Data handling

The results from the data collected during the study will be recorded in the Subjects Case Report Form (CRF). In order to maintain confidentiality, the subject will be identified only by subject number and initials.

9.1 Recording of Data

All data collected during the study will be recorded in an individual, subject specific CRF. All CRF should be completed legibly in black ink by an appropriate member of the study team who should be identified and agreed by the Principal Investigator before the start of the study. A delegation of responsibility signature log identifying personnel who can enter data and/or sign off a CRF will be maintained by the Principal Investigator.

Corrections to the data on the CRF will only be made by drawing a single line through the incorrect data (so as not to obscure the original entry) and inserting the correct data next to the original entry. The incorrect data must never be obliterated using correction fluid (e.g. Snopake® or Tippex®). Each correction will be initialised and dated by the person making the correction.

CRF should be kept current to enable the study monitor to review the subject status throughout the course of the study. CRF will be completed, reviewed and signed off by the Investigator within two weeks of the last subject visit.

9.2 Source Documentation and Study Records

The subject's number and date of entry into the study, along with a study identifier, should be recorded in the subject's study records. The following should also be recorded in the study records; confirmation of written and oral consent, the subject's clinical status, date of every study visit, date study medication was started and stopped, concomitant medications, copies of all relevant reports and laboratory tests, comments on results and reference to any adverse events**.**

Euglycaemic clamp data will not be entered into the subject notes and the formula spreadsheet for this procedure will be considered to be source.

9.3 Data Management

Data will be entered into a study specific database by designated staff on a regular basis from completed Case Record Forms (CRF). Data will be entered on a regular basis to ensure it is up to date. The database will be kept by SMART-TRIAL. Access to the database will be given to authorised personnel only and a log of authorised personnel will be stored in the trial master file.

9.4 Archiving and storage of data

Following completion of the study, subject records, CRF and other study documentation will be retained by the Investigator in accordance with Good Clinical Practice (GCP) and applicable regulatory requirements.

10 Quality Control and Quality Assurance

10.1 Monitoring Arrangements

The purpose of monitoring is to verify the rights and well-being of human subjects are protected; that trial data is accurate, complete and verifiable with source data; that the trial is conducted in compliance with the protocol, GCP and the applicable regulatory requirements.

A monitor will conduct regular site visits for the purpose of monitoring various aspects of the study. The Investigator must agree to allow the study monitor and authorised representatives of the sponsor to inspect all CRF and corresponding source documents, e.g. original medical records, subject records and laboratory raw data, access to the clinical supplies, dispensing and storage areas and agree to assist with their activities if requested. The Investigator should provide adequate time and space for monitoring visits.

The monitor will query any missing or spurious data with the Investigator, which should be resolved in a timely manner. A monitoring log will be maintained recording each visit, the reason for the visit, the records reviewed and any findings.

10.2 Quality Assurance

For the purpose of compliance with GCP and regulatory agency guidelines, it may be necessary for sponsor authorised Quality Assurance personnel and/or authorised personnel from an external regulatory agency to conduct an audit/inspection of an Investigational site. The purpose of an audit is to assess the quality of data with regard to accuracy, adequacy and consistency, and to assure that studies are in accordance with Good Clinical Practices, and Regulatory Agency guidelines. Having the highest quality data from studies is an essential aspect of drug development.

The Investigator will be given sufficient notice to prepare for such visits, which are planned to take usually between one and two days and may be conducted at any stage during the study. The audit will involve the review of all study related documentation, which is required by GCP to be maintained by site, review of drug storage, dispensing and return, review of all study related supplies and review of source documents against the CRF to assure the adequacy and accuracy of the information which has been recorded, including the verification of any AE which have occurred.

11 Administrative procedures

11.1 Ethics Approval

The study protocol, subject information and consent form, available safety information, subject recruitment procedures, information about payments and compensation available to the subjects and documentation evidencing the Investigator's qualifications should be submitted to the Ethics Committee for ethical review and approval according to local regulations, prior to the study start.

Any changes, which may need to be made, will be submitted in the form of numbered and dated protocol amendments in accordance with local regulations.

11.2 Regulatory Notification

As required by local regulations, approval of the appropriate regulatory bodies will be obtained, prior to study initiation.

11.3 Insurance Provisions

The Sponsor will take out appropriate insurance cover for this trial.

11.4 Publication Policy

A whole or part of this study results will be communicated, orally presented, and/or published in appropriate scientific journals. Full anonymity of subject's details will be maintained throughout. Subjects wanting to see the results of the trial can request a copy of the article from the investigators once it has been published.

11.5 Drug Accountability

The investigator will ensure that the investigational product will only be used in accordance with the protocol. Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions.

Dolutegravir 50mg will be provided by ViiV Healthcare. A drug accountability log will be kept with the investigational supplies for reconciliation purposes. This should be used to record the identification of the subject to whom the investigational product was dispensed, the date and quantity of investigational product dispensed and the quantity of investigational product unused/returned by the subject. This will be verified by the study monitor.

At the end of the study partially used or empty containers may be destroyed by pharmacy (in accordance with their usual procedures) after the study monitor has completed a final inventory.

Due to the nature of the study and the need to dose subjects outside of normal working hours, pharmacy may need to prepare and dispense investigational medicinal product to the unit in advance of baseline visit. A process is in place to retrieve investigational medicinal product for those subjects who are withdrawn after medication has been dispensed to the unit and prior to dosing.

Spare investigational medicinal product will be dispensed for replacement subjects in the event of withdrawals.

Due to the nature of the study and intensive euglycaemic days occurring outside normal working hour's pharmacy will dispense spare investigational medicinal product (IMP) to the unit. This IMP should only be used in the event of a subject failing to bring in their IMP. It is the responsibility of the investigator to release the IMP and to ensure that pharmacy is informed in order to document which subjects received the IMP. Any IMP not required will be returned to pharmacy immediately.

A specific risk assessment for the concomitant use of a Covid-19 vaccine and Dolutegravir within the current protocol was performed. The outcome is that a Covid-19 vaccine given to a trial subject enrolled into the study is a simple concomitant medication with no interaction that requires advice on timing of the vaccine or other aspects.

11.6 Sample shipment and processing

Biochemistry, haematology, virology and immunology samples will be analysed by the local hospital lab according the local procedures.

Pathology Laboratory
Hammersmith Hospital NHS Trust
Chelsea & Westminster Hospital
369 Fulham Road
London SW10 9NH

References:

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Appendices

Appendix 1: Study flowchart

	Screen (-28)	*Baseline*	*Day 28*	*Day 44*	*Day 72*	*Follow-up call (Day 100)*
Informed consent	√					
Physical Exam	√					
Medical history	√					
Demographics	√					
Vital signs	√	√	√	√	√	
Weight	√	√	√	√	√	
Height, waist/hip measurement	√	√	√	√	√	
Adverse Events		√	√	√	√	√
Concomitant medication	√	√	√	√	√	
Urinary analysis[1] and Urine drug testing	√	√	√	√	√	
Biochemistry [2]	√[3]	√	√	√	√	
Haematology[4]	√	√	√	√	√	
Pregnancy testing [5]	√	√	√	√	√	√
Insulin		√	√		√	
TSH, fT4		√	√	√	√	
HIV, HBV &HCV	√					

CRP		√	√	√	√	
Adoponectin, leptin, ghrelin		√	√	√	√	
Pituitary hormone tests		√	√	√	√	
Testosterone		√	√	√	√	
Euglycaemic clamp		√	√		√	
ECG	√	√	√		√	
Indirect calorimetry		√[6]	√[7]		√[8]	
Dietary Questionnaire		√	√	√	√	
Sleep Questionnaires		√	√	√	√	
Self-administered dosing		√[7] *(am)*	√[7]	√[8] *(am)*	√[8]	
Drug Dispensing		√[7]		√[8]		
Drug returns			√[7]		√[8]	

1-urinary analysis (pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes)
2-Biochemistry safety labs assessment for standard clinical chemistry (glucose, creatinine, sodium, potassium, calcium, aspartate aminotransferase, alanine aminotransferase, bilirubin), lipids (triglycerides, cholesterol, cholesterol HDL & LDL Sub-fractions) and Hb1Ac
3-screening visit is not fasting visit
4-haematology (haemoglobin, haematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets)
5- Pregnancy testing and LMP date in female subjects
6- All participants
7-only Group 1
8-only Group 2

Appendix 2: Collecting, processing, shipping & storing of blood & plasma

Processing blood for the measurement of adipocytokines

For Leptin, Adiponectin and Ghrelin

Please see the laboratory manual for full details.

Appendix 3: Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

Please reference latest version of grading scale available at below location.

https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

Appendix 4: Risk Assessment: Dolutegravir (TIVICAY)

Potential Risk of Clinical Significance	Data/Rationale for Risk	Mitigation Strategy
Investigational Product: dolutegravir Refer to the most recently approved SmPC for this trial		
Hypersensitivity and rash	Hypersensitivity reactions have been reported with dolutegravir, and were characterized by rash, constitutional findings, and sometimes, organ dysfunction, including severe liver reactions. Further information on this risk is provided in Section 7.5.	Subjects with history or presence of allergy/sensitivity to any of the study drugs or their components are excluded Specific/detailed toxicity management guidance is provided for hypersensitivity reactions and rash (Section 7.5) The subject informed consent form includes information on this risk and the actions subjects should take in the event of a hypersensitivity reaction or associated signs and symptoms. Subjects will be monitored for symptoms of hypersensitivity reactions and stop treatment immediately should they appear. These symptoms include, but not limited to, severe rash or rash accompanied by raised liver enzymes, fever, general malaise, fatigue, muscle

Potential Risk of Clinical Significance	Data/Rationale for Risk	Mitigation Strategy
Investigational Product: dolutegravir Refer to the most recently approved SmPC for this trial		
		or joint aches, blisters, oral lesions, conjunctivitis, facial oedema, eosinophilia, angioedema). Clinical status including liver aminotransferases and bilirubin should also be monitored. Delay in stopping treatment with dolutegravir or other suspect active substances after the onset of hypersensitivity may result in a life-threatening allergic reaction.
Drug induced liver injury and other clinically significant liver chemistry elevations	Non-clinical data suggested a possible, albeit low, risk for hepatobiliary toxicity with DTG. Drug-related hepatitis is considered an uncommon risk for antiretroviral therapy containing DTG regardless of dose or treatment population.	Subjects meeting any of the following criteria during the screening period are excluded from participating • Alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) ≥1.5 times the upper limit of normal (ULN) or total bilirubin ≥1.5xULN Positive for HBV (hepatitis B virus surface antigen positive [+HBsAg]) or positive HCV (positive hepatitis C antibody test) within 3 months of the Day 1 study visit Specific/detailed liver stopping criteria and toxicity management guidance are provided for suspected drug induced liver injury or other clinically significant liver chemistry elevations
Neural Tube Defects	In the ongoing observational study conducted in Botswana, preliminary results show that in babies born to women who were taking DTG when	Pregnancy testing should be performed before initiation of DTG therapy in all women of child bearing potential (negative

Potential Risk of Clinical Significance	Data/Rationale for Risk	Mitigation Strategy
Investigational Product: dolutegravir Refer to the most recently approved SmPC for this trial		
	they became pregnant there was an increased risk of neural tube defects compared with the background rate. Dolutegravir was tested in a complete package of reproductive toxicology studies, including embryofoetal development studies. No adverse development outcomes, including neural tube defects, were identified from reproductive toxicology studies. In reproductive toxicity studies in animals, DTG was shown to cross the placenta. Data available from other sources including the Antiretroviral Pregnancy Registry (APR), other cohorts and clinical trials are insufficient to confirm or refute this potential risk.	pregnancy test at screening and randomization). Women who are pregnant or who plan to become pregnant are excluded. All women of reproductive potential should use effective contraception (Appendix 6). The subject informed consent form provides information about this potential risk.

Risk Assessment: Clamp procedure

The euglycaemic clamp procedure is associated with a potential risk of hypokalemia. In order to avoid this risk, whole blood potassium will be measured along with electrocardiograph monitoring at 30 min intervals as a safety precaution to detect any tendency toward hypokalemia.

Appendix 5: Liver Chemistry Stopping and Follow up Criteria

Dolutegravir must be discontinued if:

- **AST or ALT≥3xULN.**
- **If AST or ALT≥3xULN AND bilirubin >2xULN (>35% direct bilirubin), i.e. Hy's case, report event as SAE.**

Required Actions and Follow up Assessments following ANY Liver Stopping Event	
Actions	***Follow Up Assessments***

• **Immediately discontinue DTG**
• **Subject should not be rechallenged due to the risk of a recurrent reaction**.
• Report the event to Chelsea and Westminster NHS Trust R&D by telephone within **24 hours**.
• Events of possible drug-induced liver injury with hyperbilirubinemia[2] will be reported to Chelsea and Westminster NHS Trust R&D as serious adverse events using the serious adverse event case report form.
• Complete the liver event case report form for all events meeting liver stopping criteria, and submit to Chelsea and Westminster NHS Trust R&D within one week of first becoming aware of the event
• Perform liver event follow up assessments.
• Monitor the subject until liver chemistries resolve, stabilise, or return to within baseline
• Subject may continue in the study for any protocol specified follow up assessments.
 	
MONITORING:
• Make every reasonable attempt to have subjects return to clinic within **24 hours** for repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments at the central laboratory as described to the right.
• A specialist or hepatology consultation is recommended.
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline.
	• Viral hepatitis serology, including:
	  • Hepatitis A immunoglobulin M (IgM) antibody;
	  • HBsAg and hepatitis B core antibody;
	  • Hepatitis C RNA;
	  • Hepatitis E IgM antibody.
	• Cytomegalovirus IgM antibody.
	• Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing).
	• Syphilis screening.
	• Drugs of abuse screen, including alcohol. Record alcohol use on the liver event case report form
	• Serum acetaminophen adduct High Performance Liquid Chromatography assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week [FDA, 2009]). The site must contact the medical monitor when this test is required.
	• Serum creatinine kinase and lactate dehydrogenase.
	• Fractionate bilirubin, if total bilirubin ≥1.5xULN.
	• Obtain complete blood count with differential to assess eosinophilia.
	• Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (or gamma globulins).
	• Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease. Complete Liver

	Imaging and/or Liver Biopsy case report form. • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, fatigue, decreased appetite, nausea, vomiting, abdominal pain, jaundice, fever, or rash as relevant on the AE report form • Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications, and alcohol use.

Note:
Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that subject if ALT or AST ≥3xULN **and** bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, **record presence of detectable urinary bilirubin on dipstick**, indicating direct bilirubin elevations and suggesting liver injury.
All events of ALT or AST ≥ 3xULN **and** bilirubin ≥2xULN (>35% direct bilirubin) **or** ALT or AST≥3xULN **and** INR>1.5, if INR measured which may indicate severe liver injury **must be reported as a serious adverse event**

Appendix 6:Highly Effective Methods for Avoiding Pregnancy in Women of Child Bearing Potential*

The following is the all-inclusive list of the highly effective methods for avoiding pregnancy (i.e., have a failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label).
The list does not apply to FCBP with same sex partners, when this is their preferred and usual lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

- Contraceptive subdermal implant
- Intrauterine device or intrauterine system
- Combined estrogen and progestogen oral contraceptive [Hatcher, 2011])
- Injectable progestogen [Hatcher, 2011]
- Contraceptive vaginal ring [Hatcher, 2011]
- Percutaneous contraceptive patches [Hatcher, 2011]
- Male partner sterilization with documentation of azoospermia prior to the female subject's entry into the study, and this male is the sole partner for that subject [Hatcher, 2011]. The information on the male sterility can come from the site personnel's: review of subject's medical records; medical examination of the subject and/or semen analysis; or medical history interview provided by her or her partner.

- Male participants who have partners who are women of childbearing potential must use a condom throughout the study and for a period of at least 4 weeks after the study; in addition to any other methods used to avoid partner pregnancy.

* A Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal, females under the age of 55 years must have a serum follicle stimulating hormone, (FSH**) level > 40mIU/mL to confirm menopause.
** Females treated with hormone replacement therapy, (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level.

Ref: Hatcher RA, Trussell J, Nelson AL, Cates W Jr, Stewart F, Kowal D, Policar MS, editors. Contraceptive Technology. 20th edition. Atlanta, Georgia: Ardent Media, Inc., 2011: 50. Table 3 2.