DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL TRIAL PROTOCOL SYNOPSIS

A light-label radiotracer ^{14}C-acoziborole mass balance and metabolism study in healthy male subjects using accelerator mass spectrometry: open-label, single center, single oral dose, single group design

Name of product(s)	Acoziborole
Drug Class	Antiprotozoal
Phase	Phase 1 light –labelled ^{14}C-acoziborole mass balance
Indication	*Trypanosoma brucei gambiense* Human African Trypanosomiasis (HAT)
Protocol Number	DNDi-OXA-03-HAT
Sponsor	DND*i*, Chemin Louis Dunant, 15, 1202 GENEVA Switzerland Phone: +41 22 906 9230
Global/National Coordinating Investigator/Principal Investigator	TBD
SAC approval	approved
Clinical Trial Protocol Synopsis Version / Date	Draft 1.0 / 14 August 2019

Background Information and Trial Rationale	Human African trypanosomiasis (HAT, also called sleeping sickness) is a life-threatening disease transmitted by tsetse flies and caused by a single-celled extracellular parasite that lives free in the bloodstream and other body fluids, including lymph and cerebrospinal fluid (CSF). There are many species, however, only those of the *Trypanosoma brucei* (*T. b.*) species are known to infect humans. Two subspecies are pathogenic for humans: • *T. b. gambiense* is endemic in West and Central Africa and causes over 95% of current cases. It progresse at a more indolent pace than that of *T. b. rhodesiense*, • *T. b. rhodesiense* is endemic in East and southern Africa and accounts for 5% of cases. It causes an acute, rapidly progressive infection. HAT is considered endemic in 36 countries, of which 24 are endemic for *T. b. gambiense*. In 2012, the number of new *T. b. gambiense* cases reported to World Health Organization were fewer than 8,000 (7,106). The number of yearly reported cases constantly decreases. However, care should be taken and the cases reported may underestimate the real status of the disease due to the limited effective health surveillance in some HAT endemic regions. HAT occurs in two clinical stages. During the early haemolymphatic stage (Stage 1), trypanosomes reside in the blood and lymphatic system, and clinical signs and symptoms are mild and non-specific. In the following late stage (meningoencephalitic, Stage 2) the parasites invade the central nervous system and patients exhibit the characteristic neurological manifestations of disorientation, increasing sleep disturbances and eventually coma and death (Priotto et al., 2009). Few treatment options are currently available for either disease stage: in particular, available drugs for stage 2 were so far limited to the old and toxic melarsoprol (10-day painful i.v. injections, up to 5% treatment-related mortality), and to the less toxic but difficult to manage eflornithine, which requires 4 i.v. infusions per day for 14 days. A recently developed combination of 10-day oral nifurtimox plus 7-day eflornithine, 2 i.v. infusions per day (NECT) was shown to provide similar cure rate as a full regimen of eflornithine, with an obvious practical advantage in terms of reduction of treatment duration and ease of administration. Though NECT became the most used treatment for late stage disease since 2010, the treatment is still far from ideal in the environment in which HAT patients leave. Fexinidazole Winthrop (fexinidazole) received a positive opinion from EMA's human medicines committee (CHMP) in November 2018, under Article 58 of Regulation (EC) No 726/2004, for the treatment of both stages of HAT due to *T. b. gambiense* in adults and children ≥ 6 years old and weighing ≥ 20 kg. Therefore, Fexinidazole may become the standard therapy, especially for patients who do not have the most severe form of HAT. The difficulty of diagnosis, stage determination, and increasing number of treatment failures pose additional clinical challenges. An urgent need exists to develop novel drugs for the treatment of both stages of this fatal disease. Single dose treatment offers an additional advantage over existing options as it ensures full compliance, does not require skilled health staff for administration and, if devoid of need for monitoring and well tolerated, should be easy to administer and safe in non-specialised centres, in extremely remote areas or in conflict zones, representing

	therefore an additional tool to meet WHO's goals for interruption of transmission beyond 2020. Acoziborole is an oxaborole-6 carboxamide, formulated for oral administration. Acoziborole is active *in vitro* against both *T. b. rhodesiense* and *T. b. gambiense* parasites. Acoziborole is also active in mouse models of both acute and chronic infection with *T. b. brucei* when given orally once daily for 3 days at 50 mg/kg or for 7 days at 25 mg/kg. Time kill kinetics *in vitro* are predictive of parasite clearance *in vivo*. Preclinical PK studies have indicated that acoziborole is well absorbed by the oral route and widely distributed throughout the body. The compound crosses the blood-brain barrier. In all animal species investigated, acoziborole is rapidly metabolized through oxidation, resulting in the formation of at least one non-active metabolite (SCYX-3109) (Jacobs et al., 2011). Toxicological studies, including safety pharmacology and 4-week repeated-dose toxicity (including toxicokinetics (TK)) in the rat and dog, have shown that acoziborole is well tolerated up to 40 mg/kg/day in rat, with no major toxicities identified. In both species, 15 mg/kg/day was considered as the no observed adverse effect level (NOAEL) in 4-week repeated-dose studies. After a phase I study in healthy subjects, multicentre, open-label, prospective phase II/III study to assess the efficacy and safety study of 960 mg acoziborole in patients with human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* is currently ongoing in DRC and Guinea (CT.gov identifier: NCT03087955). As of April 15th, 2019, a total of 260 patients with HAT have been enrolled and 208 HAT patients had been treated since the start of the study in October 2016 (169 late stage and 39 early and intermediate stages). One hundred ninety two patients had reached 3-month follow up visit, 157 patients reached 6-month visit, 96 patients reached 12-month visit and 61 patients completed the study. The dose studied in the currently ongoing phase II/III study (a single administration of 960 mg acoziborole) will be administered in the present study to healthy subjects. This dose had been selected following previous administration to 102 healthy subjects and to 208 male and female patients and was found safe and well tolerated. This dose is selected to potentially detect alternative metabolic pathways in humans at therapeutic plasma levels. In this study, a low dose of labelled ^{14}C-acoziborole (900 nCi) will be added to 960 mg unlabeled acoziborole (Active Pharmaceutical Ingredient). A low radioactivity dose has been selected to minimize radioactivity exposure in healthy volunteers. Since acoziborole has an estimated mean half-life of approximately 15 days, a conventional mass balance study design, using a high ^{14}C-acoziborole dose is not possible as it will result in a high radiation exposure which would be required for quantification methods. To address quantification with the envisioned lower radiolabeled dose and anticipated levels of radioactivity in biological samples, the accelerator mass spectrometry (AMS) detection method will be used.

Trial Objectives	The aim of this study is to obtain important complementary safety information on mass balance / metabolite data from humans which were not obtained in the phase 1 study. The information obtained from this study is required by regulatory authorities. **Primary objective** • To evaluate the rate and extent (mass balance) of excretion of total radioactivity in urine and faeces following administration of radiolabelled acoziborole. **Secondary objectives** • To assess the pharmacokinetics of acoziborole and of its main metabolite SCYX-3109 in plasma and urine • To assess the kinetics of total radioactivity in plasma, blood, urine and faeces • To identify, profile and wherever quantify the biotransformation pathways of other potential metabolites of acoziborole and its main metabolite SCYX-3109 in plasma, faeces and urine • To evaluate the absorption of acoziborole, based on available urinary and faecal excretion data and to evaluate further metabolite profiles • To assess the safety and tolerability of a light-labelled radiotracer 14C-acoziborole dose of 900 nCi of acoziborole together with a 960 mg acoziborole oral single dose in healthy volunteers.

Trial Endpoints	**Primary endpoints** Total radioactivity in urine and faeces at each time interval and cumulative radioactivity excretion (mass balance). For the sample collection periods following 240 hours after the dose, excretion will be interpolated between collection periods. **Secondary endpoints** •Total radioactivity in blood and plasma at each timepoint, to determine Cmax, Tmax, half-life, AUC0-t, AUC0-∞ • Plasma and urine concentrations of acoziborole and of its main metabolite SCYX-3109 to determine Cmax, Tmax, half-life, AUC0-t, AUC0-∞, CLr, V/f, CL/f • Quantitative metabolite profiling and identification of metabolites in plasma, urine and faeces, using LC/AMS in conjunction with LC/MS-MS (Pooled samples). • Safety and Tolerability Variables: • Adverse events (AEs) and concomitant medications • Physical examination findings • Body weight and BMI • Vital signs: heart rate (HR), systolic and diastolic blood pressure (BP) • 12-lead ECG (including HR, PR, QRS, QTcB, QTcF) • Clinical laboratory tests: Haematology: haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets, white blood cells (WBC) including differential, red blood cells (RBC); Biochemistry: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), creatinine, fasting glucose, urea, bilirubin (total and conjugated), total protein, sodium, potassium, calcium, chloride; Urinalysis: by dipstick – glucose, ketone bodies, specific gravity, occult blood, pH, proteins, leucocytes, bilirubin, urobilinogen, nitrites • Urinary drug screen / alcohol breath test, • Serology (hepatitis B surface antigen, hepatitis C antibody, HIV 1 & 2 antibodies). **Tertiary endpoints** • Urinary 6 beta-OH cortisol / cortisol in urine collected during 24h after radiolabeled-acoziborole administration

Trial Design	Single center, open-label, single group, single oral dose study. Safety measurements (12-lead ECG at screening, admission on day -1 and day 11, vital signs, blood chemistry and haematology) will be performed before, during and after the study and adverse events will be monitored throughout the study. Subjects will be hospitalised: - on the day before the administration, until 240 hours thereafter (i.e. day -1 to day 11), - on days 14 – 17 for a 48 hours collection period, - on days 28 – 31 for a 48 hours collection period, - on days 58 – 61 for a 48 hours collection period, - on days 88 – 91 for a 48 hours collection period, - on days 118 –121 for a 48 hours collection period. 48 hours collection periods will start on day 1 at 8:00 am the day after the hospitalisation of the subject in the evening before. Blood, plasma, faeces and urine will be collected during the hospitalisation periods.
Sample size	6 healthy subjects will be included. This number has been determined referring to the usual number of subjects included in this type of study. In case of a dropout, subjects will be replaced to ensure a minimum number of 4 (four) evaluable subjects (to be discussed on an individual basis).
Safety measures	**12-lead ECG**: at screening, on day D-1, on day D1 in the morning before administration and at day D11 in the morning and at the end of study evaluation **Safety lab (biochemistry, haematology, urinalysis**): at screening, the morning before administration and at days D2, D11, D15, D29, D59, D89 and 119 in the morning. **Physical examination, body weight and vital signs** (blood pressure after 5 min sitting, heart rate, body temperature, breathing frequency): at screening, on day D1 the morning before administration and at day D11, D15, D29, D59, D89 in the morning and at the end of study evaluation. **Alcohol breath test**: at screening, the evening before administration and at days D14, D28, D58, D88 and D118 **Urinary drug screen:** at screening, the evening before administration and randomly at days D14, D28, D58, D88 or D118 Serology (hep B, C, HIV): at screening **Adverse event and concomitant medication** recording: from screening throughout the study until the end of study evaluation

Main Entry Criteria	**Inclusion criteria:** • Caucasian healthy male subjects • Signed Informed Consent Form • 18 to 55 years old • Subjects with a body mass index (BMI; Quetelet index) calculated as weight (in kg)/height (in m^2) from 18 to 30 kg/m^2 at screening • Non-smokers. No smoking (or use of smoking substitute e.g. nicotine patch or electronic cigarette) is permitted from screening throughout the study • Blood pressure and heart rate in the supine position prior to randomisation within the ranges: 90–140 mm Hg systolic, 60–90 mm Hg diastolic; heart rate 45-100 beats/min. Normal ECG • Having a health insurance, if mandatory due to local regulation • Ability to understand the requirements of the study and willingness to comply with all study procedures • Willingness to give written consent to have data entered into the Overvolunteering Prevention System (if applicable) • Willingness to agree to the contraceptive requirements of the study until 120 days after taking the study medication: All male subjects, including those who are sterilised (i.e. vasectomy), should use a condom. Their female partner must also use at least one of the medically acceptable forms of contraception listed below. Male subjects must not donate sperm or have unprotected sex during the study and until 120 days after taking the dose of the investigational product. Medically acceptable contraception methods (subject to change depending on local authority or Ethics Committee requirements) for this study are condoms in addition to: - Intrauterine devices, - Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring), - Diaphragm with spermicidal cream or gel, - Cervical cap with spermicidal cream or gel, - Spermicidal foam. **Exclusion criteria:** • Evidence of any clinically significant acute or chronic disease, including known or suspected HIV, HBV or HCV infection, on direct questioning and physical examination • Any clinically significant abnormality following review of pre-study laboratory tests (ASAT, ALAT and alkaline phosphatase (ALP) must be within normal ranges), vital signs, full physical examination and ECG, • Unwilling to give their informed consent, • Positive laboratory test for Hepatitis B surface antigen (HbsAg), or anti-HIV 1/2 or anti- HCV antibodies • History of allergy, intolerance or photosensitivity to any drug, • History of serious allergy, asthma, allergic skin rash or sensitivity to any drug, • Known or suspected alcohol or drug abuse (more than 14 units of alcohol per week, one unit = 8 g or about 10 mL of pure alcohol),

	• Consumption of more than 8 cups daily of beverage containing caffeine, • Regular daily consumption of more than one litre (L) of beverages containing xanthine, • Positive laboratory test for urine drug screening (opiates, cocaine, amphetamine, cannabis, benzodiazepines), • Surgery within 12 weeks prior to the start of the study, • Use of enzyme-altering drugs (e.g. barbiturates, phenothiazines, cimetidine etc.) within 30 days or 5 half-lifes, whichever is longer before study day 1, • Prescription medication 14 days before their screening examination for the study or require chronic use of any prescription medication during the study, • User of over-the-counter medications, including vitamins, analgesics, or antacids, 7 days before study day 1, • Intake of grapefruit and grapefruit juice, alcoholic beverages or caffeine-containing food or beverages, such as coffee, tea, chocolate, or cola, 48 hours before study day 1, • Exposure to artificial ionising radiation in the last 12 months (e.g. x-ray investigation, isotope studies), • Surgery (eg stomach bypass) or medical condition that may affect absorption of study drug taken orally. • Any clinical condition or prior therapy which, in the opinion of the Investigator, made the subject unsuitable for the study, including evidence or history of clinically significant hematological, renal, endocrine, pulmonary, GI, CV, hepatic, psychiatric, neurological, or allergic disease • Administration of a licensed or unlicensed medicinal product as part of another clinical trial within the 3 months before, or within 5 half-lives of their first dose of study medication, whichever is longer, or is currently in the follow-up period for any clinical trial, • Loss or donation of more than 400 mL of blood within 3 months before admission.
Study Duration	The participation of each patient will last approximately 21 weeks and includes: • A selection period of up to 28 days before dosing until 48 hours before dosing • A treatment period (hospitalization and follow-up periods): Subjects will come to the clinic the evening before the dosing. After the drug intake at day 1, subjects will have regular in-house periods for specimen collection up to 121 days after the drug administration End of study visit: Day 121
Study treatments	960 mg acoziborole + 900 nCi ^{14}C-acoziborole (approximately 0.1 mg) single oral dose under fasted conditions. Details will be outlined in a pharmacy manual.

Statistics Sample size Randomisation Summary of analysis	Summary statistics will be presented for all background characteristics and safety data. Summary statistics will be presented for the pharmacokinetic parameters. Other techniques will be applied as exploratory analysis.
Study procedures	• Standard precautions will apply. • Subjects will come to the clinic the evening before dosing, i.e. on day D-1. In the morning of the treatment day (Day 1), at 8:00 am ± 1h, the study drug will be given with 240 ml of ambient temperature mineral water after fasting overnight for at least 9 hours. No food is allowed during the 4 hours following study drug administration. • A standardised lunch is served 3 hours post morning dose. • A small standardised snack is served between 4 and 5 pm and a dinner is served 12 hours post morning dose. Permitted liquid consumption will be allowed ad libitum except 1 hour before and after drug administration. • During recruitment, informed consent review and baseline period the subject will be informed and reminded the following restriction: No strenuous physical exercise, no alcohol for 72 hours before dosing until after the last PK sample collection, no grapefruit or grapefruit juice for 48 hours before dosing until 240 hours post dose.
PK Sampling	• Concentrations of acoziborole and its main metabolite SCYX-3109 will be determined in **plasma samples** collected at the following times : Pre-dose, 1, 4, 9, 12, 24, 48, 72, 96, 120, 168, and 240 h post-dose, then at D15, D29, D59, D89 and D119 post-dose (assessed by available LC/MS-MS method) • Concentrations of acoziborole and its main metabolite SCYX-3109 will be determined in **urine samples** collected at the following times : pre-dose, 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-168, and 168-240 hrs and for 48 hour collections on Days D29, D59, D89 and D119 (assessed by LC/MS-MS, method to be developed).

^{14}C-acoziborole Sampling	• Sample processing and storage will be specified in a specimen collection manual. • Concentrations of ^{14}C-acoziborole-derived total radioactivity will be determined in **whole blood and plasma samples** collected at the following times : Pre-dose, 1, 4, 9, 12, 24, 48, 72, 96, 120, 168, and 240 h post-dose, then at D15, D29, D59, D89 and D119 post-dose (assessed by AMS or if possible by Liquid Scintillation Count [LSC]) • Concentrations of ^{14}C-acoziborole derived total radioactivity will be determined in **urine samples** collected at the following times : pre-dose, 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-168, and 168-240 hrs and for 48 hour collections on Days D29, D59, D89 and D119 (assessed by AMS or if possible by LSC) • Concentrations of 14C-acoziborole derived total radioactivity will be determined in **faecal samples** collected daily for 240 hrs and for 48 hour collections starting on D15, D29, D59, D89 and D119 (assessed by AMS or if possible by LSC). If a subject cannot pass faeces during a 48 hour collection period, the subject will get a faeces collection container home for the next bowel movement collection • Metabolite profiling, metabolite identification and metabolite quantification: plasma, urine and faeces samples will be collected. As appropriate, pooled samples per matrix (i.e. a minimum of 4 pooled samples for plasma, one pooled sample for urine and faeces each) will be subjected to LC-AMS analysis in a minimum of 60 fractions for the assessment of the (semi-) quantitative contribution of the metabolite to the total radioactivity. The metabolite accurate mass will be assessed in conjunction with LC/MS-MS to assess the metabolite structure(s) and pathway(s). Optional: an attempt may be warranted to further assess the exposure of the subjects to metabolites in plasma by quantitative metabolite analysis (please indicate costs per sample and per metabolite), and/or to assess the amount of parent compound excreted in faeces for absolute bioavailability estimation (costs per sample and method development to be provided by vendors). Additional samples per matrix will collected and stored for that purpose.

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Appendix: Flow chart mass balance study

			SCHEDULE OF EVENTS															
Protocol Activities and Forms to be completed	Screening Period	Baseline	In house Period for 11 days Days											48H in house for collection period				
Tim	*D-28 to D-2*	*D-1 before dosing*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*D14-17*	*D28-31*	*D58-61*	*D88-91*	*D118-121 End of study*
In house stay *		x	→	→	→	→	→	→	→	→	→	→	→	x	x	x	x	x
Inclusion/exclusion criteria (only at first hospitalization period)	x	Check																
Demographics, medical history previous medication (only at first hospitalization period)	x	Check																
Lab test (haematology, biochemistry, urinalysis)	x	x		x									x	x	x	x	x	x
Serology (hep B, C , HIV)	x																	
Alcohol breath test	x	x												x	x	x	x	x
Urinary drug screen	x	x												Randomly				
Informed consent	x																	
Physical examination +BW+ VS	x	x	x										x	x	X	x	x	x
12 leads ECG	x	x	x										x					x
Study drug administration			x															
PK blood + plasma sampling **			x***	x	x	x	x		x				x	x	X	x	x	x
Feces sampling **													x	x	X	x	x	x
Urine sampling **			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
AEs & concomitant medication recording	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

* in house stay to be confirmed with site, ** Blood, plasma, urine and faeces sampling to be discussed with the site; *** at predose, H1, H4, H9, H12

Appendix: Safety labs

Haematology :

- Haemoglobin,
- Red blood cell (RBC),
- Hematocrit,
- Mean corpuscular volume (MCV),
- Mean corpuscular hemoglobin (MCH),
- Mean cell hemoglobin concentration (MCHC),
- White blood cell (WBC) including differential,
- Platelet counts.

Clinical Chemistry (Serum/Plasma):

- ALP,
- ALAT,
- ASAT,
- Gamma–glutamyltranspeptidase (GGT),
- Creatine phosphokinase (CPK),
- Total and direct bilirubin,
- Total protein,
- Creatinine,
- Fasting glucose,
- Urea,
- Electrolytes (Ca^{+}, Na^{+}, K^{+}, Cl^{-}).

Urine will be examined by a standard stick test for:

- Glucose,
- Ketones,
- Density,
- Occult blood,
- pH,
- Proteins,
- Leukocytes,
- Bilirubin,
- Urobilinogen,
- Nitrites.

Stick test results will be recorded directly on the urine book.

Urine will also be collected over 24h after radiolabeled-acoziborole administration to determine the ratio 6BOHcortisol/ cortisol.

A urine drug screen (opiates, cocaine, amphetamine, cannabis, benzodiazepines) will be carried out at screening, on day -1 and randomly at following hospitalization.
An alcohol breath test (using an alcohol breath analyzer) will be carried out at screening and on day -1 of each hospitalization period.

The following tests will be performed at screening only:

- Hepatitis B Surface Antigen (HBsAg),
- Hepatitis C Antibody (HCV antibody),
- HIV 1 & 2 antibodies

Planning Information

STUDY TIMELINES

Final protocol available	30 October 2019
Study treatment supply available	November 2019
FSFV	15 January 2020
Duration of recruitment period	4 weeks
Duration of follow-up period (if applicable)	Not applicable
LSLV	June 2020
Interim analysis	Not applicable
Final study report	TBD

STUDY SCOPE

Target countries	UK
Enrollment target	6 subjects
Number of sites	1 site
Number of subjects per site	6
DSMB involvement	No DSMB
Partners involvement	Radioactivity monitoring to be performed by TNO IMP preparation to be performed by Selcia
Other study special needs	Accelerated Mass Spectrometry to be performed by specialized vendor

Study Treatments Supply

Study treatments	Therapeutic dose of Acoziborole (960 mg) to be administered to which 900 nCi radiolabelled IMP will be added
Labeling instructions	Not yet available
Other information	Not applicable