CLINICAL TRIAL PROTOCOL

Safety and tolerability study of acoziborole in g-HAT seropositive non-parasitologically confirmed subjects: a multicentre randomised double-blind placebo-controlled study

Short title	OXA004	
Name of product(s)	Acoziborole	
Drug Class	Antiprotozoal	
Phase	II/III	
Indication	Human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense*	
Clinical Trial Protocol Number	DNDi-OXA-04-HAT	
EudraCT	Not applicable	
Sponsor	DND*i*, Chemin Camille-Vidart, 15, 1202 GENEVA, Switzerland	
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Principal Investigator/ International Coordinating Investigator	[redacted]	
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Clinical Trial Protocol Version / Date	V 3.0 dated of 13 June 2023	
Protocol Amendment Number / Date	Amendment#2 dated of 13 June 2023	

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I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.

I will use only the informed consent form approved by the sponsor or its representative and will fulfil all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial if required by national law.

I agree that the sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

Investigators at each trial site

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ABBREVIATIONS – GLOSSARY OF TERMS

ΔΔ	Double Delta
ADR	Adverse drug reaction
AE	Adverse Event
AUC	Area Under the Curve
BMI	Body Mass Index
CATT	Card Agglutination Test for Trypanosomiasis
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CRD	Cross reacting determinant
CRF	Case Report Form
D	Day
DBS	Dry Blood Spot
DNDi	Drugs for Neglected Diseases initiative
DRC	Democratic republic of Congo
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
ER	Excess Rate
EoS	End of Study
FFPE	Formalin-Fixed Paraffin-Embedded
GCP	Good Clinical Practice
g-HAT	Human African Trypanosomiasis caused by *Trypanosoma brucei gambiense*
GLP	Good laboratory practice
H	Hour
HAT	Human African Trypanosomiasis
HR	Heart rate
ICF	Informed Consent Form
ICH	International Committee for Harmonization
IEC	Independent Ethics Committee
IHC	Immuno-Histochemistry
INRB	Institut National de Recherche Biomédicale
IP	Investigational Product
IRB	Institutional Review Board
ISG65	Invariable Surface Glycoprotein 65
ITM	Institute of Tropical Medicine
IV	Intravenous
mAECT	Mini Anion Exchange Centrifugation Technique
mAECT-BC	Mini Anion Exchange Centrifugation Technique on Buffy Coats
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent To Treat
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NECT	Nifurtimox-eflornithine combination therapy
NNT	Needed to Treat
NSSCP	National Sleeping Sickness Control Programme

OR	Odds Ratio
PCR	Polymerase Chain Reaction
PK	Pharmacokinetics
PPV	Positive predictive values
PT	Preferred Term
qPCR	Quantitative Polymerase Chain Reaction
RT-qPCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
RR	Risk ratio
SAE	Serious Adverse Event
SAR	Severe adverse reaction
SD	Standard Deviation
SHERLOCK	Specific High-sensitivity Enzymatic Reporter unlocking
SMS	Short Message Service
SOC	System Organ Class
SS	Safety Set
SUSAR	Suspected unexpected serious adverse drug reaction
T.b.	*Trypanosoma brucei*
TBR	*Trypanosoma brucei* repeats
T. brucei s.l.	*Trypanosoma brucei* sensu lato
TL	Trypanolysis
TNA	Total nucleic acid
TgSGP	*Trypanosoma brucei gambiense*-specific glycoprotein
SSC	Set of Study Completers
TEAE	Treatment-Emergent Adverse Event
WBC	White Blood Cell
WHO	World Health Organization

PROTOCOL SUMMARY

Study Title	Safety and tolerability study of acoziborole in g-HAT seropositive non-parasitologically confirmed subjects: a multicentre randomised double-blind placebo-controlled study
Study Phase	II/III
Indication	Human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense (g-HAT)*
Protocol Number	DNDi-OXA-04-HAT Short number OXA004
Background Information and Trial Rationale	Human African trypanosomiasis (HAT), or sleeping sickness, is a neglected tropical disease. It is a vector-borne parasitic disease that is present in sub-Saharan Africa, which is transmitted by the bite of the tsetse fly and, without prompt diagnosis and treatment, is fatal. The parasites responsible for HAT are the protozoa, *Trypanosoma brucei gambiense* (*T.b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*), which are found only in foci in regions of sub-Saharan Africa where the tsetse fly is endemic. Few therapeutic options are currently available to treat HAT at either the haemolymphatic (early) stage or meningoencephalitic (late) stage. When early stage HAT is diagnosed, patients can be treated in their villages with intramuscular injections of pentamidine for 7 days. In patients with late-stage HAT, nifurtimox-eflornithine combination therapy (NECT), a combination of oral nifurtimox for 10 days plus eflornithine, two 2-hour intravenous (IV) infusions daily for 7 days, was found to provide similar cure rates to the standard regimen with eflornithine for 14 days, but with obvious practical advantages, including ease of administration and a shorter duration of treatment. In December 2018, Fexinidazole was approved for the treatment of HAT in the Democratic Republic of Congo (DRC), which is an effective 10-day oral treatment, able to cure early and late stage patients, although an increased risk of relapse on very advanced patients keeps NECT as first line treatment for patients showing more than 100 white blood cells (WBC)/µL in the cerebrospinal fluid at diagnosis. Whilst the delivery of fexinidazole has improved the management of g-HAT cases and facilitates the integration of HAT treatment into the general health system, it is expected that the current investment in acoziborole as an oral, single-dose treatment will help boost elimination efforts envisioned for all stages of g-HAT. Acoziborole (SCYX-7158) is an orally active benzoxaborole-6-carboxamide that has been shown to have in vitro and in vivo activity against *T.b. gambiense* and *T.b. rhodesiense*. Single oral doses of acoziborole ranging from 20 to 1200 mg have been administered to healthy volunteers with no major toxic effects. A phase II/III study was conducted in 208 patients with HAT due to *T.b. gambiense* administered a single dose of acoziborole 960 mg. As of 28 August 2020, 159 out of 167 patients with late-stage HAT and 41 out of 41 patients with early- or intermediate-stage HAT had performed the last scheduled visit. The proportion of patients with serious adverse events (SAEs) was low (21/208 patients [10.1%]).

	Although the treatment options have advanced with the recent approval of fexinidazole, an effective 10-day oral treatment, there are still gaps with regards to the 2030 Public Health objective of sustained HAT elimination. Acoziborole, as an oral single-dose treatment envisioned for all stages of g-HAT, is expected to help boost elimination efforts. The exploratory TrypSkin sub-study is planned to assess the presence of extravascular dermal *T.b. gambiense* in the population enrolled.
Study objectives	**Primary objective** To assess the safety and tolerability of a single dose of acoziborole compared with placebo during a follow-up period of 4 months in seropositive individuals who are not confirmed parasitologically. **Secondary objectives** • To assess safety of acoziborole versus placebo in: o Haemato-biochemistry o Electrocardiogram (ECG) data at Day (D) 5 (double delta versus baseline and time, age and sex matched comparison) • To assess blood pharmacokinetics (PK) at Day 5 and Month 1 • To assess correlation between ΔQTc measurements and acoziborole concentrations in blood at Day 5 **Exploratory objectives (TrypSkin sub-study)** • To estimate the prevalence of extravascular dermal *T.b. gambiense* in seropositive individuals non-confirmed parasitologically. • To compare the properties (sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and repeatability) of different methods of detection of *T. brucei sensu lato (s.l.)* and *T.b. gambiense* (polymerase chain reactions [PCRs], quantitative PCRs (qPCRs), multiplex quantitative reverse transcriptase PCRs (RT-qPCRs), Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK), immuno-histochemistry (IHC) in skin biopsies and blood samples. • To determine the presence/absence of extravascular *T.b. gambiense* in skin biopsies of all subjects at enrolment, and at the end of the study. • To assess the effectiveness of acoziborole on elimination of extravascular *T.b. gambiense* parasites in dermis with the methods or combination of methods, contingent on having better validity than *Trypanosoma brucei gambiense*-specific glycoprotein PCR (TgSGP-PCR) and provided that the prevalence of dermal trypanosomes by any method at baseline is greater than 2%.
Study endpoints	**Primary endpoint** Occurrence of treatment-emergent adverse events (TEAEs). **Secondary endpoint(s)** • Occurrence of adverse events (AEs) from informed consent form (ICF) signature to the end of study (EoS) • Change from baseline in ECG parameters, biochemistry and haematology • Blood concentration of acoziborole at Day 5 and Month 1

	• ΔQTc measurements at baseline and D5 • Placebo-corrected change from baseline in ECG parameters (double delta [ΔΔ] heart rate [HR], ΔΔRR, ΔΔPR, ΔΔQRS, ΔΔQT and ΔΔQTc) • Incidence of abnormal values and morphological findings in ECG at D5 **Exploratory endpoint(s) (TrypSkin sub-study)** **Primary exploratory endpoint**: Occurrence of extravascular dermal *T.b. gambiense* at enrolment and at 4 months by TgSGP-PCR in skin. **Secondary exploratory endpoints:** • Clinical parameters: occurrence of dermatitis and/or pruritus at baseline and M4. • Diagnostic parameters: occurrence of IHC+, TBR (*Trypanosoma brucei* repeats)-PCR+ and/or 18S-PCR+ in skin and blood at baseline and M4. • Novel diagnostic parameters: occurrence of positive results by multiplex qPCRs, multiplex RT-qPCRs and SHERLOCK in blood and skin at baseline and M4. • Occurrence of dermatitis, pruritus, IHC+, multiplex qPCRs+, multiplex RT-qPCRs+ and SHERLOCK+ in skin and blood at baseline and M4. • Occurrence comparisons between all diagnostic methods alone or in combination.
Study Design	This is a randomised, multicentre, double-blind, placebo-controlled, parallel-arm phase II/III study.
Main inclusion/exclusion criteria	To be included in the study, and sub-study, subjects must fulfil all of the inclusion criteria and none of the exclusion criteria **Inclusion criteria** • Signed the informed consent form (ICF) • Male or female • 15 years of age or older • Card Agglutination Test for Trypanosomiasis (CATT) test or HAT sero-K-set as Rapid Diagnostic Test (RDT) positive • Parasitology negative in blood and/or lymph (if lymphadenopathy is present) • Karnofsky Performance Status above 70 • Able to ingest oral tablets • Known address and/or contact details provided • Must be able to comply with the schedule of follow-up visits and other requirements of the study • Agreement to be hospitalised upon enrolment for at least 5 days (in order to receive in-ward post-treatment observational follow-up through the first 5 days after treatment) • Agreement to not take part in any other clinical trials during the participation in this study • For women of childbearing potential: o Must agree to have protected sexual relations to avoid becoming pregnant from enrolment up to 3 months after dosing (contraceptive protection will be advised and offered at no cost).

	○ Negative urine pregnancy tests (before dosing at site level)
	<u>Exclusion criteria</u> • Individuals parasitologically confirmed in blood and/or lymph • Previously treated for g-HAT • Severe malnutrition, defined as body mass index (BMI) <16 kg/m^2 • Pregnant or breast-feeding women • For women of childbearing potential: ○ Urine pregnancy test positive ○ Do not accept contraceptive protection (i.e. condom or sexual abstinence) from enrolment up to 3 months after dosing • Clinically significant medical condition and/or abnormal laboratory results that could, in the opinion of the Investigator, jeopardise the subject's safety or participation in the study **Additional exclusion criteria for TrypSkin sub-study** • Rejection to participate in the sub-study in the signed ICF • Known diabetes • Known haemophilia
Study duration	• Study duration for subjects is 4 months • The duration of the enrolment period is estimated to last approximately 12 months
Study treatment	The investigational product (IP) is acoziborole or matching placebo tablet: three 320-mg tablets (960 mg dose), administered by the oral route to subjects in the fasting state as single dosing regimen on Day 1 of the study
Sample size	The sample size was determined based on statistical probabilities to detect uncommon TEAEs in the active treatment (acoziborole) group. The definition for uncommon event is a relative frequency between 0.1% and 1%. The median relative frequency is therefore 5/1000 (0.5%). The probability to detect at least 1 event if the true incidence rate is 0.5% is as high as 98% with 900 subjects and the probability is 83.5% for a true incidence rate of 2/1000 (0.2%). According to the calculations, 900 subjects exposed to acoziborole shows a high probability (>99%) of detecting at least one event (if the incidence rate is 1%) with a similar probability when exposing 1200 subjects. Therefore, 900 subjects will be administered with acoziborole and 300 subjects with placebo, for an overall sample size of 1200 subjects. **<u>Sample size calculation for the TrypSkin exploratory sub-study:</u>** The primary objective is to estimate the prevalence of dermal extravascular *T.b. gambiense* trypanosomes in a population of seropositive subjects non-confirmed parasitologically. Preliminary results from Guinea, on a limited number of subjects, have shown that all seropositive individuals presenting a positive CATT test in plasma dilution ≥1:4, but non-confirmed by parasitological examination of blood and lymph, had parasites in skin biopsies. Knowing that approximately 25% of the RDT positive subjects non-confirmed parasitologically present a positive CATT test in plasma dilution ≥1:4, it is hypothesised that up to 20-25% of the population of RDT positive subjects, non-confirmed

	parasitologically, enrolled in the TrypSkin exploratory sub-study could possibly carry dermal extravascular parasites. Bearing in mind this higher limit of the skin-dwelling trypanosome prevalence, we have estimated the minimum sample size required to be able to evaluate the real prevalence with a satisfactory precision (a narrow confidence interval [CI]). Under the assumption that skin-dwelling trypanosomes could be observed in up to 20-25% of the 1200 subjects, to obtain a 95% CI with a precision of 3%, a minimum of 690 evaluable subjects is required. Based on these calculations and taking into account a provision of 5% (35 subjects) to allow for skin samples that are non-analysable, the target number of subjects to be enrolled in the TrypSkin exploratory sub-study is 725.
Planned analyses	**Study populations** For the main safety study, the study populations that will be used for the analyses are defined as below: • Safety set of subjects (SS) is a modified intent to treat (mITT) set of subjects composed of all randomised subjects who received at least one tablet of IP (placebo or acoziborole). For the TrypSkin sub-study, the study populations that will be used for the analyses are: • Primary set of subjects: for exploratory efficacy analyses is the mITT set composed of subjects, who participate in the TrypSkin sub-study and who receive at least one dose of IP (placebo or acoziborole). • Secondary set of subjects: for exploratory efficacy analyses is the set of all seropositive subjects who are positive for *T.b. gambiense* by at least one molecular diagnostic test in the skin , blood and/or DBS at baseline. **Primary endpoint analysis** Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT). Occurrence rates of each TEAE (≥1%), by MedDRA PT, will be compared. The primary outcome measure is the excess rate (ER) of TEAEs over the first 4 months post-treatment in the acoziborole group compared with placebo. The occurrence rate will be the number of subjects with at least one TEAE as the numerator and the number of subjects who receive at least one tablet of study medication (the SS) as the denominator. Loss of subjects to follow-up and withdrawal for a reason unrelated to safety or tolerance will not be considered as AEs. The ER of occurrence of any TEAE will be transformed to the number of subjects needed to treat (NNT) to observe one more subject with a TEAE in the acoziborole group compared with placebo (NNT=1/ER). The Cochran-Mantel-Haenszel test, stratified by study centre, will be used to compare the occurrence rate of TEAEs between treatment groups. The same test will be applied to the primary endpoint and each component of the composite endpoint (common TEAEs based on PTs).

	The time course of treatment effect will be analysed using the log rank test stratified by study centre and will be performed on the time to occurrence of TEAEs, taking into account the attrition rate of subjects who are being followed up. A loss to follow-up or a withdrawal will be censored at the time of the last attended visit. The Kaplan-Meier estimates of the proportion of subjects without any TEAE in each treatment group will be presented graphically to assess the time course of the response. The rate of occurrence of AEs will be analysed by the true positive status (subjects with parasites detected during the follow up period) in either treatment or placebo groups. **Secondary endpoint analysis** The secondary endpoints are the occurrence of AEs from the time that subjects provide written informed consent to the EoS and changes from baseline in ECG and laboratory safety test results (biochemistry and haematology) to the EoS. The majority of measured parameters are quantitative and the change from baseline will be the difference between two values. For parameters with binary outcomes (presence or absence), contingency tables (before versus after treatment) will describe the change (or not) of status. **Exploratory endpoint analysis** The occurrence rate of positive extravascular dermal *T.b. gambiense* at 4 months based on the primary diagnostic parameters will be the primary measure of interest because the sample size is known and it corresponds to a measure of the level of potential transmission of reservoirs. The magnitude of the treatment effect will be the odds ratio (OR) of positive dermal *T.b. gambiense*. As the OR is not easy to interpret when the rate of positive subjects in the placebo group is not small (P0 >0.05), the OR will be converted into the risk ratio (RR) using the following formula: RR = (P1/P0) = OR/[(1−P0) + (P0×OR)], where P1 and P0 are the estimated proportions of positive subjects in the acoziborole arm and placebo arm respectively. The ER (P1 – P0) will also be estimated as a secondary measure of the magnitude of the treatment as well as the NTT (number of subjects needed to treat to get one less positive subject at 4 months in acoziborole group). **Interim analysis** For the TrypSkin sub-study, an interim analysis for futility will be performed once the baseline data of 50% of subjects will be collected. If the occurrence rate of positivity by any diagnostic method in any sample at baseline lead to less than 2%, then the recruitment in the TrypSkin sub-study will be stopped.

1. Background and study rationale

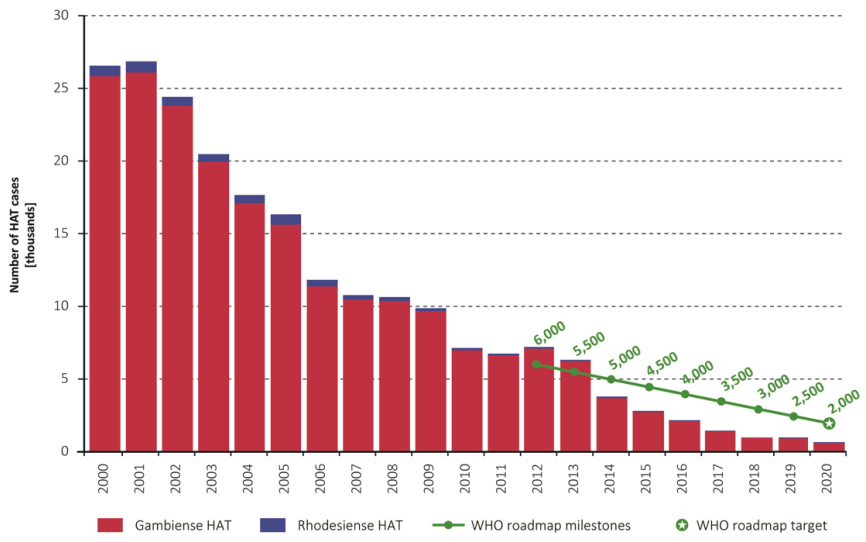
1.1. Epidemiology

Human African trypanosomiasis (HAT), or sleeping sickness, is a neglected tropical disease. It is a vector-borne parasitic disease that is present in sub-Saharan Africa, which is transmitted by the bite of the tsetse fly and, without prompt diagnosis and treatment, is fatal. The parasites responsible for HAT are the protozoa, *Trypanosoma brucei gambiense* (*T.b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*), which are found only in foci in regions of sub-Saharan Africa where the tsetse fly is endemic (1, 2, 3).

Approximately 5 million people live in areas, mainly in rural parts of 24 disease endemic countries in East, West and Central Africa, where HAT due to *T.b. gambiense* (g-HAT) is still considered a public health problem; whereas 51 million people are estimated to be at risk of infection on the African continent (4, 5). With respectively 876 and 565 cases of g-HAT reported in 2019 and 2020 (6, see Figure 1), the global goal of sustainable disease elimination by 2030, including the interruption of the transmission of g-HAT, is achievable. Consistently falling numbers of cases are attributed to efforts from national control programmes, supported by the World Health Organization (WHO), non-governmental organisations, bilateral cooperation, the private sector (including pharmaceutical companies), and philanthropic organisations. These efforts include clinical trials conducted by DNDi, which have enabled over 2 million people to be screened for HAT since 2009. The decline in the number of cases of HAT is demonstrated in the most recent data presented by WHO in 2019 displayed in Figure 1. It is to be noted that the unusual decrease in the number of people actively screened during the past year was mainly caused by the interruption for several months of active screening activities in many countries due to the pandemic of Coronavirus disease (COVID-19).

As the numbers of reported cases diminish, resources for surveillance and specialised screening will also taper. This decrease, coupled with the loss of diagnostic skills and disease management expertise, will lead to a weak and less-specialised HAT technical environment. The history of g-HAT has shown that outbreaks or re-emergence of the disease have already happened in different circumstances when surveillance was relaxed, e.g. South Sudan and the Democratic Republic of the Congo (DRC), or simply because the at-risk populations live in areas of political instability, limiting access to specialised care. Even with a steady decrease of reported incidence, no model can predict today that HAT could not re-emerge.

Figure 1. Total number of reported cases of HAT (gambiense and rhodesiense) per year (2000–2020).



HAT= Human African Trypanosomiasis; WHO=World Heath Organization.

1.2. Current therapeutic options for g-HAT

Few therapeutic options are currently available to treat HAT at either the haemolymphatic (early) stage or meningoencephalitic (late) stage (7, 8). When early stage HAT is diagnosed, patients can be treated in their villages with intramuscular injections of pentamidine for 7 days. In patients with late-stage HAT, Nifurtimox-eflornithine combination therapy (NECT), a combination of oral nifurtimox for 10 days plus eflornithine, two 2-hour intravenous (IV) infusions daily for 7 days, was found to provide similar cure rates to the standard regimen with eflornithine for 14 days, but with obvious practical advantages, including ease of administration and a shorter duration of treatment.

In December 2018, fexinidazole was approved for the treatment of HAT in DRC (9), which is an effective 10-day oral treatment, able to cure early and late stage patients, although an increased risk of relapse on very advanced patients keeps NECT as first line treatment for patients showing more than 100 WBC/µL in the cerebrospinal fluid at diagnosis. Since then, other endemic countries have been authorising the use of fexinidazole for g-HAT. Those are Angola, Central African Republic, Chad, Guinea, Equatorial Guinea Gabon, Uganda, and South Sudan.

Whilst the delivery of fexinidazole has improved the management of g-HAT cases and facilitates the integration of HAT treatment into the general health system, it is expected that the current investment in acoziborole as an oral, single-dose treatment will help boost elimination efforts envisioned for all stages of g-HAT.

1.3. Investigational product and preclinical data

Acoziborole (SCYX-7158) is an orally active benzoxaborole-6-carboxamide. Acoziborole, also referred to as AN5568, is the result of a collaboration between DNDi, Anacor Pharmaceuticals (a biopharmaceutical firm based in Palo Alto, California, United States) and SCYNEXIS (a pharmaceutical firm devoted to research and development, based in North Carolina, United States). Preclinical studies have identified the concentrations of acoziborole that must be achieved in the plasma and brain in order to be effective against late-stage g-HAT. Reversible *in vitro* assays for acoziborole showed that it is necessary to know the exposure time and area under the curve (AUC) for the minimum inhibitory concentration in order to predict efficacy.

Complete and irreversible inhibition of parasite growth was achieved with an unbound AUC of 5.81 µg•h/ml. This was confirmed in animal models considered to be predictive of HAT in humans in which a 100% trypanocidal effect was obtained with daily administration of 25 mg/kg for 7 days. On the first day of treatment, plasma concentrations reached 4.98 µg•h/ml, which is close to the critical threshold of 5.81 µg•h/ml for the unbound AUC of the minimum inhibitory concentration. From the second to the seventh day, the threshold was exceeded, and concentrations reached 7.15 µg•h/ml (10).

Preclinical pharmacokinetic (PK) studies showed that acoziborole is well absorbed by the oral route, that it is widely distributed throughout the body and that it crosses the blood-brain barrier.

The toxicology profile of acoziborole was well defined following 7 days, 28 days and 13 weeks of administration.

Several target organs/functions have been identified, mainly at high dose levels, and were not repeated during longer duration toxicity studies (13 weeks):

- Gastrointestinal tract: decrease of food consumption (40 mg/kg/day in rat and dog) and gastric/intestinal lesions at highest dose levels (80 and 40 mg/kg in rat and dog).
- Reticulocyte: increase counts mainly in rat including at the lowest dose. These increases were modest and correlated with spleen extramedullary haematopoiesis.

All the other findings in these studies were classified as minimal and in general affected only one sex or one species only.

Thus, the no-observed-adverse-effect level in rat and in dog was defined as 15 mg/kg/day in the 28 days good laboratory practice (GLP) toxicology studies.

In the 13-week toxicity studies conducted to fully cover the length of time patients are exposed, the pancreas, adrenal gland and epididymis were identified as target organs.
Compared with earlier 28-day toxicity studies, only the spermatic granulomas observed in the epididymis of some rats at 15 and 30 mg/kg was a new finding. Following the administration of acoziborole for 13 weeks, the NOAEL was 5 mg/kg and 20 mg/kg in rats and dogs, respectively.

In conclusion, the rat is the most sensitive species and should be used to estimate the therapeutic index.

In reproductive toxicity and embryotoxicity/fetotoxicity studies, including teratogenicity, acoziborole did not induce any changes in reproduction parameters in males or females, nor any abnormalities in embryos/fetuses in rats and rabbits or in treated parturient mothers, suckling neonates or the post-weaning young.

1.4. Safety in humans

As of 19 January 2021, 310 subjects, including 102 healthy male subjects of sub-Saharan African origin in DNDiOXA001, 208 patients with HAT in DNDi-OXA-02-HAT study and 6 healthy Caucasian male subjects in DNDi-OXA-03-HAT were exposed to single doses of acoziborole from 20 to 1200 mg.

In study DNDiOXA001, single doses of acoziborole from 20 mg to 1200 mg were well tolerated overall in healthy subjects of sub-Saharan African origins. The most frequently reported adverse events (AEs) after acoziborole administration were gastrointestinal disorders, abnormalities on investigations (CPK increases, transaminase increases and thyroid hormone fluctuations) and central nervous system-related disorders (mainly headache). The frequency and severity were similar for subjects receiving active and placebo in this study. In addition, some of the abnormalities could be explained by the long follow-up period, which increased the probability of detecting non-drug-related fluctuations and use of activated charcoal. One serious adverse event (SAE) judged possibly related to acoziborole and therefore, a serious adverse reaction (SAR) of transient, mild "*asymptomatic hyperthyroidism*" (preferred term [PT]: hyperthyroidism; assessed as related to acoziborole), was reported after administration of a single dose of acoziborole 240 mg, on the basis of variations of thyroid function tests. It resolved spontaneously within 24 days of onset. This event triggered additional monitoring of thyroid function in subsequent cohorts and studies, however no thyroid-related safety signal has since been observed.

In study DNDi-OXA-02-HAT, 260 patients with HAT were included, among whom, 208 patients were exposed to a single dose of acoziborole 960 mg. A total of 27 treatment-emergent SAEs were reported in 21 patients exposed to acoziborole, all of which were assessed as not related to acoziborole. The most frequently reported SAEs were in the system organ class (SOC) Infections and infestations (n=10; 9 patients) or were related to neuropsychiatric disorders (n=9; 8 patients), including one fatal SAE in the SOC Nervous system disorders ("progressive peripheral ascending polyneuropathy"). Six cases of foetal exposure to acoziborole during pregnancies were reported. The outcomes were as follows:

- Pregnancies: all pregnancies had a normal duration with no complication;
- Deliveries:
  - 5 new-borns were in good general health at birth;
  - 1 prolapse of umbilical cord leading to death 10 hours after prolapse (stillbirth)
- Follow-up:
  - Normal growth in 3 infants during the planned 24 months follow-up.
  - Neonatal infection leading to death in 1 infant at 2 days of life.
  - Probable accidental suffocation during breastfeeding on Day 12, leading to death in 1 infant.

All 6 cases were assessed as not related to acoziborole.

In study DNDi-OXA-03-HAT, the 960 mg oral dose of ^{14}C- acoziborole was well tolerated by the 6 adult healthy volunteers. No SAEs were reported. Thirteen non-serious TEAEs of mild or moderate severity were reported in 6 subjects. Among them, 3 adverse drug reactions (ADRs) were reported and consisted in headaches of mild or moderate severity. No clinically significant abnormal findings were reported for laboratory safety, vital signs, ECG, body weight or physical examination.

Overall, based on the cumulative safety data collected during the clinical development of acoziborole, the benefit-risk balance of acoziborole in g-HAT remains positive.

1.5. Choice of dose and dosing regimen

The dose of 960 mg has been confirmed as the therapeutic dose in the pivotal study DNDi-OXA-02-HAT. The single dose of the investigational product (IP) is recommended to be taken in the fasting state as a food effect study has not yet been performed.

1.6. Rationale for current study

Although the treatment options have advanced with the recent approval of fexinidazole (an effective 10-day oral treatment), there are still gaps with regards to the 2030 Public Health objective of sustained HAT elimination.

Indeed, the standard g-HAT case definition implies the demonstration of the parasite in any body fluid via microscopy. However, there are factors such as low parasitaemia and the complexity and low sensitivity of parasitological methods that make such demonstration difficult. It is known that there are some “indirect” signs of g-HAT that could indicate the presence of the parasites. The most known indirect signs of g-HAT are biological (positive antibody response to either available serological card agglutination test for trypanosomiasis [CATT], rapid diagnostic tests [RDTs; 11], or molecular tests) and clinical. In some circumstances, it has been suggested (11) to consider individuals presenting “indirect” signs, but without confirmatory observation of parasites in biological fluids (considered as g-HAT “suspected” individuals), as cases “eligible” for treatment. Only under special conditions, “suspected” g-HAT patients have been treated (12, 13, 14) since the complexity and toxicity of previously available treatment options typically precluded systematic treatment of these “suspects” due to an individual negative risk-benefit ratio. However, it has been demonstrated that a variable proportion (mainly depending on the prevalence) of such “suspects” are confirmed cases and, therefore, remaining as potential reservoirs of the parasite and a source of new infections hindering the efforts to eliminate the disease (11).

Acoziborole, as an oral single-dose treatment envisioned for all stages of g-HAT, is expected to help boost elimination efforts.

Acoziborole has been studied in an open-label pivotal Phase II/III trial (DNDi-OXA-02-HAT) in the DRC and Guinea. Eighteen months follow-up visits (primary endpoint) were successfully completed in August 2020 and study results (Tables, Figures and Listings) were issued in April 2021.

Given the encouraging safety and efficacy signs observed during the pivotal study conducted with acoziborole, DNDi opened a debate on 24 January 2019 in the frame of the ninth meeting of the sub-group “Integration of new tools into national and global policies” of the WHO g-HAT elimination network in order to define the hypothetical “preventive” use of acoziborole in the population of “suspected” g-HAT individuals. The meeting concluded that for such expanded use, there was a need to plan an additional trial to expand the safety data to complement the safety profile expected to be obtained in the pivotal trial. This would allow a better understanding of the risk-benefit of treatment with acoziborole in the population of g-HAT suspected but parasitologically unconfirmed individuals.

In the context of g-HAT elimination, the WHO HAT elimination Technical Advisory Group convened on 29 November 2019 and agreed (in the event of an effective and safe drug becoming available) to use bolder strategies coupled with case management to detect and treat any human reservoir to interrupt transmission.

A target product profile for a drug to be used in the different epidemiological scenarios was released (see Appendix 1).

The present clinical trial intends to provide responses to challenges encountered by National Sleeping Sickness Control Programmes (NSSCPs) of countries with regard to follow-up of unconfirmed g-HAT individuals (i.e. serologically positive and parasitology negative). Presently, the national policy of both DRC and Guinea is to advise this population to present regularly to health facilities for parasitological examinations until there is laboratory confirmation of parasite presence (thereby leading to treatment), or until seropositivity reverts to being negative (i.e. false seropositivity is concluded). Such an approach, dictated by the complexity of current treatment options, brings a lot of challenges and difficulties in terms of individuals' follow-up and implies that a certain number of infected (but unconfirmed) people remain as reservoirs; perpetuating disease transmission, and thereby hindering the global and national goal of eliminating HAT.

The availability of acoziborole and its unique characteristic of single oral dosing opens the door to put in place strategies to manage serologically positive but parasitologically unconfirmed populations, as long as an acceptable benefit-risk balance in the management of the referred population can be demonstrated.

In addition to this study, a sub-study named 'TrypSkin' is planned to assess the presence of extravascular dermal *T.b. gambiense* in the population enrolled. In an observational cohort study conducted in Guinea, the presence of extravascular dermal trypanosomes has been observed in individuals presenting with CATT positive results in plasma dilution ≥1:4 but not confirmed by parasitological examination of blood and lymph (15). If these dermal trypanosomes correspond to *T.b. gambiense* subspecies and are able to infect vectors, these individuals could act as reservoirs for the transmission of g-HAT, hampering the elimination goal.

Therefore, in a larger scale and in multiple transmission foci, the TrypSkin sub-study aims to determine the presence/absence of *T.b. gambiense* in skin biopsies of subjects who will be willing to participate in the TrypSkin sub-study (optional participation) at enrolment, and at the end of the study. This will allow:

- Estimation of the prevalence of extravascular dermal *T.b. gambiense* in seropositive parasitologically unconfirmed individuals
- To compare different existing and novel methods of detection in blood and/or dermal *Trypanosoma brucei sensu lato* (*T. brucei s.l.)* and *T.b. gambiense*.

An exploratory efficacy evaluation will be performed with the tests included in the TrypSkin sub-study if the prevalence of extravascular *T.b. gambiense* in the skin at inclusion is superior to 2%, and the tests involved in the study demonstrate reliable and robust results to identify presence/absence of extravascular *T.b. gambiense* in the skin.

2. Study objectives and endpoints

2.1. Objectives

2.1.1. Primary objective

To assess the safety and tolerability of a single dose of acoziborole compared with placebo during a follow-up period of 4 months in seropositive individuals who are not confirmed parasitologically.

2.1.2. Secondary objectives

- To assess safety of acoziborole versus placebo in:
  - Haemato-biochemistry
  - Electrocardiogram (ECG) data at Day 5 (double delta versus baseline and time, age and sex matched comparison)
- To assess blood PK at Day 5 and Month 1

- To assess correlation between ΔQTc measurements and acoziborole concentrations in blood at Day 5

2.1.3. Exploratory objectives (TrypSkin sub-study)

- To estimate the prevalence of extravascular dermal *T.b. gambiense* in seropositive individuals non-confirmed parasitologically (16).
- To compare the properties (sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and repeatability) of different methods of detection of *T. brucei s.l*. and *T.b. gambiense* (polymerase chain reactions [PCRs], quantitative PCRs [qPCRs], multiplex Quantitative Reverse Transcriptase [qRT]-PCRs, Specific High-sensitivity Enzymatic Reporter unlocking [SHERLOCK], immuno-histochemistry [IHC]) in skin biopsies and blood samples.
- To determine the presence/absence of extravascular *T.b. gambiense* in skin biopsies of all subjects at enrolment, and at the end of the study.
- To assess the effectiveness of acoziborole on elimination of extravascular *T.b. gambiense* parasites in dermis with the methods or combination of methods, contingent on having better validity than *Trypanosoma brucei gambiense*-specific glycoprotein PCR (TgSGP-PCR) and provided that the prevalence of dermal trypanosomes by any method at baseline is greater than 2%.

2.1.4. Collaborative objective

The blood leftover from subjects included in the TrypSkin sub-study, conserved in a nucleic acids-protective buffer (estimated at approximately 2 to 5 mL including preservative buffer per subject per time-point), will be donated to the WHO Specimen Biobank for future research on new tools for HAT control. Agreement of the subjects will be obtained through the Informed Consent Form (ICF), refusal will not be an exclusion for the participation in the TrypSkin sub-study.

2.2. Study endpoints

2.2.1. Primary endpoint

- Occurrence of treatment-emergent adverse events (TEAEs).

2.2.2. Secondary endpoint(s)

- Occurrence of adverse events (AEs) from ICF signature to the EoS
- Change from baseline in ECG parameters, biochemistry and haematology
- Blood concentration of acoziborole at Day 5 and Month 1
- ΔQTc measurements at Day 5
- Placebo-corrected change from baseline in ECG parameters (double delta [ΔΔ] heart rate [HR], ΔΔRR, ΔΔPR, ΔΔQRS, ΔΔQT and ΔΔQTc)
- Incidence of abnormal values and morphological findings in ECG at Day (D)5

2.2.3. Exploratory endpoint(s) (TrypSkin sub-study)

Primary exploratory endpoint:

- Occurrence of extravascular dermal *T.b. gambiense* at enrolment and at 4 months by TgSGP-PCR in skin.

Secondary exploratory endpoints:

- Clinical parameters: occurrence of dermatitis and/or pruritus at baseline and M4.
- Diagnostic parameters: occurrence of IHC+, TBR- PCR+ and/or 18S-PCR+ in skin and blood at baseline and M4.
- Novel diagnostic parameters: occurrence of positive results by multiplex qPCRs, multiplex RT-qPCRs and SHERLOCK in blood and skin at baseline and M4.

- Occurrence of dermatitis, pruritus, IHC+, multiplex qPCRs+, multiplex RT-qPCRs+ and SHERLOCK+ in skin and blood at baseline and at M4.
- Occurrence comparisons between all diagnostic methods alone or in combination.

3. Study design and study design rationale

3.1. Study design

This is a randomised, multicentre, double-blind, placebo-controlled, parallel-arm phase II/III study to assess the safety and tolerability of a single dose of acoziborole compared with placebo in adolescent and adult subjects seropositive to human African trypanosomiasis due to *T.b. gambiense* (g-HAT positive; not confirmed parasitologically [no demonstration of the parasite in any body fluid via microscopy]) in DRC and Guinea.

The double-blind, placebo-controlled nature of the study design will aid with minimising bias with the parallel-arm design allowing between subject comparison.

Subjects will be hospitalised at least 1 day before study treatment commences and will be randomised in a 3:1 ratio to acoziborole or matched placebo on Day 1 of the study. Following hospitalisation, subjects will attend follow-up visits at the study centre at 1- and 4-months post-treatment. At months 2 and 3, investigator contacts will be established between the site and subjects to ensure close follow-up of subjects and encourage study adherence. An on-site unscheduled visit will be proposed if deemed necessary by the investigator. Any subject with a positive parasitological test result during the study will end their participation in the study and be referred to the NSSCP for appropriate standard treatment.

In addition, a sub-study 'TrypSkin' to assess the presence of extravascular dermal *T.b. gambiense* in this population, will be proposed to subjects having given their consent to participate in the seropositive safety main study (in clinical sites participating in the sub-study only). Subjects can decide whether to participate or not in the sub-study independently of his/her participation in the main safety study.

3.2. Study duration and duration of subject participation

The duration of the enrolment period is estimated to last for approximately 12 months. The study duration for each subject will be 4 months with the EoS visit occurring at 4 months post-treatment (with a window of -10 days to +1 month).

3.3. Rationale of study design

The WHO-HAT elimination network recommended that the current study should include a sufficiently large number of subjects treated with acoziborole to reach a 95% probability to detect at least one uncommon AE. According to the sample size calculation, if 900 subjects are exposed to acoziborole, there would be a high chance (>99%) of detecting at least one AE (if the incidence rate is 1%). Therefore, it is planned that 900 subjects will be administered with acoziborole and 300 subjects with placebo, for an overall sample size of 1200 subjects.

To avoid any bias in AE/ TEAE collection, the study will be double-blinded and placebo-controlled with an allocation ratio of 3:1 (acoziborole:placebo). For more details about the rationale for the ratio decision, please refer to Section 10.1.2.

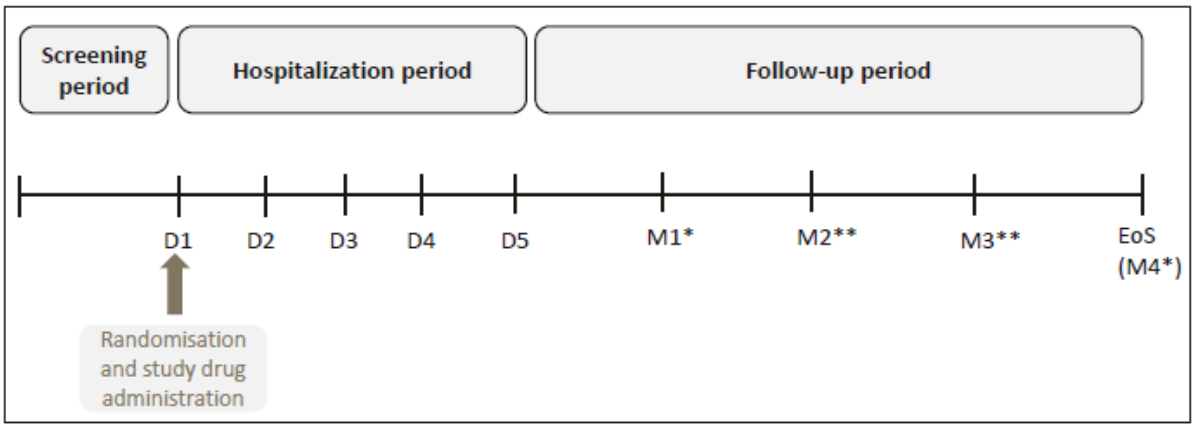
Standard safety assessments during this study will be the collection of TEAEs/AEs and laboratory safety parameters.

As the pivotal study (DNDi-OXA-02-HAT) demonstrated that 97.7% of TEAEs related to acoziborole occurred within 5 days after acoziborole administration, subjects will be hospitalised for an observational follow-up period of 5 days and will be asked to come back for on-site follow-up visits at Months 1 and 4. The visit at Month 4 corresponds to clearance of acoziborole as shown by results from the mass balance study (DNDi-OXA-03-HAT study).

Moreover, to ensure a close follow-up of subjects and encourage study adherence, investigator contacts will be established between the site and study subjects at Months 2 and 3. If deemed

necessary by the investigator, an on-site unscheduled visit will be proposed.
The overall study design is summarised in Figure 2.

Figure 2. Overall study design



D=Day; EoS=end of study; M=month;
* Study site visit; **Investigator contact.

The design of this study (double-blind and placebo-controlled) is the unique opportunity to estimate prevalence of extravascular dermal *T.b. gambiense* in a large population of seropositive individuals who are non-confirmed parasitologically and to compare the effectiveness of new methods to monitor the presence of dermal trypanosomes in seropositive unconfirmed individuals. Therefore, to allow this comparison, molecular and immuno-histochemical tests will be performed at baseline and at the month 4 (EoS) visit for all subjects, who will be willing to participate in the TrypSkin sub-study.

4. Selection of subjects

The following eligibility criteria are designed to select subjects for whom the protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Eligibility criteria may not be waived by the investigator. Any questions regarding a subject's eligibility should be discussed with DNDi medically responsible person prior to the subject's enrolment.

4.1. Inclusion criteria

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study and sub-study:

- Signed the informed consent form
- Male or female
- 15 years of age or older
- CATT test or HAT sero-K-set RDT positive
- Parasitology negative (in blood and/or lymph (if lymphadenopathy is present)
- Karnofsky Performance Status above 70
- Able to ingest oral tablets
- Known address and/or contact details provided
- Must be able to comply with the schedule of follow-up visits and other requirements of the study
- Agreement to be hospitalised upon enrolment for at least 5 days (in order to receive in-ward post-treatment observational follow-up through the first 5 days after treatment)
- Agreement to not take part in any other clinical trials during the participation in this

study
- For women of childbearing potential:
  - Must agree to have protected sexual relations to avoid becoming pregnant from enrolment up to 3 months after dosing (contraceptive protection will be advised and offered at no cost).
  - Negative urine pregnancy tests (before dosing at site level)

4.2. Exclusion criteria

The presence of any of the following criteria will exclude a subject from study enrolment:

- Individuals parasitologically confirmed in blood and/or lymph
- Previously treated for g-HAT
- Severe malnutrition, defined as body mass index (BMI) <16 kg/m^2
- Pregnant or breast-feeding women
- For women of childbearing potential:
  - Urine pregnancy test positive
  - Do not accept contraceptive protection (i.e. condom or sexual abstinence) from enrolment up to 3 months after dosing
- Clinically significant medical condition and/or abnormal laboratory results that could, in the opinion of the Investigator, jeopardise the subject's safety or participation in the study

Additional exclusion criteria for TrypSkin sub-study:

- Rejection to participate in the sub-study in the signed ICF
- Known diabetes
- Known haemophilia

5. Schedule of events

The schedule of events is presented in Table 1.

Table 1. Schedule of Events

	Hospitalisation							Follow-up	
	Screening (before dosing)	**Dosing**	**Post dosing period**					**Site visit**	
	D-2 to D1H0	**D1H0**	**D1**	**D2**	**D3**	**D4**	**D5**	**M1**	**M4 (EoS)**
Informed consent (Main study + TrypSkin sub-study[1])	X								
Serological test (CATT or HAT sero-K-set RDT)	X								X[2]
Parasitological test(s)[3]	X							X	X
Pregnancy test[4]	X							X	X
Demographics	X								
Medical History	X								
Concomitant medications record	X	X	X	X	X	X	X	X	X
Karnofsky index	X							X	X
Physical and clinical examination (including HAT signs and symptoms & vital signs)	X						X	X	X
Dermatological examination	X							X	X
Blood pressure	X		X[5]				X	X	X
Haematology /Biochemistry	X						X	X	X
Inclusion/Exclusion review	X								
Randomisation/Treatment		X							
ECG[6]	X						X		
Trypanolysis test[7]	X								
Blood PK (on DBS)							X	X	
Collection of AEs	X	X	X	X	X	X	X	X	X
TrypSkin sub-study procedures									
Blood sampling for molecular biology	X								X
2 mm-skin-punch biopsies for IHC and molecular biology	X								X

AE=adverse event; CATT=card agglutination test for trypanosomiasis; D=day; DBS=Dry Blood Spot; ECG=electrocardiogram; EoS=End of Study; H= Hour; IHC=immunohistochemistry; M=month; PK=Pharmacokinetics; RDT=rapid diagnostic test.

1. For sites participating in the TrypSkin sub-study only
2. At M4, same serological test than baseline to be repeated
3. mAECT or mAECT-BC and lymph node puncture if suspicious enlarged cervical lymph nodes are detected.
4. For women of childbearing potential only.
5. At D1, blood pressure will be taken 1 hour before dosing, at D1H4 and at D1H9
6. ECG triplicate recording at pre-dose on D1 and D5 according to acceptable time window described in section 8.3.6 .
7. Not for inclusion but as a reference test for follow-up.

6. Enrolment procedures

The study is planned to be conducted in study sites located in regions of the DRC and Guinea, where HAT disease is endemic, and personnel is trained and has experience in HAT clinical trials and in treating patients with the disease.

In order to select a more uniform study population across sites and countries in terms of HAT suspicion, only subjects referred by mobile teams (i.e. active case-finding) will be included in the study. For more details about algorithm for subject's identification and screening process, please refer to Appendix 2.

6.1. Identification at the village level (under the responsibility of NSSCP)

During the course of their usual active case-finding activities, the mobile teams of the NSSCPs of DRC and Guinea identify seropositive individuals (by positive result to one of the serological tests: CATT or HAT Sero-K-Set RDT) that are not parasitologically confirmed at the diagnostic point (village). Those individuals will be notified about the study. Those interested in participating in the study and that have not been previously treated for g-HAT, are not pregnant (a urine pregnancy test could be performed at the discretion of the mobile teams to avoid unnecessary displacements to clinical sites), or breastfeeding, will be invited to attend a visit at the clinical site. Note that all people seropositive (by CATT or HAT Sero-K-Set RDT) and parasitologically confirmed will be taken under NSSCP responsibility for appropriate treatment. Those presenting any of the serological test positive and presenting a pregnancy test positive or breast-feeding or pregnant women will be regularly followed-up by the NSSCP.

6.2. Screening and enrolment procedures at clinical site level

(i) The individuals referred to clinical sites by the NSSCP will be invited to participate in the safety main study and eventually in the TrypSkin sub-study by signing the ICF. The subject can decide to participate or not in the TrypSkin sub-study independently of his/her participation in the main study. His/her declining to participate in the sub-study will not impact his/her participation in the main study.

(ii) After the ICF signature process, all inclusion/exclusion criteria will be checked. If any of the requirements for inclusion are not confirmed, the participants will be classified as a screen failure subject and will be referred to the NSSCP for appropriated management.

(iii) For those who agree to participate in the safety main study, dry blood spots (DBS) will be obtained for a Trypanolysis (TL) test. The TL test is only performed in reference laboratories and receipt of test results could be delayed for several weeks. Therefore, TL result will not be a criterion for inclusion but will be used to classify subjects at the end of the study for addition in the final data analysis. If the TL test is positive, there is a higher probability of the subject being a true case. Upon exiting the study and as soon as TL results are available, it will be ensured that TL positive individuals are referred by the clinical sites to the NSSCP for appropriate surveillance. Upon study unblinding, the treatment arm will be communicated to the NSSCP to ensure the TL positive individuals having received placebo have the appropriate parasitology follow-up assessment. Those who are lost to follow-up will be reported to NSSCP.

(iv) For subjects that will be enrolled in the TrypSkin sub-study: five additional DBS, a 3 mL blood sample and two skin punch biopsies will be obtained for molecular analyses.

(v) Enrolled subjects will be randomised (double blind, unbalanced 3:1 randomisation) into the acoziborole or placebo arm.

7. Study treatments

7.1. DNDi study treatment

The IP is acoziborole 320 mg or matching placebo tablets.

The IP will be provided by DNDi, 15 Chemin Camille-Vidart, 1202 Geneva, Switzerland.

For more details about acoziborole, please refer to the last version of the Investigator Brochure.

7.2. Comparator study treatment

No active comparative treatment will be used in this study.

7.3. Doses and treatment regimens

Acoziborole or matching placebo will be administered to subjects by the oral route in the fasting state according to the following dosing regimen:

- 960 mg (three tablets) in a single intake on Day 1 of the study

7.4. Study treatments labelling, packaging

The IP will be packaged in aluminium-aluminium blister packs. Each pack will contain the number of tablets required for one administration (three tablets) and will be packaged in an individual treatment kit of each subject.

The labelling on the treatment kit will display the following information:

- Name of the Sponsor* and name and contact details of the Coordinating or Principal Investigator
- Study number*
- Name* and dose strength of the IP
- Dosage form*, route of administration*, and number of dose units
- Instructions for use
- The statements “for clinical study use only” and “keep out of the reach of children”
- Batch number* and number of treatment kit*
- Expiry date and storage conditions

The information items marked with an asterisk (*) will also be displayed on the immediate packaging of the IP.

Information on acoziborole will be provided in the Investigator’s Brochure, attached to the protocol submitted to the Competent Authorities.

7.5. Accountability

The study-specific forms must be used for accountability of the IP. Appropriate records concerning receipt, use, return, loss, or other disposition of the IP must be maintained by the Investigators at the study centres or by their delegates, under the supervision of the Principal Investigator. In addition, the study monitors must check accountability of the IP during their on-site monitoring visits.

In the study centres, the IP must be stored in a locked room or a locked cabinet with access limited to the person in charge of the pharmacy or authorised study personnel.

The IP must not be used for purposes other than the present protocol. The Investigator and the study personnel may not, under any circumstances, provide other investigators or healthcare services with the IP attributed to their study centre or allow IP to be used other than as described in this protocol without prior written approval from DNDi.

7.6. Storage

The IP must be transported and stored at a temperature not exceeding 30ºC. It is recommended to store IP at room temperature. The IP should not be used beyond the expiry date indicated on the label.

The storage conditions, including the temperature, at the study centres must be monitored by the study personnel and appropriate records must be available. Any temperature excursion should be recorded and reported to DND*i*.

7.7. Blinding and procedures for unblinding

7.7.1. Blinding Measures

Investigators, personnel of the clinical sites, the Sponsor (including biostatistics and data management personnel and the study monitors), and the participants will remain blinded during the study (i.e. until database lock). To ensure the double blind conditions of the study, acoziborole and matching placebo tablets will be identical in appearance. The packaging and labelling will be designed to maintain blinding of the Sponsor, study personnel and the subjects. The study data will remain blinded until the study database lock, and authorisation of data release according to standard operating procedures.

The randomisation list will be generated by Creapharm (packaging and labelling service providers) by respecting the ratio 3:1 using blocks of 16 kits.

The randomisation list is kept at Creapharm, who was in charge of assigning the kit number for the replacement of the 8 first subjects lost to follow-up.

Randomization data will be kept strictly confidential until the time of final database lock and will not be accessible by anyone else involved in the study, with the following exceptions:

- SGS, the bioanalyst for PK analysis (to avoid the unnecessary analysis of placebo samples)
- The independent statistician from PhinC, who will be in charge of analysing the correlation between ECG measurements and acoziborole concentrations at Day 5
- The designated person from DNDi Pharmacovigilance (or delegate), who will be in charge of unblinding of suspected unexpected serious adverse reaction (SUSARs) in case of exceptional request from the Health Authorities for regulatory safety reporting.
- The designated DNDi unblinded statistician, who will receive a scratching list to be used for individual unblinding in case of request from DSMB members

To ensure maintenance of the blinding during the study, a specific analytical code for PK and ECG analysis will be generated.

7.7.2. Unblinding Measures

If unblinding is required in the interest of the safety of a subject, the site Investigator will discuss the matter with the Sponsor/Study Principal Investigator and/or the National Coordinating Investigator before scratching the individual code-break card for that subject, unless in a medical emergency, when the site Investigator or delegate may open the individual code-break card for that subject without prior consultation with the Sponsor/Study Principal Investigator and/or the National Coordinating Investigator. The site Investigator or delegate will notify the Sponsor as soon as possible, i.e. within 24 hours if possible, that the randomisation code has been broken for the subject with the justification and the participant number.

If the randomisation code is broken by the Investigator, the reason will be fully documented in the appropriate document provided with the individual code-break card.

The DSMB may exceptionally request unblinding of certain subject(s) (e.g. in case of a major safety concern), but only if absolutely necessary, and always in accordance with the DSMB Charter, therefore preventing unblinding the sponsor personnel.

Treatment allocation of all trial participants will be unblinded after the database lock.

7.8. Concomitant treatments

7.8.1. Concomitant medication

If the subject requires treatment after administration of the IP, the Investigator should be informed and approve the proposed medication. Any medication used after inclusion in the study (defined as the date of the ICF signature) and/or during the follow-up period must be recorded in the case report form (CRF) specifying the reason for use.

Any medication used to treat an AE or a SAE must be recorded in the CRF and on the SAE reporting form.

Any essential medicine required during the study participation will be provided to the subject free of charge. The WHO List of Essential Medicines will be used as a reference guide for the treatment of any concomitant condition. For any chronic condition, the study team will take all necessary measures to ensure that the subject is referred to the most appropriate healthcare facility in the region.

7.8.2. Contraception

Preclinical studies showed that acoziborole has no effect on fertility or on post-natal development. However, as a precautionary measure in the absence of sufficient human pregnancy data, women of reproductive age will be advised to have protected sexual relations during the screening period and up to 3 months after receiving the study drug. Contraceptive methods will be made available to subjects free of charge throughout the duration of their participation in the study.

7.9. Medical cares after trial ended

At the end of the study, after unblinding, the NSSCP will be informed of the subjects' study status (TL results at baseline, treatment arm and parasitology results at EoS) for appropriate follow-up according to NSSCP policy as follows:

a) those included in the study and treated with acoziborole and TL positive
b) those included in the study and treated with acoziborole and TL negative
c) those included in the study treated with placebo and TL positive
d) those included in the study treated with placebo and TL negative.

Since the inclusion time-period is expected to last for 12 months and the follow-up is planned for 4 months after treatment, the first study subjects included in the study may have to wait 1 year before knowing whether he/she has been treated with acoziborole or placebo. Therefore, while waiting for the end of the study, and the unblinding, subjects who conclude their 4 months of follow-up study visit (and especially those with TL positive results) will be referred to the NSSCP for the continuation of the NSSCP's standard follow-up management.

For follow-up of women who become pregnant during the study and their newborn babies after the end of their trial participation, please refer to Section 8.5.9.

8. Study Assessments

Eligible subjects will be enrolled after they provide informed consent(s). Subjects will be hospitalised at least 1 day before study treatment commences and will be randomised in a 3:1 ratio to acoziborole or matched placebo arms on Day 1 of the study. Following hospitalisation, subjects will attend visits at the study centre at 1- and 4-months post-treatment and investigator contact between the site and study subjects will be established at 2- and 3-months post-treatment to encourage study adherence and ensure close follow-up of subjects. If deemed necessary by the investigator, an on-site unscheduled visit will be proposed to the subject. Any subject with a positive parasitological test result during the study will end their participation in the study and be referred to the NSSCP for appropriate standardised treatment.

Any premature discontinuation of a subject will be recorded with the reason in the CRF.

8.1. Timing of assessments

The timing of all required assessments is presented in the schedule of activities (Section 5, Table 1).

- Days -2 to D1H0 (before dosing): signature of the ICF (main study and possibly TrypSkin sub-study) and baseline assessments
- Day 1 H0: randomisation and administration of acoziborole or matching placebo tablets
- Days 1 to 5: observation period at hospital
- Day 5: end-of-hospitalisation visit
- Out-patient follow-up visits at 1 months and 4 months (see Table 2). The timing of follow-up visits is calculated from Day 1
- Investigator contacts at 2 and 3 months to ensure close follow-up of subjects and encourage study adherence. An on-site unscheduled visit will be proposed if deemed necessary by the investigator.

Table 2. Theoretical schedule of visits and acceptable time window

Theoretical schedule of visits	Ideal timing of visits	Acceptable time window[1]
End-of-hospitalisation visit	Day 5	Day 5 + 3 days
1 month	1 month (Day 31)	1 month (Day 31) ± 10 days (i.e. between Days 21 and 41)
4 months	4 months (Day 121)	4 months (Day 121) -10 days; +1 month (i.e. between Days 111 and 151)

[1] The acceptable time window for the visit starts on the first day of the period mentioned and ends on the last day of the period mentioned.

8.1.1. Baseline assessments (Day -2 to Day 1 before dosing)

The following assessments will be conducted to confirm subjects' g-HAT seropositive status and to check conformance with inclusion and exclusion criteria:

- Collection of informed consent(s)
- Collection of demographic data
- Review of inclusion/exclusion criteria
- Recording of concomitant medications
- Recording of medical history
- Serological test (HAT Sero-K-Set RDT or CATT) to confirm g-HAT seropositive status.
- Collection of blood/lymph for parasitological testing (m-AECT or mAECT-BC and lymph node aspirate if lymphadenopathy is present).
- Urine pregnancy test (women of childbearing potential only).
- Collection of dried blood spots for TL analysis.
- Collection of blood samples for assessment of haematology and biochemistry parameters
- Physical and clinical examination (including assessment of eventual signs and symptoms that might suggest HAT disease, vital signs, body weight and height)
- Dermatological examination
- Blood pressure (will be taken 1 hour (± 1 hour) prior the administration of the study drug)

- Karnofsky index
- Triplicate ECG at 2 minutes intervals will be recorded according to acceptable time window described in section 8.3.6)
- Collection of AEs

For subjects participating in the TrypSkin sub-study, the following assessment will be performed:

- Sampling of 3 mL fresh blood (in preservative buffer) and 250 µL in 5 DBS
- Sampling of two 2 mm-skin-punch biopsies on the right back shoulder under local anaesthesia

8.1.2. Assessments post study drug administration

8.1.2.1. Assessments during observation period at the hospital

The following procedures will be performed every day throughout the hospitalisation period:

- Collection of concomitant medications
- Collection of AEs

Assessment at Day 1

- Blood pressure will be taken 4 hours (± 1 hour) and 9 hours (± 1 hour) after the study drug administration.

Assessment at Day 5 (End of Hospitalisation Day):

- Physical and clinical examination (including assessment of eventual signs and symptoms that might suggest HAT disease, vital signs, body weight and height)
- Blood sampling for assessment of haematology and biochemistry parameters, and PK
- Recording of triplicate ECG at 2 minutes intervals

8.1.2.2. Follow-up period assessments

All efforts should be made to follow-up the subjects for 4 months.

Assessments at the 1-month follow-up visit:

- Urine pregnancy test (women of childbearing potential only)
- Collection of blood/lymph for parasitological testing
- Physical and clinical examination (including assessment of eventual signs and symptoms that might suggest HAT disease, vital signs, body weight and height)
- Dermatological examination
- Karnofsky index
- Recording of concomitant medications
- Collection of AEs
- Blood sampling for assessment of haematology and biochemistry parameters, and PK

Assessments on 4-month follow-up visit (end of study visit):

- Recording of concomitant medications
- Collection of AEs
- Serological test (CATT or HAT Sero-K-Set RDT, same test than the one used at baseline to be repeated)
- Collection of blood/lymph for parasitological testing
- Urine pregnancy test (women of childbearing potential only)

- Physical and clinical examination (including assessment of eventual signs and symptoms that might suggest HAT disease, vital signs, body weight and height)
- Karnofsky index
- Dermatological examination
- Blood sampling for assessment of haematology and biochemistry parameters

For subjects participating in the TrypSkin sub-study, the following assessment will be performed:

- Sampling of 3 mL fresh blood (in preservative buffer) and 5 DBS
- Sampling of two 2 mm-skin-punch biopsies on the right back shoulder under local anaesthesia

8.2. Assessment of efficacy

This study is not designed to evaluate efficacy.

8.3. Assessment of safety

Full details regarding the laboratory procedures described in this section will be described in the laboratory manual.

8.3.1. Laboratory examinations

Blood samples will be taken for assessment of haematology and biochemistry parameters during the screening period, on Day 5 of the hospitalisation period, Month 1 of the follow-up period, and at the EoS visit at Month 4.

The following laboratory parameters will be assessed:

- Haematology:
  - Haemoglobin
  - WBC count (and formula if WBC value is found abnormal)
  - platelet count
- Biochemistry:
  - Alanine Aminotransferase
  - Albumin
  - Alkaline Phosphatase
  - Aspartate Aminotransferase
  - Calcium
  - Chloride
  - Creatinine
  - Glucose
  - Potassium
  - Sodium
  - Total Bilirubin
  - Total Carbon Dioxide
  - Total Protein
  - Blood Urea Nitrogen

Clinically significant abnormalities in laboratory safety parameters must be recorded as AEs or medical history if recorded at baseline (see section 8.5.2).

8.3.2. Parasitology assessments

Assessments for presence of parasites using mAECT or mAECT-BC will be conducted during screening period, at Month 1, and at the EoS visit at Month 4. The most sensitive parasitological test (m-AECT-BC technique [16]) will be preferred to detect the presence of trypanosomes in blood. When indicated, lymph node aspirate will also be used to

microscopically detect the presence of trypanosomes.

8.3.3. Trypanolysis tests

Two Whatman n°4 filter papers containing eight dried blood spots of 70 µL each will be collected for each subject at screening to assess the presence or absence of antibodies against *T.b. gambiense*.

The TL test is only performed in a reference laboratory and receipt of test results can be delayed for several weeks. Therefore, TL results will not be a criterion for inclusion, but to classify subjects at the end of the study for addition in the final data analysis and to ensure proper follow-up of subjects. If TL is positive, there is a higher probability of the subject being a true case. Upon exiting the study and as soon as TL results are available, TL positive individuals will be referred to the NSSCP by the clinical site for appropriate surveillance. Upon study unblinding, the treatment received by the study participants will be communicated to the NSSCP to ensure that the TL positive individuals having received placebo have the parasitology follow-up assessments conducted. Those who are lost to follow-up will be reported to NSSCP.

8.3.4. Pharmacokinetic analyses

PK analyses will be performed at Day 5 (steady state) and Month 1 (in accordance with acceptable time window of Table 3).

Whole blood (5 x 10 µL) will be deposited on filter paper using the DBS technique and shipped to the reference laboratory for analyses.

For the first 192 subjects administered with the study drug, and who have a valid ECG and a PK sample at D5, PK samples will be analysed to assess correlation between ΔQTc measurements (ECG assessment) and acoziborole DBS concentrations at D5.

The rest of PK samples will be stored and could be analysed in case of unexpected AE, if the samples are still within the stability deadline.

Table 3. Schedule of PK sample collection

Ideal timing of PK sample collection	Acceptable time window
Day 5: 96 hours after intake of IP	± 1 hour
Month 1: 30 days after intake of IP	± 10 days (between Days 21 and 41)

IP=investigational product; PK=pharmacokinetic.

8.3.5. Clinical and physical examination

Subjects will undergo clinical and physical examination including assessment of eventual signs and symptoms that might suggest HAT disease and vital signs at screening and Day 5 during the hospitalisation period, at the follow-up visit at Month 1, and at the EoS visit at Month 4.

Blood pressure of subjects will also be taken at screening, at D1 (1 hour before dosing, at H4 and at H9), at D5, at the follow-up visit at Month 1, and at the EoS visit at Month 4.

8.3.6. Electrocardiogram

ECG measurements will be made in triplicate prior to administration of the study drug (Day 1) and at Day 5 in accordance with acceptable time window described in Table 4. The tracings will be recorded after a 20-minute rest interval and before collection of the blood samples for the PK analyses.

Table 4. Schedule of ECG recordings

Ideal timing of ECG recordings (after 20-minute rest interval and before PK sampling)	Acceptable time window

Day 1: before intake of IP	within 1.5 hour before intake of IP
Day 5: 96 hours after intake of IP	± 1 hour

ECG=electrocardiogram; IP=investigational product; PK=pharmacokinetic.

Electrocardiogram for all subjects will be collected but only data from the first 192 subjects administered with the study drug and who have a valid ECG and a PK sample at D5, will be analysed to assess effect of acoziborole versus placebo at D5. If any signal is detected in this first analysis, all ECG collected will be then analysed. If no signal is detected, ECGs collected for other subjects will be used for safety documentation purposes.

8.3.7. Pregnancy test

Females of childbearing potential will undergo a urine pregnancy test at screening, at follow-up visit on Months 1, and at the EoS visit at Month 4.

8.3.8. Other assessments

The blood leftover from PK sampling at D5 will be kept and stored until the end of the study to be potentially used for genotyping in case of unexpected AEs. These samples will be destroyed at the end of the study (defined as the release of the Clinical Study Report).

8.4. Exploratory assessments

For subjects participating in the TrypSkin exploratory sub-study, the following samples will be taken at screening (baseline) and 4 months (EoS):

- 3 mL fresh blood (in nucleic acid-preservative buffer) and 5 DBS
- Two 2 mm-skin-punch biopsies on the right back shoulder under local anaesthesia

Samples will be shipped to specialised partner laboratories (Institut National de Recherche Biomédicale [INRB], Institut Pasteur Guinea and Institut Pasteur Paris and Institute of Tropical Medicine [ITM] Antwerp) for the following analyses:

1- Molecular detection of trypanosomes:
   - Common automatised extraction of total nucleic acids (TNAs) from fresh blood, DBS and skin biopsies
   - On TNAs from fresh blood and skin (main validated diagnostic methods):
     - For all samples: TBR-PCR and 18S-PCR (*T. brucei s.l.*)
     - For TBR-PCR+ and/or 18S-PCR+ samples: *T.b. gambiense*-specific TgSGP-PCR
   - On TNAs from fresh blood, DBS and skin (additional methods to be tested):
     - qPCR detection using at least 2 targets
     - Multiplex RT-qPCR detection using novel targets
     - CRISPR-derived approach (SHERLOCK)
2- Immuno-histochemical detection of dermal trypanosomes:
   - At least 2 automatised IHC on Formalin-Fixed Paraffin-Embedded (FFPE) skin sections for all subjects with *T. brucei*-specific staining (Anti-Invariable Surface Glycoprotein 65 [ISG65] and Anti-cross reacting determinant [CRD] and secondary polymer amplification
   - Automatised acquisition and double *in silico* blind reading, pictures annotation and archiving
3- Preliminary tests for the detection of trypanosomes by Raman spectroscopy:
   - For each subject at each time point, one DBS from the field and one FFPE slide generated for IHC at IP Paris will be scanned by Raman spectroscopy at the University of Glasgow in the context of an exploratory procedure based on the measurements of biophysical parameters associated to the presence of trypanosomes.

- The results will not be uploaded in the central data base as the final analyses will rely on machine learning approaches after the end of the study.

8.5. Adverse event definitions and reporting

8.5.1. Adverse event definition

An AE will be defined as "*any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product*." (17).

Definition of an AE includes worsening (in severity and frequency) of pre-existing conditions ("Medical history") before first study treatment administration and abnormalities of procedures (i.e. ECG, X-ray, ophthalmologic or neurological examination etc.) or laboratory results which are assessed as "clinically significant".

What is not an AE

- Medical conditions present at the initial study visit that do not worsen in severity or frequency during the study are NOT considered as AEs.
- Symptom exacerbation or worsening of the studied disease will NOT be considered as AEs nor captured on the AE page of the CRF if consistent with the anticipated natural progression of the disease (overall and for this given subject).
- Lack of efficacy of the IP is not considered as an AE.

8.5.2. Assessment of laboratory/procedures abnormalities

For every laboratory/procedure assessment, the investigator will evaluate if the laboratory/procedure test is normal or abnormal. If abnormal (after repeat testing), the investigator will assess if this finding is “clinically significant” or not.

An abnormal lab/procedure test must be compared with the previous value taking into account normal values in the studied population/country.

If a laboratory/procedure parameter is **abnormal AND** the abnormality assessed **clinically significant**, it should be reported as an AE.

An AE is a new event after the administration of at least one tablet of the study drug or a worsening in the condition (in the case of laboratory/procedure tests, it is an increase in severity (clinical intensity) of the abnormality) which is judged clinically significant by the investigator.

Laboratory/procedures (i.e. ECG...) abnormalities (or worsening in severity or frequency of pre-existing abnormalities) should be assessed as “clinically significant” (and therefore have to be reported as an AE) if they meet **at least one** of the following conditions:

- The abnormality suggests a disease and/or organ toxicity **AND** this abnormality was not present at the screening visit or is assessed as having evolved since the screening visit
- The abnormality requires medical intervention or concomitant therapy

When reporting an abnormal lab/procedure as an AE, a syndromic **clinical diagnosis should be recorded** (if available) rather than the abnormal value itself (e.g. acute pancreatitis instead of each finding separately: high levels of amylase, high levels of lipase, abdominal pain and vomiting; e.g. “hypokalaemia” rather than “decreased potassium levels”; “anaemia” rather than “decreased red blood cell count”).

8.5.3. Serious adverse event

An AE will be defined as serious if :

- **It is fatal:** i.e. causes or contributes to the death.
- **It is life-threatening:** in this context refers to an AE in which the subject was at risk of death at the time of the AE; it does not refer to an AE that hypothetically might have caused death if more severe.
- **It requires or prolongs hospitalisation:** i.e. the AE requires at least an overnight admission or prolongs a hospitalisation beyond the expected length of stay. Hospital admissions for surgery planned before study entry, for social reasons, for any elective surgery (i.e. plastic surgery) or per protocol or for normal disease management (including treatment adjustment) are NOT to be considered as SAE according to this criterion.
- **It results in persistent or significant disability:** i.e. the AE resulted in a substantial disruption of the subject's ability to conduct normal activities.
- **It is a congenital anomaly/birth defect:** i.e. an AE outcome in a child or foetus of a subject exposed to the Investigational Medicinal Product before conception or during pregnancy.
- **It is an important medical event:** i.e. is medically significant. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious events/reactions, such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the subject or might require intervention to prevent one of the other outcomes listed above. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious AE.

The Investigator will assess the seriousness of all AEs as serious or non-serious and record the assessment in the electronic case report form (eCRF) along with the following information:

- *SAE onset/start date:* Start date of SAE or date when the AE becomes serious (see seriousness criteria of an SAE).
- *SAE end/stop date:* SAE end date is the date of AE recovery.

8.5.4. Eliciting adverse event information

The investigator is required to report all directly observed AEs and all AEs spontaneously reported by the trial subject using concise medical terminology. In addition, to avoid bias in eliciting AEs, each trial subject will be questioned about the occurrence of AEs during hospitalisation period and study follow-up visits, with general, non-leading questions such as *"Since xxx (e.g. last visit) have you had any health problem?"* or *"How are you feeling?"*

All AEs (serious and non-serious) must be recorded on the source documents and CRFs regardless of the assumption of a causal relationship with the study drug(s). The definition, reporting period, and reporting requirements for AEs are described respectively in sections 8.5.1, 8.5.5 and 8.5.8).

Information on AEs must be evaluated by a physician.

Each AE is to be classified by the investigator as serious or non-serious (see definition of a SAE in section 8.5.3). This classification will determine the reporting procedure for the event.

In addition, the frequency, seriousness (see section 8.5.3), severity (see section 8.5.6), and causality (see section 8.5.7) assessment of AEs will be described.

Non-serious AEs are to be reported in the CRF, including description of the event, onset date, duration, severity, seriousness, relationship to all study drugs, actions taken and outcome.

In the CRF, a given AE will be recorded only once per subject, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF.

SAEs will be reported both on the AE page of the CRF and the SAE forms.

8.5.5. Adverse event reporting period

The AE reporting period begins upon subject enrolment in the trial (after signature of the informed consent) and ends at the EoS visit (Month 4).

All AEs that occur during the AE reporting period specified in the protocol must be reported to DNDi, whether or not the event is considered medication related. In addition, any AE that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational medication should also be reported as an AE.

For the screen failure subjects, only serious study-related events will be followed-up beyond the date of screening failure (to be recorded).

8.5.6. Grading of adverse event severity

For each serious and non-serious AEs, the investigator is required to assess the severity of each AE.

The severity is a clinical determination of the intensity of a specific event.

The severity of the AE will be graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.03) as a guide in the grading of the severity of AEs.

In case of an AE is not described in the CTCAE version 4.03, the investigator will use the terminology MILD, MODERATE, or SEVERE to describe the maximum severity of the AE. This information will be entered in the AE page of the CRF. For purposes of consistency, these severity grades are defined as follows:

- MILD: The subject is aware of the event or symptom, but the event or symptom is easily tolerated (e.g. no reduction in daily activities is required).
- MODERATE: The subject experiences sufficient discomfort to interfere with or reduces his or her usual level of activity.
- SEVERE: Significant impairment of functioning: the subject is unable to carry out usual activities and/or the subject's life is at risk from the event.
- LIFE-THREATENING: The subject is at significant risk of life; it does not refer to an event which hypothetically might have caused death if it were more severe (life-threatening consequences, urgent intervention required).
- DEATH: Death related to an event.

When the intensity of an AE changes over time, each change in intensity will be recorded in the source documents until the event resolves. However, only one AE and the maximum intensity will be recorded in the CRF for each separate event. If the AE resolves but then recurs, each will be recorded as a separate AE, with the appropriate start and stop times.

There is a distinction between severity and seriousness: a severe AE is not necessarily a serious event.

8.5.7. Adverse event causality assessment

For both serious and non-serious AEs, the investigator is required to assess the possible relationship between the AE and the study drug, i.e. to determine whether there exists a **reasonable possibility** that the study drug caused or contributed to the **AE(s)**.

The following categories for relationship to treatment will be used during AE reporting:

Related	There is at least a reasonable possibility of a causal relationship between an AE and an investigational medicinal product. This means that there are **facts (evidence) or arguments** to suggest a causal relationship
Not related	There is no reasonable possibility of causal relationship.

To help investigators with the decision binary tree (Related/Not related) in the evaluation of causality, the Council for International Organizations of Medical Sciences (CIOMS) VI group recommends that investigators be asked to consider the following before reaching a decision:

- Medical history (including presence of risk factors)
- Lack of efficacy/worsening of existing condition
- Study medications
- Other medications (concomitant or previous)
- Withdrawal of study medication, especially following trial discontinuation/EoS medication
- Erroneous treatment with study medication (or concomitant)
- Protocol related procedure.

8.5.8. Adverse event reporting requirements

Information on AEs must be evaluated by a physician. Each AE is to be classified by the investigator as serious or non-serious. This classification will determine the reporting procedure for the event.

All SAEs are to be notified immediately (within 24 hours of awareness of SAEs by the investigator) to the Sponsor at SAEOXA004@dndi.org. This includes a description of the event, onset date and type, duration, severity, relationship to study drug, outcome, measures taken and all other relevant clinical and laboratory data.

NOTE: If the SAE form cannot be sent immediately, the sponsor can be immediately informed (within 24 hours of SAEs awareness by the investigator) by **telephone and/or SMS or by e-mail first**, and then additional relevant information will be shared within 2 to 3 additional calendar days, with or without the SAE report form.

The SAE form must be shared once it becomes available.

Any follow-up reports should be submitted within 2 working days of awareness in case of death or 5 working days of awareness for other cases.

SAE should also be reported on the clinical trial AE page of the eCRF. It should be noted that the form for reporting of SAE (SAE form) is not the same as the AE section of the eCRF. Where the same data are collected, the two forms must be completed in a consistent manner, and the same medical terminology should be used.

In addition, SAEs will be notified by the investigator or Sponsor to Ethics Committee/ Health Authorities as per local reporting requirements.

For more details about the process of SAE notification and reporting in the clinical trial AE page of the eCRF, please refer to the last version of the study procedure related to SAE notification (procedure DNDi-OXA-04-HAT SOP04) and to the last version of the eCRF completion guideline.

8.5.9. Exposure in utero

If any trial subject becomes or is found to be pregnant (based on start date of last menstruation period) while receiving an investigational drug or within 3 months after IP single dose administration, the investigator must notify the event (together with key information) to the Sponsor by email within 24 hours of awareness.

This must be done irrespective of whether an AE has occurred. The information submitted should include the anticipated date of delivery.

The investigator will follow the pregnancy until completion of the pregnancy or until pregnancy termination (i.e. induced/spontaneous abortion). The investigator will provide pregnancy follow-up or outcome information by email or once available on a "***Pregnancy Surveillance Form***" as a follow-up to the initial pregnancy notification.

Follow-up of the children exposed to drug in utero will be proposed to the pregnant participant by the Investigator, until the children reach 2 years of age and shall be reported using the "***Child Surveillance Form***".

As full data regarding both exposures during pregnancy and follow-up of new-born babies will be available after the trial reporting period, the associated analysis will be communicated as an addendum to previous submissions to regulators and policymakers.

A pregnancy is not a SAE. However, any unfavourable outcome meeting at least one seriousness criteria i.e. in the case of unfavourable pregnancy outcome (abortion, still birth) or congenital abnormality shall be notified to the sponsor as per same process as any SAE (within 24h of awareness by investigator, refer to 8.5.8). Once available, the "***SAE form***" must be shared (in addition to the ***Pregnancy/Child Surveillance Form***).

8.5.10. Adverse event follow- up

All AEs should be followed until they are resolved; or the investigator assesses them as 'chronic' or 'stable'; or the subject's participation in the trial ends (i.e. until the EoS page in the CRF for the subject has been completed, or otherwise the last contact with the subject).

The following categories will be used to document each AE:

- Action taken: None, drug treatment, subject withdrawn, other (specified).
- Outcome: Completely recovered; recovered with sequelae; ongoing; not recovered; death; unknown.

In addition, all SAEs (related or not) and those non-serious AEs assessed by the investigator as possibly related to the investigational drug must continue to be followed even after the subject's participation in the trial is over. Such events should be followed until they resolve or until the investigator assesses them as "chronic" or "stable." Resolution of such events is to be documented on the AE page of the CRF and SAE form (if applicable).

9. Withdrawal criteria

9.1. Subject withdrawal from the study

A subject may withdraw from the trial at any time at their own request or may be withdrawn at any time at the discretion of the Investigator or Sponsor for safety, behavioural, or administrative reasons.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject.

In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data, with the exception of safety data, which should be collected if possible and in accordance with subject consent.

If a subject withdraws from the study, and the reason is provided, this should be documented in the source note.

If a subject is withdrawn from the study because of a treatment limiting AE, thorough efforts should be made to clearly document the outcome of AE.

If a subject is withdrawn from the trial, the Investigator will make all necessary arrangements

to ensure that the subject receives the appropriate treatment for any relevant medical condition that may have caused the withdrawal.

Subjects withdrawn from the study will not be replaced.

9.2. Subjects lost to follow-up

If a subject misses the 4-month visit (-10 days to +1-month window) and can no longer be contacted by the study personnel, she/he will be considered as lost to follow-up. Before the subject can be considered as lost to follow-up, the study personnel must make every effort to re-establish contact with the subject or relatives as soon as possible to advise him/her of the importance of continuing in the study and to ascertain whether or not s/he wishes to and/or should continue in the study. These contact attempts should be documented in the participant's medical record.

A subject declared as lost to follow-up was replaced in order to maintain the planned sample size. From 5 months after the start of the study, the rate of subjects lost to follow-up was regularly assessed to ensure 1200 subjects will complete the 4 months of follow-up. However, given the low rate of lost to follow-up subjects after 1.5 year of study conduct, it was agreed that not replacing the subjects lost to follow-up will not impact the statistical power of analysing the primary objective since the subjects will be included in the safety analysis set.

10. Data analysis and statistical methods

Full details of the statistical methods are provided in the statistical analysis plan.

10.1. Sample size and ratio determination

10.1.1. Sample size determination

The sample size was determined based on statistical probabilities to detect uncommon TEAEs in the active treatment (acoziborole) group (see Table 5). The definition for an uncommon event is a relative frequency between 0.1% and 1%. The median relative frequency is therefore 5/1000 (0.5%). The probability to detect at least 1 event if the true incidence rate is 0.5% is as high as 98% with 900 subjects and the probability is 83.5% for a true incidence rate of 2/1000 (0.2%). According to the calculations, 900 subjects exposed to acoziborole shows a high probability (>99%) of detecting at least one event (if the incidence rate is 1%) with a similar probability when exposing 1200 subjects. Therefore, 900 subjects will be administered with acoziborole and 300 subjects with placebo, for an overall sample size of 1200 subjects.

Table 5. Statistical probabilities for detecting uncommon treatment-emergent adverse events (TEAEs) in the acoziborole group

Objectives/sample size (safety)	N=900	N=1200
Chance of detecting at least one event if the incidence rate is 1%	99.9%	99.99%
Chance of detecting at least one event if the incidence rate is 0.1%	59.36%	68.89%
Precision: 95% exact CI if one event is observed	0.003-0.6%	0.002-0.46%
Precision: 95% exact CI if three events are observed	0.068-0.97%	0.052-0.73%

CI=confidence interval; TEAEs=treatment-emergent adverse events.
Uncommon TEAEs are those with frequency ≥1/1000 and <1/100 as per regulatory guidelines.

Sample size calculation for the TrypSkin exploratory sub-study:

The primary objective is to estimate the prevalence of dermal extravascular *T.b. gambiense* trypanosomes in a population of seropositive subjects non-confirmed parasitologically. Preliminary results from Guinea, on a limited number of subjects, have shown that all seropositive individuals presenting a positive CATT test in plasma dilution ≥1:4, but non-confirmed by parasitological examination of blood and lymph, had parasites in skin biopsies (15). Knowing that approximately 25% of the RDT positive subjects non-confirmed parasitologically present a positive CATT test in plasma dilution ≥1:4, it is hypothesized that up to 20-25% of the population of RDT positive subjects non-confirmed parasitologically enrolled in the TrypSkin exploratory sub-study could possibly carry dermal extravascular parasites. Bearing in mind this higher limit of the skin-dwelling trypanosome prevalence, we have estimated the minimum sample size required to be able to evaluate the real prevalence with a satisfactory precision (a narrow confidence interval [CI]). Under the assumption that skin-dwelling trypanosomes could be observed in up to 20-25% of the 1200 subjects, to obtain a 95% CI with a precision of 3%, a minimum of 690 evaluable subjects is required (Table 6). Based on these calculations and taking into account a provision of 5% (35 subjects) to allow for skin samples that are non-analysable, the target number of subjects to be enrolled in the TrypSkin exploratory sub-study is 725.

Table 6. Sample sizes and corresponding precision for TrypSkin exploratory study

	Estimated prevalence					
Precision	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**
0.025	300	550	780	980	1160	1300
0.03[a]	200	390	540	**690**	800	900
0.05	80	130	200	245	290	320

CI=confidence interval.
a. Width of 95% CI approximately 0.06 (6%)

10.1.2. Ratio determination

Operational properties of various allocation ratios for the following assumptions are as follows:

1. Total sample size (for safety main study): n=1200 (900 acoziborole and 300 placebo)
2. Excess rate (ER) for safety: 1% (acoziborole = 2% of AEs of interest, Placebo = 1%)
3. Probability to detect an infrequent AE (≤1% upper limit of class)
4. Probability to detect an infrequent AE (0.5% mid-class)
5. Total sample size for efficacy (TrypSkin exploratory sub-study): n=725 in order to ensure a minimum of 700 evaluable biopsies
6. Binary endpoint for efficacy: success=no tryps detected in punch skin at month 4, failure = at least one tryp-positive result in punch skin at Month 4
7. Probability to detect at least one trypanosome in derma at baseline: 20%
8. No effect of placebo on occurrence of tryps in derma at month 4: failure rate at 4 months=20%
9. Effect of 95% of acoziborole on occurrence of tryps in derma at Month 4 (efficacy): failure rate at Month 4 = 0.05 × 0.20 = 0.01 or 1%
10. Excess rate of success in acoziborole group: 19% (assuming the same efficacy as stage 2 patients)

Table 7. Presentation of different allocation ratio scenarios

	Safety			**Efficacy (for TrypSkin sub-study)**		
Allocation ratio	Sample size for safety	Exact 95% CI limits of ER	Probability to detect an infrequent AE if occurrence rate = 1% (upper limit), = 0.5% (mid-class) and = 0.1% (lower limit of class)	Sample size for efficacy	Exact 95% CI limits of ER Estimate = 20%	Power (chance to detect a difference vs PBO) if acoziborole efficacy is 95%
3:1	PBO = 300 ACO = 900	Lower = -0.92% Upper = +2.41%	Upper: 99.99% Mid: 99.90% Lower: 59.36%	PBO = 175 ACO = 525	Lower = 13.38% Upper = 25.55%	99.99%
2:1	PBO = 400 ACO = 800	Lower = -0.64 Upper = +2.42%	Upper: 99.96% Mid: 98.18% Lower: 55.06%	PBO = 233 ACO = 467	Lower =14.02% Upper = 24.62%	99.99%
3:2	PBO = 480 ACO = 720	Lower = -2.30%* Upper = +0.66%*	Upper: 99.93% Mid: 97.29% Lower: 51.34%	PBO = 280 ACO = 420	Lower = 14.51% Upper = 24.15%	99.99%
1:1	PBO = 600 ACO = 600	Lower = -0.44% Upper = +2.51%	Upper: 99.75% Mid: 95.06% Lower: 45.23%	PBO = 350 ACO = 350	Lower: 14.81% Upper: 23.48%	99.99%

ACO=acoziborole; AE=adverse events; CI=confidence interval; ER=excess rate; PBO=placebo

*Not comparable with other allocation ratio due to decimal digits in the number of subjects requiring rounding to the closest entire number.

Conclusions:

- The four allocation ratios allow detection, with sufficient probability, of an infrequent AE with an exception for the lower limit of the class (0.1%) of infrequent events. In that case, the larger the sample in the acoziborole group, the better the probability of detection.
- The upper limit of the exact 95% CI of the ER of AEs is similar irrespective of the allocation ratio.
- The lower limit of the exact 95% CI is closer to the estimate (shorter 95% CI) with a balanced (1:1) allocation ratio.
- The power of a test of success rate (success = no trypanosome observed in skin punch sample at Month 4) is excellent irrespective of the allocation ratio if the effect of acoziborole is similar to the efficacy in stage 2 (efficacy ≥95%).
- The advantage of an allocation of 3:1 is to maximise the number of seropositive subjects exposed to acoziborole. There is a small disadvantage in the exact lower limit of the 95% CI (larger CI), but no real advantage in the power of the efficacy test.
- The main disadvantage of the balanced allocation ratio (1:1) is a smaller number of exposed subjects.

10.2. Definition of study populations included in the analysis

For the main safety study, the study population that will be used for the analyses is defined as below:

- **Safety set of subjects** (SS) is a modified intent to treat (mITT) set of subjects composed of all randomised subjects who received at least one tablet of IP (placebo or acoziborole).

For the TrypSkin sub-study, the study populations that will be used for the analyses are:

- **Primary set of subjects** for exploratory efficacy analyses is the mITT set composed of subjects, who participate in the TrypSkin sub-study and who receive at least one tablet of IP (placebo or acoziborole).
- **Secondary set of subjects** for exploratory efficacy analyses is the set of all seropositive subjects who are positive for the presence of trypanosomes by at least one molecular diagnostic test in the skin, blood and/or DBS at baseline.

10.3. Subject disposition

Subject disposition data will be presented for each treatment group and will include:

- Number of subjects who have a positive g-HAT serology test
- Number of subjects randomised to study treatment
- Number of subjects treated
- Number of subjects who complete the study
- Number of subjects who discontinue the study (with the reasons for discontinuation)

10.4. Baseline

At the end of the study, subject characteristics will be presented using descriptive statistics (N, mean, standard deviation [SD], median, inter-quartile range, minimum, maximum) for continuous variables or frequencies and percentages for categorical variables. This information will be presented for the following baseline characteristics for the acoziborole and placebo groups:

- Demographic data
- Medical history
- Clinical examination
- Laboratory assessments
- Karnofsky performance status
- Concomitant medications

10.5. Treatment compliance

Information regarding the number of subjects who have fully taken the three tablets during the single dose of study treatment and the number of subjects who vomit in the hour following the administration will be presented descriptively.

10.6. Efficacy analysis

Efficacy analysis is only exploratory in nature and will be conducted under the TrypSkin sub-study described in section 10.8.

10.7. Safety analysis

10.7.1. Primary endpoint analysis

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) SOC and PT. Occurrence rates of each TEAE (≥1%), by MedDRA PT, will be compared.

The primary endpoint for this study is the occurrence of TEAEs from administration of study drug to 4 months post-treatment. It is a binary endpoint (presence or absence of any TEAE).

The primary outcome measure is the ER of TEAEs over the first 4 months post-treatment in the acoziborole group compared with placebo. The occurrence rate will be the number of subjects with at least one TEAE as the numerator and the number of subjects who receive at

least one tablet of study medication (the SS) as the denominator. Loss of subjects to follow-up and withdrawal for a reason unrelated to safety or tolerance will not be considered as AEs.

The ER of occurrence of any TEAE will be transformed to the number of subjects needed to treat (NNT) to observe one more subject with a TEAE in the acoziborole group compared with placebo (NNT=1/ER).

The Cochran-Mantel-Haenszel test, stratified by study centre, will be used to compare the occurrence rate of TEAEs between treatment groups. The same test will be applied to the primary endpoint and each component of the composite endpoint (common TEAEs based on PTs).

Generalisability of the treatment effect will be assessed using a test of homogeneity of the odds ratio (OR) across study centres. If the number of randomised subjects in each study centre reaches 100, each study centre will be considered as a stratum. If one or several study centres has fewer than 100 subjects, the smallest study centres will be pooled together to obtain strata of at least 100 subjects. If the test of homogeneity is not significant, the common OR and the 95% CI will be estimated. If the hypothesis of homogeneity is rejected, the OR per study centre will be provided.

Treatment-emergent AEs will be categorised by severity.

All statistics will be provided to ensure that the most pertinent events (serious and severe) are not “lost” amongst non-serious and non-severe events.

Treatment-emergent AEs will also be categorised by period of occurrence during the study. Two successive periods of time will be considered:

- Occurrence during the hospitalisation period
- Occurrence or prevalence after the hospitalisation period (from discharge to Month 4).

The AE can occur in both periods for the same subject (i.e. the event is still present at the second time period). However, for the period analysis the AE start date will be used to identify AEs in each period. The denominator of occurrence rate in each period will respectively be the number of subjects who receive at least one tablet of study treatment during the first time period and the number of subjects attending the 4-month visit or have reported a TEAE during the second time period. All statistics will be provided per time period to separately assess and interpret early events probably attributed to study treatment and late events that could be attributed to the disease or are persistent.

The time course of treatment effect will be analysed using the log rank test stratified by study centre and will be performed on the time to occurrence of TEAEs, taking into account the attrition rate of subjects who are being followed up. A loss to follow-up or a withdrawal will be censored at the time of the last attended visit. The Kaplan-Meier estimates of the proportion of subjects without any TEAE in each treatment group will be presented graphically to assess the time course of the response.

The hierarchy of testing reflects the sequence of testing from the more general effect to the most specific effect. There is a multiplicity of testing issue and an inflation of type I error that cannot be discarded. However, no adjustments of type I error are planned for this study as this study has been designed to detect any potential safety issues rather than confirming and investigating a safety concern.

The rate of occurrence of AEs will be analysed by the true positive status (subjects with parasites detected during the follow up period) in either treatment or placebo groups.

The rate of conversion to parasite positive and the rate of return to seronegative over 4 months will be analysed descriptively using frequency and proportion by treatment group.

10.7.2. Secondary endpoint analysis

The secondary objectives are to compare safety laboratory and ECG data among the acoziborole and placebo groups. Data will be analysed using double delta analysis versus

baseline and will use time, age- and sex-matched comparisons.

The secondary endpoints are the occurrence of AEs from the time that subjects provide written informed consent to the EoS and changes from baseline in ECG and laboratory safety test results (biochemistry and haematology) to the EoS.

The majority of measured parameters are quantitative and the change from baseline will be the difference between two values. For parameters with binary outcomes (presence or absence), contingency tables (before versus after treatment) will describe the change (or not) of status.

No adjustment for multiplicity of symptoms or laboratory results is planned. The results will therefore not be confirmatory but considered as a signal of potential effect.

Analysis of ECG:

- ECG parameters and changes from baseline (Δ) at D5 will be summarised by treatment group
- Placebo-corrected change from baseline (ΔΔ) will be calculated for each parameter using an analysis of covariance model
- Correlation between ΔQTc and acoziborole concentrations in blood at D5 will be investigated though linear or non-linear regression approaches.
- Incidence of ECG parameters meeting predefined thresholds for abnormality (absolute values of Δ) or of ECG presenting morphological abnormality will be computed by treatment group.

Subset of subjects for ECG Analysis:

- To characterise the effect of acoziborole on QTc prolongation, manually measured intervals from digitalised triplicate ECG will be used.
- Triplicate ECG measurements will be collected and stored for all subjects, but the analysis will be performed on the ECG data of the first 192 subjects, who received at least one tablet of acoziborole or placebo and have valid ECG evaluations
- According to previous studies performed with acoziborole either on healthy volunteers or HAT patients, a common SD of 12 msec and a maximum expected difference with placebo of 4 msec were assumed. The 192 subjects (accounting for the randomisation block size of 16 subjects) will ensure a power above 90% to demonstrate that the effect of acoziborole on QTc is below the threshold of regulatory concern (ΔΔQTc 90% upper bound <10 msec).
- If this criteria of no QTc prolongation is reached, other ECG collected will not be analysed, otherwise the ECG for remaining subjects will be analysed and the effect of acoziborole on QTc prolongation will be estimated at completion of the study.
- In any case, the investigators will review the automatic ECG printouts for safety evaluation during the study.

10.8. Analysis of exploratory endpoints

Primary exploratory efficacy questions of interest

Because dermal trypanosomes could contribute as reservoirs to the transmission of g-HAT hampering the elimination goal, the primary exploratory question of interest is: Does acoziborole reduce the occurrence at 4 months of extravascular dermal *T.b. gambiense* in comparison with placebo?

Primary diagnostic parameter

A subject is considered positive to extravascular dermal *T.b. gambiense* if TgSGP-PCR test in skin is positive.

Primary estimand

The occurrence rate of positive extravascular dermal *T.b. gambiense* at 4 months based on the primary diagnostic parameters will be the primary measure of interest because the sample size is known and it corresponds to a measure of the level of potential transmission of reservoirs. The magnitude of the treatment effect will be the OR of positive dermal *T.b. gambiense*. As the OR is not easy to interpret when the rate of positive subjects in the placebo group is not small (P0 >0.05), the OR will be converted into the risk ratio (RR) using the following formula: RR = (P1/P0) = OR/[(1−P0) + (P0×OR)], where P1 and P0 are the estimated proportions of positive subjects in the acoziborole arm and placebo arm respectively. The ER (P1 – P0) will also be estimated as a secondary measure of the magnitude of the treatment as well as the NTT (number of subjects needed to treat to get one less positive subject at 4 months in acoziborole group).

Comparability of treatment group

To assess whether randomisation provided comparable treatment groups, randomised groups will be compared using descriptive statistics at baseline and more precisely the proportion of dermal trypanosome positive subjects at baseline.

Interpretability of results

The knowledge of the change of status between baseline and 4 months is helpful to interpret results. If in the placebo group, a large proportion of subjects change their status from negative to positive and positive to negative then the repeatability of the test result, and consequently the predictive validity of the diagnostic test, is questionable (see secondary analysis of the primary endpoint).

Primary statistical test

A Cochran-Mantel-Haenszel test using sites as strata will compare acoziborole and placebo occurrence rate at 4 months of positive dermal. The overall OR will be estimated for each parameter as well as the ERs and NTT.

In case of small proportions, the exact Cochran-Mantel-Haenszel test will be performed.

Generalisability of the treatment effect

The Breslow-Day test of homogeneity of the OR across sites will be performed to assess the generalisability of the treatment effect. If the test is significant, a forest plot will be provided to display the heterogeneity of the magnitude of treatment effect across sites and to detect sites explaining the heterogeneity.

Sensitivity analysis

The main sensitivity analysis will be performed on the subgroup of positive dermal trypanosome subjects at entry.

Handling of missing data

Missing data at month 4 will not be imputed to avoid loss of sensitivity of test (exploratory analysis of observed cases). However, a sensitivity analysis will assess the robustness of the primary analyses after imputation of a positive diagnosis in case of missing information.

Multiplicity of testing

Due to the exploratory nature of the sub-study, no adjustment for multiplicity of testing will be done. The p-value will be used to assess the significance of the results.

Analysis of secondary clinical and diagnostic parameters

Dermal trypanosomes may be the cause of dermatitis and/or pruritus, therefore a secondary clinical and exploratory question of interest is:

Does acoziborole reduce the occurrence at 4 months of dermatitis and/or pruritus signs or symptoms in comparison with placebo?

The secondary clinical parameter will be the occurrence/presence of dermatitis and/or pruritus signs or symptoms at 4 months.

The same approach as for the primary diagnostic parameter will be used for the occurrence of dermatitis or pruritus.

Current specific molecular diagnostic methods are known to be of low sensitivity. The TrypSkin sub-study aims at testing new methods to overcome this weakness.

Secondary diagnostic parameters will be the occurrence of IHC+ results in skin, TBR-PCR+ and/or 18S-PCR+ in blood and/or skin, as well as novel diagnostic parameters corresponding to occurrence of positive results by qPCRs, multiplex RT-qPCRs and SHERLOCK in blood and/or skin.

The secondary analyses will be exactly the same as the primary diagnostic parameters.

Properties of diagnostic tests

Results of the following tests, alone or in combination, will be analysed in both groups at enrolment to assess the specificity, sensitivity, positive and negative predictive values of the new qPCRs, multiplex RT-qPCRs, and SHERLOCK in blood and/or skin:

1) TgSGP-PCR in skin (primary diagnostic parameter) and,
2) IHC in skin; TBR-PCR and 18S-PCR in blood and/or skin (secondary diagnostic parameters)

Results of all the new molecular tests, alone or in combination, will also be analysed in the placebo group at 4 months to test their repeatability.

Based on the placebo group data, the association between baseline and 4 months diagnosis results will be estimated through Cramer V coefficient for each diagnostic test and combination of diagnostic tests. The test or combination of tests providing the best association (reproducibility of baseline result) will be used for ranking tests. Other properties: sensitivity, specificity, predictive value, cost and rapidity for obtaining a response will also be used for ranking tests. Multicriteria ranking (sum of ranks) will be performed and the primary analysis will be redone on this new reference (best multicriteria rank) and each diagnostic test property will be computed with respect to this new reference.

10.9. Interim analysis

For the TrypSkin sub-study, an interim analysis for futility will be performed once the baseline data of 50% of subjects will be collected. If the occurrence rate of positivity by any diagnostic method in any sample at baseline lead to less than 2%, then the recruitment in the TrypSkin sub-study will be stopped.

10.10. Missing, unused, and spurious data

Missing data will not be imputed. An observed case approach will be used for the analyses, which will include subjects with available data for a given parameter.

10.11. Deviations from the original statistical plan

Deviations from the original planned analyses will be described and justified in the clinical study report.

11. Data safety monitoring board

A Data Safety Monitoring Board (DSMB), composed of at least 3 members independent of the investigator and sponsors, will be set up prior to study initiation. The DSMB monitors the study in order to ensure that harm is minimised and benefits maximised for the study subjects. They will review the study data at pre-determined intervals and issue recommendations about the

study. The data and intervals will be agreed prior to or soon after the study initiation and documented in the DSMB Charter.

12. Quality assurance and quality control procedures

12.1. Investigator's file

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigator's Site File, subject clinical source documents and screening/enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRF/SAE/pregnancy/Child Surveillance Forms and query forms, IEC and regulatory approvals with correspondence, sample informed consent, drug accountability records, staff curriculum vitae and authorisation forms and other appropriate documents/correspondence etc.

12.2. Case report forms (CRFs)

Data will be collected by laboratory technicians, medical doctors, clinical officers and nurses authorised by the investigator. It will be supervised by the Investigator and signed by the investigator or by an authorised staff member. Study-specific information will be entered into the eCRF and forms/documents sent to Pharmacovigilance. Data that are derived should be consistent with the source documents or the discrepancies should be explained. All eCRF data should be anonymised, i.e. identified by study subject number only.

The investigator at each trial site should ensure the accuracy, completeness, legibility, consistency, and timelines of all data reported to the sponsor in the eCRFs and any other additional information (including SAE/pregnancy/Child Surveillance Forms and queries) that is required. The investigator is responsible for keeping all consent forms, screening forms, CRF/forms sent to Pharmacovigilance and the completed subject identification code list in a secure location.

12.3. Source documents

The verification of the CRF data must be by direct inspection of source documents. Source documents include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, electroencephalogram, X-ray, pathology and special assessment reports, signed ICFs, consultant letters, and subject screening and enrolment logs. The specific laboratory source documents include individual sample tracking forms, raw and annotated PCR gel pictures, raw and annotated q-PCR and RT-qPCR data, and raw and annotated IHC pictures.

In this study, no data will be recorded directly into the eCRF and thus, be considered as source data.

The investigator must maintain source documents such as laboratory and consultation reports, history and clinical examination reports, etc., for possible review and/or audit by DNDi and/or Regulatory Authorities. The Investigator/designee will record the date of each subject's visit together with a summary of their status and progress in the study.

12.4. Record retention

The investigator must keep all study documents on file for at least 15 years after completion or discontinuation of the study. After that period of time the documents may be destroyed with prior permission from DNDi, subject to local regulations.

Should the investigator wish to assign the study records to another party or move them to another location, DNDi must be notified in advance.

12.5. Monitoring

Monitoring visits to the trial and laboratory sites will be made periodically by DNDi

representatives or designated clinical monitors to ensure that good clinical practice (GCP) and all aspects of the protocol are followed. Source documents will be reviewed for verification of consistency with data on CRFs. The investigator will ensure direct access to source documents by DNDi or designated representatives. It is important that the investigators and their relevant personnel are available during the monitoring visits.

The investigators will permit representatives of DNDi and/or designated clinical monitors to inspect all CRFs, medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each subject will be made available provided that subject confidentiality is maintained in accordance with local regulations.

The monitors will perform remote monitoring to follow the progress of the study and verify the completeness of the eCRFs.

The monitoring visits provide DNDi with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of CRFs/SAE/pregnancy/Child Surveillance Forms and queries, resolve any inconsistencies in the study records, as well as to ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four visit types are planned: pre-study, study start, during the study, and study end.

It will be the clinical monitor’s responsibility to inspect the CRF/SAE/pregnancy/Child Surveillance Forms and queries at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

12.6. Audits and inspections

The trial and laboratory sites may also be subject to quality assurance audits by DNDi or designated representatives and/or to inspection by regulatory authorities or Independent Ethics Committees (IEC). The inspections are for the purpose of verifying the adherence to the protocol and to ensure the study is conducted according to GCP.

It is important that the investigators and their relevant personnel are available for possible audits or inspections.

12.7. Data management

Processing of study data will be performed in accordance with applicable Sponsor’s standards and data cleaning procedures and study Data Management Plan, using a validated database. This is applicable for data recorded on eCRF as well as for data from other sources (e.g. PK laboratory).

For data coding (e.g. AEs, medical history, medication), internationally recognised and accepted dictionaries will be used (e.g. MedDRA and WHO Drug Global dictionary). This will be detailed in the Data Management Plan.

12.8. Confidentiality of trial documents and subjects records

The investigator must assure that subjects’ anonymity will be maintained and that their identities are protected from unauthorised parties. On CRFs or other documents submitted to the sponsor, subjects should not be identified by their names, but exclusively by an identification code. The investigator should keep a subject enrolment list showing codes, names, and addresses. The investigator should maintain documents for submission to sponsor authorised representative, and subject’s signed written consent forms, in strict confidence.

12.9. Quality control of laboratory procedures

Quality control of TL tests will be performed by ITM of Antwerp on approximatively 20% of Whatman paper filter samples.

13. Protocol amendments

The Principal investigator will ensure that the study protocol is strictly adhered to throughout, and that all data are collected and recorded correctly on the CRF.

All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the sponsor and the Principal investigator and is to be submitted to the appropriate IEC for information or approval in accordance with local requirements, and to regulatory agencies if required. Approval by IEC (and Regulatory Authority, if applicable) must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial [e.g. change in clinical monitor[s], change of telephone number[s].

The protocol amendment can be initiated by either the sponsor or by any investigator.

The investigator will provide in writing the reasons for the proposed amendment and will discuss it with the sponsor.

14. Early termination of the study

Both the sponsor and the investigator reserve the right to terminate the study at any time prior to inclusion of the intended number of subjects, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the sponsor and the investigator will assure that adequate consideration is given to the protection of the subject's interest.

Reasons for early termination by the sponsor(s) may include but not be limited to:

- Too low enrolment rate.
- Protocol violations.
- Inaccurate or incomplete data.
- Unsafe or unethical practices.
- Questionable safety of the test article.
- Suspected lack of efficacy of the test article.
- Following the recommendation of the DSMB or IEC
- Administrative decision.

Reasons for early termination by the investigator may be:

- Insufficient time or resource to conduct the study
- Lack of eligible subjects

In the event that a study is early terminated either by the sponsor or by the investigator, the investigator has to:

- Complete all CRFs to the greatest extent possible
- Return all test articles, CRF, and related study materials to the sponsor who provided them
- Answer all questions of the sponsors or their representatives related to data of subjects enrolled at the site prior to study termination
- Ensure that subjects enrolled in the study who had not yet reached a follow-up time point are followed up with the necessary medical care.
- Provide in writing the reasons for his decision to the national health authority and the sponsor.

15. Ethics

The experimental protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and International Committee for Harmonization (ICH) guidelines for GCP (ICH E6 R2). DNDi assures that it will comply with all applicable state, local and foreign laws for protecting the rights and welfare of human subjects. This protocol and any protocol amendments will be reviewed/approved by an IEC before its implementation.

It is the responsibility of the Global or National Coordinating Investigator/Investigator to apply for review to the IEC of the country where the study takes place regarding local rules and regulations. Written approval from all involved IECs must be obtained before implementation of any protocol-specified intervention /investigation provided to the subject [such as subject information sheets or descriptions of the study].

Any modifications made to the protocol after receipt of the IEC approval must also be submitted by the principal investigator or national coordinating investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

15.1. Information to communities

The study will be conducted in collaboration with the NSSCP of the DRC and Guinea. The national programmes are responsible for all prevention and treatment activities regarding HAT within the country and, in particular, supervision and coordination of the teams in charge of all HAT screening activities, including survey activities. The NSSCP are fully involved in planning and implementing the study in Guinea and in the Democratic Republic of the Congo.

Information to communities who will participate in the study will follow the procedures of the NSSCP.

The following information on the study will be disseminated at various levels within the communities:

- Routine screening and diagnostic procedures for HAT;
- Primary objective of the study, i.e. to have an oral drug that is safe to use in population at risk of g-HAT infection and that will be made available to the local population;
- Information on the new treatment
- Information on the duration of hospitalisation and the number of follow-up visits up to 4 months, on the importance of attending follow-up visits and the possibility of visits in the village by the study personnel if the subject does not attend follow-up visits at the investigational centre;
- Information on the fact that food will be provided to subjects, who will come at the study centre;
- Information on the organisation of travel and/or reimbursement of travel costs from their village to the investigational centre for subjects included in the study;
- Information on the importance of free choice, expressed by each individual subject, to decide whether or not s/he wishes to participate in the study and sub-study, after receiving full information on the study;
- Information on the need for subjects under legal age to come to the centre accompanied by a guardian/legal representative.

At the end of the study, the community will receive information on the results using the same means of communication, i.e. community mobilisers.

15.2. Informed consent process

15.2.1. General process

Inclusion in the study will occur only if the subject gives written informed consent. It should be noted that informed consent will be taken separately for subjects who agree to participate in the TrypSkin sub-study. Therefore, the subject could decide to participate or not in the

exploratory study independently of his/her participation in the main study.

It is the responsibility of the investigator/designee to obtain written informed consent from each individual participating in this study, after adequate presentation of aims, methods, anticipated benefits, and potential hazards of the study. The informed consent procedure must be done according to the GCP (ICH E6 R2) and local regulations. No study procedure will be performed before the ICF has been signed.

The information provided during the information session will address the following topics:

- Study and TrypSkin sub-study objectives and needs for scientific evaluation of a new treatment
- Information on the new drug from previous study (safety, PK…)
- Number of subjects to be enrolled and the duration of the study
- Criteria to fulfil to be eligible for inclusion in the study and sub-study
- Subjects' commitments during the study and sub-study, i.e. time, compliance with study-specific procedures and attendance at follow-up visits
- Samples to be collected for laboratory tests and purpose of tests
- Benefits and risks associated with study and sub-study participation;
- Compensation for travel costs and provision of food during hospitalisation;
- Subjects' rights regarding withdrawal, rescue treatment, additional information, etc.

Visual aids (including photographs, drawings and samples) describing the activities performed during the study will also be made available to the investigator.

The written informed consent documents will be translated into the local language or a language understood by the subject(s); and submitted to the IEC in each country for approval. If the subject does not speak the national/local language and if pre-specified and authorised staff with knowledge of the dialect/local language are present, an *ad hoc* oral translation may be acceptable. The document signed by the subject will be the form in the language of his/her country/region. The procedures for illiterate subjects should apply. The oral translation should be documented on the signed consent form, i.e. the subject who did the translation will indicate her/his name and the language/dialect used, and will sign the form.

If needed, the person will be given time to discuss the information received with members of the community or family before deciding to consent. The subject or parent/guardian will be asked to provide written and signed consent.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

15.2.2. Illiterate subjects

If the subject is illiterate or unable to write, a literate witness must sign (this person should have no connection to the research team and the sponsor, and, if possible, should be selected by the subject).

If the subject does have an appropriate witness, the team will propose someone from the hospital staff who is not working in the HAT clinical unit, or any literate person from the neighbourhood who is willing to act as a witness. The study team will take all necessary measures to prepare a list of possible witnesses before the start of the study and keep this list updated, in order to find a witness quickly, whenever necessary.

The witness will sign the consent form to attest to the completeness of the information given to the subject, and its compliance with the written information in the ICF. The witness must be present throughout the entire information session.

The witness will confirm that the subject has freely given his/her informed consent to participate in the study.

15.2.3. Subjects under legal age

For subjects under legal age (between 15 and 18 years old) considered as adolescents/young adults, the consent of one of their parents or another culturally acceptable, legal representative will be required. During field visits by the mobile team, adolescents and young adult subjects will be advised to come to the study centre accompanied by a legal representative. No specific subject information sheet or specific form will be used to collect assent from adolescents/young adults recruited to the study, since the data in the subject information sheet is considered to be understandable by both adolescents and adults.

The form must be signed by both the adolescent/young adult and his/her legal representative. If the subject or the legal representative is illiterate, a fingerprint should replace the signature. If the legal representative is illiterate, an impartial witness must attend the assent process and the consent process for the legal representative. The witness will sign the consent form to attest to the completeness of the information given to the subject and his/her legal representative, and its compliance with the written information in the ICF. The witness will confirm that the subject and his/her legal representative have freely given his/her informed consent to participate in the study.

For young adults considered as emancipated because they are already married, the legal representative may be the spouse. If they are not married, but are living on their own, they may be included with their own consent, provided an impartial witness is present during the consent process to confirm their understanding of the study, to confirm the probability that they are indeed emancipated and to sign the consent form along with them.

15.3. Ethical aspects of subject inclusion and study procedures

Only subjects identified during the NSSCPs of DRC and Guinea case-finding activities and confirmed to be g-HAT seropositive and parasitologically negative, will be eligible for inclusion in the study.

The present clinical trial intends to provide responses to challenges encountered by NSSCPs of countries with regards to follow-up of non-confirmed g-HAT individuals (i.e. serologically positive and parasitologically negative). As of today, the national policy of both DRC and Guinea is to advise this population to present regularly to health facilities for parasitological exams until there is laboratory confirmation of parasite presence (thereby leading to treatment), or until seropositivity reverts to negative (i.e. false seropositivity is concluded). Such an approach, dictated by the complexity of current treatment options, brings a lot of challenges and difficulties in terms of individuals' follow-up and implies that a certain number of infected (but unconfirmed) people remain as reservoirs, perpetuating disease transmission, and thereby hindering the global and national goal of eliminating HAT.

The availability of acoziborole and its unique characteristic of single oral dosing opens the door to put in place strategies to manage seropositive parasitologically unconfirmed people as long as an acceptable benefit risk balance in the management of the referred population can be demonstrated.

Data on acoziborole from Phase I studies in healthy volunteers and from the pivotal study conducted in 208 patients, confirmed that the benefit-risk ratio of the dose selected is acceptable for the study population.

No subject will be left without proper treatment and care during or at the end of the study. Therefore, each subject found to be parasitologically confirmed with g-HAT disease during the study will be referred to NSSCP who will ensure appropriate treatment. Upon exiting the study and as soon as TL results are available, TL positive individuals will be referred to NSSCP by clinical sites for appropriate surveillance. At the end of the study, after unblinding, the NSSCP will be informed of all subjects' study status (TL results at baseline, treatment arm, serology, and parasitology results at EoS) for appropriate follow-up according to NSSCP policy.

For subjects participating in the main safety study:

Subjects participating in this study may experience discomfort during examination and blood sampling. Blood draws/laboratory assessments will follow standard of care management.

All samples taken during the study will be used to address the study objectives and ensure the safety of subjects. An extra volume of blood could be taken from subjects at the discretion of the investigator to ensure subject safety. The volume of blood collected will be reduced to a minimum and is estimated at 25 mL in total from screening to the EoS (Month 4).

The maximal quantity of blood taken at each visit is 8 mL.

Some samples will be sent to specialised laboratories:

- PK samples will be sent to SGS in Belgium for centralised assessment of exposure to acoziborole
- Whatman filter paper samples will be sent to INRB at Kinshasa for centralised TL tests
- Approximatively 20% of Whatman filter paper samples will be sent to ITM at Anvers for quality control of TL results

Samples will only be identified by the study number and the subject number. Thus, no information identifying the subject personally will leave the country.

All samples will not be retained after the end of the study and remaining biological material will be destroyed at the latest once the final clinical study report has been validated and signed. The destruction procedure will be recorded in a certificate of destruction.

For subjects participating in the TrypSkin sub-study:

Subjects who agree to participate in the TrypSkin sub-study may experience discomfort during blood and skin-punch biopsies sampling.

In order to reduce pain and limit eventual adverse effects related to sampling, local anaesthetic cream (i.e. Emla®) will be used prior skin-punch biopsies sampling and intra-lesion cutaneous glue will be applied at the sampling site to minimise the risk of infection.

Blood and skin sampling will be performed for the purpose of detecting the presence of trypanosomes. The volume of blood collected will be reduced to a minimum (i.e. 3mL per timepoint)

Some samples will be sent out of the country where the study is being conducted for IHC at Institut Pasteur Paris, France (1 punch biopsy in formalin for secondary diagnostic test), Raman spectroscopy analyses at the University of Glasgow, UK (1 IHC slide and 1 DBS for collaborative objective), and digital droplet qPCR at IRD Montpellier, France (1 DBS for the collaborative objective).

Subject to informed consent, leftover blood will be conserved in nucleic acid-preservative buffer (total volume estimated at around 2 to 5 mL per subject per time-point according to the number of tests performed) and will be donated to WHO Specimen Biobank for further research on new tools for HAT control.

No further samples will be retained after the end of the study and remaining biological material will be destroyed at the latest once the final study report has been validated and signed. The destruction procedure will be recorded in a certificate of destruction.

15.4. Ethical aspects of study treatments

This study could allow a better understanding of the benefit-risk profile to apply treatment of acoziborole in the population of g-HAT suspected but parasitologically unconfirmed subjects (see Section 1.6).

All subjects who are screened but are ineligible or become ineligible at any time during the study will be referred to the NSSCP for appropriate standard treatment.

After unblinding, subject data will be shared with NSSCP for appropriate follow-up according to NSSCP policy.

15.5. Subject indemnity

Subjects administered with the study drug will receive an inconvenience allowance to compensate the loss of income due to their participation in the study. The total inconvenience allowance will be $40.

Subjects will be reimbursed for travel to and from the study site. Any medication that is required during the trial period will be provided free of charge to the subject. Food during the in-patient treatment phase will also be provided free of charge to the individual. This is seen as an essential part of the subject care plan bearing in mind the high prevalence of malnutrition and the poverty of this population.

15.6. Insurance and liability

DNDi is insured to indemnify the investigator against any claim for damages brought by a research subject who suffers from a research related injury during the performance of the trial according to the protocol.

16. Reporting and publication

All clinical trials will be registered with a recognised clinical trial registry such as www.clinicaltrials.gov.

17. References

1. Blum J, Schmid C, Burri C. Clinical aspects of 2541 patients with second stage human African trypanosomiasis. Acta Trop 2006;97(1):55-64.
2. Malvy D, Chappuis F. Sleeping sickness. Clin Microbial Infect 2011;17(7):986-95.
3. Stich A, Abel PM, Krishna S. Human African trypanosomiasis. BMJ 2002;325(7357) :203-206.
4. Franco JR, Simarro PP, Diarra A, Ruiz-Postigo JA, Jannin JG. The journey towards elimination of gambiense human African trypanosomiasis: not far, nor easy. Parasitology 2014;141(6):748-760.
5. Franco JR, Cecchi G, Priotto G, Paone M, Diarra A, Grout L, Simarro PP, Zhao W, Argaw D. Monitoring the elimination of human African trypanosomiasis at continental and country level: update to 2018. PLoS Negl Trop Dis 2020;14(5):e0008261.
6. Franco JR, Cecchi G, Paone M, Diarra A, Grout L, Kadima Ebeja A, et al. (2022) The elimination of human African trypanosomiasis: Achievements in relation to WHO road map targets for 2020. PLoS Negl Trop Dis 16(1): e0010047. https://doi.org/10.1371/journal.pntd.0010047
7. Astelbauer F, Walochnik J. Antiprotozoal compounds: state of the art and new developments. Int J Antimicrob Agents 2011;38(2):118-124.
8. Lutje V, Seixas J, Kennedy A. Chemotherapy for second-stage Human African Trypanosomiasis. Cochrane Database Syst Rev 2010;(8):CD006201.
9. Fexinidazole, the first all-oral treatment for sleeping sickness, approved in Democratic Republic of Congo. https://dndi.org/press-releases/2019/fexinidazole-sleeping-sickness-approved-democratic-republic-congo/.
10. Wring S, et al. Pharmacokinetics and pharmacodynamics utilizing unbound target tissue exposure as part of a disposition-based rationale for lead optimization of benzoxaboroles in the treatment of Stage 2 Human African Trypanosomiasis. Parasitology 2014;141:104-118.
11. WHO (2013). Control and surveillance of human African trypanosomiasis, Technical Report Series. World Health Organisation 2013;984:1-250. https://www.who.int/neglected_diseases/resources/who_trs_984/en/
12. Chappuis F et al. Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African trypanosomiasis (Trypanosoma brucei gambiense) among serological suspected individuals in southern Sudan. Am J Trop. Med Hyg 2004;71:313–317.
13. Simarro PP et al. Attitude towards CATT-positive individuals without parasitological confirmation in the African trypanosomiasis (T.b. gambiense) focus of Quicama (Angola). Trop Med Int Health 1999;4:858–861.
14. Simarro PP et al. The elimination of Trypanosoma brucei gambiense sleeping sickness in the focus of Luba, Bioko Island, Equatorial Guinea. Trop Med Int Health 2005;11(5):636–646.
15. Camara M, Soumah AS, Ilbouldo H, Travaillé C, Clucas C, Cooper A, et al. Extravascular dermal trypanosomes in suspected and confirmed cases of gambiense Human African Trypanosomiasis. Clin Infect Dis 2020;ciaa897
16. Camara M, Camara O, Ilboudo H, et al. Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. Trop Med Int Health 2010;15(7):796-799.
17. ICH E2A - CPMP/ICH/377/95, Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. June 1995.

18. Appendix 1: WHO target product profile for a single-dose oral medicine for extended use in *gambiense* HAT

Outline:

- Table 1 presents the general Target Product Profile.
- Table 2 displays the escalating needs of drug safety (in the last column) that correspond to the escalating extension of use in people with lesser and lesser probability of being infected, for whom the benefit/risk ratio is lower and lower.

Table 1: Target-Product-Profile for a HAT medicine to treat individuals without confirmed infection (extended use)

Characteristics	Ideal profile	Acceptable profile	Comments / Questions
Indication	*T.b gambiense* and *T.b rhodesiense*, both stages. Infection confirmed, suspected, or at-risk.[1]	*T.b gambiense*, both stages. Infection confirmed, suspected, or at-risk.	If active against both sub-species, it would cover hypothetical overlap.
Target population	All population (no subgroups excluded)	Adults including pregnant / lactating women, children >5 years old.	In extended use, if there was need to exclude young children, it's still feasible.
Efficacy	98% in confirmed HAT patients Assumed at least as high in suspects	94% in confirmed HAT patients Assumed at least as high in suspects	Methods to measure efficacy in suspects are not developed
Safety *(see escalating profiles in Table 2)*	Safe in presence of any coexisting pathology or condition. No drug interaction with commonly used medicines.	Few and identifiable conditions where exclusion is recommended (contraindications).	If too many criteria need to be applied, it complicates implementation in extend use.
Route of administration	Oral	Oral	Other route of administration not practical in extended use
Formulation	Adult, and paediatric (e.g. solids for reconstitution or chewable tablets)	Adult, adaptable to children (e.g. crushed or fragmented)	
Need for monitoring adverse events	No need for monitoring	Need to monitor for 1-2 days	The monitoring could be done by health staff in ambulatory
Mode of administration	Oral. Food independent	Oral. Manageable food effect	Practical implications of required food, or fasting condition, must be pondered
Duration of administration	Single dose	Single dose	
Setting of administration	Anywhere, including out of health structures (given by health staff)	In health structures, given by health staff	
Stability	No cold chain. 5 years in Zone 4[2]	No cold chain. 3 years in Zone 4	
Cost	Available for free for patients trough national programs	Available for free for patients trough national programs	Assumes donation by manufacturers and/or subvention by donors
Distribution	Centralized and controlled by WHO	Centralized and controlled by WHO	Ensures appropriate use and monitoring of HAT elimination process.

[1] Confirmed: trypanosomes seen in microscopy. Suspected: definition needs to be developed (see note below). At risk: based on epidemiological markers.
[2] Climatic zone with storage conditions of 30 ± 2°C/65 ± 5% relative humidity (www.ich.org/page/quality-guidelines)

HAT= Human African Trypanosomiasis; WHO=World Health Organization.

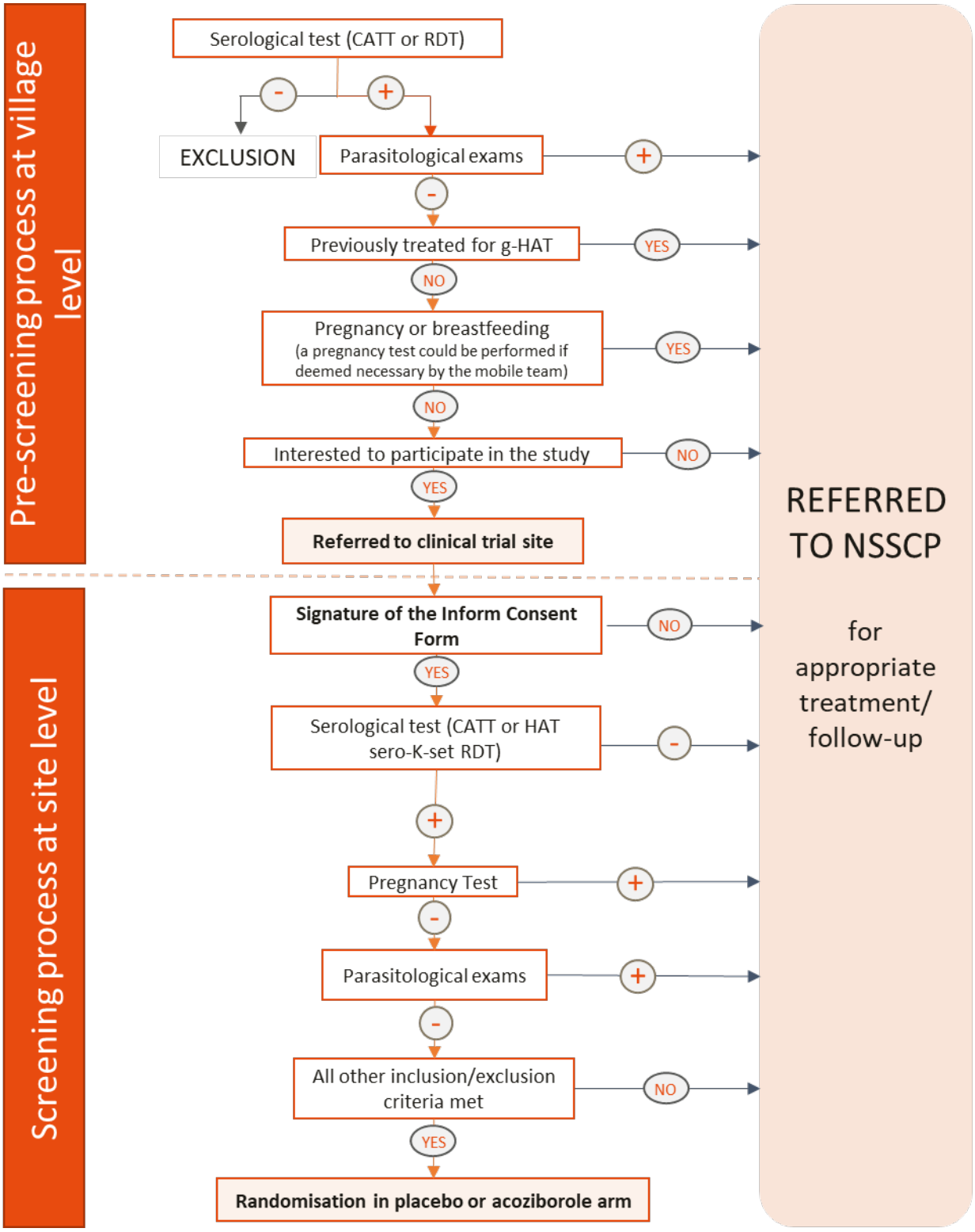
Table 2: Target Safety Profile for escalating extension of use of a single-dose oral medicine (scenarios 1 to 5)

Use of medicine	Advantages / Opportunities	Disadvantages / Challenges	TPP safety
1. Treatment of confirmed g-HAT cases	- Clear benefit for people treated - Known number of true cases - Good control of drug use - Very limited drug pressure averts drug resistance	- Requires parasite exam for confirmation (hinders integration) - A certain number of infected (but unconfirmed) people are not identified and remains as reservoir	Comparable to current treatments (pentamidine, NECT, fexinidazole)
2. Treatment of confirmed g-HAT cases + suspects[3]	- Coverage of confirmed cases and other human parasite carriers - Reduces transmission better/faster than scenario 1 - Good control of drug use - Limited drug pressure averts drug resistance - Known number of true cases, hence it's easier to know when to stop such extended use	- Still requires parasite exam to study suspects in order to confirm cases (hinders integration) - Some people receive unneeded medicine (depending on the PPV[4] of the diagnostic method used) - Benefit/risk ratio[5], for individuals treated, lower than scenario 1	- More robust safety data, on larger cohorts (e.g. from pharmacovigilance) - Safety data in pregnancy - Safety data in children (ideally all ages, but at least in >5 yrs.)
3. Treatment of g-HAT suspects[3] without parasitological exams	- Coverage of many human parasite carriers including cases, depending on diagnostic method used - May reduce transmission better/faster than scenario 1 - No need of parasitology work - Facilitates integration in the health system - Good control of drug use	- Some people receive unneeded medicine (depending on the diagnostic PPV) - Benefit/risk ratio, for individuals treated, lower than scenario 1, and with time, lower than scenario 2 - Uncertainty about HAT epidemiological situation (data on confirmed cases), hence difficult to know when to stop this approach	Strong safety data - larger studies detecting uncommon (1/100 to 1/1,000) AE - Safety data in pregnancy - Safe in children (ideally all ages, but at least in >5 yrs.)
4. Collective use in well-defined populations[6]	- Broader coverage of human parasite carriers including cases - Reduces transmission even faster than scenario 1, 2 and 3 - No need of screening or confirmation tests for the intervention itself - Facilitates integration with other community-based health activities	- Most people receive unneeded medicine - Benefit/risk ratio for individuals treated even lower than scenario 1, 2 and 3 - Possible risk of uncontrolled use of the drug - Requires population acceptability - Involves outreach work - Poor visibility of epidemiological situation	Requires larger studies and specific studies to complete safety knowledge - Rare adverse events (1/1,000 to 1/10,000) studied and characterized - Good safety in pregnancy - Safe in children (ideally all ages, but at least in >5 y/o) - -No interactions, with other drugs or pathogens, causing toxicity or threatening efficacy of other drugs
5. Mass administration in endemic areas	- Treatment of all parasite carriers - Should strongly reduce transmission - No need of screening or confirmation tests for the intervention itself - Facilitates integration with other community-based health activities	- Most people receive unneeded medicine - Benefit/risk ratio is very low - High drug pressure. Risk of uncontrolled drug use and resistance - Requires population acceptability - Involves complex outreach work - No epidemiological visibility	- Proven very low toxicity - No attributable SAE - Very rare adverse events (< 1/10,000) studied and characterized - No toxicity in children exposed in utero - No threat in pregnancy - Safe in children and elderly - No drug interactions causing toxicity or threatening efficacy of other drugs

[3] Suspects' definition needs to be developed, and may include or combine clinical, serological, molecular, immunological, epidemiological methods, and should be adaptable to different settings.
[4] PPV (positive predictive value): probability that a subject testing positive truly has the infection. It depends on the specificity of the test and on the prevalence of the infection in the population.
[5] Benefit/risk for an individual taking the medicine.
[6] e.g. all inhabitants in villages with confirmed cases or with defined lab markers, or sharing space/activity risks; areas with difficult access.

AE=adverse event; g-HAT=Human African Trypanosomiasis caused by *Trypanosoma brucei gambiense*; NECT=Nifurtimox-eflornithine combination therapy; PPV=positive predictive values; SAE=serious adverse events.

19. Appendix 2: Algorithm for subjects' identification and screening



CATT= Card Agglutination Test for Trypanosomiasis; g-HAT=Human African Trypanosomiasis caused by *Trypanosoma brucei gambiense*; NSSCP= National Sleeping Sickness Control Programme; RDT=rapid diagnostic test.