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 **<u>Exploratory endpoint(s) (TrypSkin sub-study)</u>** **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 **<u>Inclusion criteria</u>** • 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)
	Exclusion criteria • 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. **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. 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.