

CLINICAL TRIAL SYNOPSIS

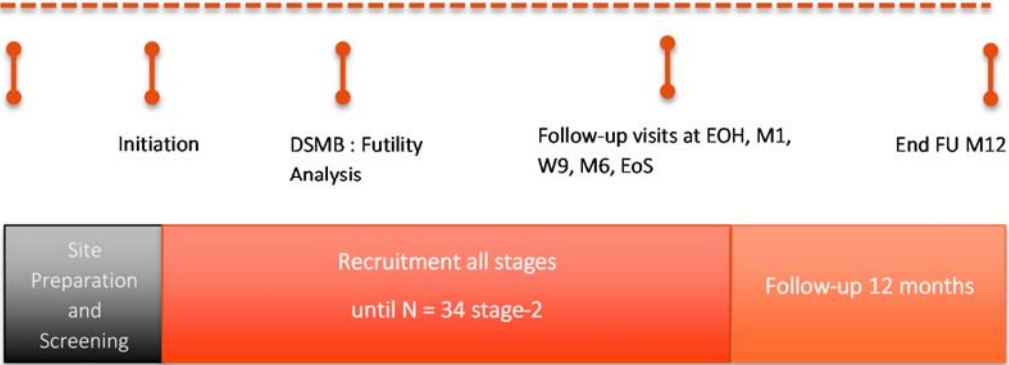
Efficacy and safety of fexinidazole in patients with Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei rhodesiense*: a multicentre, open-label clinical trial

Product Name	Fexinidazole
Drug Class	Antiprotozoal
Phase	II-III
Indication	<i>Trypanosoma brucei rhodesiense</i> (<i>T.b.rhodesiense</i>) Human African Trypanosomiasis (HAT)
Protocol Number	DNDi-FEX-07-HAT
Sponsor	Drugs for Neglected Diseases <i>initiative</i> (DNDi) 15 chemin Louis Dunant, 1202 Geneva, Switzerland
Study Management	Drugs for Neglected Diseases <i>initiative</i> (DNDi) 15 chemin Louis Dunant, 1202 Geneva, Switzerland
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Study Title	Efficacy and safety of fexinidazole in patients with Human African Trypanosomiasis (HAT) due to <i>Trypanosoma brucei rhodesiense</i> : A multicentre, open-label clinical trial
Protocol Synopsis Version / Date	Version 6.0; 16-Nov-2018
Trial Registration	The study will be registered with www.clinicaltrials.gov registry

Background and Rationale	<p>Rhodesiense human African trypanosomiasis (r-HAT) is the zoonotic, acute form of sleeping sickness in Eastern Africa. The disease is rapidly lethal if untreated and has caused large epidemics in the past century. Over the past 15 years, efforts by the national HAT control programmes in all disease-endemic countries and key stakeholders have brought down the patient number to less than 100 per year. Even though these numbers are encouraging, approximately 1.5 million people live in areas still with moderate to high risk of contracting r-HAT [1, 2]. East Africa is affected by sleeping sickness in different foci, which tend to remain spatially stable over time, although the transmission intensity waxes and wanes over the years [3]. To date, Uganda and Malawi reported the highest number of cases worldwide. Latest WHO available data for the 5 years between 2012 and 2016 showed 222 cases in Uganda (51% of all) and 150 in Malawi (34%). Disease severity appears to be very different across East African countries. Disease severity appears to be very different across East African countries. In a cohort of patients treated in Lwala, Uganda between 2004-12; 42.8% were identified as 1st stage (306/715) (18)</p> <p>In Zambia, <i>T.b. rhodesiense</i> infection has shown two different patterns of progression (acute and chronic), whereas an acute disease with rapid progression to late-stage infection is observed in Uganda [7] and more chronic seems to be prevalent in Malawi [23]. Variation in HAT disease severity could be explained by a genetic variation in trypanosome virulence and/or differences in host response to trypanosomes infection [8]. Indeed, two genetically distinct strains of parasite (<i>busoga</i> and <i>zambezi</i>) have been associated with separate geographical locations: the strain group <i>busoga</i> appears to be associated with infections in northern areas, while the <i>zambezi</i> group is associated with the southern semi-acute form of the disease [9].</p> <p>To date, only one drug, melarsoprol, is available for late-stage (meningoencephalitic stage) r-HAT. The use of this arsenic-based drug is associated with severe adverse drug reactions, the most important being an encephalopathic syndrome, which occurs in an average 8.0% of <i>T.b. rhodesiense</i> patients, with a case fatality rate of 57% [10]. Patients treated with melarsoprol need to be hospitalized. Furthermore, melarsoprol-monotherapy could be prone to the development of parasite resistance to the drug in the long term, as already observed in the <i>T. b. gambiense</i> endemic region of northwestern Uganda [11]. Suramin, a sulphated naphthylamide, remains the treatment of choice for early haemolymphatic stage of <i>Tb rhodesiense</i> infection as it does not penetrate the CSF. Its half-life in Plasma can be between 44-54 days. Its relapse levels have been described between 6.9 -31% of 1sts stage <i>Tb rhodesiense</i> patients, increasing up to 64% in patients with borderline CSF anomalies (WBC count between 7-10/μL). It has sometimes been recommended as pre-treatment before giving melarsoprol, but its effect in improving the outcome has not been demonstrated [26]</p> <p>In a declaration for the elimination of HAT due to <i>T.b. rhodesiense</i>, WHO stakeholders urged for a safe, effective and preferably oral treatment [2]. Fexinidazole was identified by DNDi out of hundreds of nitroimidazole compounds as a promising anti-protozoal drug candidate for the treatment of sleeping sickness. <u>The ultimate goal of this study is to provide evidence for the safety and efficacy of fexinidazole for <i>Tb rhodesiense</i> HAT.</u></p> <p>Fexinidazole is a 2-substituted 5-nitroimidazole, formulated for oral administration. It has been shown to possess <i>in vitro</i> and <i>in vivo</i> activity against both <i>T.b. rhodesiense</i> and <i>T.b. gambiense</i> parasites [12].</p> <p>Fexinidazole was shown to be safe in healthy volunteers given single or repeated doses for 14 days [13]. Study participants administered with fexinidazole have shown mild to moderate headache, vomiting and/or other gastrointestinal symptoms [13]. Some</p>
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	<p>reversible elevations in liver enzymes and plasma creatinine levels have been observed in few volunteers after receiving fexinidazole [13]. Changes in ECG parameters consisting of an increased heart rate and prolongation of the QT interval have been observed across several dose levels, with no clear dose effect seen. None of these changes are considered clinically relevant. Predicted CSF concentrations reached target levels after repeated dosing.</p> <p>Fexinidazole efficacy and safety in patients with early- and late-stage HAT due to <i>T.b. gambiense</i> has been tested in three clinical trials (DNDiFEX004, DNDiHATFEX005, DNDiHATFEX006). Overall tolerance in all three studies is similar to healthy volunteers' studies and treatment has not been aborted in any patient. Patients presented nausea/vomiting and CNS/psychiatric AEs (dizziness, headache, tremor, anxiety or insomnia). The majority of AEs were mild or moderate in severity and only two led to permanent treatment discontinuation. Also, a mild and reversible increase in creatinine levels was observed in some patients without any clinical consequence. Variations in ECG parameters were observed within normal ranges including asymptomatic QTc prolongation. There is a new ongoing trial DNDi-FEX-09-HAT testing fexinidazole for <i>Tb gambiense</i> in additional population groups, including pregnant and breastfeeding women, as well as outpatients taking the treatment at home. Preclinical testing has shown that fexinidazole has no genotoxicity in mammalian cells. It is present in breast milk, in similar concentration as in blood, but no relevant toxicity has been predicted for breastfed children [25]</p> <p>Efficacy and safety of fexinidazole as a treatment in other kinetoplastid diseases has been tested. The dose finding, 7-arm placebo-controlled trial for American trypanosomiasis (Chagas disease) DNDi-CH-FEXI-001 was interrupted for safety and tolerability after including 47 patients, using higher dosages than the one used for HAT. The key safety events were: 1) reversible and asymptomatic neutropenia grade 3 and 4; 2) hepatotoxicity, defined as an acute or chronic reversible increase in liver enzymes without bilirubin change; 3) neuropsychiatric events (including a case of completed suicide) and 4) reversible skin hyperpigmentation in sun-exposed areas were reported. Following conclusion of 12 months of follow-up, data review showed high sustained parasite clearance rates of fexinidazole even at the lowest dose tested (1200 mg 2 weeks), including in patients that received < 3 days treatment, with no evidence of relapse. This led to a second clinical trial DNDi-FEX-12-CH, a proof-of-concept evaluation of low doses (600 and 1200 mg) and short treatment duration (at 3, 7 and 10 days) to determine the minimal efficacious and safe dose for the treatment of adult patients with chronic indeterminate CD. This trial is ongoing.</p> <p>The proof-of-concept trial DNDiFEXIVL001 for visceral leishmaniasis, which used the same dose as for HAT, was suspended for lack of efficacy. Overall, fexinidazole was well tolerated by the 14 visceral leishmaniasis patients enrolled in the trial. No SAEs have been reported during this clinical trial.</p>
<p>Objectives</p>	<p><u>Ultimate Objective</u></p> <p>To show that fexinidazole offers an alternative over melarsoprol in stage-2 r-HAT patients and over suramin in stage-1 r-HAT patients</p> <p><u>Primary objective</u></p> <p>To show that the <u>fatality rate</u> (r-HAT or treatment related death) at the end of hospitalisation in stage-2 patients treated with fexinidazole is smaller than a threshold of unacceptable rate of 8.5%</p> <p><i>Rationale: The major issue with melarsoprol is not the cure rate if the patient survives at</i></p>

	<p><i>EoH, but the fatality rate due to toxicity</i></p> <p><u>Secondary Objectives</u></p> <ol style="list-style-type: none"> 1. To show that the <u>failure rate</u> (r-HAT or treatment related death according to DSMB or presence of trypanosomes) at the <u>end of hospitalisation</u> in stage-2 patients treated with fexinidazole is smaller than a threshold of an unacceptable rate of 9% <i>Rationale: A new compound can show low mortality rate but still be not efficacious.</i> 2. To show that the proven failure rate (r-HAT related death according to DSMB or relapse) at <u>12 months</u> (or before) in stage-2 patients treated with fexinidazole is below an unacceptable rate of 12% <i>Rationale: A new compound can be non-toxic and initially efficacious (trypanosomes no longer observed at EoH), but the sustainability of effect can be poor (i.e. no complete elimination of trypanosomes or persistence of high WBC in CSF during follow-up).</i> 3. To estimate the failure rate at EoH and at 12 months in stage-1 r-HAT patients treated with fexinidazole and to verify whether the estimates are smaller than that of suramin 4. To estimate the fatality rate and success rate at 12 months in the overall population (late- and early-stage r-HAT patients) treated with fexinidazole <i>Rationale: 1) It is the same parasite; the same population of patients and the ultimate objective is to yield one treatment regardless of the stage of advancement of the disease. 2) Stage-1 patients are rare hence the estimates for stage 1 alone will not be very informative.</i> 5. To evaluate the safety profile of fexinidazole in late- and early-stage r-HAT patients and to compare it to the one of melarsoprol and suramin as reported in the literature [14]. <p><u>Exploratory Objective</u></p> <ol style="list-style-type: none"> 6. To estimate the time course of relapse of fexinidazole from EoT to 12 months after the end of treatment 7. To assess the PK of fexinidazole and its main metabolites in the blood 8. To assess the reduction in the number of trypanosomes in the blood until the end of study visit
<p>Endpoints</p>	<p><u>Primary Endpoint</u></p> <p>Possibly Related fatality rate at the end of hospitalisation in stage 2 r-HAT patients treated with fexinidazole (death possibly related to r-HAT or treatment according to DSMB; since at the study sites anatomopathological techniques are not available, the completion of the WHO verbal autopsy questionnaire will be requested in case of death)</p> <p><u>Secondary endpoints</u></p> <ol style="list-style-type: none"> 1. Success rate at the end of treatment in stage 1 and stage 2 r-HAT patients, where success is defined as: patient alive and no trypanosomes at end of hospitalization. Failure is defined as: death or presence of trypanosomes in any body fluid at end of hospitalization. Deaths to be considered are defined as possibly related to r-HAT or treatment according to DSMB. Unrelated deaths are neither success nor failure. 2. Success and failure outcomes at the test-of-cure (ToC) visit 12 months after the end of treatment (EOT). A modification of the WHO recommendations [15] is used

	<p>to determine success and failure for stage-1 and stage-2 r-HAT patients (Appendix I - Evaluation criteria of efficacy endpoints)</p> <ol style="list-style-type: none"> 3. Occurrence of adverse events, including abnormal laboratory or ECG findings, during the observation period (until the end of hospitalisation scheduled up to 7 days after EOT) and those considered as possibly related to r-HAT or treatment, among those detected until the end of the follow-up period (12-month visit). All serious adverse events (SAE) whether they are considered as possibly related to r-HAT treatment or not. 4. Unsatisfactory clinical and parasitological response, defined as the compound analysis of the evolution of signs and symptoms as well as laboratory tests during the observation period and at the end of treatment visit. <p>Exploratory Endpoints</p> <ol style="list-style-type: none"> 5. Earliest time to detect a relapse from EOT to 12 months after the end of treatment 6. PK parameters for fexinidazole and its metabolites in whole blood 7. Semi quantification of trypanosomes in blood until end of study and trypanosomes genetic analysis until EoT
<p>Study Design</p>	<p>Multicentre, open-label, non-randomized, clinical trial of patients with r-HAT on efficacy/ tolerability of fexinidazole</p>  <p>From 2012 to 2017, the annual number of patients treated for <i>Tb rhodesiense</i> in all endemic countries was decreasing almost every year: 110, 86, 118, 71, 54 and 27 (source: WHO Data repository). This rare occurrence/incidence of disease raises the issue of the best use of available patients. A positive control randomized trial is not applicable because a randomized study based on the failure rate at EoH requires 124 patients per arm to get a power of 80%. A randomized study based on fatality rate at EoH would require a similar number of patients. With 20 patients per group and using the same expectations (failure rate of 9% and mortality rate of 1%), the exact power is equal to 2.39% This is actually equal to the type I error. In this case, the working hypothesis (smaller fatality rate with fexinidazole) cannot be validated through a frequentist statistical test. In order to retain a null hypothesis that can be rejected by data with a small and realistic sample size, a <u>benchmark study</u> comparing the observed fatality rate to an unacceptable rate (that of melarsoprol in the recent years) was designed. Moreover, a non-inferiority trial is not applicable because the non-inferiority margin is a possible difference in fatality rate in favour of the positive control (melarsoprol) and the goal of the trial is to verify whether fexinidazole could decrease the mortality rate at EOH. For this reason, this trial was designed as a single-arm trial treating patients with fexinidazole only.</p>
	<p>Patients must meet all the inclusion criteria and none of the exclusion criteria.</p>

	<p><u>Inclusion criteria</u></p> <ul style="list-style-type: none"> • Signed Informed Consent Form (plus assent for children) • ≥ 6 years old • ≥ 20 kg body weight • Ability to ingest at least one complete meal per day (or at least one Plumpy'Nut® sachet) • Karnofsky index ≥ 40 • Parasitological confirmed of <i>T.b. rhodesiense</i> infection • Having a permanent address or being traceable by others and willing and able to comply with follow-up visit schedule • Agreement to be hospitalised for a minimum of 12 days and to receive the study treatment <p><u>Exclusion criteria</u></p> <ul style="list-style-type: none"> • Active clinically relevant medical conditions other than HAT that may jeopardize subject safety or at the investigator discretion may interfere with participation in the study. • Compromised general health or severely deteriorated general condition, such as severe malnutrition, cardiovascular shock, respiratory distress, or terminal illness • Any contraindication to nitroimidazole class (known hypersensitivity to imidazoles) or to any of the excipients • Patients previously enrolled in the study or having already received fexinidazole • First trimester of pregnancy • Patients with severe hepatic impairment
<p>Measurements and Procedures</p>	<p><u>Screening</u>: Subjects will undergo blood sampling and/or lymph node aspirate collection followed by microscopic examination to detect trypanosomes. In case of a positive result, a diagnostic lumbar puncture will be performed to evaluate the disease stage..</p> <p><u>Enrolment</u>: Only patients who have given written informed consent and who comply with all the inclusion and none of the exclusion criteria will be included in the study.</p> <p><u>Treatment and follow-up</u>: Participants will undergo study related procedures for efficacy and safety assessment throughout the trial (from baseline until last follow-up visit). During treatment, blood samplings will be drawn for PK and qPCR analysis. During treatment and at 9-week follow-up visit procedures will include: vital signs, physical and neurological examinations, ECG recordings, adverse events recording, history of concomitant medication, haematology, biochemistry, parasitology (mAECT will be used for a semiquantitative comparison of the number of parasites at D0, D5 and D11), and pregnancy test. Blood samples will also be taken at W9, M6 and M12 for qPCR analysis. ECG and laboratory exams other than parasitology will be optional during later follow-up visits, according to the patient status.</p>
<p>Study Product / Intervention</p>	<p>Fexinidazole, 600 mg tablets, given orally, once daily for 10 days, right after the main meal and preferably at the same time every day and only during hospitalisation.</p> <p>The daily doses are adjusted based on body weight and day of treatment, as follows:</p> <ul style="list-style-type: none"> • All adults and children with a body weight ≥ 35 kg: <ul style="list-style-type: none"> – 1800 mg (3 tablets) from day 1 to 4 – 1200 mg (2 tablets) from day 5 to 10 • Children with a body weight ≥ 20 and < 35 kg: <ul style="list-style-type: none"> – 1200 mg (2 tablets) from day 1 to 4

	<p>– 600 mg (1 tablet) from day 5 to 10</p> <p>Allocation of treatment</p> <p>All consenting individuals will be assigned to treatment with fexinidazole.</p>
<p>Risks and benefits of study intervention</p>	<p>First-in-man studies showed that fexinidazole is safe and well tolerated when given in the same doses used in this study [13]. The drug is well absorbed when administered in tablet form and its bioavailability depends on concomitant food intake. Drug-related adverse events are generally mild to moderate, and include headaches, nausea, vomiting, reversible rise in markers of the liver function and psychological problems (anxiety) with high doses [13]. Data in patients with HAT due to <i>T.b. gambiense</i> confirmed that fexinidazole is overall well tolerated. Higher doses studied in Chagas disease patients showed transient neutropenia and raised transaminases.</p> <p>This trial will be conducted in hospitals experienced in the treatment of HAT patients, where qualified staff, equipment and other agents will be available in case of severe adverse reactions to fexinidazole or lack of efficacy. Patients will be followed up to 12 months after hospital discharge to detect potential relapses.</p> <p>If proven to be safe and effective, fexinidazole could replace the toxic melarsoprol for late-stage and suramin for early-stage HAT caused by <i>T.b. rhodesiense</i>. Furthermore, fexinidazole could be used even in community health centres because it is easy to administer.</p> <p>If proven to be safe and effective in both stages, fexinidazole could then overcome the need for staging the disease. This would not only save patients from a painful lumbar puncture but also reduce the overall costs of equipment, infrastructure and trained personnel.</p> <p>If an early failure is observed, the DSMB may propose to continue the study until 34 stage-2 patients or until further decision whichever comes first, assuming that no statistical conclusion will be drawn.</p>
<p>Unacceptable Thresholds</p>	<p>Unacceptable fatality rate at end of hospitalisation</p> <p>Annual fatality rates reported in the four most endemic countries together (Malawi, Uganda, Zambia and Zimbabwe) for stage-2 patients treated with melarsoprol are the following: 12.5% (12/96) in 2010, 12.4% (11/89) in 2011, 20% (14/70) in 2012, 12.1% (8/66) in 2013, 10.4% (10/96) in 2014 and 14.3% (6/42) in 2015. On average, the fatality rate in these 6 years in those 4 countries was 13.3% (61/459).</p> <p>The average (13%) cannot be retained as a threshold because the fatality rate is clearly dependent upon the site and the rate is probably lower in clinical studies. For example, in 2014, the observed fatality rate was 23.3% at the Rumphi site (Malawi) and 6.7% at the Lwala site (Uganda). Moreover, in the Impamel III clinical study the observed death rate was 8.4% (9/107). The retained threshold for the unacceptable fatality rate is therefore 8.5%.</p> <p>Unacceptable failure rate at end of hospitalisation</p> <p>The rate of failure at the end of hospitalisation due to presence of trypanosome is quite infrequent. In the Impamel III study, all failures at end of hospitalisation were due to death.</p> <p>The unacceptable rate of failure (death or presence of trypanosomes) at the end of hospitalisation for stage-2 patients is therefore set at 9%. This threshold was proposed by HAT experts and WHO representatives in a meeting held in December 2016.</p> <p>Unacceptable failure rate at 12 months follow-up</p> <p>The rate of failure at 12 months in the IMPAMEL III study for stage-2 patients was 10.3%</p>

	<p>(9 deaths at EOH + 1 death and 1 relapse during FU). The unacceptable rate of failure set by HAT experts and WHO representatives in December 2016 was 12% and this threshold was retained in this study. The slightly higher failure rate is justified by the fact that a relapse with a new drug can be treated with melarsoprol as a rescue medication.</p>
<p>Number of Participants</p>	<p>A scientific hypothesis is a hypothesis that can be rejected in an experimentation through a statistical test or a given observed fact. If the sample size is too small, the null hypothesis is not rejected even if no deaths are observed (whatever the observed death rate, H_0 is not rejected). The minimal sample size to get a possible rejection of H_0 (fatality rate at EOH = 8.5% or more) in favour of H_1 (fatality rate < 8.5%) is 34 <u>evaluable stage-2 patients</u> for alpha = 0.05 one-sided. In that case if no patients died during treatment the exact one-sided test becomes significant ($p = 0.0488$).</p> <p>Consequently, the sample size is not set according a minimal power of 80%, but according to the possible rejection of the null hypothesis. The second consequence of such an approach is: as soon as one death is observed at EOH, the study may be stopped for futility and failure (inconclusive result). To accept one failure and get a rejection of the null hypothesis once one failure is observed, the minimal sample size becomes 53 patients ($p = 0.04967$, for 1/53 deaths). Because this sample size cannot be reached within two years, the study is unfeasible if this hypothesis is applied.</p> <p>Even if the minimal sample size ($n = 34$) was retained to get a working hypothesis that can be rejected statistically, the power of the study remains quite reasonable. Fexinidazole was administered to 361 stage 2 patients suffering from HAT <i>T.b gambiense</i>. Two deaths were observed before the end of hospitalisation, considered unrelated to the treatment or disease. The fatality rate for any cause was therefore equal to 0.554%. These deaths were probably not due to treatment but considering the case of deaths regardless the cause, the power of the study in patients infected by <i>T.b gambiense</i> would be 82.79%. Using MSF data (M. Humberlin's thesis), the fatality rate in r-HAT stage 2 patients treated with melarsoprol was twice (exactly 1.98) larger in <i>T.b rhodesiense</i> than in <i>T.b gambiense</i> stage 2 patients. If we consider it is due to the fragility of patients infected by rhodesiense trypanosomes, then the expected fatality rate for any cause with fexinidazole could reach 1.097%. Using the corrected estimate (1.097%) as true then the exact power of the study is 68.73 %. Considering deaths possibly related to treatment or disease, the power should be larger than 70% that is a reasonable power in a rare and neglected disease.</p> <p>If one death attributable to the treatment or disease-may occur, the DSMB will rule whether or not the use of fexinidazole shall continue until the sample size of 34 patients is reached but no statistical inference will be made with regards to the unacceptable limit of 8.5%.</p> <p>N.B. In case of non-evaluable patients, the sample size will be increased to reach 34 evaluable patients that are required to get a testable hypothesis in the primary analysis (see primary analysis).</p> <p>The recruitment will be stopped once 34 stage-2 patients are recruited</p>
<p>Study Duration</p>	<p>The entire study should last about 36 months.</p> <p>The enrolment period should be at least 24 months. The participation of each patient will last 12-13 months, and will include:</p> <ul style="list-style-type: none"> • Hospitalisation period:

	<ul style="list-style-type: none"> ○ Pre-treatment: up to 7 days ○ Treatment: 10 days ○ Observation: 2 to 8 days ● Follow-up period: 12 months after the first dose of treatment. <p>If the recruitment of 34 stage-2 patients is shorter than 2 years, the duration of the study can be shortened.</p>
Study Schedule	<p>The participation of each patient will last 12-13 months, and will include:</p> <ul style="list-style-type: none"> ● Screening and informed consent: D-7 to D-1. It includes: diagnosis of <i>T.b. rhodesiense</i>, treatment of concomitant helminthiasis and malaria, ECG and laboratory screening ● Baseline assessment: D -4 to D -1 ● Treatment: D1 to D10 ● End of treatment visit (EoT): D11 ● Observation period (D11-D18) ● End of hospitalization visit (EoH): between D12 and D18 ● Follow-up visits: M1 (home visit) – W9 – M6 – M12 (Test of Cure). Timing for follow-up visits is calculated from D1 (first dose of treatment)
Study Centres	<p>Subjects will be recruited among the patients reporting to Lwala Hospital (Uganda) and Rumphi District Hospital (Malawi). If feasible, r-HAT patients from other hospitals and centres in Kaberamaido/Dokolo Districts (Uganda) and Rumphi/Mzimba North District (Malawi) and possibly from Chama province in Zambia will be referred to Lwala and Rumphi Hospitals, respectively, for treatment.</p>
Case detection	<p>Given the expected low number of study participants, the clinical study will be advertised using local radio. Furthermore, r-HAT experienced people with good knowledge of the area/local culture and good communication skills will visit the respective local authorities/village chiefs before the start of screening activities and inform them about the trial and the related activities.</p> <p>Patients who are not eligible for fexinidazole treatment or who decide not to participate in the trial for any reason will be treated with melarsoprol or suramin according to the national guidelines.</p>
Statistical Consideration	<p>Populations</p> <p>The population of evaluable patients is composed of patients who took at least one dose of fexinidazole excluding those whose death (if any) is documented and clearly attributable to other causes than r-HAT or treatment according to the DSMB and those who escape from the hospital and were not retrieved later to know their status on the primary outcome. This is the primary population. The choice of this set as the primary one is justified by the fact that the study is inconclusive if only one death occurred. If a death is, according to the DSMB, clearly unrelated to r-HAT or treatment such as poisoning, it is not reasonable to stop the trial for failure due to such an unrelated event. The secondary population is the modified intent to treat (mITT) set of patients consisting of all patients who took at least one dose of the study drug. This set will also be the safety set of patients.</p> <p>The intent to treat population (ITT set of patients: all recruited patients who signed the informed consent and were eligible for treatment) will be used to describe the disposition of patient.</p> <p>The fexinidazole treatment completer set of patients will consist of all patients who</p>

terminate the 10 days of treatment with fexinidazole.

Primary analysis

The primary population is the evaluable set of stage-2 patients.

The primary outcome is survival (alive or dead) at EoH.

The primary parameter of interest is death rate at the EoH.

In case of escape of a patient from the hospital and if the patient is not retrieved, the patient will be excluded from the population of evaluable patients and will be considered as a failure, but not a proven death.

A one-sided exact test with respect to 8.5% (unacceptable fatality rate under H_0) will be performed ($\alpha = 0.05$ one-sided).

The one-sided test is justified by the very small number of available patients.

Sensitivity analyses

The same analysis will be performed on the mITT set of patients and on the set of treatment completers if at least 34 patients belong to these sets.

Sample size

Recruitment of both stages will stop once 34 evaluable stage-2 patients are reached.

Futility analysis

Because it is a one arm design and because only one death will lead to a failure of the trial (inconclusive results), the DSMB will meet as soon as one death occurs and the study can be stopped depending on the DSMB recommendations (the DSMB can recommend continuing the trial for exploratory purposes).

Presence of trypanosomes or persistence of high WBC in CSF at any time from the end of hospitalisation until the end of follow up will also be considered as failure and could also help the DSMB in their recommendation to stop the trial.

In case of unsatisfactory clinical and/or parasitological response during the treatment period and if the patient's survival is at risk for an assumed lack of efficacy of the study treatment, the investigator may introduce rescue treatment with suramin for stage 1 and melarsoprol for stage 2 patients. These cases may also be taken into consideration by the DSMB in their recommendation.

Secondary analyses

The first secondary analysis will consist of calculating the failure rate at end of hospitalisation in stage-2 patients and to compare it with the threshold of unacceptable failure rate 9%) through a one-sided exact test ($\alpha = 0.05$ one-sided).

The second secondary analysis will consist of estimating the failure rate at 12 months in stage-2 patients and comparing it with the unacceptable threshold of 12%. A one-sided exact test of comparison will be performed if the observed rate is below 12%.

The fatality rate at EOH, failure rate at EOH and failure rate at 12 months will be estimated. Because the sample size will be very small, no null hypotheses will be testable.

The estimate of fatality rate at EOH, failure rate at EOH and failure rate at 12 months for stage-1 and stage-2 combined will be calculated and tested against the unacceptable limit of 8.5%, 9% and 1%, respectively.

Stopping rules

	The study can be stopped prematurely after recommendation by the DSMB if the DSMB considers that the tolerance to treatment or efficacy is insufficient.
GCP Statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP, as well as all relevant national legal and regulatory requirements.

Appendix I – Evaluation criteria of efficacy endpoints

Category Visit	Death	Relapse	Probable Relapse	Lost to Follow up	Uncertain Evolution	Favourable Evolution	Probable Cure	Cure
EoH	All deaths during hospitalisation, after the start of treatment or resulting from an event that started within that period, which cannot be clearly attributed to reasons other than HAT or the treatment itself	Trypanosomes detected in any body fluid	Tryps-negative patient who refuses lumbar puncture OR whose CSF is haemorrhagic AND who requires rescue treatment		Tryps-negative patient whose clinical condition requires, in the opinion of the Investigator, a close follow-up	Patient alive with no evidence of trypanosomes in any body fluid		
Intermediate follow up visits	All deaths with a clinical picture certainly or possibly compatible with HAT, with the treatment or unknown, to which no alternative cause can be clearly attributed	Trypanosomes detected in any body fluid	Tryps-negative patient with increased WBC/ μ l in CSF from previous values, whose WBC count is unlikely due to causes other than HAT OR who refuses lumbar puncture OR whose CSF is haemorrhagic AND who requires rescue treatment		Tryps-negative patient who, in the opinion of the Investigator, requires a close follow-up exam because of a rising CSF WBC count OR a deterioration of clinical condition that might be due to HAT	Tryps-negative patient with ≤ 5 WBC/ μ l in CSF in a not haemorrhagic sample OR decreased from previous values AND for whom there is direct evidence of satisfactory clinical condition OR whose clinical status is unlikely due to HAT		
12-month	All deaths with a clinical picture certainly or possibly compatible with HAT, with the treatment or unknown, to which no alternative cause can be clearly attributed	Trypanosomes detected in any body fluid	<ul style="list-style-type: none"> Tryps-negative patient with >20 WBC/μl in CSF in a non-haemorrhagic sample, whose WBC count is unlikely due to causes other than HAT Tryps-negative patient who refuses lumbar puncture OR whose CSF is haemorrhagic AND who requires rescue treatment 	Patient who did not attend the 6 and the 12 months visits AND for whom there is no direct evidence of satisfactory clinical condition OR there is direct evidence of a clinical status likely due to HAT		<ul style="list-style-type: none"> Tryps-negative patient who refuses lumbar puncture OR whose CSF sample is haemorrhagic AND whose clinical condition is satisfactory OR whose clinical status is unlikely due to HAT Patient who did not attend the 6 and the 12 months visit AND for whom there is direct evidence of satisfactory clinical condition OR whose clinical status is unlikely due to HAT 	Tryps-negative patient whose CSF sample is not haemorrhagic with ≤ 20 WBC/ μ l	
Analysis	Failure	Failure	Failure	Failure	To be assessed by an Independent evaluation committee	To be assessed by an Independent evaluation committee	Success	Success

Appendix II - Schedule of events

Protocol Procedures and Forms to be completed	Screening and Baseline	Treatment Period										EoT Visit	EoH Visit	Follow-up Visits			Unscheduled Visit ¹		
		D-7 to D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9			D10	D11 ²	D12-D18 ²		M1 ³	W9
Thick/thin blood smear	x																		
mAECT ⁴	x					x						x			x	x	(x) ⁵		
Lymph node aspirate microscopic examination	x											x			x	x	(x) ⁵		
Lumbar Puncture	x											x			x	x	(x) ⁵		
Patient informed consent	x																		
Inclusion/Exclusion criteria review	x																		
Demographics	x														x ⁶	x ⁶	(x) ^{5,6}		
Medical history	x																		
Concomitant medications record	x	x	x	x	x	x	x	x	x	x	x	x	x	x					x
Karnofsky index	x					x						x	x	x	x	x	x	x	x
Treatment of helminthiasis	x																		
Malaria rapid test and/or thick blood smear	x																		
Pre-treatment of malaria ⁷	x																		
Vital signs	x ⁸	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x) ⁵	x	

¹ Additional tests can be performed at investigator's discretion, if medically needed

² EoT could be at D12 and EoH from D13 and D19 if there was a temporary interruption of treatment (max 24hours)

³ M1 consists of a home visit to the patient by the investigator. In case of clinical concerns, the investigator could request parasitological test

⁴ If parasites show negative or very low number, mAECT-BC will also be performed

⁵ If patient is unable or unwilling to attend M12 visit, direct evidence of a satisfactory patient's clinical condition by the Investigator or a delegated staff from the trial team is sufficient. If the clinical condition is unclear, patient should be brought back to the hospital for parasitological exams

⁶ Applied only to children

⁷ Only if malaria test is positive

⁸ Tests to be performed at baseline (D -4 to D -1)

Protocol Procedures and Forms to be completed	Screening and Baseline	Treatment Period										EoT Visit	EoH Visit	Follow-up Visits			Unscheduled Visit ¹		
		D-7 to D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9			D10	D11 ²	D12-D18 ²		M1 ³	W9
Biochemistry	x ⁸					x						x			x				
Haematology	x ⁸					x						x			x				
PK sampling		H0.5 H3.5 H6			H3.5 H6 H12							D10 H24	D10 H48						
Blood sampling for qPCR	x ⁸	H6			H12							D10H 24			x	x	x		
ECG recording (safety)	x ⁸		x ⁹	x ⁹	x ⁹														
ECG recording (triplicate)	x ⁸				H3 H12							D10 H23			x				
HAT signs and symptoms questionnaire	x ⁸					x							x	x	x	x	(x) ⁵	x	
Physical examination	x ⁸					x						x	x	x	x	x	(x) ⁵	x	
Neurological examination	x ⁸					x						x	x	x	x	x	(x) ⁵	x	
Pregnancy test	x ⁸												x		x				
Fexinidazole administration		x	x	x	x	x	x	x	x	x	x								
Adverse events record	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x ¹⁰	x ¹⁰	x ¹⁰	(x) ^{5,10}	x
Serious adverse events (SAEs) record	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x) ⁵	x

⁹ Pre-dose

¹⁰ From M1 to M12: only AEs considered related to fexinidazole are to be reported

Appendix III – Planning information

a. Study Timelines

Final protocol available	Q3 2018
Study treatment supply available	Q4 2018
First Subject First Visit	Q1 2019
Duration of recruitment period	24 months
Duration of follow-up period	12-13 months
Last Subject Last Visit	Q1 2022
Interim analysis	The DSMB will perform a futility at any time during the recruitment period
Final study report	Q1 2022

b. Study Scope

Target countries	Uganda and Malawi
Enrolment target	34 patients in second stage of r-HAT
Number of sites	2
DSMB involvement	A DSMB will regularly review safety data information
Partners involvement	Epicentre, MoH in Malawi and Uganda, Makerere University. IHMT Lisboa, IRD and SwissTPH in training.
Other study special needs	

Appendix IV – Biological assessments

The following biological assessment methods will be performed:

- **Biochemistry tests**

Piccolo[®] chemistry analyser (Abaxis) will be used. 14 parameters will be analysed and recorded in the eCRF.

Albumin (ALB)	Blood Urea Nitrogen (BUN)
Alkaline phosphatase (ALP)	Sodium (Na ⁺)
Alanine Aminotransferase (ALT)	Potassium (K ⁺)
Aspartate Aminotransferase (AST)	Calcium (Ca ²⁺)
Total Bilirubin (TBIL)	Chloride (Cl ⁻)
Total protein (TP)	Glucose (GLU)
Creatinine (CRE)	Bicarbonate (tCO ₂)

- **Haematology tests**

Depending on the equipment available at the trial site laboratories:

- HemoCue[®] Hb 301⁺ (HemoCue[®] AB) may be used to measure haemoglobin content
- Total Leucocytes count. Differential counting will be performed at all haematology timepoints.
- Platelets count.

- **Urine pregnancy test**

- **CSF tests**

- Modified Single Centrifugation (MSC): parasite detection
- Fuchs-Rosenthal chamber: WBC count

- **Blood parasitological tests**

- Thick/Thin blood smear
- mAECT
- In case of unavailability of the mAECT, it can be replaced by the WOO (CTC) test.

- **PK sampling (DBS)**

- a catheter will be inserted for serial sampling during the first 48 hours
- then additional samples will be drawn on day 4 and day 11

- **Blood sampling for qPCR**

- Additional blood from the same PK sampling timepoints and at W9, M6 and M12

Appendix V - Karnofsky scale

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment [16, 17]. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the more serious the impairment is.

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

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