DNDi
Drugs for Neglected Diseases *initiative*

CLINICAL STUDY PROTOCOL

Efficacy and Safety of Fexinidazole compared to Nifurtimox-Eflornithine Combination Therapy (NECT) in patients with late-stage Human African Trypanosomiasis (HAT) due to *T.b. gambiense*: a pivotal, non-inferiority, randomised, open-label, multicentre study

Name of Product(s)/ Project Code	Fexinidazole
Drug Class	Antiprotozoal
Phase	II/III
Indication	Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* at the meningoencephalitic stage (late-stage HAT)
Protocol Number	DNDiFEX004
Sponsor	DND*i*, 15 chemin Louis Dunant, 1202 Geneva, Switzerland Tel: +41 22 906 9230
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In this public version of the protocol, the signature pages have been removed.

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ABBREVIATIONS – GLOSSARY OF TERMS

ACT	Artemisinin-based Combination Treatment
AE	Adverse Event
ALAT	Alanine Aminotransférase
ASAT	Aspartate Aminotransférase
BMI	Body Mass Index
CAR	Central African Republic
CATT	Card Agglutination Test for Trypanosomiasis
CIOMS	Council for International Organisations of Medical Science
Cmax	Maximum Concentration
CSF	Cerebrospinal Fluid
CTC	Capillary Tube Centrifugation
CTCAE	Common Toxicity Criteria for Adverse Events
DBS	Dry Blood Spot
DNDi	Drugs for Neglected Diseases *initiative*
DSMB	Data Safety Monitoring Board
e-CRF	electronic Case Report Form
e.g.	for example
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EOH	End of Hospitalisation
EOT	End of Treatment
FA	Futility Analysis
FDA	Food and Drug Administration
Fexi	Fexinidazole
GGT	Gamma Glutamyl Transpeptidase
H	Hour
HAT	Human African Trypanosomiasis
ICH	International Conference on Harmonisation
INRB	*Institut National de Recherche Biomédicale* (Democratic Republic of the Congo)
ITT	Intention to Treat
IV	Intravenous
J	Day
LP	Lumbar Puncture
M	Months
M1	Metabolite 1 - fexinidazole sulfoxyde
M2	Metabolite 2 - fexinidazole sulfone
mAECT	mini-Anion Exchange Column Test
mAECT-BC	mini-Anion Exchange Column Test - Buffy Coat
MIC	Minimal Inhibitory Concentration
MSC	Modified Single Centrifugation

MSF	*Médecins Sans Frontières*
NCI	National Cancer Institute
NECT	Nifurtimox-Eflornithine Combined Therapy
PACTR	Pan African Clinical Trials Registry
p_{Fexi}	Success rate for fexinidazole
PK	Pharmacokinetic
p_{NECT}	Success rate for NECT
PNLHAT	National HAT Control Programme *(Programme National de Lutte contre la Trypanosomiase Humaine Africaine)*
QT	QT interval on ECG (interval of time between electrical depolarisation and repolarisation of the right and left cardiac ventricles)
QTcF	QT interval corrected by heart rate, according to the formula proposed by Fridericia
RDC	Democratic Republic of the Congo
RDT	Rapid Diagnostic Test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SNC	Central Nervous System
SOT	Start of Treatment
SUSAR	Suspected Unexpected Serious Adverse Reaction
Swiss TPH	Swiss Tropical and Public Health Institute
T.b.	*Trypanosoma brucei*
TOC	Test of Cure
ULN	Upper Limit of Normal
vs.	versus
WHO	World Health Organisation
δ	delta (difference)
µL	Microlitre

PROTOCOL SUMMARY

Study Title	Efficacy and Safety of Fexinidazole compared to Nifurtimox-Eflornithine Combination Therapy (NECT) in patients with Late-stage Human African Trypanosomiasis (HAT) due to *T.b. gambiense*: a pivotal, non-inferiority, randomised, open-label, multicentre study
Study Phase	II/III
Indication	Human African Trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* at the meningoencephalitic stage (late-stage HAT)
Protocol Number	DNDiFEX004
Study Rationale	Human African Trypanosomiasis (HAT) is a potentially fatal, neglected disease. There are currently few treatment options for late-stage HAT, i.e. at the meningoencephalitic stage. Nifurtimox-Eflornithine Combination Therapy (NECT) has been the most widely used since 2010. Although NECT constitutes a significant improvement over other current treatments, it is far from ideal given the conditions under which patients with HAT generally live, i.e. in poor, remote areas with little or no healthcare infrastructures and problems with logistics. There is an urgent need to develop less toxic and easier-to-use products for the treatment of this fatal disease. Fexinidazole is a 2-substituted 5-nitroimidazole, formulated for oral administration. It has been shown to possess *in vitro* and *in vivo* activity against both parasites, *T.b. rhodesiense* and *T.b. gambiense*. Single and repeated doses of fexinidazole have been administered to healthy volunteers for 14 days with no harmful effects (15). Target concentrations in the cerebrospinal fluid can be reached on repeated dose administration. It is now necessary to demonstrate the efficacy and safety of fexinidazole in patients with late-stage HAT.
Study Objectives	The objective of the study is to assess the efficacy and safety of an oral regimen of fexinidazole, once daily for 10 days, as compared to NECT in the treatment of late-stage sleeping sickness due to *T.b. gambiense*. **<u>Primary Objective</u>** ▪ To demonstrate that the success rate at 18 months after the end of treatment is in favour of fexinidazole, or that the difference as compared to the success rate with NECT is acceptable. **<u>Secondary Objective</u>** ▪ To compare the time course of the failure rate with fexinidazole and NECT from the end of treatment visit to 24 months.

	▪ To compare the safety of fexinidazole with that of NECT in the treatment of late-stage sleeping sickness due to *T. b. gambiense*. ▪ To assess concentrations of fexinidazole and its metabolites in the plasma and cerebrospinal fluid, and the correlations between the concentration and the efficacy and safety of fexinidazole. ▪ To compare the pharmacodynamic impact of fexinidazole and of NECT on QT/QTcF interval. **Exploratory Objectives** ▪ To validate the predictive value of three logistic models for treatment success at 18 months. ▪ To compare the efficacy of fexinidazole to that of melarsoprol, eflornithine and NECT using historical data as comparators.
Primary Endpoint	**Efficacy** Outcome, i.e. either success or failure, of treatment assessed at the test-of-cure visit, 18 months after the end of treatment, based on adapted WHO criteria (55).
Secondary Endpoints	**Efficacy** Outcome, i.e. either success or failure, of treatment assessed at each visit between the end of treatment and 24 months.
	Safety Occurrence of adverse events, including laboratory or ECG abnormalities during the observation period, i.e. from D1 to D8, and occurrence of serious adverse events between the first treatment intake and the end of the follow-up period, i.e. 18 months. Long-term safety will be assessed up to 24 months.
	Pharmacokinetics Pharmacokinetic analyses will be performed on all patients in the fexinidazole group using serial dry blood spots and one dry cerebrospinal fluid spot.
	Electrocardiogram (ECG) Categories of QT/QTcF and changes at various timepoints.
Exploratory Endpoint	**Surrogate Efficacy Endpoint** The surrogate endpoint is the actual or predicted outcome (using logistic models, if the outcome is missing) at 18 months, based on the outcome at the last visit that took place at 6 months or later
Futility Analyses	Futility analyses (FAs) assessing the response rate are planned in order to stop exposure to the investigational product if efficacy is below an acceptable limit (see Section 9.11 Futility Analyses; p 67).

Study Design	This is a pivotal, phase-II/III, randomised, open-label, multicentre study with two unbalanced parallel groups (2:1 ratio), comparing fexinidazole and NECT. At least 6 centres will participate in the study. Each centre will recruit up to 140 patients.
Inclusion Criteria	To be eligible for inclusion in the study, patients must fulfil all of the inclusion criteria and none of the exclusion criteria presented in Section 4, Selection of Study Population; p 33). <u>Study Population:</u> ▪ Male or female (pregnant or breast-feeding women will not be included) ▪ 15 years of age or older ▪ Late-stage HAT due to *T.b. gambiense* with parasitological confirmation ▪ Signed informed consent form ▪ Able to ingest at least one complete meal per day ▪ No factors that could aggravate the prognosis or hinder assessment or follow-up in the study
Study Duration	Each patient's participation will last approximately 25 months and will include: • pre-treatment period • treatment period of 10 days • hospitalisation for 3 to 8 days after treatment • out-patient follow-up for 18 months, until the test-of-cure visit • additional follow-up for 6 months, until M24

Study Treatment and Reference Treatment	**Study Treatment** Fexinidazole, 600 mg tablets, administered by the oral route after the main meal of the day: • 1800 mg (3 tablets) in a single daily intake for 4 days • followed by 1200 mg (2 tablets) in a single daily intake for 6 days The total duration of treatment will be 10 days. **Reference Treatment** Nifurtimox-Eflornithine Combination Therapy (NECT). Nifurtimox tablets, administered by the oral route three times daily, at the dose of 15 mg/kg/day, for 10 days (D1 to D10). Eflornithine, administered twice daily as a 2-hour IV infusion, at the dose of 400 mg/kg/day, for 7 days (D1 to D7). The total duration of treatment will be 10 days. **Treatment Assignment** Patients will be randomised at a 2:1 ratio, with 2 patients in the fexinidazole group for 1 patient in the NECT group. The randomisation process will be centralised.
Study Schedule	▪ Screening: D-15 to D-1 ▪ Baseline assessment: D-4 to D-1 ▪ Randomisation: D-1 ▪ Treatment: D1 to D10 ▪ End of Treatment Visit: D11 ▪ End of hospitalisation Visit: D13 to D18 ▪ Observation Period: D1 to D18 (for safety) ▪ Additional Follow-up Visit: 9 weeks after D1 (D64 to D70) ▪ Follow-up Visits: M3 – M6 – M12 – M18 (Test of Cure) – M24 (Additional Follow-up Visit) after D11
Statistical Analyses	**Populations** The primary analysis will be performed on the Intention-to-Treat population. Sensitivity analyses will be performed on patients who completed the treatment, evaluable patients and the per protocol population. **Interim Analysis** An interim primary analysis for success will be performed on the first 195 randomised patients who have theoretically reached the 12-month follow-up visit and will exclude patients for whom no post-treatment data are available because the patient fled the area strictly for reasons of armed conflict. The analysis will be identical to the final primary analysis with the exception of the threshold for significance, the rule for imputing missing data (see Adjustment of Type-I Error for Multiplicity) and the assessment visit, which will be 12 months instead of 18 months.

A sensitivity analysis, including all randomised patients, will be performed and will consider all patients who fled for reasons of armed conflict as failures.

If the interim primary analysis for success should not prove to be significant, no further interim secondary or sensitivity analyses will be performed. If the analysis proves to be significant, all of the interim secondary and sensitivity analyses will be performed in accordance with the approach described for the final analyses, i.e. those initially planned. In addition, the success rate at 18 months will be compared, using a superiority test, to the historical success rate for melarsoprol at 18 months, i.e. 73.7%. No adjustment of the threshold for significance will be made in this analysis. The time course of the success rate, i.e. EOT, 6 months, 12 months and 18 months, will be studied using the Kaplan-Meier approach. The cumulative failure rate at each visit will be presented graphically including historical data for melarsoprol and eflornithine.

The study will be continued to completion regardless of the result of the interim analysis, and all of the analyses planned in the protocol and SAP will be performed.

Primary Analysis

The primary analysis will be performed using a Blackwelder non-inferiority test, based on the difference in the success rates at the Test-of-Cure Visit at 18 months with the maximum margin of non-inferiority set at 13%.

The interim primary analysis will be performed using the same test, but with the timepoint of 12 months.

Sample Size

The sample size, initially calculated at 510 patients with statistical power of 87.9% has been reduced to 390 patients in total, with 260 patients in the fexinidazole group and 130 patients in the NECT group. Thus, the overall power is 80% if the actual success rate for NECT is 94% and 89% for fexinidazole.

Futility Analyses

Futility analyses will be performed in order to inform decisions about possible study discontinuation, based on pre-determined stopping rules. The aim is to limit the number of patients exposed to the investigational product in the event that the latter should prove to have limited efficacy.

Of note: data handling and data review will be performed in a blinded manner.

Interim Secondary Analyses

In the interim analyses, the success rate for NECT and for fexinidazole will be calculated on patients who have reached the 18-month follow-up visit and the rates will be compared to those calculated at the 12-month visit in the same patients.

The time course of the success rate will be analysed on the data available, using the same approach as for the final secondary analysis.

1. Background and Study Rational

1.1. Epidemiology

Human African trypanosomiasis (HAT), or sleeping sickness, is a vector-borne parasitic disease found in sub-Saharan Africa. It is transmitted by the bite of the tsetse fly (genus *Glossina*) and, if untreated, it almost invariably leads to death. HAT is caused by the protozoan parasites *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, which are found only in foci in sub-Saharan Africa where the tsetse fly is endemic (3, 33, 49). Since 2005, human cases of HAT due to *T.b. gambiense* have been reported in 13 countries and cases due to *T. b. rhodesiense* in 8 countries. Uganda is the only country where both species of the parasite are found. A total of 7,139 cases worldwide were reported to the WHO in 2010, 75% of which in the Democratic Republic of the Congo (DRC) alone (35, 47, 55). The largest most comprehensive and accessible database of treated HAT cases was set up by MSF and is managed by Epicentre. The database compiles information from 18 programmes to control HAT in 6 countries and describes the distribution by age and by sex of a total of 31,817 patients treated for HAT in 2010. Overall, children under 5 years of age account for 4.22% of patients, those between 5 and 9 years of age for 6.63% and those between 10 and 15 years of age for 12.82%. In total, children under 15 years of age account for 23.67% of the total treated population.

1.2. Clinical Presentation of HAT

There are two successive stages in the clinical course of HAT. In the first stage, called the haemolymphatic stage or early stage, trypanosomes are present in the blood and lymphatic system. The clinical signs and symptoms are mild and non-specific, including intermittent fever, headache, pruritus and lymphadenopathy. The signs and symptoms are very similar to those of malaria, which explains why patients with early-stage HAT are often misdiagnosed with malaria. If it is not diagnosed and treated, HAT will progress to the next stage, called the meningoencephalitic stage or late stage, in which parasites invade the central nervous system. At this stage, patients display the classic neurological signs associated with HAT, including mental confusion, worsening sleep disturbances and, eventually, coma and death (3, 33, 49).

1.3. Diagnosis

Current treatment options are different for early- and late-stage HAT and, for this reason, it is crucial to distinguish between the two stages before initiating treatment. HAT is diagnosed by detecting the parasite in the blood using thick or thin smear tests, or in a lymph node aspirate. Testing for *T.b. gambiense* is performed using a card agglutination test for trypanosomiasis (CATT). Patients

who test positive on CATT (diluted to 1/4, 1/8, 1/16 and 1/32) and/or who have enlarged cervical lymph nodes undergo further investigations (see Figure 1, p 19).

The diagnosis of late-stage HAT relies on lumbar puncture. The presence of parasites and/or of more than 20 white blood cells (WBCs) per µL in the cerebrospinal fluid (CSF) confirms late-stage HAT. In the national programmes in most countries, the threshold of positivity is a CSF WBC count of 5/µL.

The presence of trypanosomes in the blood or any other bodily fluid cannot be assessed in an independent central laboratory because fixing the sample affects the sensitivity of microscopic detection. The most sensitive methods of detection are those that use concentration techniques to examine fresh centrifugated samples for motile trypanosomes.

Cure is defined as the absence of parasites in any bodily fluid and a CSF WBC count below a predetermined threshold. As a result, patients with late-stage HAT must undergo additional lumbar punctures to confirm that they are cured. The current recommendation is to assess cure 18 to 24 months after the end of treatment (8).

1.4. Routine Case Detection and Management

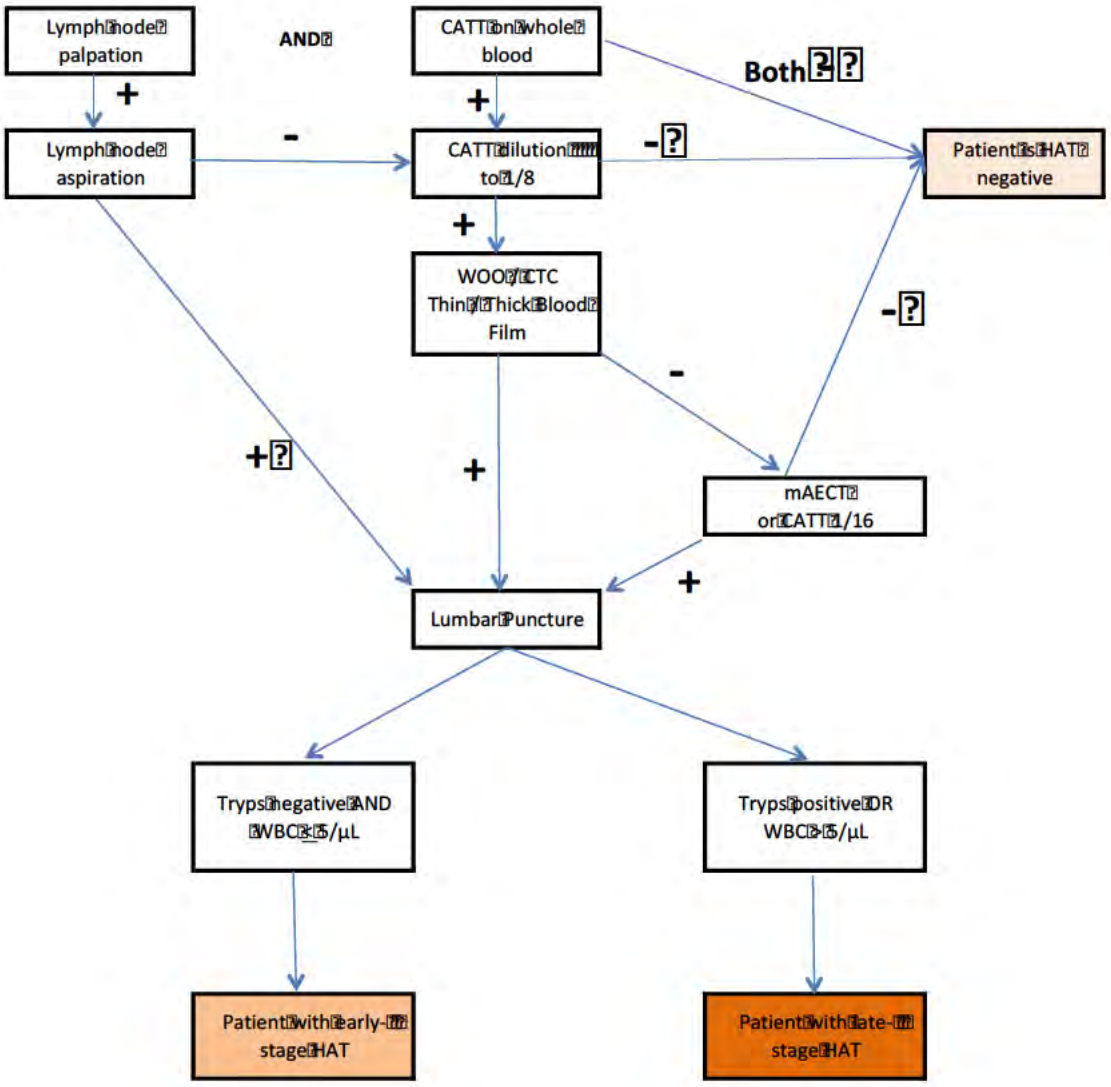
The routine procedure for case detection varies from one country to another and from one hospital/investigational centre to another. Patients may either present spontaneously to the hospital/investigational centre or be diagnosed in the field by mobile teams working in the context of active screening programmes.

In the DRC, the mobile teams in the National HAT Control Programme usually comprise 7 people, including 3 laboratory technicians and one person who acts as a community mobiliser. The mobiliser travels through the villages a few days in advance to announce to the community that HAT screening is on the way.

The routine activities of the mobile teams in charge of screening and diagnosis in the DRC are described below:

- In the field or at hospital, patients initially undergo a serological assessment, i.e. CATT. Testing is then carried out to detect the parasite 1) in the blood using various methods, including the Woo test, also called the capillary tube centrifugation (CTC) test, thick or thin blood smears, and mini-anion exchange centrifugation tests (mAECT), or 2) in a fresh lymph node aspirate using a microscope, and 3) in the CSF collected on lumbar puncture for staging of the disease. CSF samples are collected only by specially trained personnel and only under specific conditions, i.e. at sites particularly remote from hospital. The mobile teams in the National HAT Control Programme or other partners are experienced and well trained.

Figure 1 – Decision tree for routine diagnosis of HAT



CATT = Card Agglutination Test for Trypanosomiasis
WOO test = microhaematocrit centrifugation test = QBC test
mAECT = mini-Anion Exchange Centrifugation Test
WBC = White Blood Cell Count

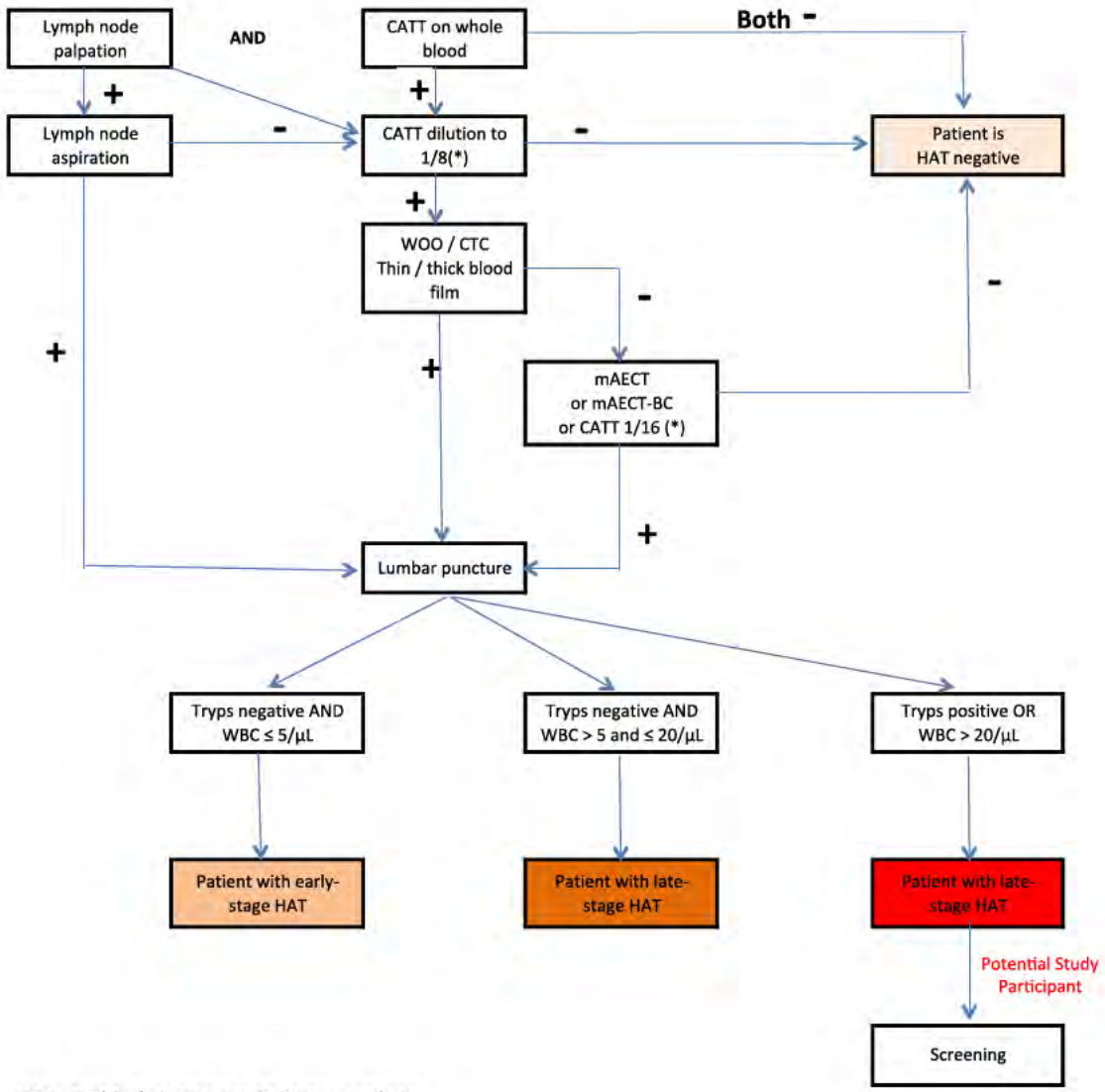
When patients are diagnosed with early-state HAT, they can be treated in their villages with intramuscular injections of pentamidine for 7 days.

When patients are diagnosed with late-state HAT, they are referred to hospital by the mobile team and remain in hospital for pre-treatment, if they have concurrent malaria and/or helminthiasis, and for treatment of HAT.

In the present study, mAECT or the more sensitive, improved technique called mini-anion exchange centrifugation test – buffy coat (mAECT-BC) (7), will be used whenever possible to screen CATT-positive patients, in addition to the routine diagnostic procedures presented in the decision tree (Figure 1). In order

to ensure that only late-stage HAT patients are included, patients in whom no parasites are found in the CSF must have a CSF WBC count of at least 20/µL, and only patients in whom parasites are found in at least one bodily fluid will be included (see Section 4.2 Pre-inclusion/ Pre-exclusion Criteria; p 35).

Figure 2 – Decision tree for routine diagnosis of HAT



CATT = Card Agglutination Test for Trypanosomiasis
WOO test = microhematocrit centrifugation test = CTC test
mAECT = Mini Anion Exchange Centrifugation Test
mAECT-BC = Mini Anion Exchange Centrifugation Test on Buffy Coats
(*) CATT 1/16 positive patients without parasitological confirmation will not be eligible for inclusion in the study (routine care)
CSF = Cerebrospinal Fluid WBC = White Blood Cell count

If the WBC count is 19, 20, 21 or 22/µL on the first analysis, the count will be performed again using another smear and checked by another examiner to determine whether the WBC is above or below the cut-off value. If no second examiner is available, the results may be considered as questionable, and the

patient may be asked to undergo another lumbar puncture when s/he arrives at the investigational centre. If s/he refuses, s/he cannot be included in the study.

1.5. Current Therapeutic Options for Late-stage HAT

Few therapeutic options are currently available to treat HAT at either stage (1, 31). Until recently, the drugs available to treat late-stage HAT were limited to an older toxic product, melarsoprol, administered in painful IV injections for 10 days with up to 5% treatment-related mortality, and eflornithine, less toxic but difficult to manage since it requires four IV infusions daily for 14 days. NECT, a recently developed combination of oral nifurtimox for 10 days plus eflornithine, two 2-hour IV infusions daily for 7 days, was found to provide similar cure rates to the standard regimen with eflornithine, but with obvious practical advantages, including ease of administration and a shorter duration of treatment.

In May 2009, the WHO Expert Committee added NECT to the 16th Edition of the WHO List of Essential Medicines for adults, as an alternative to single-drug therapy with melarsoprol or eflornithine.

In keeping with the WHO recommendation, several National HAT Control Programmes have already adopted NECT as first-line treatment for late-stage HAT due to *T.b. gambiense* and, in 2010, NECT became the most widely used treatment for late-stage disease (43, 56).

NECT represents a significant improvement over current therapies, however it is far from ideal given the conditions under which patients with HAT generally live, i.e. in poor, remote areas with little or no healthcare infrastructures and problems with logistics. Thus, there is an urgent need to develop less toxic and easier-to-use products for the treatment of this fatal disease, ideally a simplified, short-course treatment that can be administered orally at a primary healthcare facility.

1.6. Investigational Product and Preclinical Data

Fexinidazole is 2-substituted 5-nitroimidazole, formulated for oral administration. Fexinidazole has been shown to possess *in vitro* and *in vivo* activity against both parasites, *T.b. rhodesiense* and *T.b. gambiense*. In mouse models of both acute and chronic infection (the latter mimicking late-stage disease), oral administration of fexinidazole at doses of 100-200 mg/kg/day was shown to be curative and to significantly prolong survival (30). Preclinical pharmacokinetic (PK) studies have indicated that oral fexinidazole is well absorbed and is widely distributed throughout the body, including in the brain (15).

In all animal species studied, fexinidazole was rapidly metabolised through oxidation, resulting in the formation of at least two active metabolites, fexinidazole sulfoxide and sulfone, which have a trypanocidal activity similar to that of the parent molecule, and account for most of the pharmacodynamic activity. Toxicological studies were performed, including safety pharmacology and 4-week

repeated-dose toxicokinetic studies in rats and dogs. In both species, 200 mg/kg/day was considered as the no observed adverse effect level (NOAEL) (50). Fexinidazole was well tolerated up to 800 mg/kg/day, and no major toxicity was identified. While fexinidazole, like many nitroheterocycles, was shown to be mutagenic in the Ames test, it is not genotoxic to mammalian cells *in vitro* or *in vivo*, and is therefore not expected to pose a genotoxic risk in humans (50).

1.7. Safety and Tolerability in Healthy Volunteers

Fexinidazole has been investigated in three phase-I studies in healthy volunteers of sub-Saharan African origin (15). In all studies, the subjects were closely monitored, with physical examinations, vital signs, electrocardiogram (ECG) and safety laboratory tests, as well as pharmacokinetic sampling performed before, during and after dosing. A total of 118 subjects were randomised in these studies. Single doses ranged from 100 mg to 3600 mg, and multiple doses from 1200 mg to 3600 mg for a maximum of 14 days.

Administration of fexinidazole in healthy volunteers was safe when given as a single dose or as repeated doses for 14 days. As with all nitroimidazole drugs, fexinidazole primarily affects the central nervous system (CNS) and gastrointestinal system, and can cause headache and/or vomiting or other mild to moderate gastrointestinal symptoms. At the highest dose, two subjects experienced prolonged anxiety with an episode of panic attack, associated with nausea and vomiting. No specific treatment was needed. Both subjects returned to normal within 2 to 3 days after stopping the investigational product (IP).

Fexinidazole is highly metabolized. As expected with this pharmacological class, a marked, transient, quickly reversible increase in liver enzymes was observed in one volunteer just after 14 days treatment at the highest dose. Data from these studies also suggest that there is a relationship between the dose used, as well as overall exposure (dose and duration), and the frequency and severity of abnormalities on liver function tests. These effects will be closely monitored during the study.

Another important laboratory finding was an increase, within the normal range, in plasma creatinine levels in subjects receiving the active treatment. This effect was not dose-dependent and is believed to be related to inhibition of renal tubular creatinine secretion, an effect that has previously been reported with nitroimidazole drugs (21). It is always reversible and is not accompanied by any other abnormalities on kidney function tests.

ECG recordings in studies in humans showed a limited number of cases of QTcF interval prolongation, usually in the 30 ms range. QT interval corresponds to the time it takes for the left and right heart ventricles to depolarise (Q wave) and to repolarise (T wave). QT interval is corrected using a mathematical formula to take into account the heart rate (QTcF for the Fridericia formula). If the interval is abnormally prolonged or shortened, there is a risk of developing ventricular

arrhythmia. In earlier studies, only one out of more than 4000 ECG recordings showed an increase of 60 ms compared to baseline. The absolute value remained within the normal range (<450 ms), however, a 24-hour Holter recording showed a mean QTcF prolongation of 16 ms compared to baseline. Even though the increase remains within the normal range, ECGs recordings will be performed throughout the first study in patients to continue assessment of QT interval prolongation.

1.8. Pharmacokinetics and Metabolism in Healthy Volunteers

Fexinidazole is well absorbed by the oral route, and rapidly metabolised to sulfoxide (M1) and sulfone (M2), which account for the largest proportion of drug exposure, regardless of the dose used. Elimination of the second active metabolite is a slow process. C_{max} values for both fexinidazole and M1 are reached in 3 to 6 hours, while M2 levels peak at approximately 24 hours. The mean elimination half-life of fexinidazole and M1 is approximately 10 hours versus approximately 24 hours for M2.

Plasma levels of fexinidazole, M1 and M2 increase in a non-dose-proportional manner with no major changes in their pharmacokinetic profiles.

Urinary excretion of unchanged fexinidazole, M1 and M2 is very low. Fexinidazole, M1 and M2 together account for only 3% of the total dose.

The effects of concomitant intake of food were assessed in two separate studies. In the first study, administration after a standard high-fat meal (FDA recommended) was compared to administration under fasting conditions, and in the second study, two different field meals, i.e. Plumpy'Nut®[1] and a traditional meal of rice and beans, were compared to administration under fasting conditions. The observed bioavailability of fexinidazole, M1 and M2 was multiplied by approximately 4-fold with the high-fat meal, and by 2.5- to 3.0-fold with the two field meals (15).

1.9. Choice of Regimen and Dose

All pharmacokinetic data from the first-in-man study were incorporated into a PK model simulating several regimens using different doses in order to calculate the optimal exposure. The primary objective was to achieve exposure in the brain at least twice as high as the minimum inhibitory concentration (MIC) for M2, and the secondary objective was to shorten the duration of treatment to 10 days in order to avoid exposure-related increases in liver enzymes. The food effect was used to maximise exposure over a short period of time and to reduce the dose.

[1] Peanut-based paste in a plastic wrapper for treatment of severe acute malnutrition, easily available in the field.

The analysis came out in favour of using a once-daily dosing regimen after the main meal, starting with a loading dose for 4 days followed by a maintenance dose for 6 days.

This regimen was tested in a second study (49) conducted in healthy subjects who received a single daily dose for 10 days after a main meal of rice, beans and oil. The study population was divided into two cohorts: Cohort 1 received 1800 mg (from D1 to D4) and 1200 mg (from D5 to D10), Cohort 2 received 2400 mg (from D1 to D4) and 1200 mg (from D5 to D10) in a sequential and ascending manner. The first dose was well tolerated overall with only a few gastrointestinal adverse events and headaches, however the second dose had to be stopped due to nausea, vomiting and prolonged anxiety.

On the basis of the plasma concentrations with the first regimen (1800 mg for 4 days followed by 1200 mg for 6 days), it would appear that CSF concentrations compatible with the objective of treatment of late-stage HAT will be achieved. The predicted concentration of the M2 metabolite (fexinidazole sulfone) in the CSF indeed corresponds to more than twice the MIC from Day 2 to Day 10, and more than three times the MIC from Day 4 to Day 6. The prediction of the CSF concentration is based on a free fraction of 0.4 for M2, as measured in the DNDiFEX002 study.

No clinically significant laboratory abnormalities were observed with the first regimen.

Thus, the best tolerated regimen that consistently yielded plasma concentrations within the target range, as well as CSF concentrations within the predicted range, was 1800 mg (3 tablets) once daily for 4 days followed by 1200 mg (2 tablets) once daily for 6 days (15). This dosing regimen was therefore chosen for the phase-II/III study.

The preclinical and clinical data summarised above support the rationale for conducting a study with fexinidazole in patients with late-stage HAT.

1.10. Rationale for the Study Design

Conducting clinical studies in patients with HAT entails several specific difficulties including the need to perform repeated lumbar punctures to confirm diagnosis and cure, the long post-treatment follow-up required to confirm cure (18 months in comparative studies, as recommended by WHO experts in 2004, 54), the lack of a validated early surrogate marker for cure and the extreme poverty of the patients, who live in remote areas with no access to healthcare infrastructures. Given the scarcity of patients and of resources, as well as the lengthy follow-up required, it was decided that a single phase-II/III pivotal study would be performed. NECT was recently shown to be the most effective therapy currently available (43), and was chosen as the reference treatment since it was the latest

treatment to be added to the List of Essential Medicines and is widely recommended by the National HAT Control Programmes.

In order to collect as much safety data on fexinidazole as possible, and considering that the data would come from only one study, an unbalanced randomisation ratio of 2:1 was chosen in order to increase the number of patients exposed to fexinidazole in the study.

A double-blind double-dummy study design was deemed to be too complex and unethical, and could have compromised the study feasibility. Bias in patient selection and treatment allocation will be avoided using centralised randomisation. No one in the Sponsor's team will have access to the randomisation codes as this information will not be visible in the electronic case report form (e-CRF). Only the monitoring team will have access to unblinded data.

An interim analysis of the primary objective will be conducted by a statistician independent of the statistician in charge of the study. If the result of this analysis is not significant, no further analyses will be carried out.

If the interim analysis of the primary objective is significant, it will be validated by the study statistician, who will then be responsible for conducting all the other interim analyses required. The study statistician will no longer be able to participate in decisions taken based on subsequent blinded data reviews or in any amendments introduced after the interim primary analysis. The patient's initial code will not appear in individual data listings. A new ID code will be assigned by the statistician. The blind will be maintained in the Sponsor's team, with the exception of the person in charge of Pharmacovigilance for the Sponsor. This person will be responsible for checking the listings.

1.11. Target Population

Patients with late-stage HAT, 15 years of age and older, will be included in the study.

Based on epidemiological data, patients 15 years of age and older account for approximately 77% of the overall population treated for HAT. From a clinical standpoint, adolescents between 15 and 17 years of age are considered to be physiologically mature, particularly as concerns hepatic and renal maturation. Thus, the PK profiles of fexinidazole and its two main active metabolites are not expected to differ in this age group as compared to patients over 18 years of age.

By including adolescents between 15 and 17 years of age, the efficacy and safety of fexinidazole will be assessed in the same population as that used for the study on NECT (43). This point is of particular relevance since, in a non-inferiority study, it is especially important to closely adhere to the design of the historical studies, in accordance with European directives (11). In addition, the minimum age of patients included in other studies on HAT conducted in Africa was also 15 years

of age, such as the phase-II study on pafuramide maleate (or DB289), a compound developed for the treatment of early-stage HAT (25).

In the on-going NECT-Field study assessing the clinical safety, feasibility of use and efficacy of NECT under real-life conditions (16), 3.7% of the patients included are between 15 and 17 years of age. As in prior clinical studies on HAT, the inclusion of adolescents over 15 years of age with the adult population will help facilitate recruitment of patients with late-stage HAT and, assuming that fexinidazole proves to be effective, will allow for immediate recommendation of its use in adolescents.

Moreover, adolescents over 15 years of age are well able to understand the study objectives and constraints, and can give their assent to participation in the study (see Section 14 Ethics; p 77).

2. Study Objectives and Endpoints

2.1. Objectives

2.1.1. Overall Objective

The overall objective of the study is to assess the efficacy and safety of an oral regimen of fexinidazole, once daily for 10 days, as compared to NECT in the treatment of late-stage sleeping sickness due to *T. b. gambiense*.

2.1.2. Primary Objective

- To demonstrate that the success rate at 18 months after the end of treatment (EOT) is in favour of fexinidazole, or that the difference as compared to the success rate with NECT is acceptable.

2.1.3. Secondary Objectives

- To compare the time course of the failure rate with fexinidazole and NECT from the EOT visit to 24 months, i.e. the end of the long-term follow-up period.
- To compare the safety of fexinidazole with that of NECT in the treatment of late-stage sleeping sickness due to *T. b. gambiense*.
- To assess plasma and CSF concentrations of fexinidazole and its metabolites in all patients who receive fexinidazole, and to investigate the correlation between the concentration and the efficacy and safety of fexinidazole in patients with late-stage sleeping sickness due to *T. b. gambiense*.
- To compare the pharmacodynamic impact of fexinidazole and of NECT on QT/QTcF interval, as measured on ECG.

2.1.4. Exploratory Objectives

- To validate the predictive value of three logistic models having as covariables age, sex and CSF WBC count at baseline, and at 6 and/or

12 months, when used as surrogate markers of treatment success at 18 months or for imputation of missing data (final sensitivity analysis).

- To compare the efficacy of fexinidazole to that of melarsoprol, eflornithine and NECT using historical data as comparators.

2.2. Study Endpoints

2.2.1. Primary Endpoint – Efficacy

The primary endpoint is the outcome, either success or failure, of treatment assessed at the test-of-cure (TOC) visit, 18 months after the EOT, based on adapted WHO criteria (54).

An interim analysis will be performed after 195 patients have theoretically reached 12 months of follow-up. The criteria below will apply to the interim analysis.

Success at 12 months is defined as:

- Patient cured, i.e.
  - patient alive,
  - AND no evidence of trypanosomes in any bodily fluid,
  - AND CSF WBC ≤ 20/µL.

OR

- Patient probably cured, i.e.
  - no parasites in blood or lymph,
  - AND refusal to undergo lumbar puncture, OR whose CSF sample is haemorrhagic but with no trypanosomes,
  - AND whose clinical status is satisfactory, i.e. with no clinical signs or symptoms, OR whose clinical status is unlikely to be related to HAT
  - Patients with no lumbar puncture at 12 months, but having one at 6 months will be considered as successes if the CSF WBC is ≤ 5/µL

Failure at 12 months is defined as:

- Patient relapsed, i.e.
  - evidence of trypanosomes in any bodily fluid,

OR

- Patient probably relapsed, i.e.
  - Either no evidence of trypanosomes in any bodily fluid,
    - AND CSF WBC > 20/µL,
    - AND whose WBC cannot be explained by a disease other than HAT,

- Or no evidence of parasites in the blood or lymph,
  - AND refusal to undergo lumbar puncture, OR whose CSF sample is haemorrhagic but with no trypanosomes,
  - AND who, in the opinion of the Investigator, requires rescue treatment because of marked deterioration in his/her clinical status that is unlikely to be related to a disease other than HAT,
- Or patient who refuses lumbar puncture at 12 months and in whom the outcome had already been assessed as unfavourable at an earlier time (see Table 1 – Definitions of Early Efficacy Endpoints; p **Error! Bookmark not defined.**),
- Or withdrawal from the study due to probable relapse at any time earlier than 12 months (see Table 1 – Definitions of Early Efficacy Endpoints; p **Error! Bookmark not defined.**),
- Or patient with no lumbar puncture at 12 months and whose CSF WBC at 6 months was > 5/µL,
- Or patient with no lumbar puncture at 6 months or 12 months,

- Or death, whatever the cause,
- Or lost to follow-up,

Patients living in an area of armed conflict and who, according to those close to him/her and/or inhabitants of the village, fled the area for a safer region and for whom no post-treatment data are available after several attempts to obtain such information, will not be taken into account in the primary efficacy analysis or in the interim analysis, but will be included in the safety analysis.

Success at 18 months is defined as:

- Patient cured, i.e.
  - patient alive,
  - AND no evidence of trypanosomes in any bodily fluid,
  - AND CSF WBC ≤ 20/µL.

OR

- Patient probably cured, i.e.
  - no parasites in blood or lymph,
  - AND refusal to undergo lumbar puncture, OR whose CSF sample is haemorrhagic but with no trypanosomes,
  - AND whose clinical status is satisfactory, i.e. with no clinical signs or symptoms, OR whose clinical status is unlikely to be related to HAT.

Failure at 18 months is defined as:

- Patient relapsed, i.e.
    - evidence of trypanosomes in any bodily fluid,

OR

- Patient probably relapsed, i.e.
    - Either no evidence of trypanosomes in any bodily fluid,
        - AND CSF WBC > 20/µL,
        - AND whose WBC cannot be explained by a disease other than HAT,
    - Or no evidence of parasites in the blood or lymph,
        - AND refusal to undergo lumbar puncture, OR whose CSF sample is haemorrhagic but with no trypanosomes,
        - AND who, in the opinion of the Investigator, requires rescue treatment because of marked deterioration in his/her clinical status that is unlikely to be related to a disease other than HAT,
    - Or patient who refuses lumbar puncture at 18 months and in whom the outcome had already been assessed as unfavourable at an earlier time (see Table 1 – Definitions of Early Efficacy Endpoints; p **Error! Bookmark not defined.**),
    - Or withdrawal from the study due to probable relapse at any time earlier than 18 months (see Table 1 – Definitions of Early Efficacy Endpoints; p **Error! Bookmark not defined.**)
- Or death, whatever the cause,
- Or lost to follow-up,

Patients living in an area of armed conflict and who, according to those close to him/her and/or inhabitants of the village, fled the area for a safer region and for whom no post-treatment data are available after several attempts to obtain such information, will not be taken into account in the primary efficacy analysis or in the interim analysis, but will be included in the safety analysis.

2.2.2. Secondary Efficacy Endpoints or Exploratory Endpoints

Early Efficacy Endpoints

The early efficacy endpoints used in the futility and secondary analyses vary depending on the timepoint and analysis.

Futility analyses (FAs) assessing the response rate are planned in order to stop exposure to the IP if efficacy is below an acceptable limit (see Section 9.11 Futility Analyses; p 67).

All cases of probable relapse and all deaths will be included as relapses in all analyses.

Table 1 - Definitions of Early Efficacy Endpoints, for statistical purposes only

Timepoint	*Ideal timing of visit after EOT*	*Analyses*	*Responder / Favourable outcome*	*Non-responder / Relapse*	
				Probable Relapse	*Confirmed Relapse*
24 hours after EOT	*Within 2 days*	*FA1* n_1*=20 Fexi* *FA2* n_2*=70 Fexi*	• Patient alive with no evidence of trypanosomes in any bodily fluid (54)		• Evidence of trypanosomes in any bodily fluid
3 months	*3 months ± 1 week*	*FA3* n_3*=70 Fexi*	• Patient alive with no evidence of trypanosomes in any bodily fluid (no lumbar puncture at 3 months unless Investigator suspects relapse)	• Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid
6 months	*6 months ± 2 weeks*	*FA4* n_4*=70 Fexi*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL • If uncertain (CSF WBC between 20 and 50/µL), request additional visit in 1-3 months	• CSF WBC ≥ 50/µL (36) • Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid
12 months	*12 months ± 4 weeks*	*FA5* n_5*=70 Fexi*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL • CSF WBS between 20 and 50/µL, and lower as compared to previous value(s)	• CSF WBC ≥ 50/µL • CSF WBC > 20/µL and higher as compared to previous value(s) • Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid

12 months	*12 months ± 4 weeks*	*Interim n =195*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL • No evidence of trypanosomes in blood or lymph and refusal to undergo lumbar puncture or whose CSF sample is haemorrhagic but with no trypanosomes and whose clinical status is satisfactory, i.e. with no clinical signs or symptoms, or whose clinical status is unlikely to be related to HAT • No lumbar puncture at 12 months, but one at 6 months with CSF WBC ≤ 5/µL	• No evidence of trypanosomes in any bodily fluid and CSF WBC > 20/µL and whose WBC cannot be explained by a disease other than HAT • No evidence of trypanosomes in blood or lymph and refusal to undergo lumbar puncture or whose CSF sample is haemorrhagic but with no trypanosomes and intake of rescue treatment • Refusal to undergo lumbar puncture at 12 months and outcome assessed as unfavourable at previous visits • Withdrawal from study due to probable relapse at a previous visit • No lumbar puncture at 12 months, but one at 6 months with CSF WBC > 5/µL • No lumbar puncture at 12 or 6 months	• Evidence of trypanosomes in any bodily fluid

For statistical purposes, an unfavourable outcome is any situation that does not fulfil the criteria for favourable outcome in Table 1.

Late Efficacy Endpoint (24 months)

The same definitions as those for the primary endpoint will be used. The ideal timing of this visit should be 24 months ± 4 weeks after EOT.

Surrogate Efficacy Endpoint

The surrogate endpoint is the actual or predicted outcome (if the outcome is missing) at 18 months, based on the outcome at the last visit that took place at 6 months or later, and for which data are available.

The actual outcome, i.e. success or failure, is the same as that defined in the primary endpoint.

The predicted outcome at 18 months is obtained based on logistic models using CSF WBC at baseline, 6 months and/or 12 months, age and sex.

Unlike the primary endpoint, the surrogate endpoint is used to estimate the success rate in the final analysis, i.e. sensitivity analysis of the primary analysis.

In the final analysis, the surrogate endpoint may be much more accurate than the primary endpoint if the predictive value is as good as the results obtained in the set of patients used to construct the models (see Section 9.2 Handling of Missing Values and Lost to Follow-up; p 58).

Safety Endpoints

Adverse events will be graded according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.03.

- Occurrence of adverse events, all grades combined, during the observation period, i.e. from D1 to D8, including:
    - any worsening of the clinical symptoms listed among the signs and symptoms to be checked prior to inclusion,
    - laboratory abnormalities ≥ grade 2,
- Occurrence of any adverse events ≥ grade 3 during the observation period,
- Occurrence of any treatment-related adverse events, ≥ grade 3 and all grades combined, during the observation period,
- Occurrence of any serious adverse events between the first treatment intake and the end of the follow-up period (18 months), and between Month 18 and Month 24.

Potentially significant ECG abnormalities will be reported after centralised ECG review.

Pharmacokinetic Endpoints

PK sampling in patients in the fexinidazole group will only be performed at the first 6 investigational sites.

Concentrations of fexinidazole, M1 and M2 in whole blood and in CSF, as well as PK parameters derived from a population PK model.

- Samples of whole blood will be collected using dry blood spot (DBS) testing on a finger-prick sample at the following timepoints:
    - on Day 8, 3 hours and 15 minutes after intake of the IP,
    - on Day 9, 3 hours after intake of the IP,
    - on Day 10, 3 hours and 7 hours and 15 minutes after intake of the IP,
    - on Day 11, 24 hours after intake of the IP,

- on Day 12, 48 hours after intake of the IP.

- Lumbar puncture will be performed on Day 11, 24 hours after intake of the IP for the EOT efficacy assessment. A dry CSF spot will be collected at the same time for PK analyses for all patients until the end of July 2013.

ECG Endpoints

Triplicate ECG tracings will be recorded at 2-minute intervals to assess QT interval at the following timepoints:

- At baseline for all patients
- Thereafter:
  - in patients in the fexinidazole group: on Day 4, 4 hours (H4) after intake of the IP, on Day 4 Hour 23, and on Day 10 2-3 hours after intake of the IP,
  - in patients in the NECT group: on Day 7 2-3 hours after treatment intake in the morning and on Day 10 2-3 hours after treatment intake in the morning.

Triplicate ECGs and a safety ECG will be collected for different purposes and will be analysed separately (see Section 6.3.2 ECG Recordings; p 47).

3. Study Design

This is a pivotal, phase-II/III, randomised, open-label, multicentre study with two unbalanced parallel (2:1 ratio) groups, comparing fexinidazole and NECT as the active control.

4. Selection of Study Population

To be eligible for inclusion in the study, patients must fulfil all of the inclusion criteria and none of the exclusion criteria.

4.1. Enrolment/Inclusion Procedures

The study will be conducted in at least 6 centres in the DRC and the Central African Republic (CAR).

Patients will be recruited in one of three ways:

a. Patients may present spontaneously at the investigational centre and will undergo routine assessment at the centre, including testing for *T.b. gambiense* in the blood, lymph and CSF.

b. Patients may be referred to the investigational centre after being diagnosed with late-stage HAT by one of the mobile teams in the field (see Section 1.4 Routine Case Detection and Management; p 18). In that case, patients will not undergo a second lumbar puncture. They will receive a staging certificate from the mobile team and may participate in the study,

if they so desire, without undergoing a second lumbar puncture. For ethical reasons, the lumbar puncture planned at inclusion will not be performed in these patients. However, if the staging of HAT is questionable, i.e. negative findings on CSF testing for parasite and CSF WBC between 19 and 22/µL inclusive, the patient will be asked to undergo a second lumbar puncture to confirm the stage. If the patient refuses, s/he will not be included in the study and will be treated in accordance with usual practice in the centre.

c. Patients may be referred to the investigational centre by a less well-equipped mobile team that was not able to determine the stage of HAT and will then undergo assessment as described in point (a) above.

d. A new microscope has been provided on each site (February 2015). This microscope allows picture or video capture (maximum 5 sec. of video) of the trypanosomes on the slide as well as the cells counting in the sampling (blood, node, LCR). The photographic and video recordings are identified per patients, date and time of recording. Before stored in a specific database, all the recording will be anonymized as the other data in order to maintain the confidentiality. They will be saved and kept on site as well as on a specific data base (powerfolder). This procedure will be performed for the follow-up visits too.

During the screening process prior to inclusion and before the informed consent form is signed, patients will be considered as “pre-screened patients”. Patients who fulfil the pre-inclusion criteria, i.e. not requiring any study-specific procedures, will be invited to participate in the study and will receive the necessary information before they give their informed consent.

For patients to be enrolled in the study, they must sign an informed consent form after the Investigator or a delegate has provided explanations and prior to any study-specific procedures. Patients will then be considered as “screened patients”.

As previously mentioned, the initial study-specific procedures do not include lumbar puncture unless it is required in order to confirm the staging of HAT, when the staging based on CSF examination by the mobile team in the village is questionable.

The Investigator or a delegate must record the date of the screening visit, the patient’s initials, the patient’s number, referral source, status at the time of the screening visit, date of collection of informed consent, date of enrolment/ inclusion and the date of randomisation, as well as the treatment number or reason for non-enrolment, if appropriate.

The enrolment/inclusion procedures must be carried out between 1 and 15 days before the planned start of the study treatment. The procedures are described in Section 6 Schedule of Assessments (p 44). The procedures include a complete

history-taking, a physical examination, testing and treatment for malaria, treatment for helminthiasis, haematological and biochemical assessments, ECG and urine pregnancy test.

4.2. Pre-inclusion / Pre-exclusion Criteria

The pre-inclusion/pre-exclusion criteria can be checked before informed consent is obtained since the process does not involve any study-specific procedures.

Pre-inclusion Criteria

- 15 years of age or older
- Male or female
- Able to ingest at least one complete meal per day (or at least one sachet of Plumpy'Nut®)*
- Karnofsky score > 50 (see Appendix 2 – Karnofsky Performance Scale; p 90)
- Late-stage HAT due to *T.b. gambiense* with confirmed evidence of parasite in blood and/or lymph and/or CSF, certified by the report from the mobile team, with details on examinations performed and CSF WBC count, or performed at the investigational centre.

  If testing for parasites in CSF is negative, a CSF WBC > 20/µL will be required to confirm late-stage HAT (54).*
- Having a permanent address and able to comply with the schedule of follow-up visits.

Pre-exclusion Criteria

- Severe malnutrition, defined as Body Mass Index < 16.
- Unable to take medication by the oral route.*
- Pregnancy or breast-feeding.
- Clinically significant medical condition that could, in the opinion of the Investigator, jeopardise the patient's safety or participation in the study, including, but not limited to significant liver or cardiovascular disease, suspected or proven active infection, CNS trauma or seizure disorder, coma or consciousness disturbances.
- Severely deteriorated general status, including as a result of cardiovascular shock, respiratory distress or end-stage disease.
- Any condition that affects the patient's ability to communicate with the Investigator as required to complete the study.

* Criteria to be checked prior to randomisation.

- Any contraindication to imidazole drugs, i.e. known hypersensitivity to imidazoles, or to NECT, i.e. known hypersensitivity to eflornithine.
- Prior treatment for HAT.
- Prior enrolment in the study.
- Foreseeable difficulty complying with follow-up, including migrant worker, refugee status, itinerant trader.
- History of alcohol or drug addiction.

4.3. Inclusion and Exclusion Criteria

Once the Investigator has checked that the patient fulfils the pre-inclusion/pre-exclusion criteria, s/he will invite the patient to participate in the study and initiate the process for collecting the patient's informed consent (see Section 14.2 Informed Consent Process; p 80). Treatment for any concurrent disease will be initiated, if necessary, and further investigations will be performed.

The inclusion and exclusion criteria will then be checked, just prior to randomisation. The pre-inclusion/pre-exclusion criteria marked with an asterisk (*) will be checked again before randomisation.

inclusion Criteria

- Signed informed consent form.

Exclusion Criteria

- Clinically significant laboratory test abnormality, including for example:
  - aspartate aminotransferase and/or alanine transferase more than 2 times the upper limit of normal (ULN)
  - total bilirubin more than 1.5 x ULN
  - severe leukopenia at < 2000/mm^3
  - potassium (K+) < 3.5 mmol/L
- Pregnancy confirmed by a positive urine pregnancy test within 24 hours prior to the start of treatment (see Section 5.8.3 Contraception; p 41)
- Unstable abnormalities on ECG
- QTcF ≥ 450 msec, on automatic reading on two successive ECGs in resting position, performed 10 to 20 minutes apart
- Patients not tested for malaria and/or not having received appropriate treatment for malaria (see Section 5.8.1 Malaria; p **Error! Bookmark not defined.**).
- Patients not having received appropriate treatment for soil-transmitted helminthiasis (see Section 5.8.2 Helminthiasis; p **Error! Bookmark not defined.**)

The following criteria are considered to be temporary exclusion criteria

- Traumatic lumbar puncture, i.e. red blood cells visible in CSF; repeat lumbar puncture can be performed 48 hours later.
- Recovery period after treatment for malaria and/or treatment for helminthiasis, i.e. approximately 3 days.
- Abnormalities on laboratory tests or ECG that can be controlled within a few days after the initial assessment. If the value returns to normal or is not considered clinically significant, the patient can be included in the study.

Patients who fulfil the inclusion and exclusion criteria will be referred to as "included patients" and can be randomised.

Patients who are excluded will be treated in accordance with usual practice in the centre, and the reasons for exclusion from the study will be recorded.

5. Treatments

5.1. Investigational Product

Fexinidazole, 600 mg tablets, administered by the oral route **after** the main meal of the day, i.e. within 30 minutes after the start of the meal, in accordance with the following dosing regimen:

- 1800 mg (3 tablets) in a single daily intake for 4 days,
- followed by 1200 mg (2 tablets) in a single daily intake for 6 days.

The total duration of treatment will be 10 days.

Fexinidazole will be provided by DND*i*, 15 chemin Louis Dunant, 1202 Geneva, Switzerland.

5.2. Reference Treatment

Nifurtimox-Eflornithine Combination Therapy (NECT):

- Nifurtimox tablets, administered by the oral route three times daily, at the dose of 15 mg/kg/day, for 10 days.
- Eflornithine, administered twice daily as a 2-hour IV infusion, at the dose of 400 mg/kg/day, for 7 days.

NECT will be provided by DND*i*, 15 chemin Louis Dunant, 1202 Geneva, Switzerland.

5.3. Treatment Allocation

Patients will be randomised at a 2:1 ratio, with two patients in the fexinidazole group for 1 patient in the NECT group.

Each patient who fulfils the inclusion criteria will enter the randomisation process. Treatment will be allocated using a centralised internet-based system in order to avoid bias.

After randomisation, the patient will be assigned to one of the two treatment groups, and the number of the treatment pack will be recorded in the patient's file and on the pharmacy log sheet (included in the pharmacy manual).

The patient will be considered to have completed his/her participation in the study once the last protocol-planned visit, i.e. the TOC visit at Month 18, has taken place. The visit at Month 24, at the end of the additional follow-up period, has been planned in order to comply with current recommendations in the National HAT Control Programmes.

5.4. Labelling and Packaging of Treatments

Fexinidazole tablets will be packaged in aluminium-aluminium blister packs. Each blister pack will contain the number of tablets necessary for one day of treatment, i.e. 3 tablets for the first 4 days, and 2 tablets for the next 6 days. The 10 blister packs will be packaged in an individual treatment pack for each patient.

NECT will be provided in its commercial packaging, i.e. one bottle containing 100 tablets of nifurtimox (Lampit®) per patient and 14 bottles of eflornithine (Ornidyl®) per patient. The two products will be specially labelled for the study. The 15 bottles will be packaged in an individual treatment pack for each patient.

The material required for the sterile infusions will be packaged in separate packs.

The labelling of the secondary packaging will display the following information:

- Name of Sponsor*, name and contact details for the Coordinating or Principal Investigator
- Study number*
- Drug name* and dosage strength*
- Dosage form*, route of administration*, number of dosage units*
- Instructions for use
- Statements "For clinical study use only"* and "Keep out of reach of children"
- Batch number* and treatment pack number*
- Expiry date and storage conditions

The information items marked with an asterisk (*) will also be displayed on the primary packaging of the study treatments.

Information on fexinidazole will be provided in the Investigator Brochure attached to the protocol submitted to the National Authorities. Information on NECT can be obtained from the product information for Ornidyl® and Lampit®.

5.5. Accountability of Treatments

The study treatments will be shipped to the coordinating study site, i.e. DND*i* Kinshasa for the DRC, or directly to the investigational centres.

Study-specific forms will be used for accountability of the study treatments. Appropriate records concerning receipt, use, returns, loss and any other disposition of the treatments will be maintained by the Investigators on site, or their delegates, under the supervision of the Principal Investigator. Study monitors will check accountability of the study treatments during on-site monitoring visits.

All study treatments must be stored in a locked room, or a locked cabinet if no specific room is available, at each investigational site, with access restricted to the nurse in charge of the pharmacy or to authorised study personnel.

The supplies of fexinidazole and NECT for the study must not be used for purposes other than the present protocol. The Investigator and the site staff may not, under any circumstances, provide other Investigators or healthcare services with the study treatments, or allow the treatments to be used other than as described in this protocol without prior written approval from DND*i*.

5.6. Storage of Treatments

Neither the IP nor the reference treatment requires shipping or storage under refrigerated conditions.

Eflornithine should be stored at room temperature prior to dilution in water for injections. Fexinidazole and nifurtimox should be stored at a temperature not exceeding 30°C. NECT is frequently stored in pharmacies in the rural setting that have no air conditioning. Long-term stability studies have shown that fexinidazole, stored in the bottle, remains stable at 30°C under conditions of high humidity.

Fexinidazole and nifurtimox must be protected from light. This condition is ensured by the fact that they are packaged in aluminium-aluminium blister packs and in amber-glass bottles.

The stability of the tablets in the aluminium-aluminium blister packs and amber-glass bottles will be monitored in the context of regulatory stability studies.

The storage conditions, including the temperature, must be monitored by the study personnel and appropriate records should be available.

5.7. Blinding and Procedure for Unblinding

The study is being conducted using an open-label design because the route of administration and dosing regimen are different for the two study treatments. Thus, the patients and the Investigators will necessarily know which treatment is administered. Treatment compliance will, however, be recorded in the e-CRF in such a way that neither the Sponsor nor its representatives can identify the treatment administered.

- **Persons involved in the study who are blinded to treatment received by the patient:** the Sponsor, persons working on data management, the study statistician, the committee in charge of centralised reading of triplicate ECGs and the Data Safety Monitoring Board (DSMB). The latter may request lifting of the blind at any time upon written request;
- **Persons not blinded to treatment:** patients, Principal Investigators and their teams, monitors, independent statistician in charge of FAs, members of the Independent Steering Committee (ISC) at their request on a case-by-case basis, persons responsible for the interactive response system and persons working in Information Technology.

Data management will be carried out under blinded conditions. DND*i* personnel will not have access to information regarding which treatment was received or to detailed data on compliance, with the exception of the person in charge of Pharmacovigilance, if necessary.

The blind will be lifted for the independent statistician in charge of analysing the primary efficacy endpoint for the interim analysis.

If this analysis proves to be significant, the blind will then be lifted for:

- the statistician in charge of the study, who will validate the interim primary analysis and then proceed with all of the planned analyses in the interim analysis,
- the person in charge of preparing the clinical study report (CSR).

In that case, the study statistician and the person in charge of the CSR will no longer participate in decisions taken during data review for the subsequent analyses.

In addition, in order to maintain the blind among members of the Sponsor's team, the patient's initial code will not appear in individual data listings in the CSR. A new ID code will be assigned by the statistician. Only the person in charge of Pharmacovigilance for the Sponsor and the person in charge of the CSR will have access to the corresponding patient codes.

If the analysis of the primary efficacy endpoint for the interim analysis proves not to be significant, no other analyses will be performed and the blind will be maintained for the study statistician and the person in charge of preparing the CSR until the time of data review for the final analysis.

FAs will be performed by an unblinded independent statistician who is not involved in preparing the study, in the blind review of data or in the final statistical analyses. S/he will provide recommendations to the DSMB/ISC with regard to stopping the study for reasons of futility or to continuing the study. The magnitude of the treatment effect will not be revealed. If the study is allowed to continue, this will signify that efficacy is deemed sufficient from an ethical standpoint, but does

not inform about the study's chances of success. Indeed, the endpoints in the FAs are different from the primary endpoint: not only do the conditions for stopping the study differ from those that define treatment success, but the size of the sample in the FAs is small.

The logistic models will be validated in a fully blinded manner, regardless of the treatment group.

After the data base lock on the primary analysis (18 months data), the sponsor, the data management and the statistician will be unblinded. Once all patients reached their 24 months follow up, a sensitivity analysis could be performed on request.

5.8. Concomitant Treatment

5.8.1. Malaria

All patients will undergo a test to detect malaria. All patients with a positive thick smear and/or rapid diagnostic test (RDT) should receive treatment.

Prior to starting treatment for HAT, malaria will be treated with Coartem®, unless there are individual contraindications such as hypersensitivity to one of the components or severe malaria. All existing artemisinin-based combination therapies against malaria have effects on the QT interval. Coartem® was chosen because its effects on QT-interval prolongation are well known, moderate and well quantified (19). The choice was also made in order to minimise confounding factors regarding the assessment of fexinidazole-related QT-interval prolongation. The Sponsor will provide Coartem® free of charge.

In patients with a contraindication to Coartem®, the Investigator can choose another antimalarial agent. The choice must be documented.

Treatment for malaria will be followed by a recovery period of at least 3 days between the last dose of the antimalarial agent and the first administration of treatment for HAT, as per usual practice in the investigational centres.

5.8.2. Helminthiasis

Treatment for helminthiasis, with mebendazole or albendazole, will be provided free of charge by the Sponsor for use as per usual practice in the investigational centres.

Treatment for helminthiasis will be followed by a recovery period of at least 3 days between the last dose of the antimalarial agent and the first administration of treatment for HAT, as per usual practice in the investigational centres.

5.8.3. Contraception

Women of child-bearing potential will be advised to use a method of contraception or to abstain from sexual relations during the treatment period and, if possible,

until cure is confirmed. Medically proven methods of contraception, i.e. hormonal contraception and condoms, will be available to patients free of charge during the 24-month follow-up period.

5.8.4. Other Medication

Unless there is an urgent medical need, patients should refrain from using any medication required to treat concurrent conditions until after the end of the treatment for HAT.

Any medication used during the hospitalisation period must be recorded in the e-CRF, specifying the reason for use.

Information on any serious adverse event (SAE) that may occur during the follow-up period will be collected at an unscheduled visit and recorded in the e-CRF, specifying any treatment(s) received. Other medical events will be recorded only in the patient's medical file.

Any essential medicine required during the 24-month follow-up period in the study will be provided to the patient free of charge. The WHO List of Essential Medicines and the MSF reference guide entitled *Essential Medicines* (2010 edition) will be used as a basis for treatment of any concurrent condition. For any chronic condition, the study team will take all necessary measures to ensure that the patient is referred to the most appropriate healthcare facility in the region.

5.9. Rescue Treatment

Patients who show no clinical response to treatment at the EOT visit, as well as patients with evidence of relapse, i.e. *T.b. gambiense* found in any bodily fluid, or of probable relapse at any time during follow-up will receive an alternative treatment for HAT, as per usual practice in the investigational centre.

Rescue treatment for HAT will not be recorded as concomitant treatment in the e-CRF, but will be recorded in the Comments section with the date of treatment start.

Probable relapse at the 6-month, 12-month or 18-month follow-up visit is defined as follows:

Table 2 – Clinical Classification of Patients

Visit	*Ideal timing of visit after end of treatment*	*Favourable outcome*	*Uncertain outcome*	*Probable relapse*	*Proven relapse*
24 hours after EOT	*Within 2 days*	• Patient alive with no evidence of trypanosomes in any bodily fluid (54)			• Evidence of trypanosomes in any bodily fluid
3 months	*3 months ± 1 week*	• Patient alive with no evidence of trypanosomes in any bodily fluid (no lumbar puncture at 3 months unless Investigator suspects relapse)	• Any reason prompting the Investigator to request an additional follow-up visit	• Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid
6 months	*6 months ± 2 weeks*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL	• CSF WBC between 20 and 50/µL and additional visit requested within 1-3 months • Any reason prompting the Investigator to request an additional follow-up visit	• CSF WBC ≥ 50/µL (36) • Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid
12 months	*12 months ± 4 weeks*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL • CSF WBS between 20 and 50/µL, and lower as compared to previous value(s)	• CSF WBC > 20/µL and non-significant increase from a clinical standpoint in relation to prior value(s) • Any reason prompting the Investigator to request an additional follow-up visit	• CSF WBC ≥ 20/µL • CSF WBC > 20/µL and significant increase from a clinical standpoint in relation to prior value(s) • Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid
18 months *and 24 months*	*18 months ± 4 weeks* *24 months ± 4 weeks*	• Patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/µL • Patients with no signs of HAT and refusing to undergo lumbar puncture and who, in the opinion of the Investigator do not require rescue treatment or an additional follow-up visit	• Any reason prompting the Investigator to request an additional follow-up visit	• CSF WBC > 20/µL • Neurological signs or symptoms leading to use of rescue treatment	• Evidence of trypanosomes in any bodily fluid

An uncertain outcome may be a discrepancy between the clinical picture and the laboratory findings, or any situation that leads the Investigator and the study team to consider that an additional follow-up visit is required to decide whether or not to provide rescue treatment. This classification will not be used directly for statistical purposes.

6. Schedule of Study Procedures and Assessments

6.1. Timing of Assessments

See Table 4 - Timing of Assessments (p 99)

- D-15 to D-1: Patient screening with detection of *T.b. gambiense,* pre-treatment of concomitant malaria and/or helminthiasis, if any, ECG and laboratory screening tests
- D-4 to D-1: Baseline assessment
- D1 to D10: Treatment period
- D8 to D12: PK sampling for patients in the fexinidazole group
- D11: End-of-Treatment (EOT) visit
- between D13 and D18: End-of-Hospitalisation (EOH) visit; if hospitalisation is prolonged beyond D18, the EOH visit should be recorded on D18 and an unscheduled visit should take place at the actual time of discharge from hospital
- Additional Follow-up Visit: in Week 9 after D1, i.e. D64 to D70, for patients who reach this visit starting in mid-December 2014
- Follow-up Visits: at M3, M6, M12, M18 and M24 after D11, see Table 3 – Theoretical Schedule of Visits and Acceptable Leeway). The timing of follow-up visits is calculated from D11 (EOT).

Table 3 - Theoretical Schedule of Visits and Acceptable Leeway

Theoretical schedule of visits	Ideal timing of visits	Acceptable leeway*
End-of-Treatment visit (EOT)	D11 after the Start of Treatment (SOT) (D1 after EOT)	Between D11 and D12 after SOT (D1 or D2 after EOT)
End-of-Hospitalisation visit (EOH)	Between D13 and D18 after SOT (D3 and D8 after EOT)	D18 at the latest
Week 9 after D1	D64 to D70	
3 months	3 months ± 1 week after EOT	2-4 months after EOT
6 months	6 months ± 2 weeks after EOT	5-9 months after EOT
12 months	12 months ± 4 weeks after EOT	10-16 months after EOT
18 months	18 months ± 4 weeks after EOT	17-21 months after EOT
24 months	24 months ± 4 weeks after EOT	≥ 22 months after EOT

***The acceptable leeway for the visits starts on the first day of the period mentioned and ends on the last day of the period mentioned.**

For the purposes of the study, patients will be hospitalised from their arrival at the hospital/investigational centre until D18. They will be permitted to leave hospital from D13 onwards, if their clinical status allows it.

Any additional unscheduled visits that may take place must be recorded in the e-CRF.

6.2. Screening and Baseline Assessment

6.2.1. Diagnosis of HAT

The mobiliser of the mobile team will inform the communities in the villages of the HAT screening activities, as is usually the case (see Section 14.1 Information of Communities; p 78). Specific information concerning the study should be provided to the community, i.e. a brief description of the aim of the study, explanation of the process for collecting informed consent, duration and importance of follow-up.

HAT case detection may be carried out by the mobile team.

A physical examination and, potentially, tests may be performed to collect sufficient information to suggest a diagnosis of late-stage HAT. Whenever possible, CSF sampling will be performed at the investigational centre, or by the mobile team of the National HAT Control Programmes (see Section 4.1. Enrolment/Inclusion Procedures; p 33).

6.2.2. Pre-screening and Screening

The following assessments will be performed to confirm the diagnosis of HAT, to collect historical safety data and to check the inclusion and exclusion criteria.

- Sampling to test for trypanosomes in the blood using Woo test/CTC, thick and thin blood smears, mAECT and mAECT-BC with INRB kits, as well as in lymph using microscopic examination of fresh lymph node aspirate;
- CSF sampling for diagnosis of late-stage HAT based on WBC and detection of trypanosomes using Fuchs-Rosenthal/Fast-Read 102® counting chamber and Modified Single Centrifugation (MSC) with INRB kits;
- Collection of full medical history, with special attention to HAT;
- Demographic data and prior treatment;
- Karnofsky Performance Score, body weight, height and vital signs including body temperature, blood pressure, heart rate and respiratory rate;
- Screening for malaria using RDT and/or thick blood smear;
- Review of inclusion and exclusion criteria;

- Assessment of laboratory safety parameters (see Appendix 3 – Laboratory Tests; p 96) – whenever possible, laboratory tests will be performed on fasting patients:
    - Haemoglobin
    - Haematocrit[2]
    - Haematology: WBC, platelet count
    - Biochemistry: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), blood urea nitrogen (BUN), chloride (Cl-), creatinine (CRE), glucose (GLU), potassium (K+), sodium (Na+), calcium (Ca2+), total bilirubin (TBIL), bicarbonates (tCO_2), and total protein (TP)
    - Urine analysis, i.e. WBC, pH, protein, urobilinogen, blood, nitrites, glucose, ketone bodies and bilirubin
- Digital ECG recording (CarTouch®). The tracings will be uploaded to the e-CRF. Each upload of an ECG tracing will be accompanied by a request for centralised reading by the cardiologist. This will be recorded in the e-CRF and will be available for consultation by the Investigator.

6.2.3. Baseline Assessment

The following assessments will be performed before randomisation to assess the patient's baseline status just before the start of treatment. The assessments will also provide confirmation of the patient's eligibility to participate in the study, just before randomisation:

- Review of inclusion and exclusion criteria
- Vital signs, including body temperature, blood pressure and heart rate
- Verification of clinical signs and symptoms of HAT
- Physical and neurological examinations
- Collection of concomitant treatment
- Urine pregnancy test for women of child-bearing potential
- Laboratory safety assessments (see Appendix 3 – Laboratory Tests; p 96) – whenever possible, all laboratory tests will be performed on fasting patients:
    - Haemoglobin
    - Haematology: WBC, platelet count
    - Biochemistry: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), blood urea nitrogen (BUN), chloride (Cl-), creatinine (CRE), glucose

[2] Only patients at sites where PK sampling will take place.

(GLU), potassium (K+), sodium (Na+), calcium (Ca2+), total bilirubin (TBIL), bicarbonates (tCO_2) and total protein (TP)

- Urine analysis, i.e. WBC, pH, protein, urobilinogen, blood, nitrites, glucose, ketone bodies and bilirubin

- Digital ECG recording (CarTouch®): triplicate tracings to assess QT interval. The tracings will be uploaded to the e-CRF for specific analysis of QT interval.
- If the assessments mentioned in Section 6.2.2, i.e. haemoglobin, haematology, biochemistry and urine analysis, were performed within 4 days prior to the first administration, the findings will be considered as baseline values and the tests will not be repeated at the Baseline Assessment.

A treatment pack will be assigned to each randomised patient using the internet-based randomisation process accessible via the e-CRF. Patients will receive treatment over 10 days in the hospital/investigational centre.

6.3. Assessments during Hospitalisation

6.3.1. Efficacy, Clinical Signs and Symptoms

Patients will be questioned on a daily basis with regard to potential adverse events during hospitalisation and then at each subsequent visit (see Section 6.5 Safety Assessments, Definitions and Reporting of Adverse Events; p **Error! Bookmark not defined.**).

- Physical examination and neurological assessment, including signs and symptoms of HAT, will be performed prior to treatment intake (D-1/D1), and on D5, D8, D11 (EOT visit) and D18 (EOH visit, between D13 and D18)
- Testing for *T.b. gambiense* in blood, lymph and CSF at the EOT visit (D11).

6.3.2. ECG Recordings

ECG recordings will be performed at the following timepoints:

To ensure the patient's safety

- At the baseline assessment: a single ECG tracing will be analysed by the cardiologist to assess potential rhythm or conduction disorders and to assist the Investigator in screening patients.
- On D2, D3 and D4 prior to dosing in the fexinidazole group (along with the triplicate ECG tracings on D4H23): a single ECG will be analysed by the Investigator to check the QTcF interval (QT interval corrected using Fridericia's formula), calculated automatically. If the value for the QTcF

is higher than 500 ms, a second ECG tracing must be collected after a 10-to-20 minute rest. If the value is confirmed, the patient must be withdrawn from the study, and no further doses of fexinidazole are to be administered. The ECG tracings will not be reviewed systematically by the cardiologist.

- During hospitalisation and the follow-up period, additional ECG tracings may be collected at the discretion of the Investigator.

To assess QT interval

- Triplicate ECG tracings, 2 minutes apart, will be recorded at the following timepoints:
  - At the baseline assessment for all patients
  - In patients in the fexinidazole group:
    - on D4 4 hours after intake of the IP;
    - on D4H23, i.e. one hour before intake of the IP on D5, this ECG should also be used for the patient's safety assessment prior to IP administration;
    - on D10 between H2 and H3 after intake of the IP.
  - In patients in the NECT group:
    - on D7 between H2 and H3 after the morning intake of NECT, i.e. within one hour after the end of the infusion of eflornithine;
    - on D10 between H2 and H3 after the morning intake of NECT, i.e. 2-3 hours after intake of nifurtimox.

Repeat ECG

- If the cardiologist issues an alert over the last ECG examination, related to prolonged QT interval or to any aspect of the ECG morphology, the Investigator will collect a repeat ECG recording at the EOH visit, prior to discharge. If the alert is issued again at the EOH, the Investigator must collect a repeat ECG recording at the M3 follow-up visit.

6.3.3. Laboratory Tests

A repeat urine pregnancy test will be performed at discharge, i.e. between D13 and D18.

Blood haematology and biochemistry assessments will be repeated on D5, D8 and D11 (EOT visit). Safety biochemistry and haematology assessments will be performed 9 weeks after intake of the first dose on D1, only in patients who reach this visit from mid-December 2014 onwards. Blood samples will be analysed in the laboratory in each centre using standard equipment specifically provided for the study by the Sponsor. Whenever possible, the samples should be collected from patients in the fasting state.

Parasitology testing requires 5 mL of whole venous blood or 4 mL of CSF. The blood sample should be analysed on an mAECT column within 30 minutes after collection. The CSF sample should be examined after MSC within 15 minutes after collection. The WBC in the CSF sample should be performed within 15 minutes after collection. It is necessary to collect 5 mL of CSF to perform the two tests.

Full haematological and biochemistry analyses require at least 0.5 mL of capillary or venous blood. Capillary blood collection considerably reduces the total quantity of blood collected as compared to classical techniques. However, because it is not always feasible, collection of venous blood is permitted. Each sample corresponds to 2 mL per tube, one tube containing EDTA for Haematology and one tube containing heparin lithium for Biochemistry.

The urine analyses performed at screening, at the baseline assessment and at the EOT visit, as well as on D18 and at 3 months for female patients, require only a few millilitres (5-10 mL).

During hospitalisation, additional safety assessments such as haematology, biochemistry or urine tests may be performed at the discretion of the Investigator in order to monitor for abnormalities.

The volume and the number of samples required for each patient are presented by visit in Appendix 3 – Laboratory Assessments (p 96).

6.3.4. PK Assessments

All patients in the fexinidazole group, included in the first 6 investigational centres opened, will undergo PK assessments.

The sampling procedure and assay method will be described in the corresponding laboratory manual.

- Approximately 300 µL of whole blood will be collected on filter paper by finger-prick for DBS testing at each of the following timepoints:

Ideal timepoint for sampling	Leeway
D8 3 hours 15 minutes after intake of the IP	± 5 minutes
D9 3 hours after intake of the IP	± 5 minutes
D10 3 hours and 7 hours and 15 minutes after intake of the IP	± 15 minutes
D11 24 hours after the last intake of the IP	± 1 hour
D12 48 hours after the last intake of the IP	± 1 hour

- The timing of sample collection was optimised with WinPOPT® software using the parameters of population PK modelling pooled from the DNDiFEX001 and DNDiFEX002 studies. The timepoints for sampling were chosen in order to obtain the best predictions of pre-dose M2 concentrations.

- Lumber puncture will be performed on D11 24 hours after the last intake of the IP, and the CSF sample will be analysed using dried spot sampling. Dried spot sampling of CSF will be performed on all patients up to the end of July 2013.

The CSF dried spot samples will be stored under the same conditions (see laboratory manual).

6.4. Assessments at Follow-up Visits

Should a patient move residence during the course of the study, the follow-up visits can be performed at Kinshasa by the coordination team (PNLTHA and INRB) or at the new location by the site investigator together with his/her required staff. Related documentation will be forwarded to the initial clinical site who will enter the data into the CRF".

6.4.1. At the Additional Visit 9 Weeks after D1

- Haematology: haemoglobin, WBC count and differential, platelet count
- Biochemistry
- Physical examination including vital signs
- Neurological examination

6.4.2. At the 3-month Follow-up Visit

- Physical examination, HAT symptoms since discharge, neurological assessment including signs and symptoms of HAT
- Testing for *T.b. gambiense* in blood and lymph for diagnosis of potential relapse
- CSF sampling and analysis (MSC with INRB kits) only if symptoms suggesting disease progression are present
- Urine pregnancy test in female patients of child-bearing potential to investigate possible exposure *in utero* (see Section 6.5.8. Exposure *in utero;* p 55)
- Additional safety assessments such as ECG, haematology, biochemistry or urine tests may be performed at the discretion of the Investigator. An ECG recording may be required at the M3 visit if the cardiologist issued an alert at the EOH visit.

6.4.3. At the 6, 12 and 18-month Follow-up Visits

- Physical examination, HAT symptoms since the previous visit, neurological assessment including signs and symptoms of HAT
- Testing for *T.b. gambiense* in blood and lymph for diagnosis of potential relapse

- CSF sampling to test for potential relapse, with WBC count and testing for trypanosomes (Fuchs-Rosenthal/Fast Read 102® counting chamber, MSC with INRB kits)
- Additional safety assessments such as ECG, haematology, biochemistry or urine tests may be performed at the discretion of the Investigator.

6.4.4. At the 24-month Follow-up Visit

- Physical examination, HAT symptoms since hospital discharge, neurological assessment including signs and symptoms of HAT
- Testing for *T.b. gambiense* if relapse is suspected or if required according to current local standard of care.
- Additional safety assessments such as ECG, haematology, biochemistry or urine tests may be performed at the discretion of the Investigator

6.4.5. At Unscheduled Visits

If a relapse is suspected on the basis of physical examination findings or CSF WBC count, at any visit, the patient must attend a return visit within 1 to 3 months, at the discretion of the Investigator.

The patient must also return to the investigational centre if s/he does not feel well, even if there is no apparent relationship with treatment and/or HAT.

The following assessments will be performed:

- Physical examination, HAT symptoms since the last visit, neurological assessment, including signs and symptoms of HAT
- Investigation of any concomitant condition that may have led to the visit
- Testing for *T.b. gambiense* in blood and lymph, if indicated
- CSF sampling (Fuchs-Rosenthal/Fast Read 102® counting chamber, MSC with INRB kits): only if symptoms suggesting disease progression are present
- Additional safety assessments such as ECG, haematology, biochemistry or urine tests may be performed at the discretion of the Investigator.

6.5. Safety Assessments, Definitions and Reporting of Adverse Events

The safety and tolerability of treatment will be assessed through routine monitoring of adverse events (AEs). During the observation period, the study personnel will collect AEs every day.

The **observation period** will extend from the start of treatment (D1) to hospital discharge, planned on D18, but potentially taking place as early as D13 if the patient's status allows it.

In addition, patients will be advised to return to the hospital/investigational centre at any time during the follow-up period if they experience any AEs, in order to undergo additional safety assessments.

Safety data will be reviewed at each meeting of the DSMB/ISC.

Safety endpoints will be classified as follows:

- Occurrence of any adverse events ≥ grade 3, including laboratory abnormalities, during the observation period. Adverse events will be graded according to the NCI CTCAE, Version 4.03 (38)
- Occurrence of any adverse events, including laboratory abnormalities, (all grades combined) during the observation period
- Occurrence of any treatment-related adverse events, including laboratory abnormalities, (≥ grade 3 and all grades combined) during the observation period
- Occurrence of any serious adverse events, including laboratory abnormalities, between the first treatment intake and the end of the follow-up period (18 months)
- Occurrence of any serious adverse events, including laboratory abnormalities, between the first treatment intake and the end of the additional follow-up period (24 months).

6.5.1. Definition of AE

An AE is defined as any untoward and unintended medical occurrence (sign, symptom or disease), including a clinically significant abnormal laboratory or ECG finding, or worsening of any pre-existing condition during the study, whether or not it is considered to be study related.

Any abnormal laboratory result on haematology, biochemistry or urine analysis will be reported as an AE if it occurs or worsens after the start of the study treatment, and if the CTCAE grade is > 1, unless it is associated with a previously reported clinical event.

The Investigator or appropriate study personnel will examine any patient who experiences an AE as soon as possible. The Investigator will do whatever is medically necessary for the patient's safety and well-being. The patient will remain under observation as long as s/he is receiving study treatment and up to the last day of the observation period (between D13 and D18), or longer if medically indicated in the opinion of the Investigator. All AEs observed or reported following administration of the study treatment will be followed until resolved or until the Investigator considers them to be "chronic" or "stable".

All identified AEs will be recorded in the appropriate AE section of the e-CRF using concise medical terminology, and avoiding vague, ambiguous or colloquial language. SAEs will be reported by telephone, Short Message Service (SMS) or email to Swiss TPH (see Section 6.5.5 Requirements for AE Reporting; p 54).

6.5.2. Definition of SAE

An SAE is any AE that:

- results in death,
- is life-threatening,
- requires in-patient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above.
- In this study, ALAT or ASAT levels more than 3 x ULN associated with a total bilirubin level more than 2 x ULN will be considered as SAEs.

SAEs also include any other events defined in the present protocol or by the regulatory authorities in the country in which the event occurs.

For the purposes of this study, hospitalisation for uncomplicated delivery will not be considered as an SAE.

6.5.3. Collection of Information on AEs

The Investigator is required to report all AEs s/he observes directly, as well as all AEs spontaneously reported by the patient, using concise medical terminology. In addition, during the observation period, i.e. from D1 to D18 (or earlier in the event of early discharge) and at each follow-up visit, the patient will be asked a series of questions, and a targeted physical examination will be performed, to investigate any potential AEs.

6.5.4. AE Collection Period

The periods for collection of AEs that occur in the context of the present study are defined as follows:

- For **all non-serious adverse events**, the **observation period** extends from the first intake of the study treatment on Day 1 until hospital discharge between D12 and D18;
- For **all serious adverse events**, the **observation period** extends from enrolment of the patient in the study, i.e. after signature of the informed consent form, **until the end of follow-up** (24 months). SAEs will be analysed initially up to 18 months, and then up to 24

months for the additional follow-up period.

All AEs that occur during the AE collection period defined in the protocol must be recorded in the e-CRF, whether or not they are considered to be treatment related. In addition, all AEs that occur after the AE collection period, and that the Investigator considers as possibly related to the study treatment, must also be reported.

6.5.5. Requirements for AE Reporting

Information on AEs must be assessed by a physician. The Investigator must determine whether the AE is considered as serious or not, if necessary with the help of the Coordinating Investigator and the study monitor. This classification will determine the reporting procedure for the event.

All SAEs must be reported immediately, i.e. no later than 24 hours after the Investigator becomes aware of the SAE, to the Swiss TPH clinical study monitor, first by telephone and/or SMS, then by email using the SAE reporting form. This report must include a description of the event, onset date and type, duration, severity, relationship to study treatment (with the help of the Coordinating Investigator as needed), outcome and measures taken, as well as any other relevant clinical or laboratory data. Any additional information must be sent on an SAE follow-up form as it becomes available. Follow-up reports should be submitted as soon as possible, and, if possible, within 5 working days after the new information becomes available. A close-out follow-up report must be sent after the final assessment of the case as “recovered”, “recovered with sequelae” [chronicity], “death” etc.

SAEs must also be reported in the AE section of the e-CRF. It should be noted that the reporting form for SAEs (SAE form) is not the same as the form in the AE section of the CRF. The two forms must be completed in a consistent manner, using the same medical terminology.

All AEs must be recorded on the e-CRF.

For the purposes of this study, the Coordinating Investigator will be in charge of reporting Suspected Unexpected Serious Adverse Reactions (SUSARs) considered to be related to the study treatment and SAEs to the Ethical Committees in the countries and other relevant ethical committees. DND*i* will be responsible for reporting SUSARs and SAEs to the regulatory authorities.

6.5.6. Grading of AE Severity

The severity of the AE must be graded according to NCI CTCAE, version 4.03 (38). For some biological parameters, the NCI CTCAE criteria have been adapted (i.e. hemoglobin, leucocytes, sodium, calcium, creatinine). If the AE is not described in the CTCAE, version 4.03, the Investigator will use the terms “mild”, “moderate” or “severe” to describe the maximum severity, as defined below:

Mild	does not interfere with the patient's usual activities
Moderate	interferes to some extent with the patient's usual activities
Severe	significantly interferes with the patient's usual activities

The information concerning AE grading must be recorded in the AE section of the e-CRF.

It is important to distinguish between the severity and the seriousness of AEs: a severe AE is not necessarily an SAE.

6.5.7. Assessment of AE Causality

For all AEs, the Investigator is required to assess the possible causal relationship between the study treatment and the AE, with the help of the Coordinating Investigator as needed, in order to determine whether there is a reasonable possibility that the study treatment caused or contributed to the AE.

The causal relationship between the study treatment and the AE is assessed by the Investigator after a detailed analysis of the event in terms of the biological plausibility, taking into account possible unrelated causes, pre-existing medical conditions, concomitant treatments, the temporal relationship between intake of the study treatment and onset or worsening of the event, and known patterns of response to fexinidazole and NECT in general.

The two types of relationships are defined as follows:

- Unrelated: there is no temporal relationship between intake of the IP or reference treatment and the event, and/or there is a plausible alternative explanation;

- Possibly related: any AE that is not considered as unrelated to the study treatment and/or for which there is no plausible alternative explanation.

The decision to interrupt, resume or permanently discontinue study treatment due to an AE will be left to the discretion of the Investigator, except in situations described in Section 8 Withdrawal Criteria (57).

6.5.8. Exposure *in utero*

Three pregnancy tests are planned during the study: prior to randomisation, at discharge and at the 3-month visit. Among women of child-bearing potential, only those who have a negative result on the pregnancy test prior to randomisation will be eligible to participate in the study.

The Investigator must report any pregnancy that occurs during the observation period of the study, or that is diagnosed at the 3-month follow-up visit, using the appropriate pregnancy reporting form. This must be done irrespective of whether an AE occurred or not. If known, the due date must be specified.

The Investigator will monitor the patient until the term of the pregnancy, i.e. full term or preterm, in the event of a miscarriage. The Investigator will provide information on the outcome of the pregnancy using the pregnancy follow-up reporting form.

A physician, preferably a paediatrician, should examine the infant at birth and submit a report using a pregnancy follow-up reporting form. The Investigator will offer the parents follow-up on infants exposed to the study treatment *in utero* until they reach 24 months of age. As far as possible, stillborn infants should be examined by a physician to assess the cause of death.

6.5.9. Follow-up on AEs

All AEs must be followed until resolution, or until the Investigator considers them to be “chronic” or “stable”, or until the patient’s participation in the study ends, i.e. until the final report is completed for the study in which the patient was participating.

If the AE is a laboratory abnormality with a toxicity grade ≥ 3, the test must be repeated 2 to 4 days later, and then at regular intervals until the parameter reaches toxicity grade ≤ 1, or until it returns to the baseline level (see Baseline Assessment).

In addition, all SAEs and all events that the Investigator (and the Coordinating Investigator or the monitor, as needed) considers as possibly related to the study treatment must continue to be followed even after the end of the patient’s participation in the study. Such events should be followed until their resolution, or until the Investigator considers them as “chronic” or “stable.” The resolution of such events must be documented in the e-CRF, and if they are SAEs, on an SAE follow-up form.

7. Study Duration

The enrolment period is expected to last 18-20 months (see Table 7 – Expected Enrolment Rate; p 104). The duration of treatment will be 10 days in both treatment groups.

Each patient’s participation will last approximately 25 months and will include:

- pre-treatment period (pre-screening and screening, treatment of concurrent disease)
- treatment period of 10 days
- hospitalisation for 3 to 8 days after treatment
- out-patient follow-up for 18 months, until the TOC visit

- additional follow-up for 6 months, until M24

The total duration of the study, including the preparative phase, is expected to be 48 months.

8. Withdrawal Criteria

8.1. Rules for Temporary Interruption of Treatment

Temporary interruption of treatment will not necessarily lead to withdrawal of the patient from the study. In some cases, treatment may be interrupted for a maximum of one day, i.e. one missed dose of fexinidazole or 3 missed doses of NECT, and treatment will therefore be delayed. Treatment may be reintroduced at the discretion of the Investigator responsible for the patient. One additional day of treatment will be added to make up for the missed dose (or doses as concerns NECT). The patient should continue the visits and study procedures as planned, taking into account the delay. The reasons for interrupting treatment must be recorded in the appropriate source documents and e-CRF.

8.2. Rules for Definitive Discontinuation of Treatment

The Investigator will discontinue the study treatment in the following cases:

- Severe skin reaction
- ALAT or ASAT exceeding 8 x ULN
- ALAT or ASAT exceeding 3 x ULN associated with total bilirubin > 2 x ULN
- ALAT or ASAT exceeding 3 x ULN accompanied by fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)
- pre-dose QTcF measurement ≥ 500 ms on D2, D3, D4 or D4H23, confirmed by a second ECG recorded after at least a 10-20 minute rest
- Any condition that, in the opinion of the Investigator, requires treatment discontinuation for medical reasons.

If a patient is withdrawn from the study before the end of treatment, the physician will make all necessary arrangements to ensure that s/he receives the appropriate treatment for the condition in question.

8.3. Patient Withdrawal from the Study and Replacement of Patients

A patient may be withdrawn from the study in the following cases:

- withdrawal of consent by the patient or his/her legal representative,
- study termination by the Sponsor.

If the patient or his/her legal representative withdraws his/her consent, no further evaluations will be performed, with the exception of safety data, which must be collected whenever possible.

If a patient decides to withdraw from the study, the reason must be recorded in the e-CRF. If a patient is withdrawn from the study due to an AE, all measures must be taken as needed to clearly document the outcome of the AE.

Data collected prior to withdrawal of the patient will be taken into account in the PK, efficacy and safety analyses.

Patients withdrawn from the study will not be replaced.

If a patient is lost to follow-up for reasons related to armed conflict, after several unsuccessful attempts have been made to contact him/her, and if no data are available after the treatment period, the patient will be removed from the primary analysis and another patient will be included.

8.4. Patients Lost to Follow-up

If a patient does not attend a protocol-planned visit, all necessary measures must be taken to contact him/her. In all cases, all necessary measures must be taken to document the outcome of the patient's condition, if possible.

9. Data Analysis and Statistical Methods

A draft of the full Statistical Analysis Plan (SAP) will be prepared prior to conducting the first FA. It will provide a more detailed description of the statistical methods. The SAP will be reviewed and, if necessary, amended prior to final database lock.

9.1. Sample Size Determination

The calculation of the sample size has been revised. The total size of the sample is now calculated to be 390 patients, instead of 510, with the same 2:1 allocation ratio, i.e. 260 patients in the fexinidazole group and 130 patients in the NECT group. If the expected success rates are 94% with NECT and 89% with fexinidazole[3] then the power of the final analysis is 80% with an acceptability ("non-inferiority") margin of 13%. The calculations were made for a one-sided type-1 error of 0.025.

No provision will be made for patients lost to follow-up since the missing outcome will be imputed, except for patients in areas of armed conflict (see Section 8.3).

[3] The observed response rate for NECT was as high as 0.97 in the per protocol population and 0.965 in the ITT population (43). However, the definition of success was not the same as in this protocol, and the success rate for NECT would have been 0.95 or 0.94 if the same definition had been used. If fexinidazole has the same true success rate as NECT, and is equal to 0.95 or 0.94, then the power of the study will be greater than 99%. However, NECT is a co-administration of two drugs and there is no reason to expect fexinidazole to be more effective than eflornithine alone. Available data show that the success rate with eflornithine depends on the project and ranges between 0.89 and 0.90. If the expectations are 0.90 for fexinidazole and 0.95 for NECT, the exact power of the final primary analysis is 91.6%.

9.2. Handling of Missing Outcomes and Patients Lost to Follow-up

The primary endpoint is based on the outcome at 18 months. If outcome assessed at the final visit (M18) is missing, the **primary imputation method** will consist in imputing a probable success (considered as a success) to patients who attended the 18-month visit but who refused the lumbar puncture planned for the visit, who showed no signs or symptoms of HAT at 18 months, who did not subsequently report any symptoms of relapse and for whom the outcome was considered as favourable at the last available assessment (42). If these criteria are not fulfilled, the patients will be considered as treatment failures. The outcome for patients lost to follow-up from the 18-month visit onwards will also be considered as a failure, except for patients who fled an area of armed conflict.

Three additional methods for imputing missing data will be used.

The **second method** will consist in imputing failure if the lumbar puncture at 18 months is not performed (see 5th sensitivity analysis).

In the **third method**, missing data will not be imputed. All patients for whom an assessment is available at a given timepoint will be included in the estimate of the success rate at that timepoint. If a value (number of CFS WBC) is missing at an intermediate visit, followed by a success or a failure at one of the subsequent visits, the status at the subsequent visit will prevail over the previous one to determine the status at the intermediate visit. In the Kaplan-Meier approach, the observation is censured at the first missing outcome, provided that all subsequent outcomes are also missing.

The **fourth method of imputation** will consist in using the predicted probability of relapse. If the predicted probability of relapse at 18 months, as derived from logistic models based on 1821 patients (data from MSF), is below a cut-off value that balances the proportions of false positives and false negatives, the patient will be imputed as a probable relapse, otherwise the patient will be imputed as a probable success[4].

The cut-off value will not be the value derived from the models built with the data from MSF, but will be redefined in a blinded manner in order to perfectly balance the proportions of false positives and false negatives in patients for whom the outcome at 18 months is unknown[5]. However, all efforts must be made to contact

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[4] Three logistic models were fitted to MSF data: one using the baseline and 6-month CSF WBC counts, as well as the age and sex of the patient (significant explanatory variables); another using 6-month and 12-month CSF WBC counts; and the last one using the CSF WBC count at 12 months, age and sex. The rate of false positives was 3.3% and the rate of false negatives was 3.0% for patients having a WBC count at 6 and 12 months after EOT. These models provide the probability of being cured at 24 months. The outcome is assessed at 24 months instead of 18 months because most patients in the MSF database had a missing CSF WBC count at 18 months. It is assumed that if cure at 24 months can be adequately predicted using the model, the prediction of cure at 18 months should be conservative due to possible relapses between 18 and 24 months.

[5] This approach assumes that the predicted probability of success is not dependent on whether or not patients are lost to follow-up in the study. This assumption seems realistic since, in most

patients at 18 months or later, in order to establish whether the outcome is a success because the primary analysis is not based on this rule. Cases that do not comply with the protocol but in which the outcome is known at any time after 18 months will be used in the efficacy analyses. The models will be validated in a blinded manner once all randomised patients have reached the 18-month visit after the EOT (primary endpoint).

Analysis	**Method of imputation**
Primary	Primary method (method similar to Priotto)
1st sensitivity analysis	Fourth method: surrogate endpoint (predicted or actual outcome)
2nd sensitivity analysis (treatment completers)	Primary method
3rd sensitivity analysis (evaluable patients)	Primary method
4th sensitivity analysis (per protocol population)	Fourth method
5th sensitivity analysis	Second method (patient lost to follow-up = failure)
Planned secondary analysis (K-M analysis)	Third method

For the interim analysis, if the lumbar puncture is missing at M12:

- if a lumber puncture was performed at M18, the result at M18 will be used,
- if a lumber puncture was performed at M6, success will be imputed if the WBC count is ≤ 5/µL, no trypanosomes are detected and no symptoms related to HAT are present at 12 months,
- if data for lumbar puncture are available at M6 and at M18, the outcome at M18 will prevail.

All other cases in which the lumbar puncture is missing will be considered as failures.

9.3. Handling of Centres

The primary analysis will not be stratified by centre. However, a logistic model with random intercepts using the centre and treatment as covariables will be used to check whether there is an interaction between the centre and the treatment, and whether there is a main centre effect. If there is a significant centre effect, the primary analysis may be slightly biased because of the dependency among observations. The odds ratio of success will provide an estimate of the treatment effect, and the odds ratio margin will be calculated *a posteriori* based on the

cases, patients who choose not to attend follow-up visits do so because they are doing well.

success rate with NECT minus 13%. If there is a significant interaction, it will not be possible to generalise the estimate of the treatment effect. The centre(s) responsible for the interaction will be examined.

The analysis stratified by centre is a secondary analysis. This secondary analysis provides an estimate of the odds ratio of success and the predicted success rate of each treatment, with a 95% confidence interval, but not the excess of the success rate.

9.4. Definition of Study Populations included in the Analyses

Populations used in the analyses

Type	Aim	Definition
Primary: ITT patients	Analysis of safety and primary efficacy analysis	All randomised patients who received at least one dose of study treatment. Except patients who fled an area of armed conflict and for whom no post-treatment data are available (these patients will be included in the population as failures for a sensitivity analysis).
Secondary: treatment completers	Sensitivity analysis on efficacy	All randomised patients who completed the treatment period and who received all protocol-planned doses.
Secondary: evaluable patients	Sensitivity analysis on efficacy	ITT patients, except those who died due to causes clearly unrelated to efficacy or safety. Exclusion will be documented and decided during the blind data review.
Secondary: per protocol patients	Sensitivity analysis on efficacy	ITT patients with no major protocol violations. Major violations will be provided for each patient, and exclusion will be decided during the blind data review.

9.5. Patient Disposition

At the end of the study, patient disposition will be presented in terms of:

- Number of patients screened
- Number of patients who fled an area of armed conflict and for whom no post-treatment data are available
- Number of patients not included in the study because they did not fulfil the eligibility criteria, and reason for non-inclusion
- Number of patients randomised, total and by treatment group
- Number of randomised patients who received at least one dose of study treatment (ITT patients), total and by treatment group
- Number of randomised patients who completed the treatment period and who received all protocol-planned doses (treatment completers), total and by treatment group

- Number of randomised patients who received at least one dose of study treatment, except those who died due to causes clearly unrelated to the efficacy or safety of treatment (evaluable patients), total and by treatment group
- Number of randomised patients with no major protocol violations (per protocol patients), total and by treatment group
- Number of randomised patients who attended the following visits: screening visit, baseline assessment, visits during treatment period (D1 to D10), EOT visit, PK sampling visit, EOH visit, and follow-up visits at 3, 6, 12, 18 and 24 months, total and by treatment group
- Number of randomised patients who were withdrawn from the study, classified by reason for withdrawal, total and by treatment group
- Number of randomised patients with at least one protocol violation, classified by nature of violation (minor or major), total and by treatment group

Patient disposition will take into account the method of imputing missing data (see Section 9.2 Handling of Missing Outcomes and Patients Lost to Follow-up; p 58).

9.6. Demographic and Baseline Data

Descriptive summary statistics (n, mean, standard deviation, median, minimum, maximum) or frequencies and percentages will be presented for the following baseline characteristics of the study population:

- Demographic data
- Medical history
- Physical examination
- Vital signs
- Urine pregnancy test (only for women of child-bearing potential)
- Laboratory assessments
- ECG
- Karnofsky Performance Score
- Neurological examination
- Concomitant medication

9.7. Treatment Compliance

The dosing regimen of the study treatments, i.e. the IP and reference treatment, are fully described in Section 5 Treatments (p **Error! Bookmark not defined.**).

Treatment compliance will be analysed by describing, by treatment group, the number and percentage of patients who completed the treatment period. Patients in whom the treatment period was extended by one day (missed dose made up for at end of treatment) will be considered as having completed the treatment period. For patients who prematurely discontinued treatment, the duration of exposure will be presented.

9.8. Efficacy Analysis

9.8.1. Primary Efficacy Analysis

Primary Efficacy Endpoint

The primary efficacy endpoint is the outcome at 18 months. Outcome will be considered as a success if there is no evidence of trypanosomes in any bodily fluid and if the CSF WBC is ≤ 20/µL. Death and use of rescue treatment will be considered as a relapse, i.e. a failure. A patient who attends the 18-month visit or who is found again after an active search, who refused to undergo lumbar puncture but who has no signs or symptoms of HAT, and for whom the outcome was assessed as favourable at the latest assessment will be considered as a probable cure, i.e. a success, unless signs of relapse are detected on any assessment up to 24 months.

Primary Assessment Criterion

The primary assessment criterion is the success rate at 18 months.

Primary Test

The primary test is the Blackwelder non-inferiority test, based on the difference in the success rates and a margin of 13% (see Margin for "Acceptable Difference"; p 63).

Primary Measure of Size of Effect

The size of the treatment effect will be measured by the excess rate of success. The excess success rate is equal to the difference in the success rates between the two groups: size of effect = $p_{fexi} - p_{NECT.}$ A negative success rate corresponds to a treatment effect in favour of NECT.

Margin for "Acceptable Difference"

The margin for an "acceptable difference" is set at 13%[6].

[6] A survey was carried out by e-mail in the small population of local and international physicians who are experienced in treating patients with HAT, in order to assess the limit of an acceptable difference and the limit of an unacceptable difference in efficacy between an oral drug and NECT. Between the two limits, there is an "uncertainty zone". The median response for an unacceptable difference was 17% and the median response for an acceptable difference was 9%. A margin of 13% was chosen and is equal to the first tertile of the distribution of an unacceptable difference. It means that the unacceptable limit was above 13% for 2/3 of respondents, i.e. for 6/19 respondents, the unacceptable limit was below 13% and, for 12/19, the limit was above 13%. The margin of 13% is also at the mid-point between the median of an acceptable difference (9%) according to respondents and the median of an unacceptable difference (17%) for the same respondents (see results of the survey in Figure 4; p 108).

In life-threatening diseases, any margin of difference is clinically and ethically relevant, even if there may be differences in efficacy between treatments that are more or less interchangeable.

The observed absolute mean difference in the rate of favourable outcomes between the estimated treatment effects in various programmes/clinical studies is 17%. This would explain why a margin of 15% was used in a phase-III study on the efficacy of pafuramidine maleate (**Error! Reference source not found.**). B ecause products are not necessarily interchangeable, and efficacy is largely dependent on the project or programme, the margin of non-inferiority is not based on the limit of a non-pertinent difference, but rather on a difference in efficacy that is acceptable for an easily administered oral drug that can be used to treat HAT in primary healthcare facilities in outlying areas with little or no healthcare infrastructure (see Figure 4; p 107).

If the non-inferiority test is significant, this will not mean that fexinidazole is equivalent or superior to NECT, but rather that the practical advantage of an oral treatment counterbalances or outweighs a potential difference in efficacy in favour of NECT for the majority of experienced physicians. Interpretation of the size of the effect as concerns NECT and of the 95% confidence interval will prevail over interpretation of the test result in assessing the benefit-to-risk balance.

9.8.2. Sensitivity Analyses

Several sensitivity analyses will be performed to assess the robustness of the result of the primary analysis and to facilitate interpretation. Additional sensitivity analyses may be described in the SAP.

		Population	Efficacy endpoint	Test
Primary analysis		Primary = ITT	Primary	Primary
Sensitivity analysis	1st	ITT	Surrogate: actual or predicted outcome for patients with missing data for CSF WBC at 18 months	Primary
	2nd	Treatment completers	Primary	Primary
	3rd	Evaluable patients	Primary	Primary
	4th	Per protocol patients	Primary	Primary
	5th	ITT	Success or failure, but all patients with no lumbar puncture at 18 months are considered as failures	Primary
	6th	Study completers with a known CSF WBC at 18 months	Primary (with no imputation)	Primary

The excess failure rate at 18 months based on the K-M estimator can also be considered as a sensitivity analysis.

Analyses exploring the centre effect and interaction between the centre and treatment will be performed primarily to assess the possible generalisabilty of the size of the effect, i.e. no potential interaction between the centre and treatment, as well as potential bias in estimating the confidence interval of the excess success rate if a significant centre effect is present (see Section 9.3 Handling of Centres; p 60).

9.8.3. Secondary Efficacy Analyses

The curve representing the cumulative failure rate over time will be plotted using the Kaplan-Meier estimates for the fexinidazole and NECT groups.

The difference in the failure rates at 18 months will be estimated. The hazard ratio with respect to NECT will be estimated using a Cox model along with the 95% confidence interval.

No imputation of missing data will be performed. Data on patients lost to follow-up will be censured just after the last available assessment.

The time course of the failure rate for fexinidazole and for NECT will be compared using a mixed-effect logistic regression model for repeated measures. Treatment, centre, time, baseline CSF WBC, age and sex will be used as candidate covariables. The linear predictor will include treatment-by-time and treatment-by-centre, as well as age and sex interactions. A backwards selection procedure will be used to identify significant terms, excepting those that involve the primary effect of centre and treatment. No imputation of missing data will be performed, except if outcome is missing at an intermediate visit and the outcome before and after the missing outcome were favourable.

The time course of the failure rate for melarsoprol and eflornithine will be based on historical data (see Figure 5; p 107). No statistical tests will be performed for comparison with historical controls.

9.9. Safety Analyses

All patients who received at least one dose of study treatment will be included in the safety analyses.

The percentage of patients with SAEs and/or AEs leading to treatment discontinuation will be presented for each treatment group and by system-organ class, using appropriate MedDRA preferred terms, according to the NCI CTCAE, version 4.03.

The percentage of patients with at least one AE will be described. Patients in whom an AE with the same preferred term occurred several times will be counted only once. If a patient experienced several AEs described using the same preferred term, the AE with the maximum severity will be used in the analysis. In addition, each SAE will be presented in a narrative describing all aspects of the medical event with a causality assessment.

AEs not leading to treatment discontinuation will be presented for each treatment group using the same classification as presented above.

The incidence of SAEs and AEs, along with the respective 95% confidence intervals will be presented for each treatment group, by category and by frequency. In other cases, only descriptive statistics will be presented.

Laboratory safety parameters, i.e. haematology and biochemistry, will also be presented for each treatment group, indicating the percentage of patients, the size of the increase in the value in relation to the ULN and baseline value, and changes in blood levels over time. Shift tables will be presented. A listing of patients with laboratory abnormalities will be provided.

ECG abnormalities will be presented for each treatment group and for each timepoint within each treatment group.

9.10. Analysis of Other Endpoints

PK Data

For patients in the fexinidazole group:

- Concentrations of fexinidazole, M1 and M2 in whole blood and in CSF will be measured, with PK parameters derived using a population PK model.
- Analysis of all PK samples will be centralised. A population PK model will be developed using Nonmem software. The model will be fitted on rich sampling data from phase-I studies and used for sparse sampling data from phase-II studies. The model will be used to estimate population PK parameters, including clearance (CL) and volume of distribution (Vd). All of the analyses and statistical methods, including data conventions, will be described in detail in a separate SAP, which will be finalised prior to database lock.
- Correlations between blood and CSF concentrations will be described on samples collected at the EOT visit. Correlations between blood and CSF concentrations, on the one hand, and the success rate/AE rate, on the other hand, will be described.

Information on the PK data from the study will provide confirmation of phase-I data on a larger sample size, will allow for analysis of individual failures or intolerance, and will help predict the dose for a future study in early-stage HAT.

ECG Data

- QT/QTcF interval (mean, range and categories) at the various timepoints.
- Prolongation of QT/QTcF interval in relation to baseline.

All of the analyses and statistical methods, including data conventions, will be described in detail in a separate SAP, which will be finalised prior to database lock.
An interim analysis, involving ECG data from 195 patients, will be performed.

9.11. Interim Analyses

Two types of interim analyses will be performed:

- interim futility analyses, the aim of which is to discontinue the study if the efficacy of fexinidazole is markedly inferior to expectations, and
- an interim analysis of success, the aim of which is to accelerate the process for submission of the registration dossier if the efficacy of fexinidazole is superior to expectations. Unlike with usual interim analyses of success, this study will be continued to completion regardless of the result of the interim analysis in order to obtain a precise idea of the relapse rate and rate of intercurrent events in the long term.

Interim Futility Analyses

Futility analyses will be performed in order to inform decisions about possible study discontinuation, based on pre-determined stopping rules. The aim is to limit the number of patients exposed to the IP in the event that the latter should prove to have limited efficacy.

An independent statistician with no involvement in preparing the study, in the blind review of data or in the final statistical analyses will be responsible for performing the FAs. Based on the analyses, s/he will be able to recommend continuing the study or stopping it due to futility. The independent statistician will be the only person able to access the randomisation code at the time of the FAs.

No information concerning the magnitude of the treatment effect will be provided. The ISC/DSMB may use additional or more stringent rules to stop exposure to fexinidazole, particularly in the event of safety concerns. The ISC/DSMB may decide to disregard recommendations based on the FAs if death is not related to treatment failure, but to accident, crime, etc.

The FAs will be performed on raw data on the ITT population.

The FAs will be performed as soon as data are available at a given timepoint. However, since the size of the sample increases at each timepoint, additional analyses may be performed at the written request of the ISC/DSMB to improve the statistical power of the analyses (see Table 5 – Overview of Protocol-planned Futility Analyses, and Table 7 – Expected Enrolment Rate).

Information concerning statistical power, criteria and the rules for the FAs is presented in Table 5 – Overview of Protocol-planned Futility Analyses (p 107).

The quantitative thresholds for decisions provided in the table are correct if the actual sample size is equal to the planned sample size.

Futility Analysis 1: EOT responder rate (n = 30). Performed if fewer than 27 out of 30 patients are responders at the EOT visit.

The 1st FA will be performed under certain conditions, once the outcome at the EOT visit is known for 30 patients, i.e. 20 patients exposed to fexinidazole and 10 patients exposed to NECT.

The blind will be lifted for the first 30 patients, and the analysis will be performed if and only if the number of responders is less than 27 out of 30 patients, based on the assumption that no deviations from randomised treatment assignment occur.

The study will be stopped if the actual percentage of responders in the fexinidazole group is significantly lower than 95%, which corresponds to the historical rate for melarsoprol at the end of treatment (published responder rate, p = 95.2%; n = 6840).

The test will be significant if the number of responders in the fexinidazole group is ≤ 16 out of 20 patients.

Futility Analysis 2: EOT responder rate (n = 105). Performed if fewer than 98 out of 105 patients are responders at the EOT visit.

The 2nd FA will be performed under certain conditions, once the outcome at the EOT visit is known for 105 patients, i.e. 70 patients in the fexinidazole group and 35 patients in the NECT group.

The blind will be lifted for the first 105 patients, and the analysis will be performed if and only if the number of responders is less than 98 out of 105 patients.

The study will be stopped if:

- Rule 1: the actual percentage of responders in the fexinidazole group is significantly lower than 95%, which corresponds to the historical rate for melarsoprol at the end of treatment (published responder rate, p = 95.2%; n = 6840),
  AND the percentage of responders in the NECT group is compatible with 99%.
  The first test will be significant if the number of responders in the fexinidazole group is ≤ 62 out of 70 patients. The second condition will be met if the number of responders in the NECT group is ≥ 33 out of 35 patients.

- OR Rule 2: the percentage of responders in the NECT group is not compatible with 99%,
  AND the difference in the percentage of responders between fexinidazole and NECT is significantly higher than 4%, which corresponds to the historical difference at the end of treatment between melarsoprol and eflornithine (δ = 4%) (see Table 5 – Overview of Protocol-planned Futility Analyses; p 107).

The test for comparison of the difference between the rates with a margin of 4% will be performed using a modified Blackwelder test, i.e. difference greater than the margin instead of smaller than the margin.

Futility Analysis 3: responder rate 3 months after the EOT (n = 105). Performed if fewer than 94 out of 105 patients are responders at 3 months.

The 3rd FA will be performed under certain conditions, once 105 patients have performed the 3-month visit.

The blind will be lifted for the first 105 patients, and the analysis will be performed if and only if the number of responders is less than 94 out of 105 patients at 3 months.

The study will be stopped if:

- Rule 1: the actual percentage of responders in the fexinidazole group is significantly lower than 91%, which corresponds to the historical rate for melarsoprol at 3 months (see Table 5 – Overview of Protocol-planned Futility Analyses; p 107);
  AND the actual percentage of responders in the NECT group is compatible with 99%.
  The first test will be significant if the number of responders in the fexinidazole group is ≤ 59 out of 70 patients. The second condition will be met if the number of responders in the NECT group is ≥ 33 out of 35 patients.
- OR Rule 2: the percentage of responders in the NECT group is not compatible with 99%,
  AND the difference in the percentage of responders between fexinidazole and NECT is significantly higher than 7%, which corresponds to the rounded historical difference between melarsoprol and eflornithine at 3 months (see Table 5 – Overview of Protocol-planned Futility Analyses; p 107).

Futility Analysis 4: responder rate 6 months after the EOT (n = 105). Performed if fewer than 84 out of 105 patients have a favourable outcome at 6 months.

The 4th FA will be performed under certain conditions, once 105 patients have performed the 6-month visit.

The blind will be lifted for the first 105 patients, and the analysis will be performed if and only if the number of responders at 6 months is less than 84 out of 105 patients.

The study will be stopped if:

- Rule 1: the actual percentage of responders in the fexinidazole group is significantly lower than 80%, which corresponds to the historical rate for melarsoprol at 6 months,
  AND the percentage of responders in the NECT group is compatible with 95%.
  The first test will be significant if the number of responders in the fexinidazole group is ≤ 49 out of 70 patients. The second condition will be met if the number of responders in the NECT group is ≥ 30 out of 35 patients.

- OR Rule 2: the actual percentage of responders in the NECT group is not compatible with 95%,
  AND the difference in the percentage of responders between fexinidazole and NECT ($p_{NECT} – p_{fexi}$) is significantly higher than 12%.

  The 95% rate of favourable outcomes for NECT is lower than the historical rate (42), and slightly higher than the historical rate with eflornithine. The 12% threshold corresponds to the rounded difference between the rate for eflornithine and the rate for melarsoprol (see Table 5 – Overview of Protocol-planned Futility Analyses; p 107).

Futility Analysis 5: Favourable outcome rate at 12 months after EOT (n = 105). Performed if fewer than 80 out of 105 patients have a favourable outcome at 12 months.

The 5th FA will be performed under certain conditions, once 105 patients have performed the 12-month visit.

The blind will be lifted for the first 105 patients, and the analysis will be performed if and only if the number of responders at 12 months is less than 80 out of 105 patients.

The study will be stopped if:

- Rule 1: the actual percentage of responders in the fexinidazole group is significantly lower than 75%, which corresponds to the historical rate for melarsoprol at 12 months,
  AND the percentage of responders in the NECT group is compatible with 95%.
  The first test will be significant if the number of responders in the fexinidazole group is ≤ 44 out of 70 patients. The second condition will be met if the number of responders in the NECT group is ≥ 30 out of 35 patients;

- OR Rule 2: the percentage of responders in the NECT group is not compatible with 95%,
  AND the difference in the percentage of responders between fexinidazole and NECT ($p_{NECT} – p_{fexi}$) is significantly higher than 13%.

This threshold is slightly lower than the historical difference between eflornithine and melarsoprol at 12 months, i.e. ≈ 15%. It should be noted that, at this stage, the study will be stopped if the result is significantly worse than the margin of an unacceptable difference, whereas the final analysis will be positive if the result is significantly better than the margin of an unacceptable difference.

Interim Analysis for Success

An interim primary analysis for success will be performed on the first 195 randomised patients who have theoretically reached 12 months of follow-up, excluding patients for whom no post-treatment data are available because the patient is known to have fled the area strictly for reasons of armed conflict. The analysis will be identical to the final primary analysis with the exception of the threshold for significance (see Adjustment of Type-I Error for Multiplicity) and the assessment visit, which will be 12 months instead of 18 months.

A sensitivity analysis, including all randomised patients, will be performed and will consider all patients who fled for reasons of armed conflict as failures.

If the interim primary analysis for success should not prove to be significant, no further interim secondary or sensitivity analyses will be performed. If the analysis proves to be significant, all of the interim secondary and sensitivity analyses will be performed in accordance with the approach described for the final analyses, i.e. those initially planned. In addition, the success rate at 18 months will be compared, using a superiority test, to the historical success rate for melarsoprol at 18 months, i.e. 73.7%. No adjustment of the threshold for significance will be made in this analysis. The time course of the success rate, i.e. EOT, 6 months, 12 months and 18 months, will be studied using the Kaplan-Meier approach. The cumulative failure rate at each visit will be presented graphically including historical data for melarsoprol and eflornithine.

Adjustment of Type-1 Error for Multiplicity

No adjustment will be made for the FAs, because the study cannot be prematurely discontinued for success based on an FA. In this case, Chen has shown that performing such analyses slightly reduces the final type-I error (9).

An adjustment of the type-I error will be made in view of the interim analysis for success. The Pocock approach will be used to determine the threshold of significance of the interim analysis as well as that of the final analysis. As a reminder, the final analysis will be performed on the planned number of patients.

10. Independent Steering Committee/Data Safety Monitoring Board

An Independent Steering Committee (ISC or DSMB) composed of at least 3 members independent of the Investigators and the Sponsor, will be set up prior to study initiation. The ISC will monitor the study in order to minimise any risk of harm to the patients included in the study. At predetermined intervals, the ISC will analyse safety data and all information related to SAEs or AEs leading to treatment discontinuation, and will put forward recommendations regarding the study if the benefit-to-risk balance for patients seems to be in jeopardy. The data and the intervals for review will be decided before, or soon after study initiation and documented in the ISC Charter.

The results of FAs will be presented to the ISC, who will decide whether or not the study can continue and whether the enrolment period should be extended.

The organisation of the ISC will be described in the ISC Charter, which will be prepared and approved prior to the first planned FA.

Additional *ad hoc* members may be invited to join the ISC if any safety concerns emerge, in order to give additional support to the competencies already present.

11. Quality Assurance and Quality Control Procedures

The Investigator must maintain appropriate accurate records to ensure that all aspects of conducting the study are fully documented, and that study data can be verified at the end of the study. These documents include the Investigator Site File, the patients' clinical source documents, screening/enrolment logs and other study-specific forms.

11.1. Investigator Site File

The Investigator's Site File must contain the protocol and protocol amendments, Independent Ethics Committee (IEC) and regulatory approval with all correspondence, a copy of the patient information and informed consent form, drug accountability records and curriculum vitae for study personnel, as well as authorisation forms and any other relevant documents or correspondence.

11.2. Case Report Forms

Data will be collected by laboratory technicians, physicians, nursing staff or care-givers authorised by the Investigator. Data collection will be supervised by the Investigator. Study-specific information will be entered in an electronic Case Report Form (e-CRF). Data generated from this information must be consistent with the source documents and any discrepancies must be explained. All data that are recorded directly in the e-CRF must be rendered anonymous, i.e. such that they can only be identified by the patient's code.

The Investigator must ensure the accuracy, completeness, legibility and timely entry of all data reported to the Sponsor via the e-CRF, and any other additional information that is requested. The Investigator is responsible for ensuring that all informed consent forms, screening forms and results of randomisation for all patients are stored in a secure location. Data will be entered in the e-CRF, saved and sent by Internet after each visit. The e-CRF will be signed electronically by the Investigator at the time of the interim analysis and at the end of the study, prior to closing the database.

11.3. Source Documents

The data in the e-CRF must be verified by direct inspection of the source documents. The source documents are the patient's medical files, the physicians' and nursing staff's notes, appointment books, originals of laboratory test results, ECG tracings, reports on specific assessments, signed informed consent forms, and patient screening/enrolment logs.

The Investigator must keep the source documents up to date, i.e. reports on laboratory tests and consultations, records of medical history and physical examination reports, so that they can be examined and/or audited by DND*i* or its designated clinical monitors and/or by the Regulatory Authorities.

11.4. Retention of Documents

The Investigator must retain all essential documents for at least two years after approval of the last marketing authorisation is obtained, and until there are no on-going or planned applications for marketing authorisation, or until at least 15 years after the official stop date of clinical development of the study treatment. However, study documents may need to be retained for a longer period of time if required by local regulations in effect or by agreement with DND*i*. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained. After that date, the documents may be destroyed with prior permission from DND*i*, subject to local regulations.

DND*i* must be notified in advance if the Investigator plans to assign the study records to another party or move them to another site.

11.5. Monitoring

Clinical monitors will perform regular monitoring visits during which they will verify source data, Informed Consent Forms, medical records, laboratory results, imaging reports, e-CRFs, drug dispensing logs and protocol violations. The monitors will be given access to the corresponding source documents for each patient on condition that that the patient's confidentiality is maintained in accordance with local regulations.

Monitoring visits at the investigational sites will be performed periodically by *DNDi* representatives or designated clinical monitors to ensure compliance with Good Clinical Practice and all aspects of the protocol. Source documents will be reviewed for verification of consistency with the data in the e-CRFs. It will be the clinical monitor's responsibility to inspect the e-CRFs at regular intervals. The Investigator will ensure that DND*i* designated representatives have direct access to source documents. It is important that the Investigators and the personnel concerned are available during monitoring visits. The Investigator agrees to cooperate with the clinical monitor to ensure that any problems detected during monitoring visits are resolved.

The monitoring visits provide DND*i* with the opportunity to assess progress of the study, to verify the accuracy and completeness of the e-CRFs and to resolve any inconsistencies in the study records, as well as to ensure compliance with all protocol requirements, applicable regulations and Investigator obligations.

Four types of visit are planned: site evaluation visit, site initiation visit, monitoring visit and site closure visit.

11.6. Audits and Inspections

The investigational site may also be subject to quality assurance audits by DND*i* or designated representatives, and/or to inspection by regulatory authorities or IEC members.

The purpose of the inspections is to verify adherence to the protocol and to ensure the study is being conducted in accordance with Good Clinical Practice. It is important that the Investigators and the personnel concerned are available for any audits or inspections.

11.7. Data Management

An e-CRF must be completed for each patient who gives informed consent to participate in the study. A validated e-CRF will be used in the study. The study data will be stored in a computer database that ensures confidentiality of the data, in accordance with national legislation on data protection.

All data are entered in the e-CRF under the responsibility of the Investigator or a designated qualified staff member. The Investigator will provide a written document at the start of the study attesting that his/her electronic signature is equivalent to his/her written signature from a legal standpoint.

Data will be checked regularly by the clinical monitor. Data queries will be generated, documented and resolved on a regular basis throughout the study.

11.8. Confidentiality of Information, Study Documents and Patients' Files

The Investigator will ensure that the anonymity of patients is maintained and that their identity is protected from unauthorised third parties. Patients must not be identified by their names in the e-CRF or on any other documents submitted to the Sponsor. Only the patient number should appear. The Investigator must keep a patient enrolment log containing the number, name and address of patients. The Investigator must ensure the confidentiality of all documents submitted to the Sponsor's authorised representatives, including the signed informed form.

The findings of any assessments, including laboratory tests, will remain strictly confidential to the patient him/herself. This includes patients under legal age and vulnerable patients. Particular attention will be paid to the confidentiality of the results of pregnancy tests and tests related to concomitant diseases.

12. Protocol Amendments

The Investigators will ensure that the study is conducted in strict compliance with the protocol, and that all data are collected and recorded in the e-CRF.

All protocol modifications must be documented in writing. A protocol amendment can be initiated by either the Sponsor or any Investigator. The Investigator will provide the reasons for the proposed amendment in writing and will discuss it with the Sponsor and the Principal Investigator.

Any protocol amendment must be approved and signed by the Sponsor and the Principal Investigator, and must be submitted to the appropriate IEC for information and approval in accordance with local requirements, and to regulatory agencies, if required. Approval must be received from the IEC, and the regulatory authorities, if applicable, before any changes can be implemented, with the exception of changes required to avert an immediate risk for study participants, or when the change involves only logistical or administrative aspects of the study, e.g. changes in telephone numbers.

13. Early Termination of the Study

Both the Sponsor and the Principal Investigator will have the right to terminate the study early, i.e. at any time prior to inclusion of the planned number of patients, but they may exercise this right only for valid scientific or administrative reasons. If this is necessary, the two parties will define the procedures for terminating the study after consultation. The Sponsor and the Principal Investigator will ensure that early termination of the study takes place in such a way as to protect of the patients' interests.

Reasons for which the study may be terminated by the Sponsor include, but are not limited to:

- insufficient enrolment rate,
- protocol violations,
- inaccurate or incomplete data,
- dangerous or unethical practices,
- on recommendation from the DSMB or IEC.

Reasons for which the study may be terminated by the Investigator include, but are not limited to:

- insufficient time or resources to conduct the study,
- lack of eligible patients.

If the study is terminated early by the Sponsor or the Investigator, the latter must:

- complete all e-CRFs to the largest extent possible,

- return all study-related articles and equipment to the Sponsor who provided them,
- answer all queries from the Sponsor, or delegated representatives, related to data on patients enrolled by the site prior to study termination,
- ensure that patients enrolled in the study who have not yet attended any follow-up visits receive all necessary medical care,
- provide the IEC, the regulatory authorities and, if appropriate, the Sponsor with a written explanation of the decision to terminate the study.

14. Ethical Considerations

The protocol for this study was prepared in accordance with the general ethical principles set out in the Declaration of Helsinki of the World Medical Association (see Appendix 1 – Declaration of Helsinki; p 90) and ICH guidelines for Good Clinical Practice (ICH Harmonised Tripartite Guideline - Guideline For Good Clinical Practice E6(R1) - current step 4 version, dated 10 June 1996). DNDi commits to respect all applicable laws for the protection of the rights and welfare of human subjects.

The study was submitted to a panel of international experts who were invited, by the WHO, to provide an opinion on ethical aspects of the study, under the supervision of the French and Francophone Society for Medical Ethics (SFFEM) presided by Professor Christian Hervé. The minutes of this meeting, as well as the responses from the Sponsor, are presented in appendix to the dossier for submission of the protocol to the various IECs.

The protocol will also be submitted by the National Investigator for official approval from the IEC in each country:

- the Ethics Committee of the Ministry of Health in the DRC,
- the Scientific Committee in charge of validating study protocols and results at the University of Bangui (Faculty of Health Sciences) in the CAR.

In addition, the protocol will be reviewed by the MSF Ethics Review Board prior to study initiation, since MSF is involved in the study as an implementation partner.

Formal approval must be obtained in each country prior to undertaking any protocol-specific procedure in any patient in the country concerned.

Any modification made to the protocol after receipt of the IEC approval must also be submitted in writing by the National Investigator to the IEC, in accordance with local procedures and regulatory requirements (see Section 12 Protocol Amendments ; p 76).

The protocol will be submitted along with appendices relevant to the information and safety of patients, such as the patient information sheet & consent/assent form, and the Investigator Brochure. The set of images provided to the

Investigators as visual aids to explain the study procedures will be presented to the IEC. The patient information sheet and consent/assent form should be formally agreed upon by each IEC separately. If necessary, during the protocol review process, the specificities of each country may be incorporated in a specific version of the protocol for the countries concerned.

14.1. Information of Communities

14.1.1. Democratic Republic of the Congo

The Primary Investigator for the study is the Head of the National HAT Control Programme (PNLHAT) in the DRC. The programme is responsible for all prevention and treatment activities regarding HAT within the country, and in particular for the supervision and coordination of the mobile teams in charge of HAT screening activities. PNLTHA is fully involved in the design and implementation of the fexinidazole study in the DRC.

Information of the communities participating in the study will be provided at three different levels.

Firstly, the study will be presented to the public health representatives of the provinces concerned, namely the Provincial Medical Inspectors (*Médecins Inspecteurs Provinciaux*) and District Medical Officers (*Médecins Chefs de Zones*), as well as the District Administrators prior to any study-related activity in their respective geographic area of responsibility. Information on the study will be provided by the Provincial Coordinators of the PNLTHA (*Médecins Coordinateurs*), if possible in conjunction with a DNDi representative. The information will be based on the study protocol summary, the patient information sheet and consent form, and a summary of the Investigator Brochure.

Secondly, and before starting screening activities for the study in a given area or health zone, an adequate, HAT-experienced person with good knowledge of the area and local culture and good communication skills, i.e. either a member of a mobile team and/or a community mobiliser, will visit the local authorities, and tribal or village chiefs a few days before arrival of the mobile team, and inform them about the study and the related activities. In agreement with the local chiefs, an additional information session for the local population may be held, possibly during the usual community information session, which routinely takes place just before the start of screening activities by mobile teams. HAT is endemic in the regions where the study is to be conducted, and therefore, individuals already have a basic knowledge of the disease. In addition, most of the community mobilisers and many mobile team members have undergone specific training in community communication and HAT and will also receive additional study-specific training. Their experience and knowledge will therefore be highly useful in promoting a good understanding of the study.

The following information on the study will be disseminated at the community level:

- Routine procedures for detection and diagnosis of HAT;
- Primary objective of the study, i.e. to develop a safe oral drug to treat HAT that will be made available to the local population;
- Information on the new drug, the reference treatment as an alternative outside the study, the availability of a rescue treatment and on concomitant treatments as needed;
- Information on the duration of hospitalisation, number of follow-up visits up to 18 months as compared to routine treatment, importance of attending follow-up visits and possibility of visits by study staff at village level if the patient does not attend the follow-up visits at the centre;
- Information on provision of food to all HAT patients treated at the sites, regardless of whether they are included in the study;
- Information on organisation of transport and/or reimbursement of transport costs for patients included in the study;
- Importance of the freedom of each individual to accept or to refuse to take part in the study, after full explanation of the study. Availability of treatment in either case;
- Need for minors and patients with impaired cognitive capacities to come to the centre accompanied by a legal guardian/representative.

The third level of information concerns the individual consent of each patient (see Section 14.2. Informed Consent Process; p **Error! Bookmark not defined.**).

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

14.1.2. Central African Republic

In the CAR, a similar three-level approach will be used for communication and dissemination of information concerning the study on fexinidazole.

MSF Spain, in its role as operational partner for the study, will select a representative and/or ask the National or Local Investigator to inform the administrative and medical authorities in the district. The PNLHAT in the CAR is fully involved in the design and implementation of the fexinidazole study in the CAR, through its Coordinator, who is acting as National Investigator.

Contrary to the DRC, in the CAR there are no mobile teams specialised in HAT screening in the vicinity of the investigational centre chosen for the study, in Batangafo. Members of the laboratory staff of the hospital will be sent out to actively search for cases. The composition of the mobile teams is, however, the same as in DRC, with community mobilisers and other qualified personnel who are responsible for raising awareness of HAT among tribal chiefs, religious leaders, local authorities and the population.

Information sessions will be organised in public places, such as churches, mosques and markets, a few days before the start of screening activities, to sensitise the population to the advantages of screening. The overall objective of the study will be presented along with information on the study, as described above in Section 14.1.1 concerning the DRC.

The screening activities will receive funding from the Sponsor throughout the duration of the study. The activities will be organised by MSF Spain in Batangafo.

14.2. Informed Consent Process

14.2.1. General Process

The patient will not be included in the study until after s/he has given informed consent in writing. It is the responsibility of the Investigator to obtain, for each individual who participates in the study, voluntary written informed consent after having provided adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. This task can be performed by a designee, referred to below as a "facilitator", who may be a study nurse.

The written informed consent document will be translated into the local language or a language understood by the patients, and submitted to the IEC in each country for approval.

A written guide, more extensive than the patient information, will be used as a tool for the facilitator to provide oral explanations. The facilitator will be chosen within the team for her/his good knowledge of the patients' preferred local language, and for his/her skills in interacting with patients. More than one facilitator may be chosen in each centre to cover all local languages and dialects.

Visual aids, including photographs, drawings and samples, will also be made available to the facilitator, describing the activities performed during the study, i.e. lumbar puncture, finger pricks, ECG, etc., and will be submitted to the IEC for approval.

The patient will be invited to attend the information session alone or together with family or friends if s/he wishes. The session will be held in a separate room in order to ensure patient confidentiality, with only one facilitator present.

The patient will first be informed about the disease, i.e. HAT, with a clear description of the signs and symptoms.

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

- currently available treatments;
- study objective and need for scientific evaluation of a new drug;
- information on the new drug from previous studies (efficacy, safety…);
- number of patients to be enrolled and the duration of the study;
- criteria to fulfil to be eligible for inclusion in the study;

- patient's commitments during the 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 participation, for both treatment groups;
- compensation for travel costs and provision of food during hospitalisation;
- patients' rights regarding withdrawal, rescue treatment, additional information, etc.

If the patient wishes, s/he will be given time to discuss the information received with members of his/her community or family before giving consent. Written consent will be given after the information session (or later) by signing the form, provided the facilitator is convinced that the patient has fully understood what was explained.
All informed consent forms have been translated into the following local/national languages/lingua franca spoken in the areas where the study is being conducted: Lingala, Kituba/Kikongo and Tshiluba. If the patient does not speak any of the national/local language/lingua franca and if pre-specified and authorized staff with knowledge the dialect/local language are present, an *ad hoc* oral translation may be acceptable. The oral translation will be supported by the use of the available visual aids. The document signed by the patient will be the form in the lingua franca of his/her country/region. The procedures for illiterate patients should apply. The oral translation should be documented on the signed consent form, i.e. the person who did the translation will indicate her/his name and the language/dialect used, and will sign the form.

14.2.2. Impartial Witness

The presence of an impartial witness is mandatory when illiterate patients are recruited and/or the legal representative is illiterate (see Section 14.2.2. Impartial Witness; p 81). Other situations may also require such a witness (see Section 14.2.3. Illiterate Patients; p 82 and Section 14.2.4. Patients Unable to Give Consent; p 82)

The witness should have no connection with the research team, and, whenever possible, should be chosen by the patient. The witness must be literate, i.e. able to read. If the patient does not know any 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 patient, and its compliance with the written information in the patient information sheet. The witness must be present throughout the entire information session.

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

14.2.3. Illiterate Patients

If the patient is illiterate, an impartial witness must be present throughout the information session.

The facilitator will explain the information contained in the written document to the patient and ask whether he/she gives his/her consent to participate. The patient's consent will be documented with his/her fingerprint on the form, and the witness will sign the form.

14.2.4. Patients Unable to Give Consent

On arrival at the investigational centre, some patients with late-stage HAT may already have impaired cognitive capacities or behavioural abnormalities that preclude them from giving free and informed consent.

Considering the frequency of such symptoms in HAT, exclusion of these patients could jeopardise the capacity to complete the study.

Consequently, for patients who present with symptom(s) of psychological or behavioural disturbances and/or with impaired mental status, such as memory, alertness, disorientation, etc., consent will be requested from an accompanying family member, acting as legal representative. It should be noted that patients with very advanced late-stage HAT will not be invited to participate in the study (see exclusion criteria).

As is the case with minors, the eventual non-consent of the patient will prevail if s/he refuses to participate in the study.

In addition, the consent process should be conducted in the presence of an impartial witness who will attest that the patient's will and best interests have been respected.

As soon as the patient has recovered his/her capacity to decide, s/he will be asked to confirm his/her desire to participate in the study, usually during the hospitalisation period, attested by the signature of an additional consent form.

14.2.5. Patients Under Legal Age

For patients under legal age, i.e. 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 in addition to their own personal assent. During field visits by the mobile team, adolescents/young adult patients will be advised to come to the study centre accompanied by a legal representative.

No specific patient information sheet or specific form will be used to collect assent from adolescents/young adults recruited to the study, since the data in the patient information sheet is considered to be understandable by both adolescents and adults.

The form will be signed by both the adolescent/young adult and his/her legal representative. If the patient 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 (see Section 14.2.2. Impartial Witness; p 81).

For young adults considered as emancipated because they are already married, the legal representative may be the husband or wife. 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.

14.2.6. Changes in the Benefit-to-Risk Assessment during the Study

If new safety information results in significant changes in the benefit-to-risk ratio, the patient information sheet and consent form will be reviewed and updated. Patients currently being treated will be informed of the new information, given a copy of the revised patient information and asked to renew their consent to continue the study.

14.3. Ethical Aspects of Study Treatments and Sampling for Laboratory Tests

Experimental data suggest that fexinidazole has significant potential for the treatment of *T.b.* gambiense infections. Phase I studies in healthy volunteers who received fexinidazole suggest that the benefit-to-risk ratio of the dose selected is acceptable.

An active comparator has been chosen for the study. The therapeutic efficacy of fexinidazole will be tested against the reference treatment.

No screened patients will be left without treatment. Patients not eligible for the study will be offered alternative treatment.

Sampling will be performed only for the purposes of safety assessments and PK analyses. The volume of blood collected will be reduced to a minimum. The discomfort of blood collection can be reduced using capillary sampling instead of venous blood sampling. However, if the skin is thick and hard, which is often the case in rural population, this sampling method may become painful due to the need for several pricks. In such cases, venous sampling may be preferable.

The PK analyses will be performed on samples collected using the DBS technique, making it possible to reduce the volume of blood collected to a minimum (5 drops of blood for each analysis, i.e. 30 drops of blood in total per patient for the entire study) (see Section 6.3.3. Laboratory Tests; p 48).

CSF samples will be collected in order to assess exposure to fexinidazole in the brain. This analysis will be performed on the sample used in the efficacy analyses, collected as per usual practice in the investigational centre. The PK analyses will require 5 drops of de CSF.

The samples collected on filter paper will be sent out of the countries where the study is being conducted for centralised assessment of exposure to the study treatments. The samples will only be identified by the study number and the patient's code. Thus, no information identifying the patient personally will leave the country.

None of the samples will be retained after the end of the study. No bank of biological material will be set up. All remaining biological material will be destroyed, and the procedure will be documented with a certificate of destruction.

14.4. Costs for Patients

Patients will be reimbursed for their travel costs to and from the investigational site, but will not receive any payment for participation in the study. During the in-patient treatment phase, food will be provided to the patient free of charge. Following usual practice at each investigational site, food will be cooked or not. If not, the family will prepare it. Enough food will be provided in order to cover the needs of the relatives accompanying the patient during hospitalisation.

For follow-up visits, the patients' travel costs will be covered by the Sponsor, based on site-specific procedures, i.e. payment of taxi, use of specific study vehicle, transport by mobile teams, reimbursement at flat rate, etc. Food will be provided for the patient during his/her stay in hospital. The lost days of work due to travel for follow-up visits may be compensated, depending on requirements from local IECs.

Any essential medication that is required during the study will be provided free of charge to the patient. The WHO essential medicine list and the MSF guide *Essential Medicines* (2010 edition) will be used as a reference for the treatment

of any concurrent condition. For any chronic disease, the study team will take all necessary measures to have the patient referred to the most appropriate local medical centre.

To ensure that participation in the study is voluntary, all HAT patients sent to hospital for treatment will be given food during the in-patient treatment phase, even if they are not included in the study, whether this is because they do not fulfil the selection criteria or because they do not wish to participate in the study.

Patients diagnosed with early-stage HAT on lumbar puncture during the screening period at the hospital/investigational centre will be treated at the hospital/investigational centre, in accordance with current guidelines for treating early-stage HAT. The patients may be hospitalised, if necessary, for treatment and will also receive food during this period.

15. Insurance and Liability

DND*i* will take out an insurance policy to cover any claims arising from the study, except for claims that arise from malpractice and/or negligence, in which case the Investigator or the institution will be held liable. In addition, DND*i* will cover the costs of treating patients in the study in the event of study-related injuries, in accordance with applicable local regulatory requirements.

16. Reports and Publications

The study will be registered with a recognised international clinical trial registry, such as www.clinicaltrials.gov (NCT 01685827) or the Pan African Clinical Trials Registry (PACTR).

The results of the study may be published or presented at scientific meetings. If this is the case, the Investigator agrees to submit all manuscripts or abstracts to DND*i* prior to publication.

In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of the results of multicentre studies only in their entirety and not as individual centre data. Any formal publication on the study in which input from DND*i* personnel exceeded that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate DND*i* personnel. Authorship will be decided by mutual agreement.

17. References

1. Astelbauer F, Walochnik J. “Antiprotozoal compounds: state of the art and new developments”. Int J Antimicrob Agents. 2011;38(2):118-24. doi: 10.1016/j.ijantimicag.2011.03.004.
2. Blackwelder W.C. "Proving the Null Hypothesis" in Clinical Trials. Contr Clin Trials 1982;3:345-353.
3. Blum J, Schmid C, Burri C. «Clinical aspects of 2541 patients with second stage human African trypanosomiasis”. Acta Trop 2006;97(1): 55-64
4. Brun R, Blum J, Chappuis F, Burri C. “Human African trypanosomiasis”. Lancet 2010; 375 (9709):148-59. doi:10.1016/S0140-6736(09)60829-1.
5. Bucheton B, MacLeod A, Jamonneau V. “Human host determinants influencing the outcome of Trypanosoma brucei gambiense infections”. Parasite Immunol. 2011;33(8):438-47. doi: 10.1111/j.1365-3024.2011.01287.x.
6. Büscher P, Mumba Ngoyi D, Kaboré J, Lejon V,Robays J, Jamonneau V, Bebronne N, Van Der Veken W, Biéler S. “Improved Models of Mini Anion Exchange Centrifugation Technique (mAECT) and Modified Single Centrifugation (MSC) for Sleeping Sickness Diagnosis and Staging”. PloS Negl Trop Dis 2009;3(11):e471.
7. Camara M, Camara O, Ilboudo H, Sakande H, Kaboré J, N'Dri L, Jamonneau V, Bucheton B. “Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test”. Trop Med Int Health. 2010;15(7):796-9.
8. Checchi F, Chappuis F, Karunakara U, Priotto G, Chandramohan D. “Accuracy of Five Algorithms to Diagnose Gambiense Human African Trypanosomiasis”. PloS Negl Trop Dis 2011;5(7):e1233. Doi: 10.1371/daynal.pntd.0001233.
9. Chen YH, DeMets DL, Lan KK. “Increasing the sample size when the unblinded interim result is promising”. Stat Med. 2004;23(7):1023-38.
10. CIOMS. Management of Safety Information from Clinical Trials (Report of CIOMS Working Group VI). CIOMS c/o WHO, Geneva, 2005. ISBN 92 9036 079 8.
11. Committee for Medicinal Products for Human Use (CHMP). “Guideline on the choice of the non-inferiority margin”. The European Medicines Agency 2005. EMEA/CPMP/EWP/2158/99.
12. Committee for Proprietary Medicinal Products (CPMP). “Points to consider on switching between superiority and non-inferiority”. The European Agency for the Evaluation of Medicinal Products 2000. CPMP/EWP/482/99.
13. Deborggraeve S, Büscher P. “Molecular diagnostics for sleeping sickness: what is the benefit for the patient?” Lancet Infect Dis. 2010;10(6):433-9.
14. Deborggraeve S, Lejon V, Ali Ekangu R, Mumba Ngoyi D, Pati Pyana P, Ilunga M, Mulunda JP, Büscher P. “Diagnostic Accuracy of PCR in gambiense Sleeping Sickness Diagnosis, Staging and Post-Treatment Follow-Up: A 2-year Longitudinal Study”. PloS Negl Trop Dis 2011;5(2):e972.
15. DNDi – Data on file – Phase 1 studies
16. DNDi. “Clinical Study to Assess the Tolerability, Feasibility and Effectiveness of Nifurtimox and Eflornithine (NECT) for the Treatment of Trypanosoma Brucei Gambiense Human African Trypanosomiasis (HAT) in

the Meningo-Encephalitic Phase (NECT-FIELD)". Accessed on January 30th, 2012, at http://www.clinicaltrial.gov/ct2/show/NCT00906880?

17. Doyle D, Hanks G, MacDonald N. "Oxford Textbook of Palliative Medicine". Oxford University Press. 845 pp, illustrated New York 1993;109. ISBN: 0-19-262028-2.
18. Durrant JD, McCammon JA. "Towards the development of novel Trypanosoma brucei RNA editing ligase 1 inhibitors". BMC Pharmacol 2011;11:9.
19. Falade C, Manyando C. "Safety profile of Coartem®: the evidence base". Malaria Daynal 2009, 8(Suppl 1):S6
20. Friede T, Kieser M. "Blinded sample size reassessment in non-inferiority and equivalence trials". Stat Med. 2003;22(6):995-1007.
21. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. Antimicrob Agents Chemother. 2009 Sep;53(9):3726-33. doi:10.1128/AAC.00112-09
22. Harrington JM, Widener J, Stephens N, Johnson T, Francia M, Capewell P, Macleod A, Hajduk SL. "The Plasma Membrane of Bloodstream-Form African Trypanosomes Confers Susceptibility and Specificity to Killing by Hydrophobic Peptides". J Biol Chem 2010; 285(37):28659-66. doi: 10.1074/jbc.M110.151886.
23. Hasker E, Lutumba P, Mumba D, Lejon V, Büscher P, Kande V, Muyembe JJ, Menten J, Robays J, Boelaert M. "Diagnostic Accuracy and Feasibility of Serological Tests on Filter Paper Samples for Outbreak Detection of T.b. gambiense Human African Trypanosomiasis". Am J Trop Med Hyg 2010;83(2):374-9. doi: 10.4269/ajtmh.2010.09-0735.
24. Hasker E, Mitashi P, Baelmans R, Lutumba P, Jacquet D, Lejon V, Kande V, Declercq J, Van der Veken W, Boelaert M. "A new format of the CATT test for the detection of Human African Trypanosomiasis, designd for use in peripheral health Facilities" Trop Med Int Health. 2010;15(2):263-7.
25. Immtech Pharmaceuticals, Inc. "Phase IIb Trial of DB289 for the treatment of stage 1 African Trypanosomiasis". ClinicalTrials.gov 2008. Consulted on January 30th, 2012, at http://www.clinicaltrial.gov/ct2/show/NCT00803933?
26. Inojosa WO, Augusto I, Bisoffi Z, Josenado T, Abel PM, Stich A, Whitty CJ. "Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case finding strategies". BMJ 2006;332(7556):1479.
27. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. "ICH Harmonised Tripartite Guideline". Accessed on www.ich.org on November 24th, 2011.
28. Jacobs RT, Plattner JJ, Nare B, Wring SA, Chen D, Freund Y, Gaukel EG, Orr MD, Perales JB, Jenks M, Noe RA, Sligar JM, Zhang YK, Bacchi CJ, Yarlett N, Don R. "Benzoxaboroles: a new class of potential drugs for human African trypanosomiasis". Future Med Chem 2011; 3(10): 1259-78.
29. Kagira JM, Maina N, Njenga J, Karanja SM, Karori SM, Ngotho JM."Prevalence and Types of Coinfections in Sleeping Sickness Patients in Kenya (2000/2009)". J Trop Med 2011;2011:248914. doi:10.1155/2011/248914.
30. Kaiser M, Bray MA, Cal M, Trunz BB, Torreele E, Brun R."Anti-trypanosomal activity of Fexinidazole – A New Oral Nitroimidazole Drug Candidate for the Treatment of Sleeping Sickness". Antimicrob Agents

Chemother. 2011. doi: 10.1128/AAC.00246-11

31. Lutje V, Seixas J, Kennedy A. “Chemotherapy for second-stage Human African Trypanosomiasis”. Cochrane Database Syst Rev. 2010;(8):CD006201.
32. Lutumba P,Kande V, Boelaert M, Kayembe JM,Mampunza S. “Research capacity strengthening in the DRC”. Lancet. 2010;375(9720):1080.
33. Malvy D, Chappuis F. “Sleeping sickness”. Clin Microbiol Infect. 2011;17(7):986-95. doi: 10.1111/j.1469-0691.2011.03536.x.
34. Ministère de la Santé de la République Démocratique du Congo. « Lignes directrices pour l’évaluation éthique de la recherche impliquant des sujets humains en R. D. CONGO ».
35. Mumba D, Bohorquez E, Messina J, Kande V, Taylor SM, Tshefu AK, Muwonga J, Kashamuka MM, Emch M, Tidwell R, Büscher P, Meshnick SR. “Prevalence of Human African Trypanosomiasis in the Democratic Republic of the Congo”. PloS Negl Trop Dis 2011;5(8):e1246..
36. Mumba Ngoyi D, Lejon V, N’Siesi FX, Boelaert M, Büscher P. "Comparison of operational criteria for treatment outcome in gambiense human African trypanosomiasis." Trop Med Int Health 2009;14(4):438-44.
37. Nare B, Wring S, Bacchi C, Beaudet B, Bowling T, Brun R, Chen D, Ding C, Freund Y, Gaukel E, Hussain A, Jarnagin K, Jenks M, Kaiser M, Mercer L, Mejia E, Noe A, Orr M, Parham R, Plattner J, Randolph R, Rattendi D, Rewerts C, Sligar J, Yarlett N, Don R, Jacobs R; “Discovery of Novel Orally Bioavailable Oxaborole 6-Carboxamides HATt Demonstrate Cure in a Murine Model of Late-Stage Central Nervous System African”. Antimicrob Agents Chemother. 2010;54(10):4379-88.. doi: 10.1128/AAC.00498-10.
38. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) V4.0. Published: May 28, 2009 (v4.03: June 14, 2010). Accessed on January 13th, 2012 at http://evs.nci.nih.gov/ftp1/CTCAE/About.html
39. Ndem Amin D, Mumba Ngoyi D, Nhkwachi GM, Palomba M, Rottenberg M, Büscher P, Kristensson K, Masocha W. “Identification of Stage Biomarkers for Human African Trypanosomiasis”. Am J Trop Med Hyg 2010;82(6):983-90. doi: 10.4269/ajtmh.2010.09-0770
40. Opigo J, Woodrow C. “NECT trial: more HATn a small victory over sleeping sickness”. Lancet. 2009;374(9683):7-9. doi:10.1016/S0140-6736(09)61163-6.
41. Paliwal SK, Verma AN, Paliwal S. “Neglected Disease – African Sleeping Sickness: Recent Synthetic and Modeling Advances”. Sci Pharm 2011;79(3):389-428. doi: 10.3797/scipharm.1012-08.
42. Priotto G, Chappuis F, Bastard M, Flévaud L, Etard J.-F. “Early prediction of treatment efficacy in second-stage gambiense Human African Trypanosomiasis”. PLoS Neglected Tropical Diseases, *in press*.
43. Priotto G, Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U, Ghabri S, Baudin E, Buard V, Kazadi-Kyanza S, Ilunga M, Mutangala W, Pohlig G, Schmid C, Karunakara U, Torreele E, Kande V. “Nifurtinox-eflornithine combination therapy for second stage African Trypanosoma Brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non –inferiority trial”. Lancet 2009;374 (9683):56-64. doi:10.1016/S0140-6736(09)61117-X
44. Radwanska M. “Emerging trends in the diagnosis of Human African Trypanosomiasis”. Parasitology. 2010;137(14):1977-86. doi:

10.1017/S0031182010000211.

45. Rodgers J, Jones A, Gibaud S, Bradley B, McCabe C, Barrett MP, Gettinby G, Kennedy PG. “Melarsoprol Cyclodextrin Inclusion Complexes as Promising Oral Candidates for the Treatment of Human African Trypanosomiasis”. PloS Negl Trop Dis 2011;5(9):e1308..
46. Schag CC, Heinrich RL, Ganz PA. “Karnofsky performance status revisitd: reliability, validity, and guidelines”. J Clin Oncology. 1984;2:187-193.
47. Simarro PP, Diarra A, Ruiz Postigo JA, Franco JR, Jannin JG. “The Human African Trypanosomiasis Control and Surveillance Programme of the World Health Organisation 2000–2009: The Way Forward”. PloS Negl Trop Dis 2011;5(2):e1007.
48. Sokolova AY, Wyllie S, Patterson S, Oza SL, Read KD, Fairlamb AH. “Cross-Resistance to Nitro Drugs and Implications for Treatment of Human African Trypanosomiasis”. Antimicrob Agents Chemother. 2010;54(7):2893-900. doi: 10.1128/AAC.00332-10
49. Stich A, Abel PM, Krishna S. “Human African trypanosomiasis”. BMJ 2002;325(7357):203–6.
50. Torreele E, Trunz BB, Tweats D, Kaiser M, Brun R, Mazué G, Bray MA, Pécoul B. “Fexinidazole – A New Oral Nitroimidazole Drug Candidate Entering Clinical Development for the Treatment of Sleeping Sickness”. PloS Negl Trop Dis 2010;4(12):e923..
51. Vincent IM, Creek D, Watson DG, Kamleh MA, Woods DJ, Wong PE, Burchmore RJ, Barrett MP. “A Molecular Mechanism for Eflornithine Resistance in African Trypanosomes”. PloS Pathog 2010;6(11):e1001204..
52. Wastling SL, Welburn SC. “Diagnosis of human sleeping sickness: sense and sensitivity”. Trends Parasitol. 2011;27(9):394-402. doi: 10.1016/j.pt.2011.04.005.
53. WHO (2000). "Operational Guideline for Ethics Committees HATt Review Biomedical Research. " World Health Organisation. TDR/PRD/ETHICS/2000.1.
54. WHO (2007). "Recommendations of the Informal Consultation on Issues for Clinical Product Development for Human African Trypanosomiasis." World Health Organisation. WHO/CDS/NTD/IDM/2007.1.
55. WHO (2011). "New cases of human African trypanosomiasis continue to drop." World Health Organisation. Consulted on January 30th, 2012, at http://www.who.int/neglected_diseases/disease_management/HAT_cases_drop/en/index.html
56. Yun O, Priotto G, Tong J, Flevaud L, Chappuis F. “NECT is next: implementing the New Drug Combination Therapy for Trypanosoma brucei gambiense Sleeping Sickness”. PloS Negl Trop Dis 2010;4(5):e720.
57. Pohlig G, Bernhard SC, Blum J, Burri C, Mpanya A, Lubaki J-PF, et al. (2016) Efficacy and Safety of Pafuramidine versus Pentamidine Maleate for Treatment of First Stage Sleeping Sickness in a Randomized, Comparator-Controlled, International Phase 3 Clinical Trial. PLoS Negl Trop Dis 10(2):e0004363. doi:10.1371/journal.pntd.0004363

Appendices

Appendix 1 – Declaration of Helsinki

WORLD MEDICAL ASSOCIATION – DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington, USA, 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo, Japan, 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. The Declaration is addressed primarily to physicians. Nevertheless, the WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research

subjects set forth in this Declaration. The committee must have the right to monitor on-going studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other healthcare professional. The responsibility for the protection of research subjects must always rest with the physician or other healthcare professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well

as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered in the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient’s decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Appendix 2 – Karnofsky Performance Scale

KARNOFSKY PERFORMANCE STATUS SCALE
DEFINITIONS OF RATING CRITERIA (%)

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment (46, 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 worse the survival for most serious illnesses.

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.

Appendix 3 – Laboratory Assessments

Laboratory Tests and Methods

All laboratory assessments will be described in a laboratory manual. Laboratory technicians have received specific training on these standard methods.

Biochemical tests: Piccolo® chemistry analyser

14 parameters will be analysed:

Albumin (ALB)	*Calcium (CA)*
Alkaline phosphatase (ALP)	*Glucose (GLU)*
Alanine aminotransferase (ALAT)	*Bicarbonates (tCO_2)*
Aspartate aminotransferase (ASAT)	*Blood urea nitrogen (BUN)*
Total bilirubin (TBIL)	*Sodium (Na+)*
Total protein (TP)	*Chloride (Cl-)*
Creatinine (CRE)	*Potassium (K+)*

Haematological tests

- ***Hemocue® Hb 210+ or Hb301+****: to measure haemoglobin level*
- ***Haematocrit*** *(only patients at sites where PK sampling is performed)*
- ***Microscopy****: full blood cell count (visual count) using the TIC® system (Bioanalytic GmbH) and Neubauer counting chambers.*

Urine analysis

- *Urine dipstick analyses for safety parameters/COMBUR 9 TEST®*

White blood cells	*Nitrites*
pH	*Glucose*
Protein	*Ketone bodies*
Urobilinogen	*Bilirubin*
Blood	

- *Urine Pregnancy Test*

CSF Analysis

- Modified Single Centrifugation (MSC) with INRB kits: detection of parasite
- Fuchs-Rosenthal/Fast Read 102® counting chamber: WBC count

Blood parasitology tests

- Thick/thin blood smears
- Woo test/CTC
- mAECT with INRB kits
- mAECT-BC with INRB kits

Quantity of biological fluid required at each sampling timepoint

Screening Visit

	Type of test	Specific test	Number of samples	Quantity per sample (approx.)	Total quantity
Blood	Parasitology	Woo/CTC	1	100 µL	≤ 400 µL
		Thin/thick blood smear	1	≤ 300 µL	
		mAECT (±BC)	1	5 mL	5 mL
	Haematology	Haemoglobin	1	20 µL	Between 190 µL (capillary blood[7]) and 4 mL (venous blood)
		Haematocrit[8]	1	20 µL	
		WBC count	1	20 µL	
		Platelet count	1	10 µL	
		Differential WBC	1	20 µL	
	Biochemistry	14 parameters	1	100 µL	
Lymph	Parasitology	If lymph nodes detectable			
CSF	Parasitology	Modified Single Centrifugation	1	4 mL	4 mL
Urine	Pregnancy		1	5 mL	5-10 mL
	Urine analysis	*COMBUR 9 Test*®	2	5 mL	

Hospitalisation (Baseline, D5, D8, D11)

	Type of test	Specific test	Number of samples	Quantity per sample (approx.)	Total quantity (4 visits)
Blood	Parasitology	Woo/CTC	1	100 µL	≤ 400 µL
		Thin/thick blood smear	1	≤ 300 µL	
		mAECT (±BC)	1	5 mL	5 mL
	Haematology	Haemoglobin	4	20 µL	Between 630 µL (capillary blood[6]) and 16 mL (venous blood)
		WBC count	4	20 µL	
		Platelet count	4	10 µL	
		Differential WBC	4	20 µL	
	Biochemistry	14 parameters	4	100 µL	
CSF	Parasitology	Modified Single Centrifugation	1	4 mL	4 mL
Urine	Pregnancy		1	5 mL	5-10 mL
	Urine analysis	*COMBUR 9 Test*®	1	5 mL	

[7] *All haematological and biochemical analyses can be performed using a single finger-prick with a Tenderlett® device or similar.*

[8] *Only for patients at sites where PK sampling is performed*

Additional Follow-up Visit at Week 9 after D1

	Type of test	Specific test	Number of samples	Quantity per sample (approx.)	Total quantity
Blood	Haematology	Haemoglobin	1	20 µL	170 µL (capillary blood[6])
		WBC count	1	20 µL	
		Platelet count	1	10 µL	
		Differential WBC	1	20 µL	
	Biochemistry	14 parameters	4	100 µL	

Follow-up Visits (per visit)

	Type of test	Specific test	Number of samples	Quantity per sample (approx.)	Total quantity
Blood	Parasitology	Woo/CTC	1	100 µL	≤ 400 µL
		Thin/thick blood smear	1	≤ 300 µL	
		mAECT (±BC)	1	5 mL	5 mL
Lymph	Parasitology	If lymph nodes detectable			
CSF	Parasitology	Modified Single Centrifugation	1	4 mL	4 mL
Urine (only M3 Visit)	Pregnancy		1	5 mL	5 mL

Quantity of biological fluids required for pharmacokinetic analyses (only in the fexinidazole group)

Entire Study (only capillary blood, for technical reasons)

	Type of test	Number of samples	Quantity per sample (approx.)	Total quantity
Blood	PK	6	≤ 300 µL	≤ 6 mL
CSF	PK	1	Included in sample collected for detection of parasite (≤ 300 µL)	

Appendix 4 - Tables

Table 4 – Schedule of Study Procedures

Protocol-planned procedures and forms to be completed	**Pre-screening and Screening**	**Baseline**	**Treatment period**										**End-of-Treatment Visit until End-of-Hospitalisation Visit**			**Follow-up period (months)**
Timepoint	*D-15 to D-1*	*D-4 to D-1*	*D1*	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*	*D8*	*D9*	*D10*	*D11*	*D12*	*D13-D18*	*Week 9[3] 3 – 6 – 12 – 18 -24 months*
Detection of parasite in blood and/or lymph	X												x			x
Lumbar puncture (parasite and white blood cells in CSF)	X												x			x[1]
Informed consent (**before any additional medicines or study-specific procedures**)	X	Check														
Pretreatment of helminthiasis (+ 3-day recovery period)	X															
Rapid diagnostic test and/or thick blood smear for malaria	X															
Pretreatment of malaria if necessary (+ 3-day recovery period)	X															
Karnofsky score	X	Check														x
Safety ECG	X	Check														
Safety ECG (fexinidazole group only)				x	x	x										
Urine pregnancy test		x													x	x (only at 3 months)
Inclusion and exclusion criteria	X	x														
Randomisation		x														
Demographic data	X															
Medical history	X															x
Signs and symptoms of HAT		x													x	x
Vital signs	X	x					x			x			x		x	x
Physical examination		x					x			x			x		x	x
Neurological examination		x					x			x			x		x	x
Haematology and biochemistry	X	x					x			x			x			x (only at Week 9)
Haematocrit[2]	X															
Urine analysis	X	x											x			

[1] *Except for 3-month follow-up visit unless the patient presents with clinical symptoms or signs of HAT.*

[2] *Only for patients at sites where PK sampling is performed.*

[3] *Only at Week 9 after D1, perform sampling for haematology and biochemistry, physical examination and neurological examination.*

Protocol-planned procedures and forms to be completed	**Pre-screening and Screening**	**Baseline**	**Treatment period**										**End-of-Treatment Visit until End-of-Hospitalisation Visit**			**Follow-up period (months)**
Timepoint	*D-15 to D-1*	*D-4 to D-1*	*D1*	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*	*D8*	*D9*	*D10*	*D11 (EOT)*	*D12*	*D13-D18*	Week 9 3 – 6 – 12 – 18 -24 months
Triplicate ECGs –fexinidazole group		x				x D4H4 D4H23						x D10H2-H3				
Triplicate ECGs – NECT group		x							x D7H2-H3			x D10H2-H3				
Administration of fexinidazole			x	x	x	x	x	x	x	x	x	x				
Administration of NECT			x	x	x	x	x	x	x	x	x	x				
Treatment-related adverse events			x	x	x	x	x	x	x	x	x	x	x	x	x	
Treatment-related serious adverse events			x	x	x	x	x	x	x	x	x	x	x	x	x	x (up to 24 months)
Recording of concomitant medication	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Dry blood spot sampling										x D8 H3.15	x D9H3	x D10H3 D10H7.15	x D10H24	x D10H48		
CSF sampling													x D10H24			

Table 5 – Overview of Protocol-planned Analyses

Analysis	Patients (n) (ITT)	Timepoint	Efficacy endpoints	Stopping Rules ♦ **π: actual responder rate** **p: observed responder rate**	Power Chances of stopping the study due to actual responder rate (exact power)	Chances of stopping the study unduly (exact probabilities)
Futility analysis 1 Performed if the number of responders is ≤ 26/30 patients	20 (fexi) 10 (NECT)	End-of-Treatment Visit	Responder = no evidence of trypanosomes* at end of treatment	Unacceptable limit: π fexi < 95% = worse than melarsoprol (published results on responder rate at end of treatment p= 95.2% for melarsoprol; n= 6840) Stop if p fexi ≤ 80% Or if the number of responders is ≤16 out of 20 patients	58.9% if π fexi = 80% 77.5% if π fexi = 75% 89.3% if π fexi = 70%	1.59% if π fexi = 95% 0.0043% if π fexi = π NECT = 99%
Futility analysis 2 Performed if the number of responders is ≤ 97/105 patients	70 (fexi) 35(NECT)	End-of-Treatment Visit	Responder = no evidence of trypanosomes* at end of treatment	Unacceptable limit: π fexi < 95% (historical responder rate for melarsoprol) with π NECT ≥ 99% (expectation for NECT and eflornithine) Or if the difference between the responder rates is > 4% in favour of NECT: $\pi_{NECT} - \pi_{Fexi} > 4\%$ Stop if Rule 1: p fexi ≤ 88.57% (≤ 62/70) and p NECT ≥ 94.29% (≥ 33/35) OR Rule 2: p NECT − p Fexi ≥ 15.7% and p NECT < 94.2% (<33/35) (Rule 2: modified Blackwelder test)	Rule 1 40.1% if π fexi = 90% 84.3% if π fexi = 85% 98.0% if π fexi = 80% Rule 2 29% if π fexi = 85% and π NECT = 98% 63.2% if π fexi = 80% and π NECT = 98%	Rule 1 2.33% if π fexi = 95% 0.0000% (if π fexi = 99%) Rule 2 0.08% (if π fexi = 95% and π NECT = 98%) 0.02% (if π fexi = 95% and π NECT = 97%) Note: the probability of applying Rule 2 is low (0.5%) if NECT ≈ eflornithine

Analysis	Patients (n) (ITT)	Timepoint	Efficacy endpoints	Stopping Rules ♦ **π: actual responder rate** **p: observed responder rate**	Power Chances of stopping the study due to actual responder rate (exact power)	Chances of stopping the study unduly (exact probabilities)
Futility analysis 3 Performed if the number of responders is ≤ 93/105 patients	70 (Fexi) 35 (NECT)	3 months after End of Treatment	Responder = no evidence of trypanosomes and no neurological signs at 3 months	π Fexi < 91% (worse than melarsoprol at 3 months) and π NECT ≥ 99% OR π NECT – π Fexi > 7%** (responder rate at 3 months = 91.2% (n = 3477) for melarsoprol and 98.6% (n = 743) for eflornithine)¤ Stop if Rule 1: p Fexi ≤ 82.85% (≤ 58/70) et p NECT ≥ 94.29% (≥ 33/35) OR Rule 2: p NECT − p Fexi ≥ 18.57% and p NECT < 94% (<33/35)	Rule 1 35.6% if π Fexi = 85% 76.8% if π Fexi = 80% 95.6% if π Fexi = 75% Rule 2 13.1% if π Fexi = 85% and π NECT = 98% 41.3% if π Fexi = 80% and π NECT = 98%	Rule 1 0.17% if π Fexi = 95% 0.0000% (if π Fexi = 99%) Rule 2 0.02% (if π Fexi = 95% and π NECT = 98%) 0.002% (if π Fexi = 95% and π NECT = 97%)
Futility analysis 4 Performed if the number of responders (favourable outcome) is ≤ 83/105 patients	70 (Fexi) 35 (NECT)	6 months after End of Treatment	Responder at 6 months = no evidence of trypanosomes and CSF WBC ≤ 20/µL OR CSF WBC >20 and <50/µL <u>and</u> reduced in relation to previous value(s) #	π Fexi < 80% (worse than melarsoprol at 6 months) and π NECT ≥ 95% OR π NECT – π Fexi > 12% (worse than the difference between melarsoprol and eflornithine)** (responder rate at 6 months = 80.8% for melarsoprol and 93.1% for eflornithine)¤ Stop if Rule 1: p fexi ≤ 68.6% (≤ 48/70) and p NECT ≥ 85.7% (≥ 30/35) OR Rule 2: p NECT − p Fexi ≥ 24.3% and p NECT < 85.7% (≤ 30/35)	Rule 1 44.1% if π Fexi = 70% 77.2% if π Fexi = 65% 94.5% if π Fexi = 60% Rule 2 36.7% if π fexi = 70% and π NECT = 95% 83.5% if π fexi = 60% and π NECT = 95%	Rule 1 0.000% (if π fexi = 90%) 0.000% (if π fexi = 95%) Rule 2 0.007% (if π fexi =90% and π NECT = 95%) 0.08% (if π fexi = 85% and π NECT = 90%)

Analysis	Patients (n) (ITT)	Timepoint	Efficacy endpoints	Stopping Rules ♦ **π: actual responder rate** **p: observed responder rate**	Power Chances of stopping the study due to actual responder rate (exact power)	Chances of stopping the study unduly (exact probabilities)
Futility analysis 5 Performed if the number of responders (favourable outcome) is ≤ 79/105 patients	70 (Fexi) 35 (NECT)	12 months after End of Treatment	Responder at 12 months = no evidence of trypanosomes and CSF WBC ≤ 20/μL OR CSF WBC >20 and <50/μL and reduced in relation to previous value(s) #	π Fexi < 75% (worse that melarsoprol at 12 months) and π NECT ≥ 95% OR π NECT – π Fexi > 13% (worse than the limit of unacceptable difference) (responder rate at 12 months = 74.84% for melarsoprol and 90.3% for eflornithine)¤ Stop if Rule 1: p fexi ≤ 62.9% (≤ 44/70) and p NECT ≥ 85.7% (≥ 30/35) OR Rule 2: p NECT – p Fexi ≥ 25.71% and p NECT < 85.7%	Rule 1 12.1% if π Fexi = 70% 39.7% if π Fexi = 65% 72.7% if π Fexi = 60% Rule 2 33.4% if π fexi = 70% and π NECT = 95% 58.8% if π fexi = 65% and π NECT = 95%	Rule 1: 0.000% if π fexi = 90% Rule 2 0.000% (if π fexi = 90% and π NECT = 95%) 0.01% (if π fexi = 85% and π NECT = 95%)
Interim analysis	195 at 12 months	12 months after End of Treatment	Primary endpoint at 12 months	None	NA	NA
Final analysis	390 at 18 months	18 months	Primary endpoint	None		

♦ Stopping rules: π < x% means that the observed responder rate is significantly lower than x%; π = x% means that the responder rate is compatible with an actual rate of f x%. π NECT - π Fexi > z% means that the observed difference between the responder rates is significantly higher than z% in favour of NECT.

* "Responder" at the end of treatment (EOT) is defined as patient alive with no evidence of trypanosomes in any bodily fluid, i.e. blood, lymph or CSF, 24 hours after the end of treatment.

¤ Humbelin, 2006.

** The choice of the limit for the difference is based on the rounded difference between melarsoprol and eflornithine instead of NECT because more data are available on eflornithine (n = 748). Consequently, the stopping rules are defined as a difference with regard to NECT significantly larger, i.e. worse, than the difference between melarsoprol and eflornithine. ¤¤ See below time course of the response for melarsoprol, eflornithine and NECT.

∆ "Responder" at 3 months is defined as patient alive with no evidence of trypanosomes in any bodily fluid, i.e. blood, lymph or CSF or no neurological signs or symptoms requiring rescue treatment.

"Favourable outcome" at 6 and 12 months is defined as patient alive with no evidence of trypanosomes in any bodily fluid and CSF WBC ≤ 20/μL CSF, or CSF WBC > 20 and < 50/μL and reduced in relation to previous value(s). Relapse at 6 and 12 months is defined as evidence of trypanosomes in any bodily fluid, or CSF WBC ≥ 50/μL, or CSF WBC > 20 and < 50/μL and increased in relation to previous value(s), or a change in treatment.

Table 6 – Study Schedule

Final version of protocol	June 2012
Study treatments available	June 2012
First patient first visit	October 2012
Duration of enrolment period	24 months
Duration of follow-up period	18 months (primary endpoint) – 24 months (follow-up)
Last patient last visit	September 2016 (primary endpoint) – March 2017 (24-month follow-up)
Final study report	December 2016 (primary endpoint) – June 2017 (follow-up)

Table 7 – Expected Enrolment Rates (actual rates up to Q2 2013)

	Actual Enrolment	**2015**	
Study Sites	Q3 2012 – Q4 2014	*Q1*	***Total per site***
01 Bandundu	47	5	52
02 Vanga	61	5	66
03 Masi Manimba	82	4	86
04 Dipumba	51	3	54
05 Dingila	32	0	32
06 Batangafo	12	0	12
07 Mushie	19	4	23
08 Katanda	19	4	23
09 Isangi	31	4	35
10 Bagata	3	4	7
Cumulative Total	**357**	**33**	**390**

The forecast may be revised if new investigational sites are added.

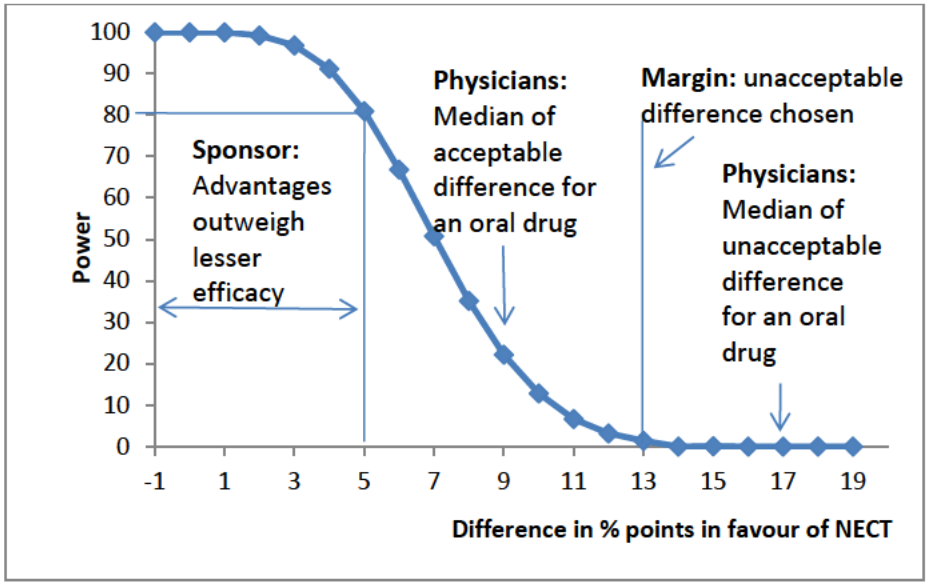
Table 8 – Overall Organisation of the Study

Target countries	Democratic Republic of the Congo (RDC), Central African Republic (CAR)		
Target enrolment rate		Screened	Randomised
	TOTAL	592	390
Number of sites	At least 6 sites		
Number of patients randomised per site	Up to 140 patients per site		
Participation of a DSMB	An independent steering committee (ISC or DSMB) will be appointed to look after safety issues.		
Partners	Swiss Tropical and Public Health Institute, Basel, Switzerland ▪ Study Coordination ▪ Monitoring ▪ Logistics (Swiss TPH/DNDi Kinshasa) Ministry of Health, Kinshasa, DRC • Primary Investigator PNLHAT, Kinshasa, DRC • Management of Investigator team • Coordination of supplies MSF • Technical support • Logistical support		
Other material required for study	Mobile teams for active case detection Equipment and supplies for biochemical analyses Renovation of hospital rooms and laboratories Telecommunication means and computer hardware/software Motorbikes for follow-up		

Appendix 5 - Figures

Figure 3 – Statistical Power of the Study

Power of the study depending on the actual difference between the success rates and assuming a success rate of 94% for NECT



The power is improved if the efficacy of NECT is greater than 94%.

The power refers to the probability that the 95% confidence interval of the difference in success rates will exclude the margin of the chosen limit of an unacceptable difference.

The interval between an acceptable difference and an unacceptable difference, as reported by those who responded to the survey, is a “grey area” or a zone of uncertainty.

The Sponsor objective is to have at least an 80% chance of being significant if the actual difference is 5% in favour of NECT, and a very limited chance (1.45% = exact power = exact one-sided type-I error) of being significant if the actual difference is 13%.

Figure 4 – Acceptable and Unacceptable Limits of the Difference in Efficacy at the Test-of-Cure Visit

Responses to two questions:

- what is the acceptable margin of difference in efficacy between an oral drug and NECT?
- what is the unacceptable margin of difference in efficacy between the two treatments?

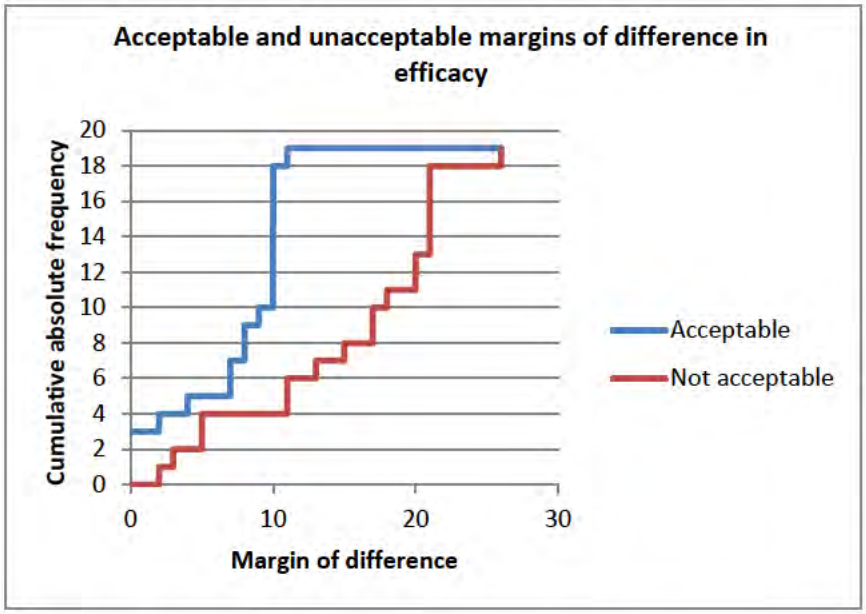


Figure 5 – Failure Rate at each Timepoint by Compound