Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial

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Summary

Background Human African trypanosomiasis (HAT; sleeping sickness) caused by *Trypanosoma brucei gambiense* is a fatal disease. Current treatment options for patients with second-stage disease are toxic, ineffective, or impractical. We assessed the efficacy and safety of nifurtimox-eflornithine combination therapy (NECT) for second-stage disease compared with the standard eflornithine regimen.

Methods A multicentre, randomised, open-label, active control, phase III, non-inferiority trial was done at four HAT treatment centres in the Republic of the Congo and the Democratic Republic of the Congo. Patients aged 15 years or older with confirmed second-stage *T b gambiense* infection were randomly assigned by computer-generated randomisation sequence to receive intravenous eflornithine (400 mg/kg per day, every 6 h; n=144) for 14 days or intravenous eflornithine (400 mg/kg per day, every 12 h) for 7 days with oral nifurtimox (15 mg/kg per day, every 8 h) for 10 days (NECT; n=143). The primary endpoint was cure (defined as absence of trypanosomes in body fluids and a leucocyte count ≤20 cells per μL) 18 months after treatment. Efficacy analyses were done in the intention-to-treat (ITT), modified ITT, and per-protocol (PP) populations. The non-inferiority margin for the difference in cure rates was defined as 10%. This study is registered with ClinicalTrials.gov, number NCT00146627.

Findings One patient from the eflornithine group absconded after receiving the first dose, without any type of assessment done, and was excluded from all analyses. In the ITT population, 131 (91·6%) of 143 patients assigned to eflornithine and 138 (96·5%) of 143 patients assigned to NECT were cured at 18 months (difference –4·9%, one-sided 95% CI –0·3; p<0·0001). In the PP population, 122 (91·7%) of 133 patients in the eflornithine group and 129 (97·7%) of 132 in the NECT group were cured at 18 months (difference –6·0%, one-sided 95% CI –1·5; p<0·0001). Drug-related adverse events were frequent in both groups; 41 (28·7%) patients in the eflornithine group and 20 (14·0%) in the NECT group had major (grade 3 or 4) reactions, which resulted in temporary treatment interruption in nine and one patients, respectively. The most common major adverse events were fever (n=18), seizures (n=6), and infections (n=5) in the eflornithine group, and fever (n=7), seizures (n=6), and confusion (n=2) in the NECT group. There were four deaths, which were regarded as related to study drug (eflornithine, n=3; NECT, n=1).

Interpretation The efficacy of NECT is non-inferior to that of eflornithine monotherapy. Since this combination treatment also presents safety advantages, is easier to administer (ie, infusion every 12 h for 7 days *vs* every 6 h for 14 days), and potentially protective against the emergence of resistant parasites, it is suitable for first-line use in HAT control programmes.

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Introduction

Human African trypanosomiasis (HAT; sleeping sickness) is fatal without appropriate treatment. About 12 000 cases of the disease are reported every year in sub-Saharan Africa,[1] but the actual number of cases could be five times higher. HAT is caused by the protozoan parasites *Trypanosoma brucei gambiense* (west and central Africa) and *Trypanosoma brucei rhodesiense* (east and southern Africa), and transmitted by the tsetse fly. The disease progresses from a haemolymphatic first stage to a meningoencephalitic second stage. Since 1949, the mainstay of treatment for second-stage HAT has been melarsoprol. This arsenical derivative causes severe adverse reactions, in particular reactive encephalopathy, which affects 5–10% of treated patients, and is fatal in 10–70% of cases.[2] Moreover, increasing melarsoprol failure rates have been reported in several sites.[3–7]

Eflornithine (α-difluoromethylornithine or DFMO) is the only new treatment registered for HAT in the past six decades. A trypanostatic drug, eflornithine acts by suicide

inactivation of ornithine decarboxylase, an enzyme essential for polyamine synthesis that is needed for cell multiplication and differentiation.[8–10] Eflornithine has proved efficacious and better tolerated than melarsoprol, and most toxic effects—mainly seizures, gastrointestinal disorders, and myelosuppression—are reversible if well managed. However, the drug is difficult to administer, requiring one slow infusion every 6 h for 14 days (56 infusions in total) because of its short half-life (1·5–5·0 h).[11,12] Efficacy of a 7-day regimen proved insufficient.[13] Despite eflornithine being available for free, the difficulty in administering it in resource-poor settings explains why melarsoprol remains the treatment of choice.

Nifurtimox is an orally administered drug used to treat American trypanosomiasis (Chagas disease, caused by *Trypanosoma cruzi*). Although not registered for the treatment of HAT, nifurtimox has been assessed in several case series and has shown varying efficacy.[14–17] It is mainly used for compassionate treatment of relapses. In Chagas disease, the dose used in adults is 8–10 mg/kg per day for 30–120 days. Toxic effects are mainly neurological (headache, sleep dysfunction, agitation, confusion) and gastrointestinal (anorexia, nausea/vomiting, dyspepsia) disorders,[18] some of which are dependent on the duration of drug intake.[14,16]

Combination treatments of these drugs with lower doses of each component might reduce overall toxicity while maintaining good efficacy. They might also prevent or delay the emergence of drug-resistant organisms and allow for simpler administration regimens. We first assessed three-drug combinations in a trial started in Uganda in 2001, which was terminated early because of excess toxicity in both melarsoprol-containing groups. By contrast, the nifurtimox-eflornithine combination seemed safer and efficacious.[19] A second study that assessed nifurtimox-eflornithine in 31 Ugandan patients showed similar results.[20] On the basis of these encouraging findings, a third study was started in 2003 in the Republic of the Congo, to assess a nifurtimox-eflornithine combination treatment (NECT) with the same doses but a simpler administration schedule. The new regimen was compared with standard eflornithine treatment in a randomised, open-label, non-inferiority clinical study design.[21] This study was extended to three new sites in the Democratic Republic of the Congo (DR Congo) from 2005, becoming the multicentre trial reported here, which includes recruited patients from the four sites.

The objectives of this study were to assess the efficacy and safety of NECT for the treatment of second-stage *T b gambiense* HAT. The study was designed to assess the non-inferiority (efficacy) of NECT against eflornithine monotherapy, the best treatment available.

Methods

Participants

This open-label, randomised, phase III, non-inferiority trial took place in four HAT treatment centres: Nkayi, (Republic of the Congo), Isangi, Dipumba, and Katanda (DR Congo). To allow comparison of our results with those of others, the methodology was aligned with recent clinical trials on second-stage trypanosomiasis[13,19,22,23] and complied with Good Clinical Practice (GCP) guidelines.

Participants were identified by routine diagnosis of HAT or during active screening campaigns. Patients aged 15 years or older with second-stage *T b gambiense* infection with trypanosomes detected in blood, lymph node fluid, or cerebrospinal fluid (CSF), and more than 20 leucocytes per μL in CSF were eligible for enrolment. Exclusion criteria included pregnancy, history of previous second-stage HAT treatment, severe comorbidities with poor chance of survival, haemoglobin concentration less than 50 g/L, and inability to complete 18 months of follow-up.

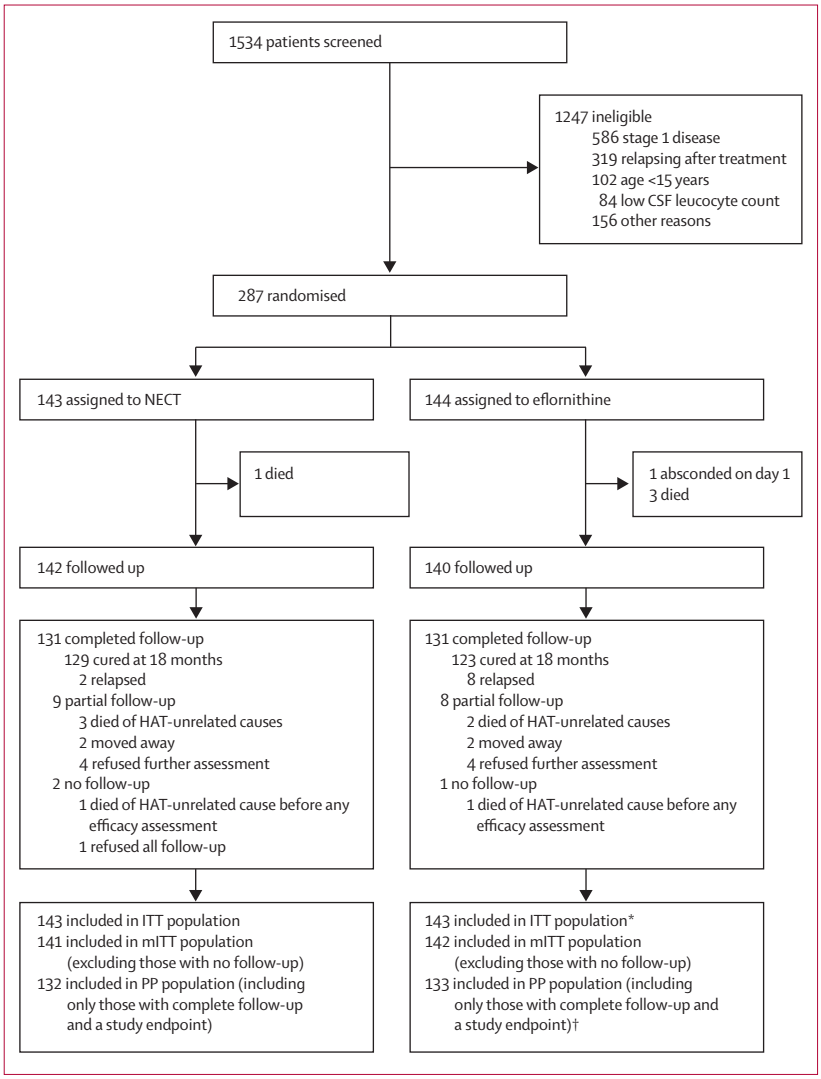

***Figure 1*: Trial profile**
NECT=nifurtimox-eflornithine combination treatment. HAT=human African trypanosomiasis. ITT=intention-to-treat. mITT=modified intention-to-treat. PP=per-protocol. *All randomised patients, apart from one who absconded on day 1. †One patient was excluded from the PP population because of a major protocol deviation.

	Eflornithine		NECT	
	N	n (%) or mean (SD)*	N	n (%) or mean (SD)*
Demographic characteristics				
Men	143	89 (62%)	143	84 (59%)
Age (years)	143	34·6 (13·5)	142	32·8 (12·5)
Weight (kg)	143	53·9 (8·7)	143	53·0 (8·3)
Height (cm)	143	165·8 (9·1)	143	165·8 (7·9)
Body-mass index <18·5 kg/m²	143	47 (33%)	143	54 (38%)
Parasitological findings				
Detected by active screening	143	51 (36%)	143	59 (41%)
Presence of trypanosomes				
in lymph nodes	143	88 (62%)	143	88 (62%)
in blood	143	74 (52%)	143	67 (47%)
in CSF	142	105 (74%)	143	103 (72%)
Leucocyte count in CSF				
6–20 cells per µL	143	2 (1%)	143	2 (1%)
21–99 cells per µL	143	26 (18%)	143	34 (24%)
≥100 cells per µL	143	115 (80%)	143	107 (75%)
Clinical characteristics				
Haemoglobin (g/L)	143	118 (18·6)	143	119 (19·2)
Malaria co-infection	142	29 (20%)	143	22 (15%)
Lymphadenopathy	143	88 (62%)	143	88 (62%)
Hepatomegaly	143	6 (4%)	143	9 (6%)
Splenomegaly	143	29 (20%)	142	21 (15%)
Coma score† <15	142	6 (4%)	142	13 (9%)
Karnofsky index‡ (median [mean, (SD)])	143	80 (77·1 [12·8])	143	80 (75·3 [15·7])
Fever§	143	29 (20%)	143	22 (15%)
Headache	142	119 (84%)	143	115 (80%)
Pruritus	142	102 (72%)	142	104 (73%)
Amenorrhoea¶	40	19 (48%)	48	23 (48%)
Impotence	86	42 (49%)	81	46 (57%)
Unusual behaviour	140	66 (47%)	141	56 (40%)
Anorexia	142	42 (30%)	142	53 (37%)
Seizures	142	16 (11%)	143	10 (7%)
Tremors	141	51 (36%)	143	51 (36%)
Diurnal somnolence	143	109 (76%)	142	96 (68%)
Nocturnal insomnia	142	65 (46%)	142	59 (42%)
Speech disorder	143	24 (17%)	142	29 (20%)
Neurological signs	143	49 (34%)	141	52 (37%)
Duration of symptoms (months)	142	8·5 (9·2)	142	8·6 (9·4)

NECT=nifurtimox-eflornithine combination treatment. CSF=cerebrospinal fluid. *Unless otherwise indicated. †Coma score uses the Glasgow Coma Score: sum of points obtained in each of three criteria (best motor response, best verbal response, eye opening). 3–8 being severe impairment; 9–12 moderate impairment; 13–14 mild impairment; and 15 normal. ‡The Karnofsky index runs from 100 to 0, where 100 is normal health and 0 is death. §Fever defined as axillary temperature 37·5°C or more. ¶Amenorrhoea assessed in women aged 51 years or younger.

Table 1: **Baseline characteristics of trial participants, by treatment group**

Four ethics committees approved the study protocol: Médecins Sans Frontières International Ethical Review Board (Geneva, Switzerland); Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (CCPPRB; Saint-Germain-en-Laye, France); WHO Research Ethics Committee (Geneva, Switzerland); and Comité d'Ethique du Ministère de la Santé (DR Congo). All participants gave signed or fingerprinted informed consent. An independent data safety and monitoring board reviewed the study regularly.

Randomisation and masking

Patients were randomised to intervention and control groups in a 1:1 ratio without any stratification. The randomisation list (in blocks of ten) was electronically generated at Epicentre headquarters (Paris, France) and concealed from the field teams that enrolled and allocated treatments. Participants were enrolled in the same order in which they were diagnosed. Sealed and numbered opaque envelopes contained the treatment allocation and were opened in strict numeric sequence. Masking was not feasible because the method of drug administration differed between the two groups.

Procedures

Drug regimens were based on the existing evidence including early data from the precursor studies.[19,20] Participants in the intervention group were given intravenous eflornithine (400 mg/kg per day, every 12 h) for 7 days, with oral nifurtimox (15 mg/kg per day, every 8 h) for 10 days (NECT). The active comparator was intravenous eflornithine (400 mg/kg per day, every 6 h), given for 14 days. Eflornithine was diluted in 250 mL of normal saline and infused over 2 h. Nifurtimox intake was directly observed, and re-administered if vomiting occurred within 30 min. Specific rules dealt with treatment interruptions in each group. Depending on the length of the interruption and the time it occurred during the treatment course, interruptions considered likely to affect efficacy were compensated with additional doses of eflornithine.

Patients with malaria received artemether-lumefantrine for 3 days, with the study treatment starting at least 1·5 days after this treatment. Drugs for other concomitant disorders were postponed until the end of hospital admission, unless immediate treatment was warranted. Patients received a food ration of 2100 kcal per day.

All patients were medically assessed daily, and remained in hospital for 7 days after the end of treatment, or longer if needed. A lumbar puncture was done 1 day after the last study dose, to examine CSF for trypanosomes and to obtain a leucocyte count. Follow-up laboratory examinations were done at 6, 12, and 18 months, and included testing for trypanosomes in the blood (capillary tube centrifugation and quantitative buffy coat[24] techniques), CSF, and lymph node fluid, and CSF leucocyte count. Trypanosome observation and CSF leucocyte counts were confirmed through two independent readings by different laboratory technicians and the mean values were recorded. Discrepancies were resolved by a third reading taken by a senior technician.

Paticipants were followed up for 18 months, as recommended by a consensus of HAT experts[25] on the basis of data suggesting that 70–90% of relapses occur

	Eflornithine			NECT			Difference Δ (%)	One-sided 95% CI*	p value
	n/N	%	95% CI	n/N	%	95% CI			
Primary analysis									
Cure rate									
ITT population	131/143	91·6%	..	138/143	96·5%	..	−4·9%	−0·3	<0·0001†
mITT population	131/142	92·3%	..	138/141	97·9%	..	−5·6%	−1·4	<0·0001†
PP population	122/133	91·7%	..	129/132	97·7%	..	−6·0%	−1·5	<0·0001†
Secondary analysis (ITT population)									
Probability of event-free survival‡	..	84·8%	70·1–92·7	..	94·3%	78·3–98·6	..	..	0·0497§
Relapse at 12 months	5/140	3·6%	1·2–8·1	0/142	0·0%	0·0–2·6¶	..	..	0·029
Relapse at 18 months	8/140	5·7%	2·5–10·9	2/142	1·4%	0·0–5·0	..	..	0·029
Parasitologically confirmed relapse	5/140	3·6%	1·2–8·1	0/142	0·0%	0·0–2·6¶	..	..	0·029
Fatality (within 30 days)	3/143	2·1%	0·4–6·0	1/143	0·7%	0·0–3·8	..	..	0·622

NECT=nifurtimox-eflornithine combination treatment. ITT=intention-to-treat (all randomised patients, apart from one who absconded on day 1). mITT=modified intention-to-treat (including patients with partial or complete follow-up but excluding those with no follow-up). PP=per-protocol (including only patients with complete follow-up and a study endpoint). *According to the Blackwelder method:[27] upper limit of a two-sided 90% CI around the difference (Δ) of cure rates. †p values testing the 10% non-inferiority margin. ‡Events included relapse of human African trypanosomiasis (HAT) and death related to HAT or to treatment. §Log-rank test. ¶One-sided, 97·5% CI.

Table 2: **Efficacy outcome by treatment group (primary and secondary analyses)**

within this period, and therefore considered sufficient for comparative efficacy evaluations in randomised trials. A relapse was diagnosed if trypanosomes were seen in any body fluid or if CSF leucocyte count increased twice consecutively by 20 cells per µL or more. Patients with a single increase were re-examined 1 month later. At 18 months, a relapse was defined as leucocyte count more than 20 cells per µL, irrespective of previous counts. No distinction was made between relapse and re-infection because of technological limitations. Within the epidemiological context of the four sites, the probability of re-infection was judged to be low.

Safety was assessed by use of the National Cancer Institute Common Toxicity Criteria,[26] which grades adverse events by intensity from 1 to 4 (mild, moderate, severe, or very severe), drug–event relation (unrelated, unlikely, possible, probable, or definite), and outcome (recovery, still present, sequelae, or death). Blood samples taken before, immediately after, and 7 days after treatment were examined for haemoglobin concentration, leucocyte count, and concentrations of creatinine, alanine aminotransferase, and total bilirubin.

The primary outcome was cure, defined as absence of trypanosomes in body fluids and a CSF leucocyte count 20 cells per µL or less at 18 months. Endpoints regarded as therapeutic failure were death in temporal relation to treatment (within 30 days of treatment start), relapse of HAT or death compatible with HAT during follow-up, and complete loss to follow-up. Deaths without a clearly established alternative cause were regarded as HAT-related. Patients missing the 18-month laboratory examination were deemed probably cured if they were seen by the investigators after 18 months and were found in good physical and mental health, or if their last laboratory examination showed a favourable evolution (this includes patients who died later of causes unrelated to HAT). Favourable evolution was defined as a decrease of CSF leucocyte count or an increase of less than 20 leucocytes per µL. Safety outcomes were clinical and laboratory adverse events temporally associated with the treatment, in particular the major adverse events: severe (grade 3) and very severe (grade 4).

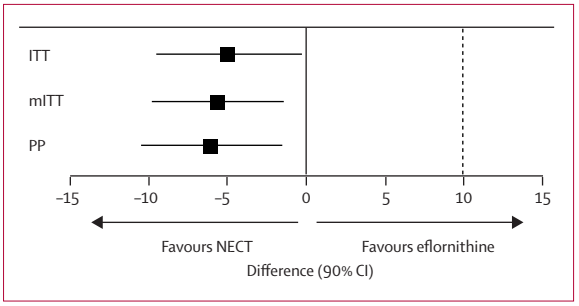

Figure 2: **Overall cure rates (non-inferiority analysis) by analysis population**
NECT=nifurtimox-eflornithine combination treatment. ITT=intention-to-treat. mITT=modified intention-to-treat. PP=per-protocol. Difference (Δ) of cure rates between the standard eflornithine regimen and NECT (eflornithine–NECT), and 90% CIs around Δ. The dotted lines indicates the non-inferiority margin (zone of non-inferiority lies to the left of Δ=10%, and the zone of superiority lies to the left of Δ=0%).

Statistical analysis

The non-inferiority margin for the difference in cure rates between groups was defined as 10% by the scientific committee, considering the high efficacy of the comparator and the crucial need of therapeutic alternatives. The control treatment (eflornithine monotherapy) has never been tested against placebo. At the time of the protocol development, available data showed effectiveness estimations with eflornithine in a

range of 89% to 97%. Assuming a 93% cure rate with the control regimen, a maximum difference in cure rates between the groups of 10%, and a similar dropout rate in the two groups, we calculated that a sample size of 280 patients (140 per group) was needed to determine non-inferiority with a power of 90% at an alpha error of 5% (one-sided test).

Data were recorded on specifically designed patient charts, and relevant trial data were extracted in case report forms. These data were double-entered electronically with EpiData version 3.0 and analysed with Stata version 9.2. The efficacy analyses were done on three populations: intention-to-treat (ITT), modified ITT, which included patients with partial or complete follow-up but excluded those with no follow-up, and per-protocol (PP), which included only those with complete follow-up and a study endpoint. Non-inferiority of NECT was tested by use of the Blackwelder[27] method by calculating the difference of overall cure rates and comparing its one-sided 95% CI (or upper limit of the 90% CI) to the non-inferiority margin. Overall cure included cure and probable cure. Secondary efficacy analyses included comparison of proportions and survival analysis (Kaplan-Meier method, comparison of distributions by log-rank test). Proportions (efficacy and safety) were compared with χ^2 or Fisher's exact test and two-sided p values. This study is registered with ClinicalTrials.gov, number NCT00146627.

	Eflornithine (N=143)		NECT (N=143)		p value
	n (%)*	Number of major events†	n (%)*	Number of major events†	
Neurological	60 (42·0%)	9	60 (42·0%)	9	1·000
Seizures	13 (9·1%)	6	18 (12·6%)	6	0·342
Confusion	2 (1·4%)	1	6 (4·2%)	2	0·282
Anxiety/agitation	11 (7·7%)	1	4 (2·8%)	0	0·109
Dizziness	24 (16·8%)	0	26 (18·2%)	0	0·756
Ataxia	2 (1·4%)	0	5 (3·5%)	1	0·447
Inner ear disturbance	7 (4·9%)	0	10 (7·0%)	1	0·365
Tremors	1 (0·7%)	0	9 (6·3%)	0	0·019
Coma	3 (2·1%)	3	1 (0·7%)	1	0·622
Amnesia	1 (0·7%)	0	1 (0·7%)	0	1·000
Insomnia	14 (9·8%)	0	14 (9·8%)	0	1·000
Hallucinations	1 (0·7%)	1	2 (1·4%)	1	1·000
Lethargy	2 (1·4%)	0	0 (0·0%)	0	0·498
Mood alteration (depression)	1 (0·7%)	0	2 (1·4%)	0	1·000
Peripheral neuropathy (motor)	2 (1·4%)	1	4 (2·8%)	1	0·684
Peripheral neuropathy (sensory)	5 (3·5%)	0	5 (3·5%)	0	1·000
Gastrointestinal	78 (54·5%)	2	106 (74·1%)	2	0·001
Anorexia	20 (14·0%)	0	36 (25·2%)	1	0·017
Dysphagia	12 (8·4%)	0	3 (2·1%)	0	0·031
Hiccups	3 (2·1%)	0	6 (4·2%)	0	0·501
Taste disturbance	2 (1·4%)	0	1 (0·7%)	0	1·000
Mouth dryness	7 (4·9%)	0	1 (0·7%)	0	0·066
Regurgitation	0 (0·0%)	0	2 (1·4%)	0	0·498
Abdominal pain	42 (29·4%)	0	35 (24·5%)	0	0·351
Diarrhoea	41 (28·7%)	2	9 (6·3%)	0	<0·0001
Constipation	6 (4·2%)	0	4 (2·8%)	0	0·749
Nausea and/or vomiting	29 (20·3%)	0	69 (48·3%)	1	0·000
Cardiovascular	49 (34·3%)	3	29 (20·3%)	0	0·008
Arrythmia	31 (21·7%)	0	27 (18·9%)	0	0·556
Hypertension	19 (13·3%)	3	6 (4·2%)	0	0·006
Oedema	6 (4·2%)	0	1 (0·7%)	0	0·120
Hypotension/shock	4 (2·8%)	0	5 (3·5%)	0	1·000
Infection	25 (17·5%)	5	14 (9·8%)	1	0·058
Tissue infection	2 (1·4%)	0	6 (4·2%)	0	0·282
Injection site infection	5 (3·5%)	0	4 (2·8%)	0	1·000
Pneumonia	2 (1·4%)	2	0 (0·0%)	0	0·498
Other infections	23 (16·1%)	5	8 (5·6%)	1	0·004

(Continues on next page)

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Enrolment at the first site started in August, 2003, and at the following three sites in 2005, and 2006, concluding in November, 2006. Follow-up was completed in June, 2008. Figure 1 shows the trial profile. One patient assigned to eflornithine monotherapy absconded after receiving the first dose, without any type of assessment done, and was excluded from all analyses.

Table 1 shows the baseline characteristics of trial participants. All patients received complete treatment. Protocol deviations were rare: four patients (two per group) were enrolled with CSF leucocyte count 5–20 cells per µL, but trypanosomes were present in the CSF. One patient (NECT group) received eflornithine for 10 days instead of 7 days. There was an error in the efficacy assessment (diagnosis of relapse) of one patient in the eflornithine group that was regarded as a major protocol violation. This patient was excluded from the PP population.

283 (99%) of 286 participants had at least one follow-up assessment or reached a study endpoint earlier, 266 (93%) completed 18 months follow-up or reached a study endpoint earlier, 17 (6%) had a partial follow-up, and three (1%) had no follow-up. All 17 patients with partial follow-up showed a favourable evolution in CSF leucocyte count at their last assessment and were deemed probably cured (eflornithine, n=8 NECT, n=9). 13 patients seen by the investigators at 18 months were in good physical and mental health but refused laboratory assessments (also regarded as probably cured; eflornithine, n=7 NECT, n=6). Thus, 15 patients in each group were judged to be probably cured. Of the three patients without follow-up, two died of unrelated causes and one refused all assessments. These patients were deemed treatment failures.

Four deaths temporally related to the treatment were judged treatment failures (eflornithine, n=3; NECT, n=1). There were ten relapses (eflornithine, n=8; NECT, n=2), five of which were detected before 18 months (range 6·1–14·5 months) and the rest at 18 months or later (18·3–20·9 months; table 2). Five relapses were diagnosed by presence of trypanosomes in CSF (all in the eflornithine group), the other five relapses were diagnosed on the basis of increases in CSF leucocyte count. Patients with relapse were treated according to national protocols and clinicians' judgment.

Table 2 shows the cure rates by treatment group and analysis population (ITT, modified ITT, and PP). In the ITT population, 131 (91·6%) of 143 patients assigned to eflornithine and 138 (96·5%) of 143 patients assigned to NECT were cured or probably cured at 18 months (difference –4·9%, one-sided 95% CI –0·3; p<0·0001). In the PP population, 122 (91·7%) of 133 patients in the eflornithine group and 129 (97·7%) of 132 in the NECT group were cured at 18 months (difference –6·0%, one-sided 95% CI –1·5; p<0·0001). Results were similar in the three analysis populations (figure 2).

Although not originally planned, we also undertook a sensitivity analysis (worst-case scenario), which included all patients (apart from the one who absconded) and judged as cured only those patients with laboratory-confirmed cure at 18 months. All other patients (relapses, all-cause deaths, lost to follow-up, probable cures) were analysed as failures. In this analysis, 116 (81·1%) of 143 controls and 123 (86·0%) of 143 patients in the NECT group were cured (difference –4·9%, one-sided 95% CI 0·02; p=0·0003). Secondary efficacy analyses (table 2) showed that relapse rates (at 12 months and 18 months) and parasitologically confirmed relapse rates were lower, and the probability of event-free survival was higher in the NECT group than in the eflornithine group.

The three deaths in the eflornithine group were attributed to septic shock (following severe neutropenia), pneumonia, and urinary infection with coma. The death in the NECT group was attributed to respiratory distress syndrome. All four deaths were judged related to study drugs. Of 1363 clinical adverse events recorded, 101 were judged by the clinicians as not related to treatment, and 1262 events were judged as drug related (table 3). There were 132 laboratory-detected adverse events (table 4). The total number of drug-related events was therefore 1394, and the number of reactions per patient was 5·3 (eflornithine) and 4·5 (NECT).

The proportion of patients who had major (grade 3–4) drug-related adverse events (table 5) was lower in the NECT group than in the eflornithine group (20/143 [14·0%] *vs* 41/143 [28·7%]; p=0·002), mainly because of a lower frequency of severe fever, infections, neutropenia, hypertension, and diarrhoea. Temporary treatment interruptions were necessary in nine (6·3%) of 143 patients assigned to eflornithine (duration ranging from 6 h to 6 days) and one (0·7%) of 143 patients assigned to NECT (duration 1 day; p=0·019) All treatment schedules were adapted (or not) according to the protocol.

	Eflornithine (N=143)		NECT (N=143)		p value‡
	n (%)*	Number of major events†	n (%)*	Number of major events†	
(Continued from previous page)					
Other clinical events	122 (85·3%)	20	101 (70·6%)	10	0·003
Fever§	61 (42·7%)	18	37 (25·9%)	7	0·003
Headache	66 (46·2%)	2	55 (38·5%)	1	0·188
Asthenia	28 (19·6%)	1	34 (23·8%)	0	0·389
Cough	14 (9·8%)	0	10 (7·0%)	0	0·394
Pruritus	27 (18·9%)	0	13 (9·1%)	0	0·017
Skin rash	20 (14·0%)	0	4 (2·8%)	1	0·001
Injection site reaction	16 (11·2%)	0	14 (9·8%)	0	0·700
Extravasation	11 (7·7%)	0	6 (4·2%)	0	0·211
Chest pain	15 (10·5%)	1	10 (7·0%)	0	0·295
Dehydration	3 (2·1%)	0	3 (2·1%)	1	1·000
Urinary incontinence	4 (2·8%)	0	4 (2·8%)	0	1·000
Urinary frequency/urgency	6 (4·2%)	0	2 (1·4%)	0	0·282
Myalgia/arthralgia	43 (30·1%)	0	43 (30·1%)	0	1·000
Dyspnoea	1 (0·7%)	0	2 (1·4%)	0	1·000
Respiratory distress	1 (0·7%)	1	1 (0·7%)	1	1·000
Epistaxis	2 (1·4%)	0	0 (0·0%)	0	0·498
Other	16 (11·2%)	0	17 (11·9%)	0	0·853

NECT=nifurtimox-eflornithine combination treatment. The total number of clinical events was 677 (48 major) in the eflornithine group and 585 (27 major) in the NECT group. *All figures indicate the number of patients with a given type of adverse event (please note, one patient might have had more than one type of event from each category [eg, neurological]). †An event was defined as major if it was classed as grade 3 (severe) or grade 4 (very severe) according to the National Cancer Institute Common Toxicity Criteria.[26] ‡Comparison for number of patients with a given event. §Fever defined as axillary temperature 37·5°C or more.

Table 3: **Number of patients with drug-related adverse events, by treatment group**

In terms of specific types of reactions, patients in the NECT group had a lower frequency of diarrhoea, hypertension, infections, dysphagia, pruritus, skin rash, fever, and neutropenia than did controls. However, nausea and vomiting, anorexia, and tremors were more frequent in patients assigned to NECT. The clinicians noted that nausea and vomiting tended to appear at least 1 h after nifurtimox intake and more frequently after the simultaneous administration of both drugs (first daily dose) than when nifurtimox and eflornithine were given at different times. Nausea did not impede the intake (supervised) of nifurtimox tablets.

Neutropenia and anaemia were the most prominent haematological reactions (table 4). The frequency of neutropenia was significantly lower in patients assigned to NECT than in controls (p=0·001). Grade 3 neutropenia (less than 1000 neutrophils per μL) developed in 12 patients (eflornithine, n=10, NECT, n=2; p=0·019). Abnormal biochemical values were rare and mild in both groups.

Of all laboratory abnormalities that developed during treatment, 70 (53%) of 132 were re-examined 7 days later. Laboratory markers returned to normal levels or improved

	Eflornithine		NECT		p value†
	n/N (%)	Number of major events*	n/N (%)	Number of major events*	
Patients with haematological reactions	58/143 (40·6%)	11	30/143 (21·0%)	4	0·0003
Anaemia‡	12/141 (8·5%)	1	10/142 (7·0%)	2	0·645
Leucopenia§	6/139 (4·3%)	0	8/142 (5·6%)	0	0·612
Neutropenia¶	46/139 (33·1%)	10	22/142 (15·5%)	2	0·001
Thrombopenia	4/93 (4·3%)	0	1/87 (1·1%)	0	0·369
Patients with biochemical reactions	9/143 (6·3%)	0	11/143 (7·7%)	0	0·643
Abnormal creatinine‖	1/129 (0·8%)	0	1/128 (0·8%)	0	1·000
Abnormal bilirubin**	6/131 (4·6%)	0	9/128 (7·0%)	0	0·398
Abnormal alanine aminotransferase††	4/131 (3·1%)	0	2/127 (1·6%)	0	0·684
Patients with any laboratory reaction	66/143 (46·2%)	11	38/143 (26·6%)	4	0·001

NECT=nifurtimox-eflornithine combination treatment. The total number of laboratory reactions was 79 (11 major) in the eflornithine group and 53 (four major) in the NECT group *An event was defined as major if it was classed as grade 3 (severe) or grade 4 (very severe) according to the National Cancer Institute Common Toxicity Criteria.[26] †Comparison for number of patients with a given event. ‡Anaemia defined as haemoglobin less than 130 g/L (men) and less than 110 g/L (women), having decreased by more than 20%. §Leucopenia defined as less than 4000 leucocytes per µL, having decreased by more than 30%. ¶Neutropenia defined as less than 2000 neutrophils per µL, having decreased by more than 30%. ‖Abnormal creatinine defined as more than 80 µmol/L (women) and 97 µmol/L (men), having increased by more than 1·5 times. **Abnormal bilirubin defined as more than 17 µmol/L, having increased by more than 1·5 times. ††Abnormal alanine aminotransferase defined as more than 12 IU/L (reagents from Randox, UK, and Roche, Switzerland) and more than 32 IU/L (women) and more than 42 UI/L (men; subgroup measured with reagents from Human, Germany), having increased by more than 2·5 times.

Table 4: **Number of patients with laboratory drug-related adverse events, by treatment group**

	Eflornithine (N=143)	NECT (N=143)	p value
Patients with major drug-related adverse events	41 (28·7%)	20 (14·0%)	0·002
Clinical	33 (23·1%)	18 (12·6%)	0·020
Laboratory	11 (7·7%)	4 (2·8%)	0·109
Patients with any drug-related adverse events	138 (96·5%)	136 (95·1%)	0·555
Clinical	134 (93·7%)	134 (93·7%)	1·000
Weight loss ≥5%	5 (3·5%)	6 (4·2%)	0·758
Laboratory	66 (46·2%)	38 (26·6%)	0·001
Patients with serious adverse events	6 (4·2%)	1 (0·7%)	0·120
Patients with temporary treatment interruptions	9 (6·3%)	1 (0·7%)	0·019
Deaths related to treatment	3 (2·1%)	1 (0·7%)	0·622

NECT=nifurtimox-eflornithine combination treatment. Data are n (%). An event was defined as major if it was classed as grade 3 (severe) or grade 4 (very severe) according to the National Cancer Institute Common Toxicity Criteria.[26] An event was defined as serious if it was fatal, life-threatening, or resulted in long hospital stay, substantial disability, or congenital anomaly.

Table 5: **Summary of safety data, by treatment group**

in most patients (53 [76%]), but remained unchanged in 14 (20%) patients. Reactions had worsened only in the eflornithine group (three of 39 [8%]: one case of neutropenia and two cases of thrombopenia).

Discussion

This multicentre study was done in four rural HAT-endemic areas that were thousands of kilometres apart. These regions are characterised by political instability, poor infrastructure, inadequate research capacity, and geographic dispersion of patients. Despite these challenges, the methods used in this trial complied with international GCP guidelines. The results show that the efficacy of NECT is non-inferior to standard eflornithine monotherapy (10% non-inferiority margin). High cure rates of 96·5% to 97·9% (depending on the analysis population) were seen in patients assigned to NECT, compared with rates of 91·6% to 92·3% in patients assigned to eflornithine. The results of the post-hoc worst-case scenario analysis were similar to the results of the primary analysis, and provided evidence that there was no potential bias related to the patients with incomplete follow-up.

Theoretically, because of the short half-life of eflornithine, 6-h infusions are needed to obtain a constant trypanostatic effect. Our data show that 12-h eflornithine infusions are highly effective when combined with oral nifurtimox. A possible explanation is that the irreversible ornithine decarboxylase inhibition caused by eflornithine arrests the parasite's defences for about 18–19 h, the time needed to replenish enzyme concentrations,[28] facilitating the trypanocidal action of nifurtimox by oxidative stress.

A common limitation of previous efficacy studies of HAT treatment has been poor follow-up, owing to contextual and behavioural factors. Follow-up compliance in this study was high, thus enhancing the robustness of the efficacy evaluation. Half of the relapses became detectable at 18 months or later, despite all patients having had interim assessments. In accordance with former studies,[29] these data suggest that follow-up in clinical studies that include these drugs should be no shorter than 18 months.

The results of this study show that this drug combination is fairly well tolerated. Notably, low fatality rates (0·7%) were associated with NECT, by contrast with the 5–6% reported fatality after treatment with melarsoprol,[2,30] the most widely used drug for treatment of HAT. Drug-related adverse events were frequent in both trial groups. However, it must be appreciated that it is difficult to discern the causality between the disease itself, comorbidities, concomitant treatments, and the study treatment. Our description is conservative: events are deemed treatment-related unless the investigators were certain to the contrary.

Because HAT is a fatal condition, and adverse events are very common during treatment, we focused the assessment on major (severe and very severe) drug-related adverse events. Half as many major adverse events were reported in the NECT group than in the eflornithine group (p=0·002). Additionally, NECT was associated with a lower frequency of infections, diarrhoea, fever, dysphagia, hypertension, pruritus, skin rash, and neutropenia than was eflornithine monotherapy, whereas nausea and vomiting, anorexia, and tremors were more frequent. Although 48% of patients assigned to NECT had nausea or vomiting, or both, which could potentially affect nifurtimox absorption, the efficacy of treatment

was remarkably good. This fact, in addition to the bedside observations on the timing of vomiting (at least 1 h after nifurtimox intake, more frequently after the concomitant administration of both drugs), suggest that vomiting was provoked at cerebral level by simultaneous peak concentrations of both drugs. On the basis of these findings, we suggest that adjustments in the timing of drug intake might alleviate nausea and vomiting. Future studies should include collection of prospective data on the timing of vomiting.

The reduced bone marrow toxicity, reflected by the lower frequency of neutropenia and anaemia in the NECT group than in the eflornithine group, is consistent with the halved eflornithine exposure (control 14 days *vs* NECT 7 days). This reduced exposure might explain the lower overall frequency of infections, an effect reinforced by a reduction in catheter-related infections expected with the substantial reduction (56 *vs* 14) in the number of infusions. In standard practice, patients return home immediately after treatment, and neutropenic cases remain vulnerable to bacterial infections for 1–2 weeks. A reduced risk of infections can be expected with the use of NECT.

Our study has potential limitations. We had to use an open-label design, which was unavoidable because of the different methods of drug administration in the treatment groups. However, efficacy outcomes were mainly established by laboratory results, which were always confirmed by two independent readings. A limitation in the safety analysis is that the hospital admission time differed per group, because of the different treatment schedules. (The longer a patient stays in hospital under observation, the more adverse events are likely to occur and be recorded.)

The overwhelming consensus among experts working on HAT and affected populations stresses the urgent need to identify feasible, safe, and effective therapies for second-stage disease.[2] This study suggests that NECT is safe and effective for the treatment of *T b gambiense* infection. The results are consistent with our two previous studies investigating this drug combination.[19,20] However, the results are not applicable to patients infected with *T b rhodesiense*; this infection would require its own specific studies.

NECT is easier to administer and less demanding on human and material resources than eflornithine monotherapy because it has a simpler administration schedule and requires shorter hospital stay. These characteristics are important because most treatment centres are in remote locations, with logistical and staffing limitations. NECT is also more affordable, mainly because the four-fold reduction of intravenous infusions implies savings in the purchase, transportation, and stocking of intravenous fluids and injection materials, in hospital staff (overall patient management but especially night duties), and in management of catheter-related infections and other adverse events. Additionally, NECT uses half the amount of expensive eflornithine while adding a small amount of inexpensive nifurtimox. The lower cost and simpler logistics of NECT could alleviate the burden on HAT control programmes.

This combination of drugs with different mechanisms of action might be protected against the development of drug resistance, as is the case for drug combinations used in other infectious diseases. There is already an established and growing parasite resistance to melarsoprol, and the increasing large-scale use of eflornithine monotherapy could see this drug exposed to the same threat. To avert the development of eflornithine resistance, full-scale implementation of the NECT regimen should be a priority for HAT control programmes.

Contributors

The authors accept full responsibility for the overall content of this report. GPr designed and coordinated the study, contributed to data analysis, and wrote the manuscript. SK set up the study in the field, undertook training, monitoring, and supervision, enrolled and managed patients, collected clinical data, and participated in writing the report. WMuto, DN, SGho, UA, VB, SK-K, MI, and WMuta enrolled and managed patients, collected clinical data, and provided daily monitoring and supervision. SGha and EB managed and analysed the data. GPo and CS participated in training, clinical monitoring, and supervision, and writing the paper. UK and ET participated in study coordination, data interpretation, and writing of the report. VK participated in project coordination, supervision, and writing of the report.

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Conflicts of interest

We declare that we have no conflicts of interest.

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