Improved melarsoprol therapy for *Trypanosoma brucei rhodesiense* sleeping sickness

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List of Abbreviations

BBB	Blood Brain Barrier
BMI	Body Mass Index
CATT	Card Agglutination Test
CDC	Centers for Disease Control and Prevention
CDM	Clinical Data Management
CI	Confidence Interval
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
DALY	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
DND*i*	Drugs for Neglected Diseases Initative
DSS	Demographic Surveillance System
EDCTP	European & Developing Countries Clinical Trial Partnership
ES	Encephalopathic Syndrome
EKBB	Ethikomission beider Basel / Ethics committee of both cantons of Basel
EMEA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
HLA	Human Leukocyte Antigen System
IPPM	Intellectual Property Protection Mechanism
ISCTRC	International Scientific Committee for Trypanosomiasis Research and Control
IDP's	Internally Displaced People
i.v.	Intravenous
LAMP	Loop Mediated Isothermal Amplification
LIRI	Livestock Health Research Institute, Uganda
LP	Lumbar puncture

HAT	Human African Trypanosomiasis
MMV	Medicines for Malaria Venture
MoH	Ministry of Health
MSF	Médecins sans Frontières/Doctors without Borders
NIH	National Institutes of Health
NIMR	National Institute for Medical Research (Tanzania)
NGO	Non-governmental Organization
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PABIN	Pan-African Bioethics Initiative
PCR	Polymerase Chain Reaction
PDP	Product Development Partnership
p.o.	per os
PPP	Public Private Partnership
R&D	Research and Development
RIME	Random Insertion Mobile Element
SAB	Scientific Advisory Board
SARETI	South African Research Ethics Training Initiative
SIDCER	Strategic Initiative for Developing Capacity in Ethical Review
SRA-gene	Serum-resistance-associated gene
SS	Sleeping Sickness
STI	Swiss Tropical Institute
USD	US Dollar
WBC	White blood cells (leucocytes)
WHO	World Health Organization

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Summary

Human African Trypanosomiasis (HAT) is a parasitic disease that occurs in a chronic form caused by *Trypanosoma brucei gambiense* in Western and Central Africa, and an acute form caused by *Trypanosoma brucei rhodesiense* in Eastern and Southern Africa.

The treatment of HAT is unsatisfactory; for over 50 years melarsoprol (Arsobal®) has been the only drug active against both forms of the disease and the only drug available to treat second stage *T.b. rhodesiense* infections. However, its use is hampered by high toxicity and lengthy and complicated treatment schedules.

Melarsoprol therapy was substantially improved by the introduction of an abridged 10-day melarsoprol schedule in *T.b. gambiense* affected areas in 2003. The new schedule was based on pharmacological investigations and was shown to be non-inferior compared to the standard regimens in the framework of the clinical trial programs IMPAMEL I & II (1997-2004). A significant reduction in overall hospitalization time from about 25 – 35 days to 13 days and a more economic use of the drug made it favorable to the patients and the health system. Subsequently, the conduct of the IMPAMEL III program in *T.b. rhodesiense* affected areas was declared a high priority by the WHO.

The presented thesis aimed at a) the assessment of the safety and efficacy of the 10-day melarsoprol schedule in *T.b. rhodesiense* patients and b) the rationalization of the suramin pre-treatment prior to melarsoprol which is proposed to control adverse drug reactions, but which is only partially implemented in East Africa.

The IMPAMEL III program consisted of the sequential conduct of a proof-of-concept trial and a utilization study using historic controls as comparator. The trials were conducted in two treatment centers in Tanzania and Uganda. Consenting patients with confirmed second stage *T.b. rhodesiense* HAT and a minimum age of 6 years were eligible for participation. Pregnant as well as unconscious or moribund patients were excluded from the trial. The primary outcome measures were safety and efficacy at end of treatment. The secondary outcome measure was efficacy during follow-up after 3, 6 and 12 months. The studies were approved by the ethics committees in Tanzania (National Institute for Medical Research/NIMR) and Uganda (Ministry of Health) and the ethics committee of both cantons of Basel (EKBB), Switzerland.

In the proof-of-concept trial a total of 60 patients were enrolled into two consecutive subgroups (2x15 in each center) of which only the first subgroup received the suramin pre-treatment. Suramin as well as steroids were administered according centre-specific guidelines. In this trial, the incidence of the encephalopathic syndrome (ES) was significantly higher in Uganda (20%)

than in Tanzania (3.3%, p=0.0444). Adverse events were more frequent in patients that received suramin (63.3%) than in patients that were directly treated with melarsoprol (23.3%, p=0.0018). Based on these results, the utilization study was designed as an extension to the arm of the proof-of-concept trial without suramin. An additional 77 patients were enrolled and directly treated with melarsoprol.

Final data analysis was performed on the pooled data set of all patients that were directly treated with the 10-day melarsoprol schedule (i.e. without suramin, n=107). These results were compared to historic controls of patients treated during past two years in the same centers. The incidence of ES in the trial population was 11.2% (CI 5-17%) and 13% (CI 9-17%) in the historic data. The respective case fatality rates were 8.4% (CI 3-13.8%) and 9.3% (CI 6-12.6%). The historic data did not allow any elucidation of the efficacy of the standard treatment regimens since systematic follow-up of patients was not routine. However, the efficacy of the 10-day melarsoprol schedule was highly satisfactory: all patients were free of parasites the day after treatment. 99% of the patients eligible for follow-up were considered clinically cured 6 months after discharge. The 12 months follow up is currently ongoing. Based on the follow-up results of the proof-of-concept trial no issues regarding treatment efficacy are expected.

Our results show that *T.b. rhodesiense* patients treated with the 10-day melarsoprol schedule were not subject to a higher incidence of serious adverse events (ES or death) than the historic controls treated with the national regimens. The hospitalization time was reduced from an average of 29 days to 13 days (p<0.0001).

In a separate analysis we compared the clinical presentation of the disease in Ugandan and Tanzanian patients as a wide spectrum of disease severity has been described for *T.b. rhodesiense* HAT.

In an ancillary study, the molecular characterization of the trypanosomes confirmed that all patients were infected with *T.b. rhodesiense*. The fear of an overlap in the *T.b. gambiense* and *T.b. rhodesiense* disease distribution areas could not be confirmed in our study area.

On the basis of our trial experience we were able to write a review on clinical research in resource limited settings. Minimal standards for sponsors and host countries were suggested in order to ensure a trial conduct in compliance with international standards.

Zusammenfassung

Die Humane Afrikanische Trypanosomiasis (HAT) ist eine parasitäre Krankheit, die sowohl in einer chronischen als auch akuten Form vorkommt. Die chronische Form wird durch *Trypanosoma brucei gambiense* verursacht und kommt in West- und Zentralafrikanischen Gebieten vor. *Trypanosoma brucei rhodesiense* verursacht die akute Form der Krankheit, die typischerweise in Ost- und Südafrika vorkommt.

Die Behandlung von HAT erweist sich als sehr unbefriedigend: seit über 50 Jahren wird Melarsoprol (Arsobal®) als einziges wirksames Arzneimittel für die Behandlung beider Krankheitsformen eingesetzt. Es ist bis heute das einzige erhältliche Medikament für die Behandlung von *T.b. rhodesiense* Infektionen die bereits zum zweiten Krankheitsstadium fortgeschritten sind. Eine hohe Toxizität, sowie langwierige und komplizierten Behandlungsverfahren erschweren jedoch die Therapie.

Durch die die Einführung eines 10-Tage-Behandlungsschemas im Jahr 2003 ist die Melarsoprol Therapie in *T.b. gambiense* betroffenen Gebieten wesentlich verbessert worden. Das neue Schema basiert auf pharmakologischen Untersuchungen und wurde im Rahmen der klinischen Studienprogramme IMPAMEL I & II (1997-2004) untersucht und erwies sich als nicht minderwertiger im Vergleich zu den Standardschemata. Die signifikante Herabsetzung der Hospitalisierungsdauer von etwa 25 bis 35 Tage auf 13 Tage kommt den Patienten zugute und der wirtschaftlichere Einsatz des Arzneimittels begünstigt das Gesundheitssystem. Als Folge wies die World Health Organization (WHO) der Durchführung des IMPAMEL III Programms in *T.b. rhodesiense* betroffenen Gebieten eine hohe Priorität zu.

Die vorliegende Doktorarbeit hatte zwei Ziele: 1) Die Bestimmung der Sicherheit und Wirksamkeit des 10-Tage-Behandlungsschemas mit Melarsoprol bei Patienten die mit *T.b. rhodesiense* infiziert sind; 2) Die Rationalisierung der Vorbehandlung mit Suramin, diese sollte der Reduzierung von schweren Nebenwirkungen der Melarsoprol Therapie dienen, ist aber zum heutigen Zeitpunkt nur teilweise in den nationalen Behandlungsschemata implementiert.

Das IMPAMEL III Programm bestand aus der sequentiellen Durchführung einer "Proof of Concept" Studie und einer Anwendungsstudie. Als Vergleich dienten historische Patientendaten. Die Studien wurden in zwei Behandlungszentren in Tansania und Uganda durchgeführt. Patienten die eine Einwilligungserklärung unterschrieben, mindestens 6 Jahren alt waren und an einer bestätigten *T.b. rhodesiense* Infektion litten, wurden zur Studienteilnahme zugelassen. Schwangere, bewusstlose oder moribunde Patienten wurden von der Studie ausgeschlossen. Der Therapieerfolg wurde zum einen anhand der Sicherheit und Wirksamkeit am Ende der

Behandlung erhoben sowie mit einem zusätzlichen Wirksamkeitsnachweis nach drei, sechs und zwölf Monaten. Die Ethikkomissionen in Tansania (National Institute for Medical Research), Uganda (Ministry of Health) sowie die Ethikkomission Beider Basel (Schweiz), genehmigten die Durchführung der Studien.

Die "Proof of Concept" Studie umfasste 60 Patienten, unterteilt in zwei Untergruppen (2x15 Patienten pro Zentrum) von der nur eine mit Suramin vorbehandelt wurde. Suramin, sowie Steroide, wurden nach zentrumspezifischen Richtlinien abgegeben. Das Auftreten des enzephalopathischen Syndroms (ES) war in Uganda (20%) signifikant höher als in Tansania (3,3%, p=0,0444). Zudem traten andere Nebenwirkungen vermehrt bei Patienten auf, welche Suramin erhielten (63,3%) als bei Patienten, welche direkt mit Melarsoprol behandelt wurden (23,3%, p=0,0018). Basierend auf diesen Ergebnissen wurde die Anwendungsstudie als eine Erweiterung der suramin-freien Untergruppe entworfen. Zusätzliche 77 Patienten wurden in die Studie eingeschlossen und direkt mit melarsoprol behandelt.

Die endgültige Datenanalyse umfasste die Datensätze aller Patienten, die direkt mit dem 10-Tage-Behandlungsschema behandelt wurden (n=107). Der Vergleich dieser Ergebnisse mit historischen Datensätzen von Schlafkrankheitspatienten, die während der vorhergehenden zwei Jahre in denselben Zentren behandelt wurden, ergab folgendes Bild: In der Studienpopulation trat ES in 11,2% (CI 5-17%) der Fälle auf und in den historischen Daten wurde ES in 13% (CI 9-17%) der Fälle nachgewiesen. Die entsprechenden Mortalitätsraten in beiden Populationen betrugen 8,4% (CI 3-13,8%) beziehungsweise 9,3% (CI 6-12,6%). Die historischen Daten gestatteten keine Beurteilung bezüglich der Wirksamkeit der Standardbehandlungsschemata, da routinemässig keine systematischen Nachkontrollen durchgeführt wurden. Die Wirksamkeit des 10-Tage-Behandlungsschema mit Melarsoprol war äusserst zufriedenstellend: bei allen Patienten waren 24 Stunden nach Abschluss der Behandlung keine Tryoanosomen mehr nachzuweisen. 99% aller Patienten, die entlassen wurden, galten sechs Monate später als klinisch geheilt. Die zwölfmonatige Nachbeobachtung dauert zur Zeit dieser Berichterstattung noch an. Jedoch sind aufgrund des Wirksamkeitsprofils der "Proof of Concept" Studie keine Probleme bezüglich der Wirksamkeit der Behandlung zu erwarten.

Im Vergleich zu den historischen Daten, die den Verlauf der Melarsoprol Therapie nach nationalen Behandlungsschemata beschreiben, zeigen die vorliegenden Studienergebnisse, dass schwerwiegende Nebenwirkungen (wie ES oder Tod) nicht häufiger auftreten, wenn die Patienten mit dem 10-Tage-Schema behandelt wurden. Jedoch führt diese Behandlungsmethode zu einer Verkürzung des Spitalaufenthalts von durchschnittlich 29 auf 13 Tage ($p<0.0001$).

Da unterschiedliche Krankheitsgrade in Ostafrika beschrieben sind, haben wir in einer separaten Analyse die Krankheitsbilder in Uganda und Tansania verglichen. Eine Zusatzstudie basierend auf der molekularen Charakterisierung der Trypanosomen bestätigte, dass alle Patienten mit *T.b. rhodesiense* infiziert waren und somit die Befürchtung einer Überlappung der Distributionsgebiete von *T.b. gambiense* und *T.b. rhodesiense* in unserem Studiengebiet nicht bestätigt werden konnte.

Basierend auf unserer Erfahrung in der Durchführung von klinischen Forschungsprogrammen in Gebieten mit limitierten Resourcen, schrieben wir einen Bericht, der Mindestanforderungen an Sponsoren und Studienländer (sog. host countries) definiert. Dies soll gewährleisten, dass die klinische Forschung auch in diesen Gebieten internationalen Qualitätsnormen entspricht.

Chapter 1

General Introduction

Cattle herders, Urambo District, Tanzania

Human African Trypanosomiasis (HAT)

Epidemiology

Sleeping sickness is the name used to describe the human form of African trypanosomiasis (*Trypanosoma spp*.), a protozoal parasitic disease that affects humans, livestock and many sylvatic species in much of sub-Saharan Africa (1). The disease is concentrated in poor and rural areas and the socio-economic impact is considered very high. The affected population suffers from economic losses due to reduced workforce and family disruption (2). Furthermore, estimates of the total losses due to trypanosomiasis range from 1.3 to 5 billion US$ depending on the methodology used (3).

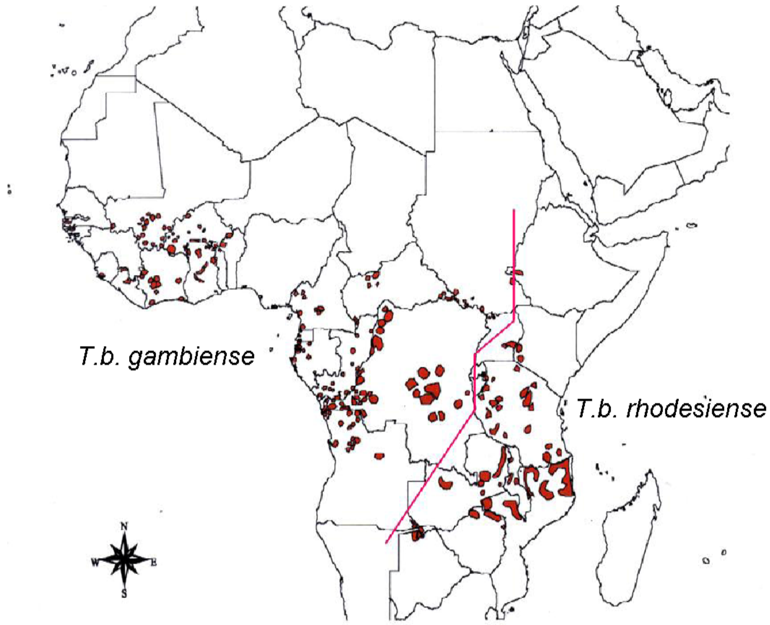
If only the incidence is considered, HAT appears to be a minor health problem compared to other parasitic diseases. But in terms of disease burden expressed in DALYs (disability-adjusted life years), HAT ranks third of all parasitic diseases in sub-Saharan Africa, just behind malaria and helminths (4).

Classically, there are three subspecies of *Trypanosoma brucei.* Two subspecies (*T.b. gambiense* and *T.b. rhodesiense)* are human pathogens and transmitted by the saliva of blood-sucking tsetse flies (*Glossina spp*.). *T.b. gambiense* and *T.b. rhodesiense* are stratified according to their geographical location but are morphologically indistinguishable (5). Their occurrence is more or less separated by the Great African Rift Valley: *T.b. gambiense* is found in West and Central Africa and *T.b. rhodesiense* in East and South Africa.

In contrast to the genetically heterogeneous *T.b. rhodesiense* of East Africa, *T.b. gambiense* exhibits very limited genetic variability. For *T.b. rhodesiense*, the serum-resistance-associated (SRA) gene is ubiquitous and conserved (6, 7) and allows an unequivocal identification. Hence, the SRA gene has gained central importance in the differentiation of the two parasites, which used to be possible only by the analysis of isoenzymes and DNA characteristics. The phylogenetic relationships between the *T. brucei* subspecies suggest two groups of *T.b. gambiense* parasites (group 1 and 2) and non monophyly for *T.b. rhodesiense*. Recent findings suggest the genetic variability of *T.b. rhodesiense* results from multiple and independent evolutions from *T.b. brucei* (8).

A characteristic feature of the epidemiology of HAT is its highly focal distribution. Today, 36 out of 53 African countries are affected with nearly 200 separate foci, and 60 million people are living in areas where HAT might be potentially transmitted.

Figure 1: Reported foci for HAT (source: WHO 1998).



Also typical for the epidemiology of HAT are long periods of endemicity interspersed with epidemics. The potential for the development of explosive epidemics is of high public health importance (9). In the past, large scale HAT epidemics were fatal for many thousand people. Between 1900 and 1920, Uganda experienced one of the most severe epidemics of sleeping sickness ever recorded. In the Buganda region along the shores of Lake Victoria and its drainage rivers, more than 200,000 people are believed to have died before the decline of the epidemic (10). The history of sleeping sickness is characterized by such waves of epidemics, resurgences and outbreaks. Nevertheless, the disease has been practically brought under control by the end of colonial times. Interventions such as continuous disease surveillance, large-scale campaigns of active case search, vector control and depopulations of affected areas have proven to be effective in reducing the prevalence and incidence of sleeping sickness. In the 1960s there was even a fair chance of disease elimination. However, after independence, the priorities shifted and the surveillance of sleeping sickness was neglected: national health authorities were not giving attention to sleeping sickness control, and civil and political unrest as well as the lack of adequate sources and competing national health priorities resulted in new epidemics, the recrudescence of many old foci and the appearance of new ones (11). The prevalence rate of trypanosomiasis rose again from 0.01 % of the population to 1-2 %. In 1998, the number of prevalent cases per year was estimated at 300'000 (12). In addition, there was also a dramatic lack of awareness about the disease situation. The neglect of HAT control and surveillance also lead to the point where physical structures and human resources were no longer available (13) and it took a long time to control the recrudescence. Increased control activities during the past 20 years have led to a substantial reduction in the number of new cases reported: in 2004, 17'500 new cases were reported, equivalent to an estimated 50'000 – 70'000 existing cases because of the significant underreporting. In 2005, the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) recommended that "WHO should launch an elimination program for sleeping sickness and adopt strategies towards this goal and advocate all partners who have permanently provided support to maintain their efforts and assistance" (14).

Epidemiology of *T.b. gambiense* HAT versus *T.b. rhodesiense* HAT

An accurate reflection of the magnitude of HAT is hampered; the rural areas are poorly covered by national health services; less than 10% of the at-risk population is under adequate surveillance and there is a significant under reporting of new cases. The available epidemiological data on HAT has to be recognized as an estimate of the real situation in the HAT affected areas.

All sub Saharan countries affected by HAT are categorized by the number of new cases reported per year. Table 1 and table 2 summarize the data from the latest available epidemiological update (WHO 2006) (14).

Table 1: Number of new cases reported per year in *T.b. gambiense* affected countries (WHO 2006)

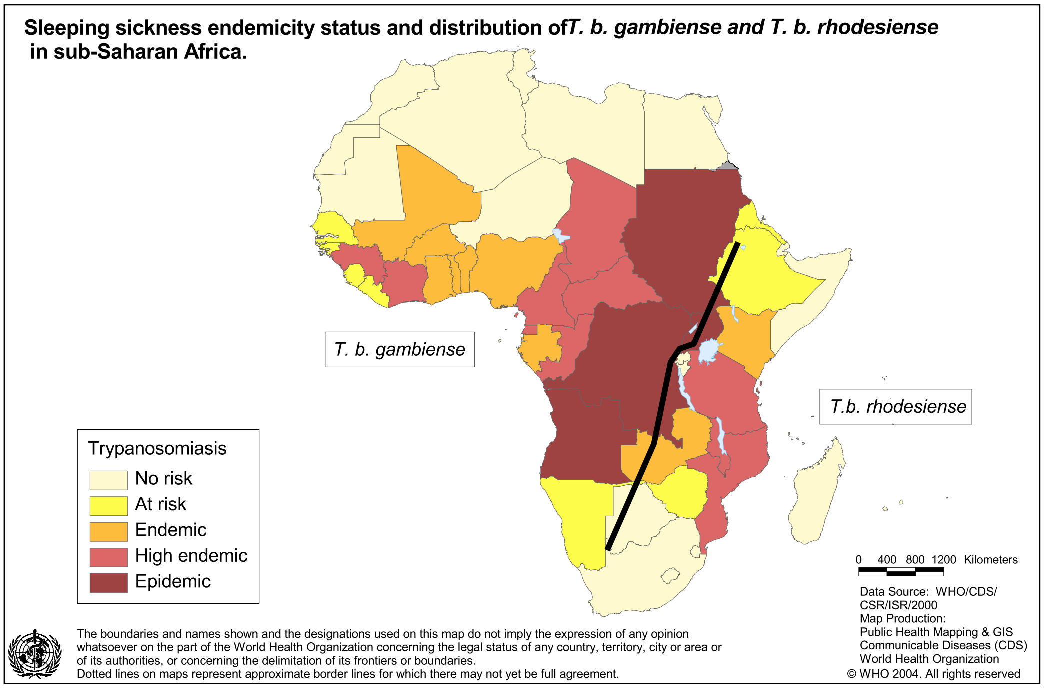
More that 1500 cases	Democratic Republic of Congo, Angola, Sudan
50-1500 cases	Central African Republic, Chad, Congo, Côte d' Ivoire, Guinea, Uganda
Fewer than 50 cases	Burkina Faso, Cameroon, Gabon, Equatorial Guinea, Nigeria
Zero cases (with surveillance activities)	Benin, Ghana, Mali, Togo
Zero cases (no surveillance)	Gambia, Guinea Bissau, Liberia, Niger, Senegal, Sierra Leone

Table 2: Number of new cases reported per year in *T.b. rhodesiense* affected countries (WHO 2006)

50-1500 cases	Malawi, Uganda, Tanzania
Sporadically fewer than 50 cases	Kenya, Mozambique, Rwanda, Zambia, Zimbabwe
Zero cases	Botswana, Burundi, Ethiopia, Namibia, Swaziland

Over 90% of the reported HAT cases are due to *T.b. gambiense* (15). Currently most of those cases originate from the Democratic Republic of Congo (DRC). In 2004, 97% of all reported cases were *T.b. gambiense* infections and only 3% were *T.b. rhodesiense* infections (14). But the overall decrease of reported HAT infections in the past 20 years has not been significant for *T.b. rhodesiense*, indicating that the focus on the human reservoir alone is insufficient (14) for effective disease control. *T.b. rhodesiense* is clearly the more neglected form of HAT. Disease awareness and surveillance is very poor and limited to passive case detection in few health centres that have the capacity to diagnose HAT. Not only the under reporting of new cases but also the under detection of death related to HAT is believed to be very high. A deterministic model has been developed to estimate the proportion of undetected *T.b. rhodesiense* cases in a given population on the basis of knowledge of the early to late disease stage ratio (16). The data used for the design of the under-detection model were obtained from an area in Uganda with a relatively low expected probability of under-detection (17), as it had better health service and better infrastructural components compared with other sleeping sickness areas in Africa. The model estimated that with passive case detection, 20% of the first stage and 42% of the second stage infections were reported and that for every reported death, 12 deaths went undetected. The model was also applied for estimating the undiagnosed deaths that may have sought health care during a HAT resurgence (2000–2002) in this same area and estimated that approximately 85% of the patients who died undiagnosed, entered the health system at some stage, and that one-third of those, died undiagnosed. It would thus be expected that less-developed areas would have an even more alarming rate of under detection, and hence mortality. These estimates of deaths due to HAT emphasize the magnitude of the burden of the disease, especially where it occurs in epidemics in areas with poor surveillance (18).

Figure 2: Distribution of HAT in sub Saharan Africa, by endemicity levels of the countries affected (© Map: Source: WHO 2004).



The location of disease transmission and the transmission cycle differ for the two forms of HAT: *gambiense*-carrying tsetse live near water in predominantly riverine vegetation associated with drainage lines, rivers and more permanent bodies of water such as lakes (19). These flies feed on aquatic reptiles and with a possible exception of domestic pig; man is the only mammalian host for *T.b. gambiense*. The cycle of transmission essentially involves man and tsetse (20). Since Gambian HAT causes a chronic infection over a long period, people can function normally for months while carrying the disease. If an infected person regularly access tsetse infested water sources, over time, this person can infect a large number of flies who might then infect other people (21).

The *rhodesiense*-carrying tsetse are savannah species, "game flies", ranging further from water and feeding on wild and domestic animals. *T.b. rhodesiense* is a zoonotic disease and humans are accidentals host in the transmission cycle, and rapidly killed. Because of the quick development of the disease, infected people are soon incapacitated and resting at home. For *T.b. rhodesiense* HAT, animals are the primary reservoir for the disease and transmission is usually from animals to people (21). Men risk infection with *T.b. rhodesiense* through physical proximity to infected livestock, farming and building on tsetse country or when passing through it as a traveller or a hunter.

Situation in Tanzania

Rhodesiense sleeping sickness, which was first reported in the 1920s and 1930s, was endemic in eight regions of Tanzania: Arusha, Kigoma, Lindi, Mbeya, Kagera, Rukwa, Ruvuma and Tabora. In 1929 a total of 2'129 cases were recorded in Kahama district. In 1939, 10 years after the original outbreak, recrudescence occurred in the districts of Tabora and Kahama. This outbreak was brought under control by re-establishing the policy of preventing families from settling themselves up in small villages scattered in the bush and by intensifying the campaign for the diagnosis and treatment of cases.

The currently active foci of HAT are located in the poorest parts of the country. And approximately 4 to 5 million people are at risk of infection, only 1% of these are under regular surveillance. In the past 30 years, the number of new cases reported has risen above 500 per year (22) which is certainly an under estimate. Transmission takes place in the three regions of Kigoma (Kibondo and Kasulu districts), Tabora (Urambo districts) and Rukwa (Mpanda and Nkansi districts). In those regions the health centres are sparse, some not accessible throughout the year and many lack trained personnel, equipment and preparedness for sleeping sickness patient management and diagnosis. Sporadically, cases are also observed in Serengeti National Park. Especially the reports of tourists who got infected with *T.b. rhodesiense* during visits in Tanzanian national parks (23) have drawn more attention to HAT and activated discussions about interventions for disease control and surveillance (24).

As a consequence of the Great Lakes Crisis in the beginning of 1994, the International Federation of Red Cross and Red Crescent Societies (IFRC) have established refugee camps in the regions of Kasulu and Kigoma (1997). The Kigoma District received a massive influx of Congolese refugees which are potentially infected with *T.b. gambiense*. With the ongoing civil movement, *T.b. gambiense* could potentially be introduced in this region. The risk of such an overlap should be carefully monitored.

Figure 3: HAT endemic regions in Tanzania (source: NIMR Tabora, 2005).

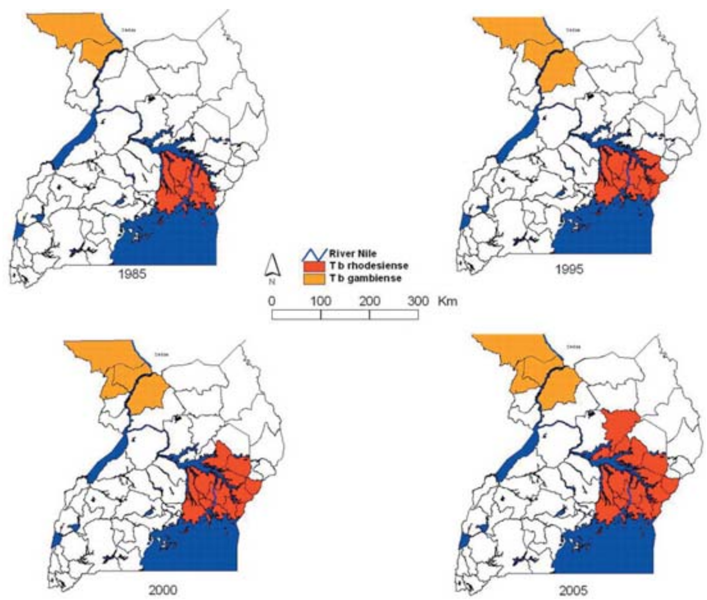
Research and control of HAT in Tanzania are under the National Institute for Medical Research (NIMR), Tabora Centre. The Tabora Research Centre is mandated to carry out, coordinate, promote and document research and control of HAT in Tanzania. It is also mandated to formulate priorities for HAT research and control as well as to monitor all aspects of sleeping sickness in the country.

Situation in Uganda

Uganda is the only country affected by both forms of the disease; *T.b. gambiense* in the North-West and *T.b. rhodesiense* in the South-East of the country. Three major epidemics of sleeping sickness were recorded in south-east Uganda since the 1890s (25). During the 1970s and 1980s Uganda experienced extensive internal displacement of the rural population, illegal human and cattle movements, growth of favourable tsetse habitats on cotton and coffee plantations, and a collapse of sleeping sickness prevention and control methods. These events likely contributed to increased human vector contact and sleeping sickness transmission. In 1976 a *T.b. rhodesiense* outbreak was detected in the western Iganga district which was the beginning of an extensive epidemic that spread throughout south-eastern Uganda (26). The peak was between 1980 and 1988, with more than 4'000 new cases alone in 1986 (27). In 1998, first reports of local sleeping sickness transmission were recorded in the Soroti district, a previously disease free area. During four years prior to this outbreak, the cattle population in the district had grown by 660% as a consequence of the country wide cattle restocking projects, which were part of the national poverty eradication action plan (PEAP). The cattle restocking activities were linked to the introduction of the parasite: 50% of the traded cattle had originated from endemic sleeping sickness areas (28). The disease has since spread to the adjacent districts of Kaberamaido, Kumi and Lira which are more remote areas and were not equipped to handle the situation. These areas had also extensively suffered from civil unrest due to the presence of rebels from the Lord Resistance Army (LRA).

Due to the growing closeness of the two disease foci (see figure 4) and continuous movement of the livestock reservoir, a potential overlap of the two yet distinct diseases areas has become very likely and must be monitored carefully (29).

Figure 4: Sequential maps of HAT affected areas, Uganda (source: Picozzi et al., 2005).



In the 1990, Uganda has established the Control Council for Trypanosomiasis under the Ministry of Agriculture. It is responsible for all activities in animal and human Trypanosomiasis. The executive body is the Coordinating Office for Control of Trypanosomiasis in Uganda / COCTU which coordinates all research and control activity in the country.

Implications of the potential overlap of disease distribution areas

Any convergence of the currently geographically distinct distributions of *T.b. rhodesiense* and *T.b. gambiense* HAT will have important implications for patient care and national control policy (30).

The treatment of sleeping sickness differs in the two forms of the disease and the infective subspecies directs the choice of drugs. So far, the identification of the infective strain is not possible under field conditions, only modern laboratories are able to perform the required PCR analysis. Proper diagnosis and treatment of the patients will impose a major public health issue to the countries at risk of an overlap in disease distribution areas. A close monitoring of the situation is crucial; relevant strategies to prevent the further spread of the disease and the likely overlap of the two disease zones should be urgently implemented.

Recent findings demonstrate that the disjunct distribution of the *Trypanosoma* parasite in Uganda can not be explained by a genetic heterogeneity of the vectors that theoretically could be responsible for an incompatibility between vector populations and parasite (31). Further, the genetic recombination of different parasite strains was successful under laboratory conditions: two clones of *Trypansoma brucei* were successfully crossed indicating that the genetic recombination between trypanosome populations transmitted within the same epidemic might occur also under natural conditions (32).

Clinical presentation

The clinical presentation of *T.b. gambiense* and *T.b. rhodesiense* HAT is remarkably different. The highly virulent *T.b. rhodesiense* causes a precipitated evolution of the disease and presents as an acute illness that is fatal within weeks or months if left untreated (33). *T.b. gambiense* is a chronic disease with elusive and mild symptoms for months, and insidious evolution towards the nervous stage (34). A wide spectrum of disease severity has been described for *T.b. rhodesiense* HAT ranging from a chronic disease pattern in southern countries of East Africa with existing reports of asymptomatic carriers (35) to an increase in virulence towards the north (36). Even though those differences were already described more than 60 years ago (37) there is a lack of data about those differences.

The onset and the first stage of both forms of the disease are similar: a sign of infection may be the development of a lesion at the site of the infective bite. Parasites proliferate and, occasionally, lead to a nodule or ulcer called a trypanosomal chancre. This trypanosomal chancre is commonly seen in white but rarely in black populations (20). Clinical symptoms commence by an uncharacteristic general malaise. Fever, headache, joint pains, transient oedema, pruritus, splenomegaly and lymphadenopathy may accompany the so-called first or haemolymphatic stage of HAT. Typical for *T.b. gambiense* HAT is the so called Winterbottom sign; enlarged, painless, rubbery cervical lymph nodes in the posterior cervical triangle. Typical for *T.b. rhodesiense* HAT are signs of myocardial involvement. In general it is very difficult to distinguish such symptoms from other tropical fevers, such as malaria, bacterial meningitis or enteric fever.

With the parasite's penetration into the central nervous system patients enter the second or meningo-encephalitic stage which ends fatal if left untreated. In the second stage of the disease neuropsychiatric signs and symptoms occur: severe endocrinological and mental disturbances, such as impotence, infertility, amenorrhea, delirium, mania, paranoia, schizoid attacks, aggressive behaviour and severe motor problems are the main signs. Compared to the *T.b. gambiense*, less demarcation between first and second stage illness is observed in *T.b. rhodesiense*. The CNS involvement in *T.b. rhodesiense* infections can be clinically limited to drowsiness and tremor (38).

In *T.b. gambiense* infections, the interval between the start of the infection and the start of the second stage is in the order of months or years (38) Recently, the mean time to reach the second stage has been estimated at over one year and the mean time to death at almost 3 years (39). The study of the duration of symptoms in *T.b. rhodesiense* showed that the disease progressed to the stage of central nervous system involvement between three weeks to two months of infection and that most (> 80%) deaths occurred within six months of illness (17), often due to cardiac failure or secondary infections.

Recently, the neuropathogenesis of second stage HAT has been reviewed. Clinical features can be grouped into categories such as psychiatric, motor, sensory and sleep abnormalities (33). The more advanced the disease the more deregulations of the 24-h distribution of the sleep-wake pattern can be observed. An alteration of the sleep structure, with frequent sleep onset rapid eye movement (REM) periods (SOREMPs) is seen in stage II patients (40). The fragmented sleep patterns are perceived as daytime somnolence and nocturnal insomnia. The name of the disease is resulting from this observation - formerly also known as the Negro lethargy.

Until today, the underlying mechanisms of CNS invasion are not known in detail. Frequently observed is meningoencephalitis. The meninges are infiltrated with lymphocytes, plasma cells and occasional morular (Mott) cells. The inflammatory cell infiltrate extends along the Virchow-Robin spaces into the substance of the brain producing the characteristic picture of perivascular cuffing (41). An immune response is certainly involved in the process; increased levels of antibodies can be detected and thus supports the inflammatory process. Further, the number of white blood cells (WBC) as well as the protein content is elevated in the cerebrospinal fluid (CSF).

Even though many hypotheses are debated, there is no detailed description of the process of CNS invasion and its direct consequences.

The ability of *T. brucei ssp.* to survive free in blood is due to its remarkable degree of antigenic variation. The surface coat of densely packed glycoproteins differentiated already when specific antibodies follow up. Thus leads to misdirection of the immune response and gradual exhaustion of the patient's immune system.

Control measures

African and colonial control measures

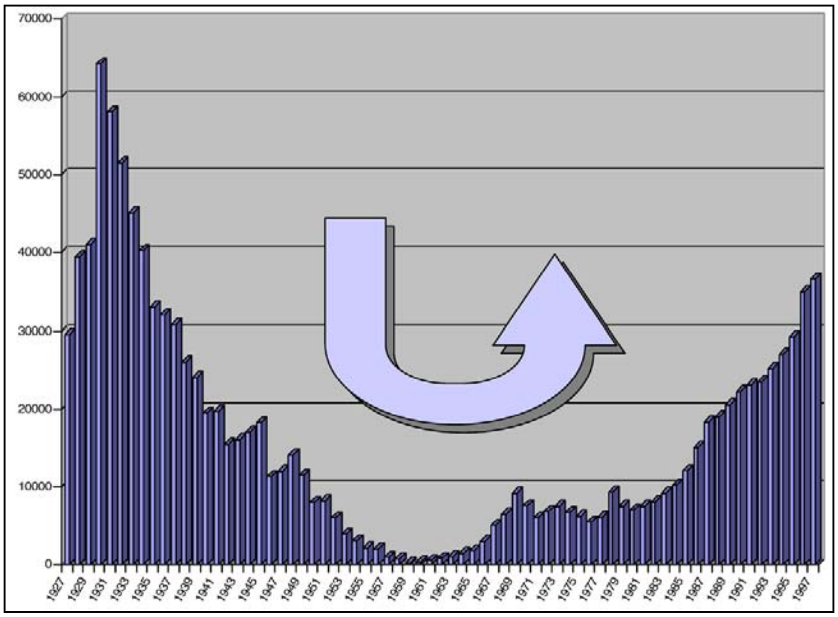
Traditionally, trypanosomiasis was controlled through bush clearing, game control, settlement patterns and careful movement of livestock and protective ointments for animals. Pastoralists in East Africa had sophisticated understanding of tsetse environments; herders in western Narok, Kenya, when expanding into fly-infested bush, first grazed goats in an area to prepare the way for the final reoccupation by cattle (21).

Colonial powers were very concerned by epidemics of the human disease and the chronic loss of livestock impeding both transport and agriculture (42). The first sleeping sickness mission by the French government to French Equatorial Africa in 1906, was sent because "the economic future of the Congo is tied to the question of human trypanosomiasis" (Congo Service de santé militaire by Martin, Leboeuf, Roubaud, 1906-08).

Colonial powers implemented a combination of targeting trypanosomes, tsetse and people through biological and medical control. Major programs for tsetse control were focused on bush-clearing (to eliminate tsetse resting sites), wild game culling (to reduce the parasite reservoirs and host availability for tsetse), and insecticide spraying of tsetse resting sites. Large-scale depopulations of tsetse infested areas were common only in East Africa. Medical interventions emphasized on the elimination of trypanosomes in people through medical examinations, the isolation of patients and drug treatment. Colonial doctors carried out patient observation, examinations, segregations and drug testing in sleeping sickness camps, clinics and hospitals. The medical control required mobile teams feeling lymph nodes, testing blood and sending infected patients to quarantined medical facilities. A further attempt in effective disease control was prophylactic treatment with pentamidine based on 6-monthyl injections (43).

The successes of the sum of those control measures were reflected in decreasing numbers of patients and disease elimination was almost achieved in the 1960ies. The rarity of HAT cases, and a decline in awareness of how the disease could return, led to a lack of interest in disease surveillance. Over time the disease slowly returned, and some thirty years later, flare-ups were observed throughout past endemic areas (figure 5) (44).

Figure 5: New cases of sleeping sickness reported for all Africa between 1927 and 1997 (source: Simarro, 2008).



Current control measures

Over the past 20 years new and more sophisticated tools for trypanosomiasis control have been developed compared to the tools available when sleeping sickness was almost eliminated. These advantages contrast with the present epidemiological situation in which the disease is increasing because of a number of socio economic and political factors (15).

HAT control relies on two principles: reduction of the human-fly contact through vector control and the reduction of the parasite reservoir through case detection and treatment. For *T.b. rhodesiense* affected areas, the control of trypanosome infections in the animal reservoir is crucial: in endemic areas, the incidence of *T.b. rhodesiense* in cattle is up to 20% (45) and the dynamics of transmission during the 1988 to 1990 epidemic in Tororo, Uganda showed that tsetse were 5 times more likely to pick up human infective parasites from cattle than from humans (46). The mass treatment of cattle has been advocated as an effective strategy for control of the spread of the disease (28) but this approach can be hindered by the emergence of cross resistant trypanosomes between the drugs in use to treat humans and animals (47). An example of current risks is the extensive human and livestock trafficking between Uganda and southern Sudan that could possibly lead to the introduction of *T.b. rhodesiense* in southern Sudan.

Tsetse control

Today, vector control strategies exist of series of control projects following administratively-defined boundaries rather than covering biologically-relevant areas (48). The destruction of the vector is possible through insecticide ground spraying and insecticide-impregnated traps and screens. Because of the colour and the shape of the traps, the tsetse is attracted and hold captive. Early traps have been barely effective but improved biconical, monoconical and pyramidal traps, also combined with olfactory baits, have improved trap efficacy. Further the development of simple screens, impregnated with insecticides, that kill tsetse flies that come in contact with them allowed a more wide spread use. Traps and screens have replaced insecticide spraying; they are effective, environmentally friendly and also suitable for use by the communities themselves. The number of traps and/or screens required for a particular location depends on the type of vegetation and the frequency and intensity of human-fly contact (15). Such vector control programs are technically successful whilst in operation but unsustainable once the formal project reached its endpoint.

Rather different was the tsetse eradication project on the island of Zanzibar sponsored by the International Atomic Energy Agency (IAEA). Although designed as a test-bed for tsetse control by large-scale release of laboratory-reared radiation- sterilized male tsetse (SIT) – and so proving rather costly – the project succeeded in complete elimination of tsetse (*Glossina austeni*) from the island (49) raising the question of whether or not similar tsetse elimination might be possible on mainland Africa.

The idea of a Pan-African initiative against tsetse and trypanosomiasis was discussed and recommended at the 25th ISCTRC (International Scientific Council for Trypanosomiasis Research and Control) in Mombasa, Kenya, in October, 1999. The recommendation was presented to the 36th summit of the African Union (AU) in Lomé, July 2000. The Heads of State and Government passed a resolution recognizing the seriousness of the tsetse and trypanosomiasis problem, and calling on member states "to act collectively… to render Africa tsetse-free within the shortest time possible". With this mandate, the AU set up the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), which is now an integral part of the AU Commission for Rural Development. PATTEC was formally launched at the 26th ISCTRC meeting in Ouagadougou in

October 2001. Within PATTEC; the responsibilities are segmented in human health (WHO), animal health (FAO) and vector control (IAEA).

Case detection

Case detection has been the corner stone of Gambian trypanosomiasis control since the beginning of the 20th century (15). Today, case finding surveys are only carried out in *T.b. gambiense* areas. Infected cases may remain asymptomatic for many months and specialized case finding teams are set up to detect patients while they are still healthy. To reach remote villages, achieve high community participation and examine large numbers of people is logistically difficult, requires skilled man power and is very expensive.

In east Africa, there is not active case detection. The limited number of patients and the lack of adequate screening tests make active case search highly cost ineffective.

Once patients are identified, definitive diagnosis is required and these patients need to be treated.

Diagnosis

The clinical profile of HAT is too diffuse to allow for a direct differential diagnosis. Accurate diagnosis is dependent on specific and sensitive methods which are still lacking today and therefore, only the proof of the parasites presence in blood, lymph nodes and/or in the cerebrospinal fluid can confirm the infection with trypanosomes.

T.b. gambiense HAT can be serologically diagnosed using the CATT test (Card Agglutination Test) (50) followed by microscopic confirmation of the parasite in blood and/or lymph. The CATT test detects specific trypanosomal antigens but the test lacks necessary sensitivity and specificity for a proof of the disease and is therefore only used for screening. The CATT is very useful for the mass screening of populations for *T.b. gambiense* area, but is not applicable in the detection of *T.b. rhodesiense* (51). The diagnosis of *T.b. rhodesiense* has to entirely rely on microscopy. The sensitivity of this method depends on the level of parasitaemia and the technicians' experience. An exception is the successful use of the CATT test in Malawi. This indicates closer genetic similarities between local *T.b. rhodesiense* stains in Malawi with *T.b. gambiense*.

The standard technique to find trypanosomes is to do thick blood smears. Staining is required and it is important to do it repetitively. Each blood smear has to be examined for at least 10 minutes. A frequently used concentration method is the microhaematocrit centrifugation technique (m-HCT) (52). After high speed centrifugation (haematocrit centrifugation) the trypanosomes concentrate on the "buffy coat", the layer of white blood cells, between the serum and erythrocytes. Microscopic examination at low magnification is done to see mobile trypanosomes. Moving microfilaria can obscure the smaller trypanosomes. This method is widely used in the field and also known as the Woo-test. In the case of high suspicion without being able to detect the trypanosome, the mini anion exchange centrifugation technique (mAECT) can be an alternative (53). By anion chromatography the trypanosomes get separated from venous blood. mAECT asks for tedious manipulation but has proved to be more sensitive than other parasitological methods of diagnosis. As a laboratory method it is only in use in centres with a high technical standard. A kit has been developed for field use (12) but due to high costs it never became an analytical standard.

Iit is estimated that 20 to 30% of patients are missed by the standard parasitological techniques (54).

A new technique in use for the detection of *T.b. rhodesiense* is the sensitive and selective polymerase chain reaction (PCR) method that amplifies the SRA (serum resistance associated) gene. This gene is found in *T.b. rhodesiense* strains only and it directly correlates to human infectivity (55). The SRA gene is a low-copy gene and therefore PCR is inadequate to amplify this target reliably in clinical samples without resources to parasite multiplication in mice. Recently, the loop-mediated isothermal amplification (LAMP) of DNA has been developed. The LAMP test is carried out under isothermal conditions of 60–65°C and produces a large amount of DNA (56).

LAMP was successfully used to detect *T.b. rhodesiense* with a sensitivity of one trypanosome/ml blood and the results can be read by visual observation of colour change. The amplification of DNA is possible by the use of a water bath only which makes the use of LAMP an efficient and robust test in the field. The availability of this test would finally allow that molecular diagnosis for case detection and confirmation of cure can become feasible in regions endemic for *T.b. rhodesiense* HAT. Currently, the validation process of the test is ongoing and the Foundation for Innovative New Diagnostics (FIND) is planning the production of a diagnostic kit that is suitable for the use in the field.

Once the presence of trypanosomes in blood and/or lymph is confirmed, a lumbar puncture has to follow for stage determination. Correct stage determination is crucial as second stage treatment is risky and the patients can suffer from serious adverse drug reactions. Either the presence of trypanosomes and/or an increase of the leukocyte count (≥ 5 cells/mm^3) in the cerebrospinal fluid indicate the second stage. Increased IgM values in CSF were found to be another marker for stage determination (57). Earlier, protein content in the CSF above 25 mg per

100 ml (method of Siccard & Cantaloube) also indicted the second stage of the disease. With time it was shown that the involvement of the CNS should be based on the WBC count only (58) as it is more reliable than the protein determination (12).

Better tools for stage determination are urgently needed. The invasiveness of the lumbar puncture is risky, even more under field conditions and exposes patients to other risks, such as bacterial infections, epidural bleeding or trauma to the spinal chord. Also, the LP is not well accepted by the local population who often see it as a threat and associate other risks like infertility and disease transmission with it (59, 60).

Part of the Foundation for Innovative New Diagnostics (FIND) portfolio is to develop new diagnostic tools for first and second stage HAT. FIND has contracted WHO for the establishment of a HAT specimen bank. Throughout sub Saharan Africa specimens of saliva, urine, plasma and CSF are collected and shipped to the Institute Pasteur, Paris, France where the specimen bank is physically located. Research groups can access those samples after an approval of request by the joint committee of FIND/WHO and use those samples for the development of sensitive and specific diagnostic tools.

Treatment

Treatment of HAT is very unsatisfactory; there is no existing vaccination and only a few effective drugs are available. Whereas new drugs for single-dose oral treatment of many parasitic diseases are obtainable, treatment of sleeping sickness mainly relies on drugs developed before 1950 which are administered parenterally in repeated doses. These drugs cause many adverse drug reactions, some of which can be even fatal. New and safe drugs for the treatment of HAT are urgently needed. At a certain point pharmaceutical companies producing anti-trypanosomal drugs decided on the cessation of the production. Only through substantial efforts by WHO and the médecins sans frontièrs (MSF) campaign to essential medicines group, contracts could be signed with and Aventis (2001) Bayer (2002). Today, both companies supply the drugs free of charge, in the quantities requested by the WHO.

First stage treatment

For first stage treatment, two drugs are in use: pentamidine, an aromatic diamidine, for *T.b. gambiense* infections and suramin, polysulphonated naphtylurea, for *T.b. rhodesiense* infections.

Pentamidine (Pentacarinat®) has a long history of clinical use. Since the beginning of the 1940's it has been used to treat African Trypanosomiasis and Leishmaniasis. The development of pentamidine stems from the research on synthalin, a potent hypoglycaemic agent that exhibited significant trypanocidal activity. Pentamidine is administered as an intramuscular injection of 4mg of pentamidine isethionate per kg bodyweight once daily for 7–10 days. Main adverse reactions include hypotension, renal and hepatic toxicity, pancreatitis and cardiac dysfunction (61). The mode of action is not fully understood. *In-vitro* studies indicate that pentamidine interferes with nuclear metabolism causing inhibition of DNA, RNA, phospholipids and proteins synthesis. Pentamidine has been widely employed for chemoprophylaxis, for instance in the former Belgian Congo (62). Applied as an intramuscular injection of 4mg/kg it was assumed to protect against *Trypanosoma* infection for several months. This practice was abandonment since the dose is sub-curative and may mask an underlying infection. Moreover, the prophylactic use has provoked resistance in several areas. Still, it is effective to treat first stage trypanosomiasis and is used even though it is toxic and inactive when given orally.

Suramin (Bayer 205, Germanin, Antipyrol, Belganyl, Fourneau 309, Moranyl, naphuride, naginin, naganol), was developed in Germany in 1916. Its development was based on the observation that the dyestuffs trypan red, trypan blue, and afridol violet cured trypanosomiasis in mice. In 1920, suramin was introduced into clinical use for treatment of African river blindness and early stage human African trypanosomiasis. It is administered intravenously as suramin sodium at a dosage of 20mg/kg every 5 or 7 days to a maximum of 5 doses. It is normal clinical practice to start with a small dose to assess the patient's tolerance of the drug. Therefore, a test dose of 5mg per kg of bodyweight is given on the first day, followed by 20mg per kg of body weight (up to am maximum of 1g) on days 3, 10, 17, 27, and 31 (12). The once a week administration schedule is sufficient, since suramin's plasma half life is reported to be 41-78 days (63) or in onchocerciasis patients even 92 days (64). Suramin is a symmetrical, polysulphonated polyaromatic urea, highly charged at the physiological pH and does not cross the BBB. After intravenous administration it circulates in the blood in tight association with serum albumin and low density lipoproteins. Suramin accumulates only slowly in trypanosomes and uptake occurs probably by receptor mediated endocytosis bound to the low-density lipoprotein. The different drug-uptake dynamics of host and parasite are believed to form the basis of the dissimilar toxicity profiles. Suramin is deposited in the renal tubes and should not be administered to patients with renal disease. Urine should be checked before and during treatment for proteinuria. The major adverse drug reactions are proteinuria, reversible liver damage, and nephrotoxicity with renal impairment. But also fever, joint pain, pruritus, exfoliative dermatitis, haemolytic anaemia, agranulocytosis, jaundice, hepatitis and diarrhea have been observed.

Since 1920 suramin has been tested against various other indications such as cancer, cardiovascular diseases, autoimmune hepatitis, incontinence and heartburn. However, treatment with suramin is not satisfactory and wouldn't pass today's standards for drug safety (65).

However, patients often get diagnosed in the second stage of the disease. Neither suramin nor pentamidine cross the blood brain barrier (BBB) sufficiently to yield anti-trypanocidal concentrations in the CNS. Therefore it disqualifies those drugs from treating second stage patients.

Second stage treatment

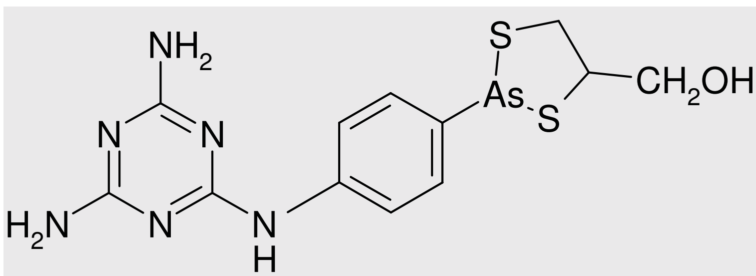
Due to the lack of drugs able to pass the BBB the second stage of the disease is more difficult to treat. Only melarsoprol and eflornithine are able to sufficiently pass the BBB to reach required drug levels in the CNS.

Melarsoprol (MelB, melarsen oxide-BAL, Arsobal®), an organic, trivalent arsenical, was introduced by Friedheim in 1949. It was the greatest advance in chemotherapy of HAT since suramin and the pentavalent arsenical tryparsamide, both introduced in the 1920ies. At the time, tryparsamide was the only effective drug once the central nervous system has become affected and this only in the Gambian disease. The Rhodesian infection were totally uncurable; a person infected in East Africa and ill for more than about one month was almost certainly doomed (20). Melarsoprol, respectively its active metabolite melarsen oxide, is effective against both, *T.b. gambiense* and *T.b. rhodesiense* infections. However, its high toxicity and frequent adverse drug reactions limit the use to second stage therapy.

Eflornithine (α-Difluoromethyl ornithine / DFMO, Ornidyl®) was first developed as a cytostatic drug and was registered for the use against HAT in 1990 (USA) and 1991 (France), (66). It is active in *T.b. gambiense* infections but for biochemical reasons it has only very limited activity in *T.b. rhodesiense* (67). The widespread use of eflornithine is hampered by the complicated application (four daily infusions for two weeks) and the high production costs of the drug. However, eflornithine is better tolerated and when administered properly, has proven to be safer than melarsoprol (68). Eflornithine is currently the first line treatment for second stage *T.b. gambiense* HAT.

Nifurtimox, a 5-nitrofuran, is not registered against HAT. It was developed for the treatment of *Trypanosoma cruzi*, the causative agent of Chagas' disease. It is a cheap, orally administered drug but not yet fully validated and not registered for use in human African trypanosomiasis (69). However, it has been in use on a compassionate basis in combination with eflornithine to treat *T.b. gambiense* cases refractory to melarsoprol. Currently, the nifurtimox-eflornithine combination therapy (NECT) is under clinical development in *T.b. gambiense* areas. Preliminary results indicate its potential as new, first line treatment for *T.b. gambiense* HAT.

Figure 6: chemical structure of melarsoprol.



The use of melarsoprol is hampered by its high toxicity and complicated dosing schemes. The treatment regimens of melarsoprol used to be empirically determined and vary considerably between countries and treatment centres and embody a real omnium-gatherum throughout Africa. Common schedules are three or four series of daily i.v. injections (with increasing or equal doses up to a maximum dose of 3.6mg/kg) for 3 or 4 days, with intervening rest periods of 7-10-day (12). Thus, total hospitalization times of up to one moth are common. The drug is administered by intravenous injection as a 3.6% solution in propylene glycol. Adverse effects such as cutaneous reactions, polyneuropathy, diarrhea, fever, or thrombophlebitis at the site of injection are quite common . The major adverse drug reaction in melarsoprol therapy is the potentially fatal encephalopathic syndrome (ES). The incidence for ES in Rhodesian sleeping sickness was found to be 8% and 5% in the Gambiense form (70), the mortality rate amongst those patients affected was approximately 50%. The concomitant use of prednisolone during melarsoprol therapy proved to reduce the incidence of ES in *T.b. gambiense* patients. A reduction of ES-associated death was also observed, but did not reach statistical significance (71). The concomitant use of steroids in *T.b. rhodesiense* patients did not reduce the incidence of ES (72, 73). Until today, the mechanism of the ES is not fully understood. The current hypothesis proposes an immunological reaction (74). In order to investigate this hypothesis in more detail, an association study was carried out where the occurrence of ES was correlated with the HLA-genotype of each patient. In a case-control study blood samples form *T.b. gambiense* patients were collected (Democratic Republic of Congo and Angola) and HLA-genotyping was performed. The association of the HLA-genotype and the incidence rates of ES showed that there is a possible correlation but the samples size is yet too small (70).

Although the drug was introduced almost half a century ago, its mode of action is not well understood. Initially, the drug was thought to act by inhibiting the trypanosomal pyruvate kinase, which is a key enzyme in African trypanosomes for production of ATP (75). Additional investigations showed that melarsen oxide, trypanothion and a major cofactor form a stable adduct. This adduct is an effective inhibitor of the trypanothione reductase. The inhibition of this enzyme leads to disturbance of the redox balance of the parasite and thus exposing the trypanosome to free radicals (76). However, this theory was questioned and it was suggested that the phospho-fructokinase, an enzyme of the glycolytic pathway and interference with energy metabolism might be the main drug target (Wang, 1995).

A further problematic progression in melarsoprol treatment is an increasing number of refectory cases observed in Gambiense sleeping sickness. Growing number of patients (up to 30%) not responding to melarsoprol treatment have been reported in Uganda, (77, 78), Angola (79), Democratic Republic of Congo, DRC (80), Sudan (unpublished data), most probable due to resistance (18). Refractory cases are treated with eflornithine or the NECT combination therapy. Repetitive melarsoprol treatment usually has a very limited success. Likely, the same scenario will sooner or later also be required for treatment of *T.b. rhodesiense* in the lack of alternatives. Melarsoprol treatment failures in *T.b. rhodesiense* patients have been reported (81) but fortunately, not yet at alarming frequencies (22).

HAT treatment requires a long-term follow-up of each patient in order to monitor long-term efficacy of the treatment. Patients are asked to present for follow-up visits after 3 or 6, 12, 18 and 24 months (12, 82). At each follow-up visit, blood and CSF samples are analyzed to confirm the absence of the trypanosomes. Due to the painful lumbar punctures and possible long geographical distances to the health centres patients are often lost to follow up. In *T.b. gambiense* areas the patient follow-up is actively supported by the mobile teams of National Control Programs or non-governmental organisations (NGOs). Without active support, follow-up attendance is approximately 40% (personal communication Christian Burri). Hence, for the monitoring of long-term efficacy in patients who participate in clinical trials substantial efforts are made to obtain high follow-up coverage rates. The follow-up of *T.b. rhodesiense* patients is hampered by the absence of mobile teams to trace and examine patients.

Second stage treatment in east Africa

For *T.b. rhodesiense* HAT, melarsoprol remains the only drug available for the treatment of second stage disease. Only in east Africa a pre-treatment with suramin is common and was introduced on purely empirical basis. It is administered in order to eliminate parasites in blood and lymph before the treatment with melarsoprol. It should prevent from (i) an initial high antigen release which might trigger major adverse reactions and (ii) the introduction of trypanosomes in the CNS while performing the diagnostic LP. However, there is no solid scientific evidence for this approach. The use of suramin in this way has been criticized, and there is evidence from West Africa (83) indicating that it is unlikely to prevent adverse reactions which may follow an injection of melarsoprol, since this is related to the degree of infection in the central nervous system rather than in the blood. Some authors believe that a high initial antigen release can trigger immunological overreactions (84). The introduction of trypanosomes during LP is theoretically possible. However, the usage of proper materials and technical skills make a LP without blood vessel damage possible. In the rare case of trypanosome introduction to the CSF, the parasites are hampered in growth and survival as the CSF is a suboptimal medium (85).

There is a wide variety of national treatment schedules in use in East Africa. In Kenya, the suramin pre-treatment is not given and in Malawi, only in some health centres. In Tanzania, two injections of suramin (one test dose, one full dose) are given over a time period of 5 days. In practice suramin is not given to critically ill patients so as to quickly reach curative melarsoprol concentrations in the CNS. In Uganda, the suramin pre-treatment is administered as a single test dose (5mg/kg) prior to the lumbar puncture. Melarsoprol is also administered according heterogeneous schedules: differences exist in the number of series and the dosages of melarsoprol. Details are shown in table 4.

Table 3: National treatment schedules in use in east Africa for the treatment of second stage *T.b. rhodesiense* HAT

	UGANDA		TANZANIA		MALAWI		KENYA	
	Day of action	Dosage	Day of action	Dosage	Day of action	Dosage	Day of action	Dosage
Suramin pre-treatment								
Application 01	1	5mg/kg	1	5mg/kg	1	5mg/kg		
Application 02			3	20mg/kg	2	20mg/kg		
Total suramin		5mg/kg		25mg/kg		25mg/kg		
Lumbar puncture (LP)	2		5		3		before melarsoprol	
Melarsoprol treatment								
Application 01	3	0.5mg/kg	5	2.2mg/kg	4	3.6mg/kg	1	3.6mg/kg
Application 02	4	0.72mg/kg	6	2.52mg/kg	5	3.6mg/kg	2	3.6mg/kg
Application 03	5	1.08mg/kg	7	2.88mg/kg	6	3.6mg/kg	3	3.6mg/kg
Resting period	5 days		7 days		7 days		7 days	
Application 04	11	1.44mg/kg	15	2.88mg/kg	14	3.6mg/kg	11	3.6mg/kg
Application 05	12	1.80mg/kg	16	3.24mg/kg	15	3.6mg/kg	12	3.6mg/kg
Application 06	13	2.2mg/kg	17	3.6mg/kg	16	3.6mg/kg	13	3.6mg/kg
Resting period	5 days		7 days		7 days		7 days	
Application 07	19	2.52mg/kg	25	3.6mg/kg	24	3.6mg/kg	21	3.6mg/kg
Application 08	20	2.88mg/kg	26	3.6mg/kg	25	3.6mg/kg	22	3.6mg/kg
Application 09	21	3.24mg/kg	27	3.6mg/kg	26	3.6mg/kg	23	3.6mg/kg
Resting period	5 days						7 days	
Application 10	27	3.6mg/kg					31	3.6mg/kg
Application 11	28	3.6mg/kg					32	3.6mg/kg
Application 12	29	3.6mg/kg					33	3.6mg/kg
Total melarsoprol		27mg/kg		28.08mg/kg		32.4mg/kg		43.2mg/kg

	UGANDA	TANZANIA	MALAWI	KENYA
Total days of treatment	13	11	12	12
Total days of hospitalization	29	27	26	33
Administration of steroids	when reaction occurs	standard during treatment	standard during treatment	standard during treatment
LP on discharge	yes	no	yes	yes
Follow up (in months)	passive 3,6,12,24	passive 3,6,12,18,24	passive 3,6,12,24	active 3, passive 6,12,24

IMPAMEL – improved application of melarsoprol

Even though melarsoprol was introduced in 1949 only after almost 50 years of use its pharmacokinetic and pharmacological properties have been investigated (86-89). Based on those findings and computer simulations, a new schedule for the treatment of second stage sleeping sickness was suggested: the alternative treatment schedule consists of a daily melarsoprol application at a dosage of 2.2mg/kg for 10 consecutive days. The IMPAMEL I program assessed the safety and efficacy of this new, abridged treatment regimen by conducting an open, randomized equivalence trial in 500 *T.b. gambiense* patients in Angola. There were no significant differences in the frequency of adverse drug reactions and efficacy, and the new schedule was found to be favourable over the Angolan standard 26-day treatment schedule. The positive impression of the new treatment schedule was corroborated by the results of a multi-national, multi-centre study, monitoring the application of the new schedule in over 2'800 patients in various different settings (IMPAMEL II program) and the effectiveness was similar to the respective standard treatment regimens (90). The IMPAMEL schedule doesn't improve the occurrence and/or frequency of adverse events which are related to the toxicity of melarsoprol. No significant clinical inferiority of the new schedule could be demonstrated. But the IMPAMEL schedule is very much favourable due to socio-economic benefits: with a similar efficacy and effectiveness over the standard regimens, the hospitalization time can be reduced by approximately 50%, the total amount of given melarsoprol by about 30%. Those factors facilitate late stage treatment on different levels: no more dose-adjusting, reduced hospitalisation time which relieves the health facilities, the patients and their families. Those factors have a high impact on the capacity of each treatment centre, on the treatment quality and compliance. Within the IMPAMEL II program a cost-effectiveness study was undertaken and the results confirmed that the new schedule reduces treatment and hospitalisation costs per patient (91). On request of WHO, the new 10-day schedule for treatment of late stage *T.b. gambiense* sleeping sickness with melarsoprol was recommended by the International Scientific Council for Trypanosomiasis Control and Research / ISCTRC at the occasion of the 27^{th} meeting in October 2003, Pretoria, South Africa.

Justification and goals

The new, abridged protocol for the treatment of *T.b. gambiense* patients with melarsoprol shows significant socio-economic benefits and better cost-effectiveness (91). Members of the Data and Safety Monitoring Board from the IMPAMEL I & II programs expressed their concerns regarding an interruption of the IMPAMEL program fearing it could lead to an uncontrolled use of the 10-day schedule in East Africa without reliable data about safety and efficacy. Also, the WHO Scientific Working Group 2001 recommended the urgent conduct of the necessary trials in *T.b. rhodesiense* HAT, a call which was repeated by a WHO Afro meeting in Kampala, 2003. Because of the differences in *T.b. gambiense* and *T.b. rhodesiense* HAT each therapeutic intervention has to be tested separately. The patient's safety can only be ensured when possible differences in pharmacodynamics or –kinetics; or different susceptibilities to the same drug are ruled out.

The current national treatment policies in east Africa are inconsistent and complicated and lead to lowered treatment compliance and quality. Due to the very long hospitalization times some patients leave the health facilities before the completion of the full melarsoprol course. This favours relapses and can possibly impact on the development of resistances. The capacities of HAT-treating health facilities are strained because of the long stay of patients and attendants. The changing dosages throughout the treatment are rarely well implemented and may lead to over or under dosed treatments. Further, the role of the suramin pre-treatment is vague; is it ineffective, beneficial or even unfavourable?

The assessment of the use of the 10-day melarsoprol schedule in East Africa is essential in order to offer the patients and the health facilities a substantial better treatment. Also it is urgently needed as basis for potential, future combination treatments. No new drug will be available in the next 5 to 8 years and it can not be excluded that melarsoprol will loose some of its efficacy against *T.b. rhodesiense*, similar to the phenomenon recently observed in some *T.b. gambiense* areas (78). However, for pharmacological reasons, combination treatments for second stage *T.b. rhodesiense* HAT will be based on melarsoprol until the mergence of new drugs.

A multinational approach is advantageous for any research activities in *T.b. rhodesiense* HAT as it considers the high strain heterogeneity which might affect the outcome of the research question.

When implementing the 10-day schedule in East Africa, several aspects have to be taken into consideration: is the pre-treatment with suramin redundant? Is the 10-day schedule safe for *T.b. rhodesiense* patients? Is the treatment efficacious? Does the short course have any impact on the incidence rate of the ES and / or the case fatality rates? The IMPAMEL schedule consists of 10 consecutive melarsoprol injections at a dosage of 2.2mg/kg. Currently, starting doses of national treatment schedules in Tanzania and Malawi are 2.2 mg/kg and 3.6 mg/kg, respectively. Only Uganda uses a lower starting dose of 0.36 mg/kg, increasing to a maximum dose of 3.6mg/kg with no better results regarding case fatality rates and/or ES reported. Late stage treatment according to the IMPAMEL schedule, reduces the total amount of given melarsoprol by 20% in Uganda, by 23% in Tanzania, by 33% in Malawi and by 50% in Kenya. Generally, there is evidence from former studies performed in *T.b. gambiense* in South Sudan (92) that the 10-day schedule leads to a comparable frequency of adverse drug reactions as the standard treatment schedule. The only exception may be skin reactions (like pruritus, maculopapular eruptions) or very rarely bullous reactions, which were observed in a higher frequency in previous trials compared to the standard treatment.

The IMPAMEL schedule does not claim clinical superiority, but the socio-economic benefits and the better cost-effectiveness make it favourable to the patients, the health facilities and the national bodies of disease control. Further, a harmonization process for all east African treatment protocols can be envisaged if the 10-day melarsoprol schedule proves to be safe and efficacious.

However, the limitations of clinical research in this field are the small number of patients and the difficulties in patient's access and diagnosis. Therefore, clinical research activities are restricted to a small sample size.

Goals

The IMPAMEL III program was designed as a series of clinical trials in Tanzania and Uganda. Its principle goal was the assessment of the safety and efficacy of the 10-day melarsoprol schedule in second stage *T.b. rhodesiense* HAT.

The objectives were

- to conduct clinical trials assessing the abridged melarsoprol treatment schedule in *T.b. rhodesiense* patients according to international standards
- to investigate the benefit of the suramin pre-treatment
- to monitoring the potential overlap in disease distribution areas of the two forms of HAT

Prior to the IMPAMEL III program, no clinical research program compliant to international standards had been conducted in *T.b. rhodesiense* affected areas. Therefore, the conduct of the IMPAMEL III program was expected to strengthen capacities at the local level, the collaboration between different *T.b. rhodesiense* affected countries and the awareness towards the disease.

After the conduct of the trial we were able to write a review on the conduct of clinical trials in resource limited settings and suggested minimal standards for sponsors and host countries in order to ensure a trial conduct in compliance with international standards.

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Chapter 2

Safety and efficacy of the 10-day melarsoprol schedule in the treatment of second stage rhodesiense sleeping sickness

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This manuscript will be submitted to the British Medical Journal

Finished product of melarsoprol

Abstract

Objective Assessment of the safety and efficacy of a 10-day melarsoprol schedule against second stage *T.b. rhodesiense* infections and the effect of a suramin pre-treatment on the incidence of adverse drug reactions during melarsoprol therapy.

Design Sequential conduct of a proof-of-concept trial (n=60) and a utilization study (n=78) using historic controls as comparator.

Setting Two trial centres in *T.b. rhodesiense* endemic regions of Tanzania and Uganda.

Participants Consenting patients with confirmed second stage disease and a minimum age of 6 years were eligible for participation. Unconscious and pregnant patients were excluded.

Main outcome measures The primary outcome measures were safety and efficacy at end of treatment. The secondary outcome measure was efficacy during follow-up after 3, 6 and 12 months.

Results The incidence of ES in the trial population was 11.2% (CI 5-17%) and 13% (CI 9-17%) in the historic data. The respective case fatality rates were 8.4% (CI 3-13.8%) and 9.3% (CI 6-12.6%). All patients discharged alive were free of parasites at the end of treatment. Six months after discharge 99% of patients were considered clinically cured. The mean hospitalization time was reduced from 29 to 13 days ($p<0.0001$) per patient.

Conclusions There is no increased risk for ES and death linked to the 10-day melarsoprol schedule compared to the national schedules in current use. In addition, there was no evidence for an increased risk of adverse events or relapses when suramin pre-treatment was omitted.

Trial registration: Current Controlled Trials ISRCTN40537886 (controlled-trials.com)

Introduction

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic disease transmitted by the bite of the tsetse fly *(Glossina spp.)*. The disease is caused by protozoan parasites of the genus *Trypanosoma* and presents as two forms (1), namely the chronic form of HAT (West and Central Africa) caused by *Trypanosoma brucei gambiense* and the acute form (East and South Africa) caused by *Trypanosoma brucei rhodesiense*. Both forms of HAT are fatal if left untreated. Time to death has been estimated at almost 3 years for *T.b. gambiense* (2) and at 6 to 12 months for *T.b. rhodesiense* infections (3).

The true prevalence of sleeping sickness among the 60 million people in the rural areas of 36 countries in sub Saharan Africa can only be estimated as less than 10% of the at-risk population are under surveillance (4). After almost having been eliminated in the 1960ies the disease re-emerged to a peak prevalence of an estimated 300-500'000 cases by the mid 1990ies (4). By an increase in control activities, the number of cases per year was reduced to an estimated 50'000 - 70'000, of which more than 95% were due to *T.b. gambiense* (5). *T.b. rhodesiense* HAT is clearly the more neglected form of HAT due to its very low prevalence. However, it has a dangerous potential for large scale epidemics which are of high public health importance (6). Between 1976 and 1998 a total of 19'974 cases were detected in south eastern Uganda (7), an area that today, has the potential for a much larger number of patients due to the expansion of HAT to previously disease free areas (8, 9). Sporadic *T.b. rhodesiense* infections in tourists have spurred discussions about disease control and surveillance, especially in National Parks (10).

Sleeping sickness has two stages, a first (haemolymphatic) stage followed by a second (meningo-encephalitic) stage defined by the presence of trypanosomes and/or elevated levels of white blood cells (≥ 5WBC/mm^3) in the cerebrospinal fluid (CSF) (4). Treatment options are limited: *T.b. gambiense* first stage infections are treated with pentamidine isethionate and *T.b. rhodesiense* with suramin (Germanin®) respectively. For second stage treatment there are currently no other registered drugs than melarsoprol (Arsobal®) and eflornithine (Ornidyl®). Melarsoprol is the only drug effective against both forms of the disease. Due to its limited activity against *T.b. rhodesiense* (11) eflornithine is only in use for the treatment of *T.b. gambiense* HAT, but its large scale use is hampered by the complicated application of the drug. Currently a new combination treatment of eflornithine with nifurtimox (NECT) is under clinical evaluation against second stage *T.b. gambiense* sleeping sickness and appears to be a promising first-line therapy (12). Still, for the treatment for *T.b. rhodesiense* HAT, melarsoprol remains the only available drug.

Since the introduction of melarsoprol in 1949 (13), treatment regimens were empirically developed and they varied considerably between countries and treatment centres. Complicated dosing schemes and repeated serial drug applications separated by 1-week drug-free intervals were common and resulted in very long hospitalization times of up to 1 month. The encephalopathic syndrome (ES) is the most severe complication of melarsoprol treatment and is observed in 5-10% of treated patients, being fatal in about 50% of those patients (4). The ES appears to be more common in *T.b. rhodesiense* HAT than in *T.b. gambiense* with reported incidence rates between 5-18% (14). The concomitant use of steroids during melarsoprol therapy proved to reduce the incidence of ES in *T.b. gambiense* patients (15) but this could not be shown in *T.b. rhodesiense* (16-18).

The suggestion of an abridged melarsoprol treatment schedule based on pharmacokinetic investigations was the first rational approach to develop a standardized treatment. The new schedule suggested daily melarsoprol injections of 2.2mg/kg for 10 consecutive days. In the IMPAMEL I & II programs (1997-2004) the use of the new schedule was validated for second stage *T.b. gambiense* HAT and was shown to be clinically non-inferior versus the standard regimens (19) and equally effective (20). It was favoured by patients and health staff due to an over 50% reduction in hospitalization time and a more economic use of the drug (21). In 2003, the 10-day melarsoprol schedule was officially recommended for use in *T.b. gambiense* affected areas by the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). In view of the increasing rates of melarsoprol treatment failures reported in *T.b. gambiense* patients (22-25) it can not be excluded that melarsoprol will also loose some of its efficacy against *T.b. rhodesiense*. Melarsoprol treatment failures have been reported in *T.b. rhodesiense* patients (26-29), but yet not at alarming rates. A reduced melarsoprol susceptibility of *T.b. rhodesiense* isolates from Tanzania was recently shown, indicating that drug resistance may be emerging (30). Hence, the 10-day melarsoprol schedule might become the basis for potential combination treatments and might allow a harmonization of all east African treatment protocols. The WHO scientific working group (2001) recommended the urgent conduct of the necessary clinical trials in east Africa, a call that was repeated by WHO Afro (2003).

The IMPAMEL schedule could not be introduced in east Africa without further testing; a much higher parasitaemia, a remarkably divergent clinical pattern and potential differences in the pharmacodynamics and –kinetics could not be disregarded as they may have an impact on treatment outcomes. Hence, separate testing of safety and efficacy of the abridged schedule was required in order to ensure its adequacy also in *T.b. rhodesiense* patients.

In East Africa the empirical treatment schedules vary considerable between countries and treatment centres. In addition, some countries (Tanzania, Uganda, parts of Malawi) administer a suramin pre-treatment prior to the diagnostic lumbar puncture (LP) and melarsoprol therapy. Suramin is intended to (i) prevent a mechanical introduction of trypanosomes into the CNS during LP and (ii) to clear trypanosomes from blood and lymph to avert initial high antigen releases at the initiation of melarsoprol therapy. The suramin pre-treatments is purely empirical (31) and there is no standardized protocol. In Tanzania it is not given to critically ill patients so as to quickly reach curative melarsoprol concentrations in the CNS. The national treatment schedules in use for second stage *T.b. rhodesiense* HAT are summarized in table 1.

Table 1: National treatment schedules in use for second stage *T.b. rhodesiense* HAT.

	Uganda	**Tanzania**	**Kenya**	**Malawi**
Suramin pre-treatment (mg/kg)				
1st dose	5	5	NA	5
2nd dose		20	NA	20
Melarsoprol treatment (mg/kg)				
1st series	0.5, 0.72, 1.08	2.2, 2.52, 2.88	3.6, 3.6, 3.6	3.6, 3.6, 3.6
2nd series	1.44, 2.80, 2.2	2.88, 3.24, 3.6	3.6, 3.6, 3.6	3.6, 3.6, 3.6
3rd series	2.52, 2.88, 3.24	3.6, 3.6, 3.6	3.6, 3.6, 3.6	3.6, 3.6, 3.6
4th series	3.6, 3.6, 3.6		3.6, 3.6, 3.6	
Total melarsoprol (mg/kg)	**27**	**28.08**	**43.2**	**32.4**
Hospitalization time (days)	**29**	**27**	**33**	**26**

NA: not applicable; i.v. melarsoprol injections at 24 hours intervals per series, each series spaced by a 5 to 7 day resting period; further variations of schedules possible at local level

Given the very limited number of patients, clinical trials on *T.b. rhodesiense* are obviously restricted to small sample sizes and must be executed in rural settings with very limited infrastructure and difficult access. This has to be accounted for in the design of trials; randomized controlled trials or other trial designs with active control groups are not feasible. The design of the IMPAMEL III program was the conduct of two sequential trials: first a proof-of-concept trial was executed to proof no harm in *T.b. rhodesiense* patients and to obtain preliminary efficacy data. Two subgroups, of which only one received suramin allowed to observe a possible substantial increase of adverse drug reactions if melarsoprol was directly administered. Based on those findings, a second trial was designed to substantiate the results in a larger patient group. The second, drug utilization trial, was designed as an extension of the selected arm of the proof-of-concept trial, i.e. without suramin, thus, allowing pooling data from both trials for final analysis. Patient records from two years prior to the IMPAMEL III program were analyzed and used as controls. The findings of the two sequentially conducted trials are reported here collectively.

Methods

Study sites

The Kaliua Health Centre (KHC), a 50-bed missionary hospital in Tanzania (Urambo District) and the Lwala Hospital, a designated 100- bed district hospital in Uganda (Kaberamaido District) participated in the IMPAMEL III program. Capacity building included the upgrade of the pharmacies and laboratories and on-site trainings in Good Clinical Practice (GCP), HAT diagnosis and informed consent procedures.

Study design

Sequential conduct of two non-randomized trials; a proof-of-concept trial (n=60) followed by a utilization study (n=78) using historic data as comparator (n=300).

Proof-of-concept trial. 60 patients were prospectively enrolled into two subgroups: participants in the first subgroup (n=30) were treated with the suramin pre-treatment followed by the 10-day melarsoprol schedule. The second sub group (n=30) was directly treated with the 10-day melarsoprol schedule. Suramin and steroids were administered according to centre-specific guidelines.

Utilization study. Additional 78 patients were treated with the 10-day melarsoprol schedule. The suramin pre-treatment was omitted and the use of steroids was adjusted to the Tanzanian standard in both centres (details below).

Eligibility criteria. Patients with confirmed second stage *T.b. rhodesiense* HAT and a minimum age of 6 years were eligible for participation. Pregnant as well as unconscious or moribund patients were excluded from the trial. Each participant gave written informed consent. For the participation of children and adolescents (below 18 years) the parents, the legal representative or the guardian gave written informed consent.

Diagnosis and staging. Diagnosis of HAT was made in blood and in the cerebrospinal fluid (CSF). Blood was examined by direct microscopy and/or the haematocrit centrifugation technique (32). If trypanosomes were present, a lumbar puncture was performed for disease staging. Patients in the first subgroup of the proof-of-concept trial received the suramin pre-treatment prior to the LP. All other patients underwent LP directly after the detection of trypanosomes in blood.

Analysis of the CSF was done by direct microscopy and/or single modified centrifugation technique and white blood cell (WBC) count using counting chambers. Second stage infections were confirmed by the presence of trypanosomes and/or ≥ 5 WBC/mm^3 in the CSF.

Patient follow-up. All patients were asked to present at the centre for follow-up examinations after 3, 6 and 12 months. At each follow-up visit blood and CSF samples were taken. These were analyzed for the presence of trypanosomes and a WBC count was performed using the CSF sample. For patients who did not present for follow-up visits, oral information on their general condition was collected.

Endpoints: safety & efficacy. Based on reported case fatality rates in the trial sites and the literature, a cut-off point of all-cause mortality was set at ≥10%. The computed stopping rule was an early discontinuation of the trials if 7 or more patients per subgroup (n=30) experienced a fatal treatment outcome (p=0.026).

The primary efficacy endpoint was cure at end of treatment. Secondary efficacy endpoint was cure after 3, 6 and 12 months. Possible outcome measures are summarized in table 2.

Table 2: possible safety and efficacy outcome measures for IMPAMEL III trials.

Cure at end of treatment	No parasites in blood and CSF
Cure during follow-up	No parasites in blood and CSF AND WBC<5/mm^3
Clinical cure at end of treatment	No parasites in blood but missing results on CSF analysis (refusal of LP, hemorrhagic LP)
Clinical cure during follow-up	Oral information on general condition of the patient No parasites in blood but missing results on CSF analysis (refusal of LP, hemorrhagic LP)
Relapse (end of treatment and during follow-up)	Trypanosomes in any body fluid
Death	Patients who died during treatment or follow-up (categorized by likely or definite cause of death): - HAT - Adverse events regarded by the investigator as possibly or probably related to treatment for HAT - Causes unrelated to HAT or the treatment of HAT - Unknown causes

A high degree of trial homogeneity was achieved by identical eligibility criteria, stopping rule and endpoints in both trials.

Historic controls. To reduce bias, historic controls were solely collected in the two trial sites and limited to a time frame of maximum two years prior to study initiation. Files that contained basic demographic data and valid information on treatment outcome were selected. If documented, information on serious adverse events (SAEs) and concomitant treatments was collected. Of almost 400 files reviewed, 300 were eligible as historic controls (153 in Tanzania, 147 in Uganda).

Sample size. No formal sample size was calculated for the proof-of-concept trial. The utilization study had a calculated sample size requirement of minimum 100 patients in order to have a precision of ±6% on the estimated endpoint.

Analysis plan. In a first step both trials were analyzed separately. Final safety and efficacy analysis was performed on the pooled dataset of all patients directly treated with the 10-day melarsoprol schedule (n=107). Those results were compared to historic controls.

Recruitment. Patient recruitment was mainly by passive case detection at the centre. Active case search was done with mobile diagnostic teams in the villages of index cases; but the outcome was poor. In Uganda, the local radio station was contracted to inform the population about the IMPAMEL III trial and invited people to present for cost-free screening for HAT at the Lwala hospital.

All trial participants were given an insecticide treated bed net (ITN). In case of need, the trial participants and/or their attendants were supported with food during the hospitalization period. Cost for transport for the patient and one attendant to present for follow-up visits was refunded.

Ethics & trial registration. For both trials, ethical clearance was obtained from the ethics committees in the host countries; the National Institute for Medical Research (NIMR), Tanzania and the Ministry of Health, Uganda. In Switzerland, ethical clearance was obtained from the ethics committee of the two cantons of Basel (EKBB). Trial registration was done in the Current Controlled Trials database prior to first patient enrolment (ISRCTN40537886). The trials were conducted in compliance with ICH/GCP.

Data management and statistical analysis. All data were double entered and verified using the EpiData Version 3.1 software (www.epidata.dk). Data analysis was done using the statistical software package STATA Version IC10.0 (STATA™, StataCorp, USA). Pearson's chi-square test and the Student's *t* test were used to test differences in proportions and means.

Trial conduct. For each patient, a case report form (CRF) was filled containing information on demographic, diagnostic, and clinical characteristics before and after treatment. The assessment of adverse events used a graded scale for the severity of the event (0 to 4) and a binary outcome for the seriousness of the event. Signs and symptoms which were spontaneously reported between the end of treatment evaluation and 30 days post-treatment were also entered in the case report form.

During the proof-of-concept trial, the blood sugar and the blood lipids were monitored daily before food intake using the whole blood test system Cardio Chek™ PA. During the proof-of-concept trial, urine analysis using COMBUR9 (Roche Diagnostics, Switzerland) was performed at baseline and discharge examinations as well as prior to the first melarsoprol injection for all patients who received the suramin pre-treatment.

During both trials, vital signs were daily monitored before drug administration. For women, a pregnancy test was performed at baseline.

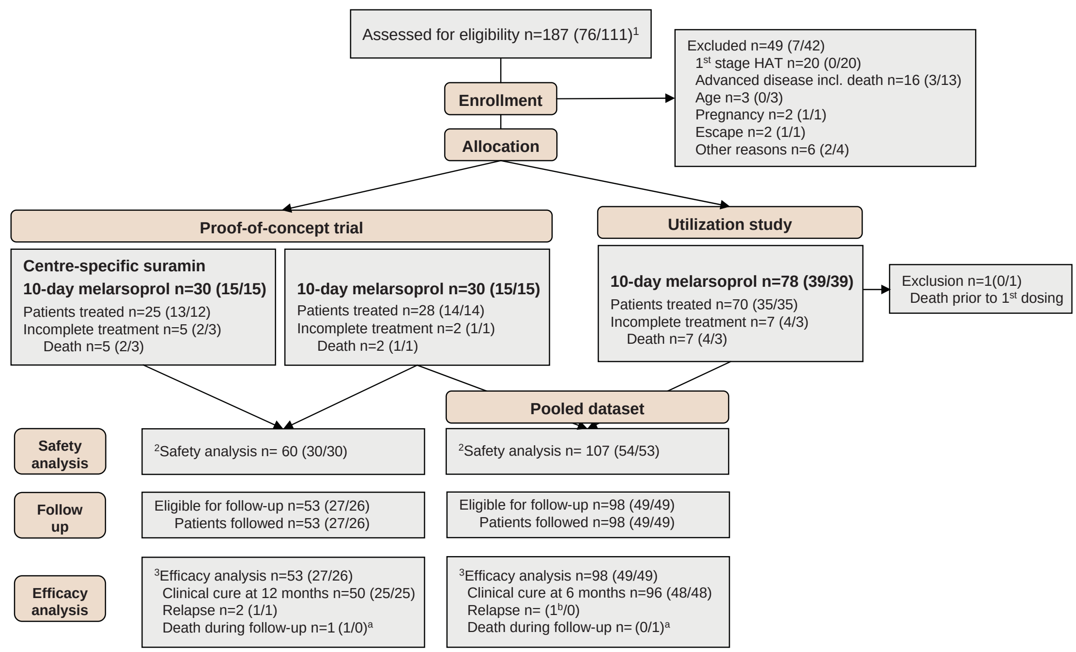
Patients were treated with anti-malarial and anti-helminth drugs prior to HAT treatment depending on baseline findings. During treatment, all patients received paracetamol (acetaminophen) 3 times per day in single doses of 1000mg for adults and 500mg or 250mg respectively for children. Suramin was administered intravenously as a 10% aqueous solution (Germanin®, Bayer). In the proof-of-concept trial, suramin as well as steroids were given according the centre-specific guidelines. In Tanzania, a suramin test dose (5mg/kg) was administered after the detection of trypanosomes in blood (day 1). After a resting day, a full dose (20mg/kg) was given on day 3. Another resting day followed before the LP on day 5. Each patient was treated with 10mg of prednisolone per os half an hour before melarsoprol injection. In Uganda, patients received one suramin test dose (5mg/kg) after the detection of trypanosomes in the blood. On the following day, the LP was performed and melarsoprol treatment was initiated in case of confirmed second stage infection. Steroids were only administered in case of adverse drug reactions to treatment. Melarsoprol treatment was for all patients 2.2mg/kg of melarsoprol for 10 consecutive days as a 3.6% solution in propylene glycol (Arsobal®; sanofi-aventis); by slow intravenous (i.v.) injection but maximally 5ml a day. In the utilization study, all patients underwent LP directly after a trypanosome-positive blood test, the use of steroids was adapted to the Tanzanian standard in both centres and the use of suramin was omitted.

If a patient developed an encephalopathic syndrome, melarsoprol treatment was interrupted and emergency treatment was initiated: i.v. hydrocortisone (100-200mg/24hours) or dexamethasone (3x15mg/24hours); if necessary, anticonvulsive drugs (diazepam, phenobarbital) were applied. The close observation and frequent monitoring of vital signs were mandatory as well as supportive feeding if necessary. For exclusion of cerebral malaria, blood was analyzed at the day of onset of the ES.

Results

First, the results of the proof-of-concept trial are presented, followed by the results of the pooled data set and the comparison to the historic data. The study flow is presented in Figure 1.

Figure 1: overall study flow chart



[1] numbers in brackets correspond to numbers in Tanzania/Uganda
[2] patients who received at least one dose of study drug
[3] Intention to treat population/ITT – at 12 months for the proof-of-concept trial and at 6 months for the pooled data set
[a] death not related to HAT
[b] same patient as already reported in proof-of-concept trial

Proof-of-concept trial

Study population and baseline characteristics

From August 2006 to July 2007 a total of 60 patients were enrolled. The age and sex distribution were similar in both settings. The median age in Uganda (31 years) was slightly lower than in Tanzania (36 years) as 10 enrolled participants were in the age of 6-15 years. The nutritional status of the patients in Uganda was poorer and a body mass index (BMI) <16.5 was more common than in Tanzania ($p=0.001$). In Tanzania, fewer patients had trypanosomes in the CSF ($p=0.0035$) but the average WBC counts were higher ($p<0.0001$). The majority of patients suffered from headaches (90%), general malaise (93.3%) and joint pains (86.7%). Fever (axillary >37.5°C) at baseline was recorded in 35% of all patients.

Safety

A total of 13 (21.6%) serious adverse events (SAE) were reported whereof 7 (53.8%) were fatal. Other SAEs were due to prolonged hospitalizations and events that required medical interventions.

In comparison, more ES were reported in Uganda than in Tanzania (6 vs. 1; p=0.0444). The time to onset of the encephalopathic syndrome was between 3 and 11 days after the first melarsoprol dose (median 6, mean 7). The overall survival rate for the ES was 28.6% (0% in Tanzania and 33% in Uganda). Other adverse events reported were headache 15% (9), vomiting 13% (8), febrile reactions 13% (8), diarrhea 8.3% (5), nausea 6.6% (4), dizziness 5% (3), skin reactions 1.6% (1) and were controlled by symptomatic treatment. 56.7% (35) patients had an event free treatment course.

Efficacy

Primary endpoint: 24 hours after treatment, all patients discharged alive (53/60) were free of parasites in blood and CSF.

Secondary endpoint: during follow-up, Tanzania and Uganda reported one relapse each: the patient from Tanzania was enrolled into the second subgroup and was treated with melarsoprol only. He did not present for the 3 and 6 months follow-up because he lived far from the centre and was in good general condition. 12 months after discharge he presented at the centre because he felt sick again and was diagnosed with second stage HAT. The patient assumed a re-infection as symptoms evolved after multiple tsetse bites. The patient from Uganda presented with trypanosomes in blood two weeks after discharge. He had been treated with suramin and melarsoprol and developed an ES after the 6th injection of melarsoprol. After an 8-day treatment interruption he resumed melarsoprol treatment for 4 more days. Both patients were successfully re-treated with melarsoprol according the national treatment schedules. Overall safety and efficacy outcomes of the proof-of-concept trial are summarized in table 3.

Blood sugar, blood lipids and urine analysis are not shown here.

Table 3: Safety and efficacy outcomes at discharge and at 12 months follow-up.

	Total	Suramin	Non-suramin
Number of patients treated	n=60	n=30	n=30
Encephalopathic syndrome	7 (11.6)	4 (13.3)	3 (10)
Death during treatment	7 (11.6)	5[a] (16.6)	2[a] (6.6)
Relapses at discharge	0	0	0
Cure at discharge	53 (88.3)	25 (83.3)	28 (93.3)
Patients eligible for follow-up at 12 months	53	25	28
Death	1 (1.8)	0	1[d]
Relapses	2 (3.8)	1(4)	1 (3.6)
Clinical cure	52 (98)	25 (100)	27(96.5)
whereof cured [b]	14[c] (26.4)	9 (36)	5 (17.9)

[a] two death (Tanzania) outside the trial centre as family seeked local treatment
[b] WBC count in CSF <5cells/mm^3
[c] all patients from Uganda
[d] not related to HAT (Tanzania)

Follow-up attendance was poorer in Tanzania, most probably due to the longer distances to the health centre. 44% of the patients presented for the 3 months follow-up and 30% and 19% for the 6 and 12 months, respectively. In Uganda, 88% presented for the 3 months follow-up and 65% for the 6 and 54% for the 12 months follow-up respectively. For all patients not seen at the centre, oral information on their general condition was collected. All patients were in good condition and working after 3, 6 and 12 months, except one patient from Tanzania who died 7 months after discharge for reasons not related to HAT. No benefit could be attributed to the suramin pre-treatment. In contrast, there were more ES and fatal treatment outcomes in the suramin group (see table 3) but this trend was not significant.

Utilization study

A total of 78 patients were enrolled from October 2007 to August 2008. One patient was excluded from the analysis as death occurred prior to first dosing (see figure 1). For final analysis data from the proof-of-concept study (without suramin) and the utilization study were pooled. Table 4 compares the two patient populations prior to data pooling. None of the parameters were significantly different.

Table 4: Baseline characteristics of patient populations prior to data pooling.

	Proof-of-concept[1]		Utilization study		Pooled dataset	
	n=30		n=77		n=107	
	n	%	n	%	n	%
Age, mean±SD	36±18		37±19		36±19	
Age, range (years)	6-67		6-72		6-72	
Male/female ratio	1.7		1.3		1.4	
Nutritional status						
BMI[2] (kg/m) - mean±SD	18.8±3.4		18.6±3.6		18.6±3.5	
BMI<16.5	8	26.6	18	23.4	26	24.3
Diagnostic findings						
Trypanosomes in blood	30	100.00	72	93.5	102	95.3
Trypanosomes in CSF[3]	28	93.33	69	89.6	97	90.7
White blood cell (WBC) count in CSF	92±57		78±64		82±62	
Clinical manifestations						
Headache	27	90.0	73	94.8	100	93.5
Fever (>37.5)	7	23.3	13	16.9	20	18.7
Oedema	6	20.0	25	32.5	31	29.0
Joint pains	29	96.7	76	98.7	105	98.1
Daytime sleep	24	80.0	63	81.8	87	81.3
Night time sleep	23	76.7	50	64.9	73	68.2
Abnormal movements	8	26.7	20	26.0	28	26.2
Walking difficulties	13	43.3	53	68.8	66	61.7
Time period of enrolment	Oct 06 - May 07		Oct 07 - Aug 08		Oct 06 - Aug 08	

Note: [1]no suramin pre-treatment; [2] body mass index; [3] cerebrospinal fluid

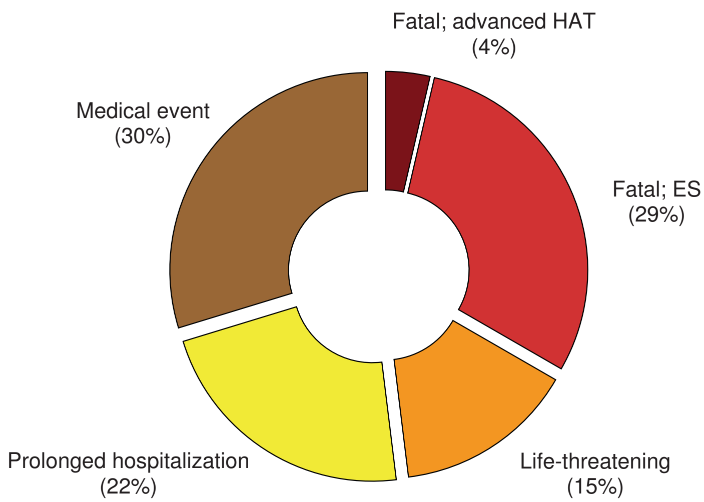
Study population and baseline characteristics

The demographic, diagnostic, and clinical characteristics of the patients were similar in Tanzania (Tz) and Uganda (Ug). 19 trial participants (Tz: 2, Ug: 17) were below 16 years of age with a mean age of 11 years (± 3 years). A poor nutritional status with a BMI<16.5 was significantly more frequent in Uganda (p<0.0001). In Tanzania, patients had less frequently trypanosomes in the CSF (p=0.0002) but significantly higher WBC counts (p<0.0001). In line with the significant higher WBC count, the neurological symptoms were more distinct in Tanzanian patients. Description of the clinical course of the disease will be published separately.

Safety

A total of 27 (25.2%) SAEs were reported, summarized by criterion in figure 2. Prolonged hospitalizations included patients who remained in the hospital due to lack of transport back to their home villages and patients who were kept for observation. Medical events were treatment of malaria (n=2), severe vomiting (n=2), severe headache (n=1), cardiac arrhythmia (n=1) and psychosis at end of treatment (n=1).

Figure 2: Serious adverse events, by SAE criterion



6 patients died during treatment; two died within 24 hours after completion of treatment; one patient was comatose for 6 days after the last injection of melarsoprol until death occurred. Overall, death occurred between 2 and 16 days (median 9, mean 8.5) after the first injection of melarsoprol. The major cause of death was the ES which contributed to 88.9% of the fatalities. One fatality (11.1%) was attributed to advanced HAT. The onset of ES was reported after an average of 7.5 days after the first dose of melarsoprol (range 3-10 days). The onset was sudden, in 58.3% preceded by headache and fever (7/12) and in 41.6% (5/12) by vomiting. In 16.6% (2/12) malaria parasites were detected at the onset of ES, which probably also caused fever and headache. Differences were observed in the duration of ES; in Tanzania they were fatal after a maximum of one day and in Uganda the ES could last for several days (range 1-8) until the patient's condition improved or deteriorated. The overall survival rate was 33.3% (Tz: 0%; Ug: 57.1%).

Other adverse events reported included febrile reactions (37%), headache (22%), vomiting (13%), dizziness (9%), skin reactions (6.5%), nausea (5.6%) and diarrhea (4%). 35.5% the patients had an event-free treatment.

Efficacy

Primary endpoint: all patients discharged alive (98/107) were free of parasites in blood and CSF 24 hours after treatment.

Secondary endpoint: Follow-up attendance rates in Tanzania were 69% at the 3 months and 97% at the 6 months follow-up. In Uganda 91% presented for the 3 months and 46% for the 6 months follow-up. For all patients that did not present at the centre oral information on their well being was collected. The 12 months follow-up is ongoing and will be concluded in June 2009 in Tanzania and in September 2009 in Uganda.

No relapses were reported from patients that were enrolled into the utilization study. 1 patient from Uganda died 2 months after discharge of unknown reasons. Table 5 summarizes the main safety and efficacy outcomes of the pooled data set at discharge and at 6 months after treatment.

Table 5: Safety and efficacy outcomes at discharge and at 6 months follow-up.

Number of patients treated	Total n=107	Tanzania n=54	Uganda n=53
Encephalopathic syndrome	12 (11.2)	5 (9.3)	7 (13.2)
Death during treatment	9 (8.4)	5(9.3)	4(7.5)
Relapses at discharge	0	0	0
Cure at discharge	98 (91.6)	49 (90.7)	49 (92.5)
Patients eligible for follow-up	98	49	49
At 6 months			
Death	3 (1.8)	1[a]	1
Relapses	1	1[a]	0
Clinical cure	97 (99)	49 (100)	48 (98)
whereof cured[b]	33 (33.7)	0	33 (62.3)

Note: [a] incident already reported in proof-of-concept trial, [b] WBC count in CSF<5cells/mm^3

Comparison trial data - historic data

Patient files from 2004-2006 were reviewed and 153 from Tanzania and 147 from Uganda were used as comparator. Files which were incomplete having missing demographic and/or treatment evolution details were excluded. Table 6 summarizes the demographics and the incidence of ES and death for the historic data.

Table 6: Descriptive analysis of historic patient files.

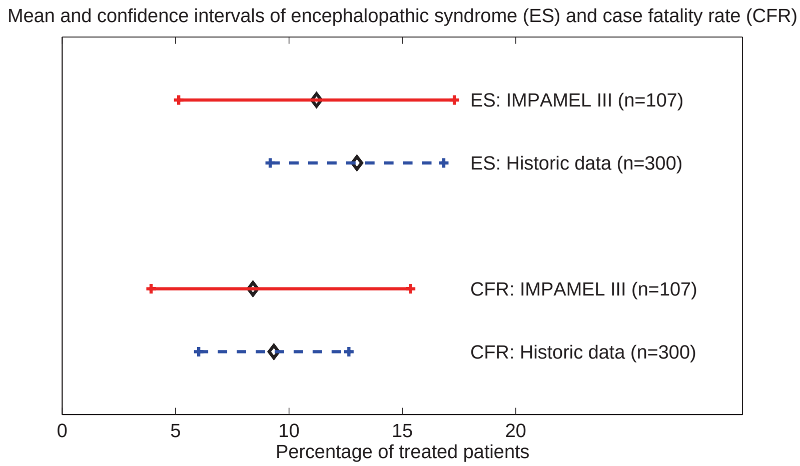
Number of patients	**Total** 300		**Tanzania** 153		**Uganda** 147	
	n	%	n	%	n	%
Age, mean ± SD	29±16		34±17[1]		25±17[2]	
Male/female ratio	1.4		2.5		0.8	
Encephalopathic syndrome	39	13.0	17	11.1	22	15.0
Death	28	9.3	12	7.8	16	10.9

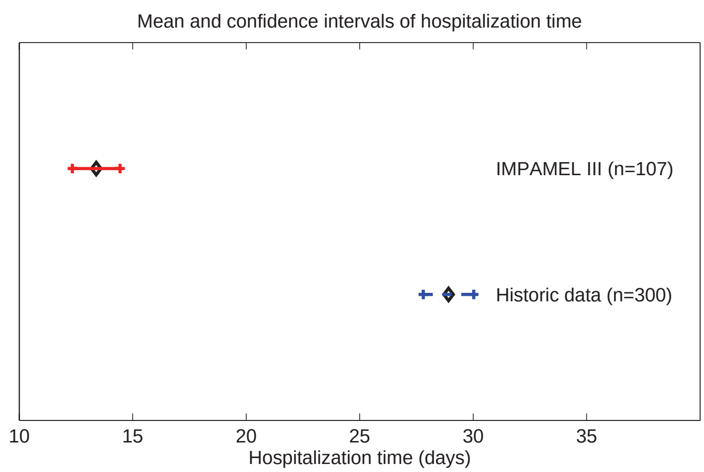
[1]missing values for age: 18, [2] missing values for age: 3

The average reported incidence of ES was 13% (Tz: 11.1%, Ug: 15%) of which 67.9% were fatal. The total hospitalization time was 27 and 32 days in Tanzania and Uganda respectively (range 3-92). Mean hospitalization time in our trials was 13 days (range 3 – 34) ($p<0.0001$). Treatment adherence was better for the current trials than the historic data (97% vs 99%).

Comparison between trial and historic data for ES, case fatality rate (CFR) and hospitalization time is shown in figure 3.

Figure 3: Mean and 95% CI for ES, CFR and hospitalization time; trial data (solid line) and historic data (dashed line).





Discussion

Given the differences between the two forms of HAT, the results of the IMPAMEL I & II programs could not be directly extrapolated to *T.b. rhodesiense* patients. In the planning of the IMPAMEL III program, treatment efficacy was not a major concern. On one hand the total exposure time of the parasite to melarsoprol is similar in the empirical schedules and in the IMPAMEL schedule: a total of 9 (3x3) or 12 (3x4) days spaced by resting periods versus a total of 10 consecutive days. On the other hand the IMPAMEL schedule was extensively tested on *T.b. gambiense* and yielded similar cure rates as empirical schedules (20). The main concerns were rather related to unexpected toxicity. Given the already higher parasitaemia and reported incidence of ES in *T.b. rhodesiense* patients, a further increase of ES under the 10-day melarsoprol schedule could not be excluded.

However, evidence from studies in *T.b. gambiense* HAT showed that the pathogenesis of ES is an immune phenomenon and dose independent (33). Hence, the 10-day melarsoprol schedule should theoretically not trigger an increase in the incidence of ES in *T.b. rhodesiense* patients.

Suramin pre-treatment. Pre-treatment with pentamidine and suramin have been given for decades in the hope of reducing the risk for ES, but this remains unproved (14). In Kenya and the northern parts of Malawi the suramin pre-treatment is not administered. In Tanzania, a test dose (5mg/kg) and a full dose (20mg/kg) are administered over a time period of 5 days. In Tanzania, the pre-treatment is not given to critically ill patients so as to quickly yield antitrypanosomal activity in the CNS. In Uganda, a test dose (5mg/kg) is applied 1 day prior to LP which, from a pharmacological point of view, is unlikely to efficiently clear trypanosomes as suramin is only taken up slowly by the parasite (34).

Because we did not want to deviate from current national rules in one step, the assessment of the ability of suramin pre-treatment to prevent adverse drug reactions became part of the study design.

In our data we observed more adverse events during the proof-of-concept trial in patients that received suramin (63.3%) than in patients that were directly treated with melarsoprol (23.3%, p=0.0018). Based on this result we decided to omit the suramin pre-treatment in the utilization study. In the pooled dataset, where none of the patients received suramin, the frequency of adverse events was 61.7%. The difference in adverse events can most likely be explained by the small sample size. However, no benefit of suramin pre-treatment was observed over the direct melarsoprol application.

Safety. 35% of the patients directly treated with melarsoprol had an event-free treatment course. Concomitant treatments were less frequently used in the trial population compared to the historic data (p=0.0001), most likely indicating a better case management. Adverse events such as vomiting, headache, skin reactions and fever were controllable with concomitant medications. Skin reactions (rashes, pruritus) were a minor problem, reported in 6.5% of the patients. This was surprisingly low given the high incidence of skin reactions in *T.b. gambiense* patients (28.4%) of which 4.2% were fatal (bullous eruptions) (21).

The most relevant safety outcome of melarsoprol treatments is the incidence of serious adverse events (ES and death). A systematic literature review on encephalopathic syndromes during melarsoprol treatment of HAT (35) reported incidence rates of ES and death in *T.b. rhodesiense* patients of 10.6% (1.5-28%) and 11.6% (CI 5.2-19%) respectively. In our historic data we found centre-specific incidence rates for ES and death of 13% (CI 9.2-16.8%) and 9.3% (CI 6.0-12.6). The IMPAMEL III data reported ES and death of 11.2% (CI 5.1-17.3) and 8.4% (CI 3-13.8). Hence the 10-

day melarsoprol schedule appears not to have a higher safety risk than the national regimens in use.

Findings from *T.b. gambiense* HAT of a higher risk for ES associated with the presence of trypanosomes or more than 100 WBC in CSF (15) could not be confirmed. An immunological background of ES was suspected for long and recent investigations indicating that a small number of alleles of the human leukocyte antigen (HLA) were associated with a significantly increased risk for ES have corroborated this hypothesis (35). During the proof-of-concept trial, patients from Uganda developed more ES than patients in Tanzania (p=0.0444). However, the incidence of ES equilibrated between the centres in the utilization study (p=0.5176) when steroids were administered in both centres according the same guidelines. The evidence for the prevention of ES with steroids in the literature is conflicting (16, 33), however, our data show a clear correlation between the frequency of ES and the use of prednisolone.

Causes of death are difficult to establish under field conditions. To avoid bias we used a composite safety endpoint of all-cause mortality, also better suited for comparison of mortality rates from literature and the historic data. We considered historic data as the most adequate source for controls even though the reporting standards were rather poor in comparison to the comprehensive documentation during the trials.

Efficacy. The historic data did not allow any elucidation of the efficacy of the standard treatment regimens since systematic follow-up of patients is not routinely implemented in East Africa. The relapse rate for the 10-day melarsoprol schedule in *T.b. gambiense* HAT was reported at 7.1% in a controlled clinical trial (21). Due to the need for repeated lumbar punctures and possible long distances to the health centres the follow-up attendance is generally very low in *T.b. rhodesiense* areas. Mobile teams for large scale population screenings are routinely present in *T.b. gambiense* endemic regions and support the follow-up activities effectively. Such teams are inexistent in East Africa, requiring other approaches to support the patient follow-up.

Follow-up visits in *T.b. gambiense* areas are scheduled after 6, 12, 18 and 24 months. In clinical trials, a test of cure visit 18 months after discharge is used to determine the efficacy of a treatment under clinical development (36). Those recommendations are not suited for the virulent and fast progressing *T.b. rhodesiense* HAT. To address this situation, the IMPAMEL III program scheduled follow-up visits after 3, 6 and 12 months. To anticipate missing data, the primary efficacy endpoint was parasitological cure at end of treatment. The secondary efficacy endpoint was parasitological cure at follow-up examinations. Given the acuteness of this disease, relapses are certainly noted by the patients and communities but not necessarily reported and re-treated due to the difficulties described. We therefore engaged local leaders and community health workers to collect information on the well-being of patients who did not present for follow-up examinations.

All IMPAMEL III trial participants will be followed for 12 months. The follow-up of the proof-of-concept trial has been completed as well as the 3 and 6 months follow-up of patients enrolled into the utilization study. The 12 months follow-up of those patients is currently ongoing. We consider the 6 months follow-up as most adequate time point for the assessment of treatment efficacy in *T.b. rhodesiense* HAT. At 12 months, follow-up attendance is very poor and the risk of re-infections can not be disregarded. Therefore we present the efficacy data of the 10-day melarsoprol schedule in East Africa after the completion of the 6 months follow up; any relevant changes until completion of the 12 months follow up would be communicated by the authors.

24 hours after treatment, all patients discharged alive (121/137) were free of parasites in blood and CSF. 96% of all participants eligible for follow-up in the proof-of-concept trial were in good condition 12 months after discharge. One patient from Tanzania died due to causes not related to HAT. In the pooled data set 97% of all participants eligible for follow-up were in good condition 6 months after discharge. 1 patient from Uganda died, due to unknown reasons. A total of 2 relapses were reported, both from patients allocated to the proof-of-concept trial (one in suramin and one in the non-suramin group). In one case the treatment was interrupted for 8 days because the onset of ES after the 6th dose of melarsoprol. This patient presented two weeks after discharge with trypanosomes in blood. For the other case, the relapse may likely be attributed to a re-infection according the patients account. Re-infections are more common in the *T.b. rhodesiense* HAT- The proximity of livestock and people increases the human-fly contact. It is also known as an occupational disease, putting hunters, fishermen, honey gatherers at higher risk of infection (37). In terms of efficacy there is no evidence from the trials conducted against the use of the 10-day melarsoprol schedule in *T.b. rhodesiense* patients. The collection of oral information proved to be a satisfactory tool as indicator for treatment efficacy in the absence of blood and CSF examinations.

The IMPAMEL III trials were the first ones on *T.b rhodesiense* conducted in compliance with Good Clinical Practice (GCP). It was of high priority by the WHO and the affected countries to offer populations at risk of *T.b. rhodesiense* HAT an improved schedule for melarsoprol and, in view of possible treatment failures the basis for potential combination treatments.

The conduct of the IMPAMEL III program strengthened local capacities especially for diagnosis, patient management and reporting. Also disease awareness rose. Whereas the follow-up activities had to be extensively pushed during the proof-of-concept trial, access to follow-up data has become easier during the second trial, most likely attributable to the better awareness among patients and staff members.

The main bottleneck of clinical research in *T.b. rhodesiense* HAT is the limited number of patients which impedes the conduct of properly powered trials. In two active foci a total of 138 second stage patients were enrolled during two years of active and passive case detection, and efforts to increase access to patients through involvement of communities and district officials for vector control and disease surveillance. In contrast, a sample size of minimum 400 patients (200 per arm) would have been required for the conduct of the IMPAMEL III program in the design of a randomized control trial. However, case detection is significantly hampered by the low sensitivity of the diagnostic tools and the prevalent lack of capacities for HAT diagnosis in the endemic regions. Many cases die undetected, there are an estimated 12 undetected deaths per each reported death (7).

Today, the biggest need for HAT affected populations is a new and safe treatment alternative. This will sadly not be the case in the near future and melarsoprol will continue to play the central role. Our results show that *T.b. rhodesiense* patients treated with the 10-day melarsoprol schedule were not subject to a higher incidence of serious adverse events (ES or death) than the historic controls treated with the national regimens (see figure 3). Hence, evidence could be provided for the improvement of the melarsoprol treatment schedule, the omission of the suramin-pre-treatment and a standardized use of steroids. The hospitalization time was reduced from an average of 29 days to 13 days ($p<0.0001$). Treatment adherence was very good; patients did not abandon treatment as frequent as reported under the national schedules in use. Further, the fixed dosing of 2.2mg/kg/day is less prone to dosing mistakes than varying dosing throughout treatment. This represents substantial advantages to the patients and the health care provider. However, as in *T.b. gambiense* HAT, ES still occur and continue to pose a major threat to the patients treated.

This study will be presented during the next 30th ISCTRC Meeting in Entebbe, Uganda, 21-25 September 2009. It is expected that WHO and the *T.b. rhodesiense* affected countries will discuss the introduction of the 10-day melarsoprol schedule in East Africa according the available data.

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Chapter 3

Molecular characterization of trypanosomes from clinical trial patients in Uganda and Tanzania

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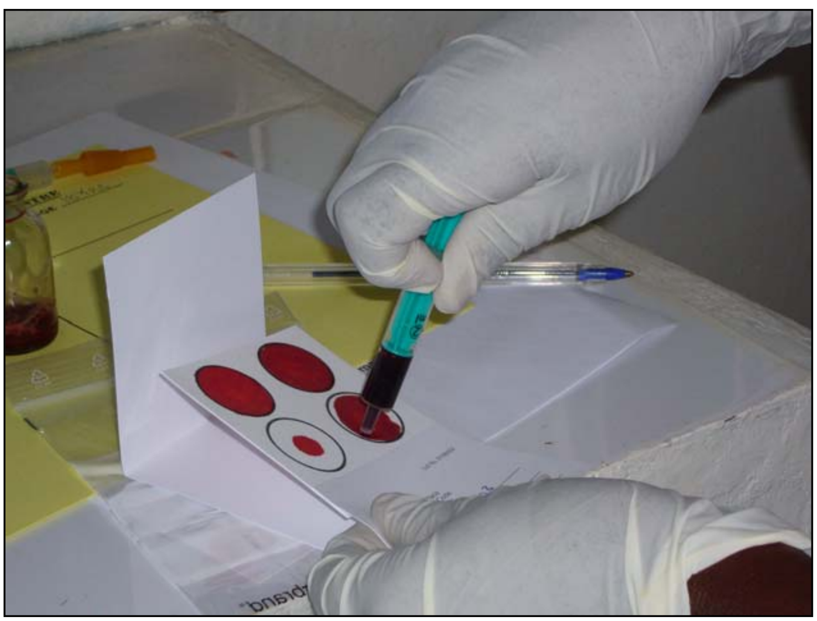

Blood sample collection at Kaliua Health Centre, Tanzania

Abstract

OBJECTIVE The samples for this study were collected in the context of the IMPAMEL III trials (Improved Application of Melarsoprol), two clinical trials assessing the short melarsoprol treatment schedule for *T. b. rhodesiense* HAT. Our aim was to characterize the infections from consenting patients as truly *T. b. rhodesiense*.

METHODS We analyzed DNA eluted from FTA cards spotted with blood from late stage HAT patients. PCR for the Serum Resistance Associated (SRA) gene specific for *T. b. rhodesiense* and the *T. gambiense* specific surface glycoprotein (TgSGP) were done. In addition, the Loop mediated isothermal amplification (LAMP) of DNA targeting the *Trypanozoon* conserved Random Inserted Mobile Element (RIME) and that for the SRA were performed.

RESULTS AND CONCLUSIONS: Out of 128 samples analyzed SRA-PCR was positive in 101 (78.9% sensitivity; 95% confidence interval of 71.1-85.1%), the SRA-LAMP positive in 120 (93.8%, with 88.2-96.8% as the 95% CI), while RIME-LAMP revealed signals in 122 (95.3%; 95% CI=90.2-97.8%). No amplification was possible from 3 samples, whereas all the 128 were as well negative for the TgSGP-PCR and a sample with known *T. b. gambiense* included as a control was positive.

All the successfully analysed samples were confirmed to contain *T. b. rhodesiense* based on the subspecies specific PCR and/or LAMP, while signals for the TgSGP were absent. Thus the results of IMPAMEL III will represent a true picture of the short concise melarsoprol treatment schedule against the acute form of HAT, at least for patients from whose FTA cards DNA was successfully amplified.

Key words: Human African trypanosomiasis (HAT), *Trypanosoma brucei rhodesiense*; *T. b. gambiense,* molecular diagnosis.

Introduction

Effective control of Human African Trypanosomiasis is still hampered by unsatisfactory diagnostics and limited options for chemotherapeutic intervention. Within health units, it is still not possible to distinguish between *T.b. rhodesiense* (the acute form of eastern and southern Africa) and *T.b. gambiense* (the chronic form of central and western Africa) infections. Thus other parameters, mainly geographical location of the patients, case history and clinical presentation, are used to determine the most probable subspecies in question. This becomes problematic in areas close to borders between traditional ranges of the two diseases and also where new, non-historical foci emerge (1, 2).

Unequivocal identification of human infective trypanosomes up to the subspecies level is only possible by molecular tools. These are PCR based, targeting the Serum Resistance Associated (SRA) gene that is specific for *T.b. rhodesiense* (3-5), or the *T.b. gambiense* Surface Glycoprotein (TgSGP) that is diagnostic for that subspecies (6, 7). The need for specialized equipment and constant supply of electricity has hindered integration of those techniques into the diagnostic algorithm. Yet the type of infection directs the choice of treatment: Melarsoprol which was the standard treatment for second stage human African trypanosomiasis (HAT) over more than 50 years frequently causes severe adverse drug reactions. *T. b. gambiense* infections are increasingly treated with eflornithine which is significantly better tolerated (8) whereas for late stage *T.b. rhodesiense* infections melarsoprol is the only choice due to innate resistance of this subspecies to eflornithine (9). Since its discovery as a late stage HAT drug in 1949, melarsoprol (10) use has for decades followed an empirical rather than rational approach. After elucidation of its pharmacokinetics (11, 12), a rational schedule was proposed that was subsequently evaluated against *T.b. gambiense* under the projects "Improved Application of Melarsoprol (IMPAMEL I and II)". At the request of the World Health Organization (WHO), by the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), it was recommended as the standard schedule (13-16). Within the framework of the IMPAMEL III program, two multiple site clinical trials were conducted in order to assess the safety, tolerability and efficacy of the 10-day melarsoprol schedule against *T.b. rhodesiense* sleeping sickness. Vicinity of one of the centres to *T.b. gambiense* endemic areas, while the other is home to refugees from central Africa, provided a possibility that both disease forms could co-exist in the same epidemics. This study aimed at the detection of potentially existing *T.b. gambiense* cases in *T.b. rhodesiense* foci and to characterize the infections from consenting patients at the two centres as truly *T.b. rhodesiense* in order to authenticate conclusions to be drawn from IMPAMEL III as a correct description of this schedule in the acute form of HAT.

Materials and methods

Study Sites

The IMPAMEL III program has been carried out at Lwala Hospital, Kaberamaido District in south-eastern Uganda and at Kaliua Health Centre, Urambo District in north-western Tanzania. Lwala Hospital (Northing 36N 0530894; Easting UTM 0205802) is located in an area over 100km outside the historic *T.b. rhodesiense* (Busoga and Tororo) focus. Over the past 5 years the disease has spread into districts previously free of sleeping sickness (Soroti, Kaberamaido, Dokolo) through the movement of infected livestock. In 2004, the Lwala Hospital diagnosed the first cases of sleeping sickness and experienced an outbreak in 2004/2005 with over 400 patients treated. Today, the area is endemic for sleeping sickness.

Kaliua Health Centre, a missionary hospital established in 1997, is located (latitude: 5.05639, longitude: 31.79462) within the major sleeping sickness endemic area of north-western Tanzania. The first cases (thought to have been *T.b. gambiense*) were reported in the 1920s. Presently, the area is a known *T.b. rhodesiense* focus that experienced an epidemic outbreak in. 2004/2005, during which about 300 cases were reported.

The IMPAMEL III program received ethical clearance from the Ministry of Health, Uganda, the National Institute for Medical Research, Tanzania and from Switzerland (Ethics Committees of both Cantons of Basel). The program was also registered with the Current Controlled Trials database (ISRCTN40537886). These clearances refer to the entire IMPAMEL III, one of whose objectives was to collect blood samples for identification of infecting trypanosomes in potential overlap areas that is here reported.

Study conduct and study population

The clinical trials were initiated in August 2006 and enrolment was terminated in August 2008. In this time period a total of 138 patients were enrolled (Uganda: 69, Tanzania: 69). All trial participants were treated with the 10-day melarsoprol schedule and asked to present for follow-up examinations after 3, 6 and 12 months in order to confirm the absence of the parasite in blood and cerebrospinal fluid (CSF). Eligible for enrolment were patients ≥ 6 years of age with confirmed late stage sleeping sickness (presence of the parasite in the CSF and/or ≥5 white blood cells (WBC) per mm^3), pregnant women and moribund or unconscious patients were excluded. Written informed consent was obtained from all trial participants. For the participation of children and adolescents (below 18 years) the parents, the legal representative or the guardian gave written informed consent.

Sample collection and DNA preparation

Blood samples were collected on FTA cards (Whatman) by making 4 spots using the blood remaining after diagnostic procedures. About 200µl was applied on each spot and the coded cards were allowed to air dry. The FTA cards were enclosed in self sealing bags containing silica and transported to the laboratory where they were stored at 4^{o}C. Trypanosome DNA adhering to the FTA cards was cleared of blood contaminants and PCR inhibitors using the FTA purification reagent following the manufacturer's instructions. A 2.0 mm disc was punched from a dried blood spot using a Harris micro punch tool (Whatman). After 3 washes with 200µl of FTA purification reagent and 5 minute incubations between washes at room temperature, the FTA reagent was removed and the disc rinsed twice in a similar manner with 200µl TE buffer. The buffer was discarded and the disc carrying purified DNA air-dried at room temperature. From each card (patient), 5 such discs were prepared and finally pooled in a single tube per patient after drying. The DNA was then eluted from the discs by incubating in 100µl of a 5% chelex suspension at 90^{o}C for 30 minutes (17). After a pulse spin at 13000g, the eluted DNA was pipetted off and used immediately, or stored at -20 for use within 3 days of its preparation. In all subsequent amplifications, 5µl of the DNA solution was added as the template.

PCR for the SRA and TgSGP genes

PCR for the SRA gene was carried out in nested manner as described by Maina *et al*. 2007 (18) using the primers SRA-outer-s 5′-CTGATAAAACAAGTATCGGCAGCAA-3′; SRA-outer-as 5′-CGGTGACCAATTCATCTGCTGCTGTT-3′ and 5µl of eluted DNA in a 25µl reaction. For the second PCR, 3µl of the first product was included as template in the reaction with the primers SRA-inner-s 5′-ATAGTGACATGCGTACTCAACGC-3′); SRA-inner-as 5′-AATGTGTTCGAGTACTT CGGTCACGCT-3′. Similarly, nested PCR for the T. b. gambiense specific gene was done with the primers TgSGP-outer-s 5′-GCGTATGCGATACCGCAGTAA-3′ and TbsGP-outer-as 5′-CTTCAACCGCCGCTGCTTCTA-3_ as well as TbsGP-s 5′-GCTGCTGTGT TCGGAGAGC-3′) and TgSGP-as 5′-GCCATCGTGCTTGCCGCTC-3′. In all cases, the annealing temperature was maintained at 60^{o}C for 1 minute, the same timing allowed for denaturation and extension. Initial denaturation was at 94^{o}C for 5 minutes. PCR products were loaded on 2% agarose and stained with ethidium bromide for UV trans-illumination.

Loop Mediated Isothermal Amplification (LAMP) of the SRA gene and the Random Insertion Mobile Element (RIME)

For the SRA-LAMP, we made use of primers recently described by Njiru *et al*. 2008 (19) namely SRA-F3 GCGGAAGCAAGAATGACC, SRA-B3 CTTACCTTGTGACGCCTG,

SRA-FIP GGA CTGCG TTGAGTACGCATCCGCAAGCACAGACCACAGC,

SRA-BIP CGCTCTTACAAGTCTTGCGCCCTTCTGAGATGTGCCCACTG,

SRA-LF CGCGGCATAAAGCGCTGAG,

and SRA-LB GCAGCGACCAACGGAGCC. The total reaction volume was 25µl into which the above primers were added to final concentrations of 0.2µM of F3 and B3, 2µM for FIP and BIP, and 0.8µM of LF and LB. In addition 200µM dNTPs, 0.8M Betaine and 8U of *Bst* polymerase (Large fragment; New England Biolabs) was added to the mix containing 1X reaction buffer supplied with the enzyme. To this 4µl of DNA eluted from the FTA cards was added to make up to 25µl total reaction volume. The reaction was incubated for 1 hour at 62^{o}C followed by inactivation of the *Bst* polymerase at 80^{o}C for 4 minutes. Two micro litres of a 1/20 dilution of SYBR Green in water was then added and the tube gently agitated as it was observed for colour change.

Similar conditions and reagent concentrations were applied for the RIME-LAMP using the primers described by Njiru *et al*.2008 (20).

Results

The content of this paper is limited to the characterization of infecting trypanosomes in patient blood spotted on FTA cards. The results of the clinical trials and the patient follow-up are reported elsewhere (Kuepfer *et al*., manuscript in preparation).

A total of 128 samples (59 from Uganda and 69 from Tanzania) were analyzed. Signals could be obtained from PCR only after nesting with products of the first round amplification. Even then, TgSGP-PCR was only positive in a laboratory strain included as a control (Figure 1.), but yielded no signal from any of the DNAs eluted from FTA cards. Overall, SRA-PCR was positive in 101 samples (78.9% sensitivity; 95% CI of 71.1-85.1%),), but was less sensitive for samples from Tanzania (49/69; 71.0% with 95% CI of 59.4-80.4%) than for Ugandan samples (52/59; 88.1% with 95% CI of 77.5-94.1%). The difference was significant (χ^2 = 5.6; P = 0.018) and could not be attributed to differences in parasitaemia. All the cases, but one Ugandan, had parasites demonstrated in stained smears at diagnosis (table 1), indicating presence of similar parasite numbers within the samples. So the observed difference could have been a result of an event happening at some stage from FTA preparation, storage, or transportation to Makerere University.

SRA-LAMP on the other hand was positive in 120 of the cases, yielding an overall sensitivity of 93.8% (88.2-96.8% as the 95% CI). Upon discrimination between the 2 centres, it was found positive in 66/69 samples from Tanzania (95.7% sensitivity; 95% CI of 88.0-98.5%) while Ugandan samples (54/59) could be detected with 91.5% sensitivity (95% CI=81.7-96.3%). The difference in sensitivity of SRA-LAMP for the two countries was insignificant ($\chi^2 = 0.9$; $P > 0.05$).

RIME-LAMP detected the highest number of cases and was positive in 122 samples, giving an overall sensitivity of 95.3% (95% CI=90.2-97.8%). It missed 2 samples from Tanzania (97.1% sensitivity; 95% CI=90.0-99.2%) and 4 from Uganda (93.2% sensitivity; 95% CI=83.8-97.3%): this was again not a significant difference in sensitivity of RIME-LAMP in samples from the 2 countries ($\chi^2 = 1.1$; $P > 0.05$).

For all the 4 methods executed, no amplification was possible in 3 of the samples from Uganda, although trypanosomes had been demonstrated in stained smears. Amplification results were consistent with those expected from the control samples: SRA-PCR and both LAMPs were positive in the laboratory *T. b. rhodesiense* strain (AL01), while RIME-LAMP but not SRA-PCR or SRA-LAMP was positive for the *T. b. brucei* strain (GVR35, table 1)

Discussion

Continued lack of new drugs is a serious problem for HAT control whose mainstay is chemotherapeutic intervention. The latest drug to have been registered is eflornithine, way back in the 1990s following its evaluation against *T.b. gambiense* (21). More recently, the clinical programme assessing pafuramidine maleate (DB289) was terminated in Phase III due to previously unobserved toxicity (22). Thus for a long time to come control will still solely depend on the existing "ancient" tools of which melarsoprol continues to play a central role particularly for treatment of *T.b. rhodesiense*. The IMPAMEL III trials were conducted to assess the safety and efficacy of the abridged 10-day schedule recently recommended for treatment of late stage *T.b. gambiense* sleeping sickness with melarsoprol (13-16). The alternative treatment schedule is advantageous because of its practical application (no dose adjustments, shortened treatment) and the apparent socio-economic advantages (shorter hospitalisation, less drug per patient, cheaper, increased hospital capacity). However, it remained uncertain whether the observation could be extrapolated to the case of the acute *T.b. rhodesiense*. Some fears could arise from the school of thought that melarsoprol accumulation in the central nervous system could be different in the two disease forms, probably resulting from differential damage to the blood-brain-barrier by the two sub-species. The major problem with melarsoprol is characteristic encephalopathic syndromes that occur in up to 10% of treated patients (23) leading to the death of about 50% of those affected (24). It was therefore essential that patients enrolled to the IMPAMEL III trials were confirmed as true *T.b. rhodesiense* infections, especially given the epidemiological circumstances at each of the two trial sites. Lwala hospital caters for the new outbreak areas stretching as far north as Lira District where a *T.b. rhodesiense* was isolated within 150km of known *T.b. gambiense* foci (2). North-western Tanzania on the other hand has over the past decade seen refugee influx from areas affected by civil strife in Central Africa. These refuges are feared to be a possible route of introduction of *T.b. gambiense* to co-exist with the east and southern African disease. In this study, we have not encountered any *T.b. gambiense* in samples taken at the two centres until August 2008, as determined by specific PCRs and LAMP. This data is welcome given the risk of the possible co-existence of the two subspecies. If they become sympatric, it will be a great challenge for HAT control and create a major public health problem. Patient management requires high-level, disease-specific expertise for proper diagnosis, treatment and follow-up of the patients. In the case of diagnosis: screening with the Card Agglutination Test for Trypanosomiasis (CATT) (25) is part of the diagnostic algorithm for *T.b. gambiense* (26), but the test misses most *T.b. rhodesiense* infections. On one hand, the latter has no field adapted serological screening and all suspicious cases are directly subjected to the laborious parasitological tests. On the other hand, if used for screening without concentration of the body fluids, parasitological tests are likely to miss more *T.b. gambiense* due to the characteristically lower parasitaemia than the acute disease. In addition, medical staff used to the prominent appearance of *T.b. rhodesiense* infections might inevitably overlook a *T.b. gambiense* infection which is characterized by rather unspecific and often subtle signs and symptoms. Thus, co-existence of the two could lead to a number of patients passing undetected by inappropriate diagnostic approaches.

For treatment, it is known that *T.b. rhodesiense* exhibits innate resistance to eflornithine (9), such that any such diagnosed in presumed *T.b. gambiense* foci is likely to be non-responsive to this intervention. Further, patients generally present in very advanced disease stages. Given the acuteness of *T.b. rhodesiense* HAT, there is not much time left to initiate treatment and major delays can be fatal.

Molecular diagnostics are theoretically of unmatched sensitivity since they involve amplification of specific targets within the parasite. Although they are of unrivalled specificity, these methods when used for HAT detection so far fall short of the expected sensitivity and usually yield much lower figures when compared to parasitological methods. The main reason behind this are the characteristically low parasitaemia in HAT and the fact that the patient tissue (blood or CSF) has to be processed to isolate DNA and get rid of potential PCR inhibitors. It is inevitable that during the elaborate purification processes, some of the scanty parasite DNA could be lost. Besides, the subspecies specific targets happen to be single copy genes whose abundance in a low parasitaemic tissue is further compromised. Thus, nested PCR has to be done to amplify the SRA and TgSGP to a level that can be visualized on a gel, adding to the duration between sampling and actual reading of the results. For those reasons and the high level technology required, it is presently inconceivable that PCR based methods will in the near future be integrated into routine point of care diagnosis.

This is the first study to publish data on such a big collection of field samples. It has generated current information on the potential strain overlap and allowed for comparison of molecular diagnostic techniques that would otherwise not have been possible. Of particular interest was the use of the recently published LAMP tests (19, 20). Although our primary aim was to identify infecting subspecies, it gave an opportunity to gain insights into the diagnostic potential of this technique. The sensitivities observed in this study (compared to PCR based methods) are the highest so far reported, yet the method is relatively easier to execute. Comparing RIME-LAMP to SRA-PCR, there was a poor agreement between the two tests (kappa 0.11) with the latter detecting only 80.3% of the samples detected by RIME-LAMP as positive (n=122). This points to a significant difference in sensitivities of the two tests ($\chi^2 = 15.3$; $P<0.001$). Similarly, there was poor agreement between SRA-PCR and SRA-LAMP (Kappa 0.08) and the former detected 80% of the samples SRA-LAMP confirmed as positive. The difference in sensitivities of SRA-PCR (78.9%) and SRA-LAMP (93.8%) was found to be significant ($\chi^2 = 11.9$; $P=0.001$). Neither was there a strong agreement between SRA-LAMP and RIME-LAMP (kappa value 0.39): however, the former detected 95.9% of what RIME-LAMP detected as positive. Their overall sensitivities were 93.8% and 95.3% respectively, and there was no significant difference between the two ($\chi^2 = 0.302$; $P>0.05$). That means that either test can be reliably used to detect HAT due to *T.b. rhodesiense*.

The advantages of LAMP can not be over emphasized: we could get results within 1 hour of initiation by adding SYBR Green and observing for colour change as previously reported (19, 20). LAMP therefore has great potential application in resource poor settings since it can as well be done in a water bath. What would be required is further reduction of required manipulations by provision of kits in which all reactants (preferably lyophilized) are included. In this way, personnel at the diagnostic centre would only need to add the reaction buffer and patient sample before starting the reaction. LAMP could also be used to confirm cure. Our results warrant bigger case-control studies to particularly generate more data on sensitivity, specificity as well as positive and negative predictive values of LAMP. This will pave the way for its implementation as a routine diagnostic which may eventually decrease the number of cases that still go undetected by the characteristically low sensitivity of parasitological tests in current use. Efforts should also be made to devise a *T.b. gambiense* specific LAMP based on TgSGP, to keep track of possible merger of the two diseases in suspect epidemics.

It has to borne in mind however, that even in presence of molecular diagnostics of unprecedented sensitivity, HAT case definition will remain demonstration of trypanosomes in some body fluid. The molecular methods will nevertheless go a long way to detect aparasitaemic cases on which extra effort will be made by health personnel to search for trypanosomes before prescriptions can be made.

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Table 1. Samples for which the SRA-PCR or LAMP were negative.

Code	Site	Stained blood	SRA-PCR	SRA-LAMP	RIME-LAMP
11007	Kaliua	+	-	+	+
11013	Kaliua	+	-	+	+
11020	Kaliua	+	+	+	-
11025	Kaliua	+	+	-	+
11035	Kaliua	+	+	+	-
11038	Kaliua	+	+	-	+
11044	Kaliua	+	+	-	+
11050	Kaliua	+	-	+	+
11051	Kaliua	+	-	+	+
11052	Kaliua	+	-	+	+
11053	Kaliua	+	-	+	+
11054	Kaliua	+	-	+	+
11055	Kaliua	+	-	+	+
11056	Kaliua	+	-	+	+
11057	Kaliua	+	-	+	+
11058	Kaliua	+	-	+	+
11059	Kaliua	+	-	+	+
11060	Kaliua	+	-	+	+
11061	Kaliua	+	-	+	+
11062	Kaliua	+	-	+	+
11063	Kaliua	+	-	+	+
11064	Kaliua	+	-	+	+
11065	Kaliua	+	-	+	+
11066	Kaliua	+	-	+	+
11068	Kaliua	+	-	+	+
12004	Lwala	+	+	-	+
12006	Lwala	+	-	+	+
12008	Lwala	-	-	+	+
12009	Lwala	+	-	+	+
12025	Lwala	+	+	+	-
12041	Lwala	+	+	-	+
12058	Lwala	+	-	+	+
12061	Lwala	+	-	-	-
12062	Lwala	+	-	-	-
12065	Lwala	+	-	-	-
AL01	Lab *T.b.rh*	n.a.	+	+	-
GVR35	Lab *T.b.b.*	n.a.	-	-	+

Trypanosomes were demonstrated by stained smears in all except 12008, indicating comparable parasitaemia that should have been detected by molecular methods. All patient samples analysed were negative for the *T. b. gambiense specific* (TgSGP)-PCR. n.a.= not applicable.

Figure 1: Representative gels to show signals obtained for the SRA- and TgSGP- PCRs from corresponding templates. Lane 1 had laboratory T. b. rhodesiense strain (ALO1) and lane 2 had the Laboratory T. b. gambiense strain (ELIANE) while lanes 3-10 had samples from patients. No TgSGP signals were obtained from any of the FTA cards spotted with patient blood.

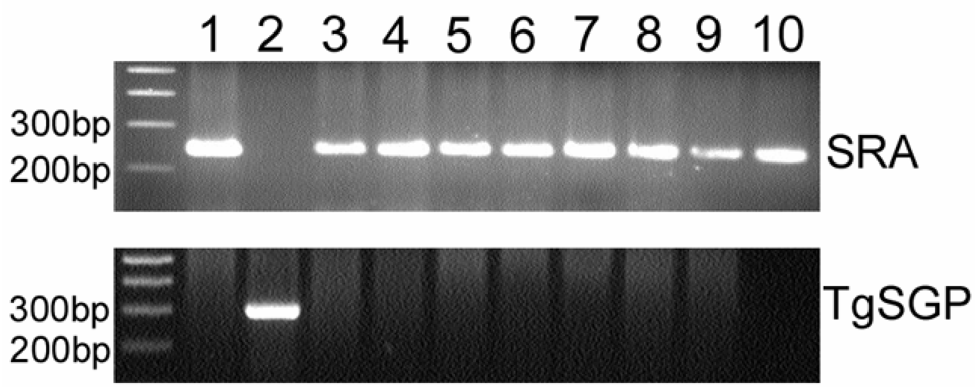

Figure 2. Representative RIME-LAMP reactions from selected patient samples. Templates in tubes 1-18 were samples from Tanzania while 19-38 contained samples from Uganda. Tube 39 had the laboratory T. b. rhodesiense (AL01), tube 40 had the laboratory T. b. brucei (GVR35) while tubes 41 and 42 were negative controls in which 5μl water was added as template.

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Chapter 4

Clinical presentation of *T.b. rhodesiense* sleeping sickness in second stage patients from Tanzania and Uganda

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Patient treated at Lwala Hospital, Uganda

Abstract

In the framework of a clinical trial program we collected the first prospective and structured data on the clinical presentation of the *T.b. rhodesiense* sleeping sickness in 138 second stage patients from Tanzania and Uganda. Significant differences in diagnostic parameters and clinical signs and symptoms were observed: the mean white blood cell (WBC) counts in Tanzania (135 cells/mm^3) was significantly higher than in Uganda (37 cells/mm^3; p<0.0001). Unspecific signs of infection (lymphadenopathy, hepatomegaly, splenomegaly) and pruritus were more common in Ugandan patients whereas neuro-psychiatric signs and symptoms such as sleeping disorders, tremor and aggressiveness were more common in Tanzanian patients. Bias due to observation, co-infections or differences in the patient cohorts appeared to be unlikely and differences in health seeking behaviour leading to a late onset of treatment could be ruled out. The two trypanosome populations could not be genetically distinguished with the currently available primers.

Introduction

Human African Trypanosomiasis (HAT), better known as sleeping sickness, is caused by the protozoan parasites *T.b. gambiense* (West and Central Africa) and *T.b. rhodesiense* (East and South Africa). The disease is solely transmitted by tsetse flies (*Glossina ssp*.). 60 Mio. people in the rural areas of most of sub Saharan Africa live at risk of infection, but less than 10% are under adequate surveillance (1), reflecting its neglected status. Sleeping sickness caused by either subspecies presents in two disease stages defined as the first, or haemo-lymphatic stage and the second, meningo-encephalitic stage. Diagnosis of HAT is made in blood and the staging of the disease by analysis of the cerebrospinal fluid (CSF). The second stage is defined by the presence of trypanosomes and/or an elevated white blood cell (WBC) count (≥5WBC/mm^3). The stage of the disease as well as the type of infection direct the choice of treatment. However, *T.b. gambiense* and *T.b. rhodesiense* can only be genetically distinguished by PCR analysis. The detection of the human serum resistance-associated (SRA) gene unequivocally identifies *T.b. rhodesiense* trypanosomes (2, 3). In the field, the type of infection is entirely determined by the geographical location of the patient. In Uganda, the only country where both forms of the disease are present, a potential geographical overlap of the two endemic areas has become likely (4) and would hamper proper diagnosis and treatment of HAT.

While often considered together, Gambiense and Rhodesiense HAT are different diseases, clinically and epidemiologically (5). While *T.b. gambiense* HAT is a chronic disease, *T.b. rhodesiense* has an acute disease progression. If left untreated, both forms of HAT are fatal. The mean time to reach the second stage has been estimated at over one year for *T.b. gambiense* (6) but only 3 weeks for *T.b. rhodesiense* HAT (7). Correspondingly, average times from infection to death are almost 3 years and 6 to 12 months, respectively (6, 7). There are no specific clinical signs and symptoms in the first stage; fever, headache and loss of appetite are common. In *T.b. rhodesiense* the presence of a chancre at the site of the infective bite may be indicative for a trypanosome infection. In the second stage of the disease characteristic neuro-psychiatric signs and symptoms occur: severe endocrinological and mental disturbances and severe motor problems are the main signs (8).

A diversity of forms of clinical progression from asymptomatic to acute have been reported for *T.b. gambiense* infections (9-11). This seems to be even more pronounced for *T.b. rhodesiense,* where a wide spectrum of disease severity ranging from a chronic disease pattern in southern countries of East Africa with existing reports of asymptomatic carriers (12) to an increase in virulence towards the north had been described (13). Even though those differences were already described more than 60 years ago (14) the first comparative study was only carried out in 2004: on the basis of the SRA gene polymorphism, trypanosomes isolates from Uganda (acute profile) and Malawi (chronic profile) confirmed to be of different genotypes. However, the clinical description of patients in this study was limited to the presence of chancre and the self-reported duration of illness (15). Another hypothesis postulates that the differences in disease severity could be attributed to differences in genetic resistance to trypanosomiasis among host populations (14).

From the estimated 50'000 to 70'00 cases per year (16) , over 97% are *T.b. gambiense* cases and only a few thousand are due to *T.b. rhodesiense* (17). Therefore, most literature concentrates on *T.b. gambiense* HAT and its clinical picture and related cardiac and endocrinological disorders have been extensively described (18-25). On the other hand, the literature on the clinical aspects of *T.b. rhodesiense* HAT is scarce. We could identify four studies describing its clinical presentation (see table 1) and only one was based on a standardized questionnaire (26).

Table 1: Published literature on the clinical aspects of *T.b. rhodesiense* HAT.

	Buyst (1977)	Boatin (1986)	Wellde (1989)	Mbulamberi (1987)
Number of patients	**385**	**60**	**96**	**3152**
Country	Zambia	Zambia	Zambia	Uganda
Disease stage of patients	2nd stage	2nd stage	2nd stage	1st stage[a]
Male/female ratio	NA	1.73	1.53	1.1
Clinical signs and symptoms (%)				
Chancre	NA	5	15.6	19
Headache	66.2	73.3	95.8	95.8
Fever	31.2	71.7	36.4	96.8
Lymphadenopathy	80.5	NA	86.4	17.6
Itching or pruritus	N.A.	35	53.1	NA
Oedema of face	30.1[b]	21.7	3.1	27.5
Swelling of legs	NA	43.3	25.3	NA
Joint pains	NA	65	88.5	95
Daytime sleep	NA	63.3	70.8	26.8[c]
Nighttime sleep	NA	28.3	NA	NA
Abnormal coordination	NA	NA	51	NA
Abnormal speech	NA	NA	38.5	NA
Mental confusion	17.4	NA	NA	NA

NA: not applicable; [a] 98.7% of the patients were in the first stage and 1.3% of the patients in the second stage of the disease; [b] reported as oedema, [c] reported as somnolence

In this paper we describe the first prospective and structured study of the clinical presentation of *T.b. rhodesiense* HAT and will compare our findings to the existing literature.

Factors that could explain the different clinical presentation in the two countries include an observation bias, concomitant infections, differences in the patient cohorts or the admission of the patients at different time points of infection and will be discussed. We will also consider differences in host and parasite genetics.

Materials and Methods

Study design and data collection. The IMPAMEL III program (2006-2009) was conducted in order to assess the safety and efficacy of the abridged, 10-day melarsoprol schedule for the treatment of second stage HAT (27, 28) in *T.b. rhodesiense* patients. Two trials, first a proof-of-concept trial in 60 patients and a utilization study in an additional 78 patients were sequentially conducted in the *T.b. rhodesiense* endemic regions of Tanzania and Uganda. The studies have been approved by the ethics committees in Tanzania (National Institute for Medical

Research/NIMR) and Uganda (Ministry of Health) and the ethics committee of both cantons of Basel (EKBB), Switzerland. Before first patient enrolment the study was registered in the database of the current controlled trials (ISRCTN40537886).

Eligible for enrolment were second stage patients with a minimum age of 6 years and confirmed second stage HAT. Moribund or unconscious patients as well as pregnant women and patients with first stage infection were excluded.

Diagnosis of HAT was made in blood and in the cerebrospinal fluid (CSF). Blood was examined using microscopy and/or the haematocrit centrifugation technique (29). If trypanosomes were present, a lumbar puncture was performed for disease staging. Analysis of the CSF was done by direct microscopy and/or single modified centrifugation technique and white blood cell (WBC) count using counting chambers. Second stage infections were confirmed by the presence of trypanosomes and/or ≥ 5 WBC/mm^3 in the CSF.

For all 138 patients enrolled, the local principle investigator recorded data in an individual set of case report forms (CRF): information on demographics, diagnosis, duration of signs and symptoms prior to the diagnosis and clinical signs and symptoms at admission were collected. The assessment of clinical signs and symptoms used a graded scale for severity (grade 1, 2).

Data management and statistical analysis. All data were double entered and verified using Epi Data 3.1 software (www.epidata.dk) and analysis was accomplished with the statistical software package STATA Version IC10.0 (STATA™, StataCorp, USA). The statistical analysis was performed comparing proportions with the Pearson chi square, means with the Student's *t* test and distributions with the Kruskal-Wallis test.

Results

A total of 138 late stage *T.b. rhodesiense* patients were enrolled. Demographic and diagnostic baseline characteristics of the study population are shown in table 2. The proportion of male (57.2%) and female (42.8%) patients was similar. 18.8% (26) trial participants were younger than 16 years whereof 88.5% (23) were enrolled in Uganda. Significantly more patients from Uganda had a body mass index (BMI) below 16.5 ($p<0.0001$). Trypanosomes were more often identified in the blood of Tanzanian patients, although the difference did not reach statistical significance (99% vs. 91%, $p=0.0524$) whereas the frequency of trypanosomes in CSF did not differ (80% vs. 86%, $p=0.3690$). Country specific differences were observed for the WBC counts in the CSF: the mean WBC count in Tanzania was 135 (±85) and 37 (±40) in Uganda ($p<0.0001$).

Table 2: Demographic and diagnostic baseline characteristics of the study population.

	Total (n=138)		Tanzania (n=69)		Uganda (n=69)	
	n	%	n	%	n	%
Age, mean ± SD	35±19		38±15		32±22	
Age, range	6-85		9-70		6-85	
Male female ratio	1.34		1.38		1.3	
Age below 16 years	26		3		23	
Nutritional status						
BMI[1] (kg/m^2) - mean ± SD	18.5±3.4		19.6±2.5		17.3±3.8	
BMI<16.5	38	28	5	7	33	48
Malaria positive on admission	57	41	55	80	2	3
Diagnostic findings						
Trypanosomes in blood	131	95	68	99	63	91
Trypanosomes in CSF[2]	114	83	55	80	59	86
WBC[3] count in CSF						
0 - 20 cells/ul - no. (%)	35	25	0		35	51
21 - 100 cells/ul - no. (%)	52	38	23	33	29	42
> 100 cells/ul - no. (%)	51	37	46	67	5	7
Median	70		134		20	
Mean ± SD	86±82		135±85		37±40	

Note: [1]Body Mass Index, [2] CSF: cerebrospinal fluid, [3] WBC: white blood cell

The clinical signs and symptoms reported at baseline are summarized in table 3. The level of significance (95%) between patients in Tanzania and Uganda is also shown.

Table 3: Clinical signs and symptoms of study population at baseline.

	Total (n=138)		**Tanzania (n=69)**		**Uganda (n=69)**		**Statistical**
	n	**%**	**n**	**%**	**n**	**%**	**test**
Self reported duration of illness							
up to 1month	40	29	15	22	25	36	
2-3 months	52	38	32	46	20	29	
more than 3 months	46	33	22	32	24	35	
Mean reporting time to health facility in months (range)	3 (0-12)		3.2 (1-12)		2.8 (0-7)		p=0.2683
Clinical manifestations							
Lymphadenopathy	27	19.6	7	10.1	20	29.0	p=0.0053
General Body Pain	132	95.7	69	100.0	63	91.3	p=0.0123
Headache	128	92.8	65	94.2	63	91.3	p=0.5114
Fever (>37.5°C)	41	29.7	20	29.0	21	30.4	p=0.8522
Fever (>38.5°C)	9	6.5	3	4.3	6	8.7	p=0.3010
Joint pains	129	93.5	67	97.1	62	89.9	p=0.0847
Diarrhea	9	6.5	1	1.4	8	11.6	p=0.0158
Pruritus	21	15.2	4	5.8	17	24.6	p=0.0003
Oedema	40	29.0	26	37.7	14	20.3	p=0.0244
Dyspnoe	10	7.2	1	1.4	9	13.0	p=0.0086
Cough	27	19.6	8	11.6	19	27.5	p=0.0183
Tremor	54	39.1	43	62.3	11	15.9	p<0.0001
Hepatomegaly	25	18.1	4	5.8	21	30.4	p=0.0002
Splenomegaly	51	37.0	11	15.9	40	58.0	p<0.0001
Walking difficulties	75	54.3	35	50.7	40	58.0	p=0.3928
Abnormal movements	36	26.1	31	44.9	5	7.2	p<0.0001
Sleeping disorder daytime	105	76.1	66	95.7	39	56.5	p<0.0001
Sleeping disorder nighttime	88	63.8	64	92.8	24	34.8	p<0.0001
Strange behaviour	25	18.1	15	21.7	10	14.5	p=0.2691
Disturbed appetite	120	87.0	60	87.0	60	87.0	p=1
Inactivity	100	72.5	57	82.6	43	62.3	p=0.0076
Speech impairment	16	11.6	6	8.7	10	14.5	p=0.2875
Aggressiveness	45	32.6	43	62.3	2	2.9	p<0.0001

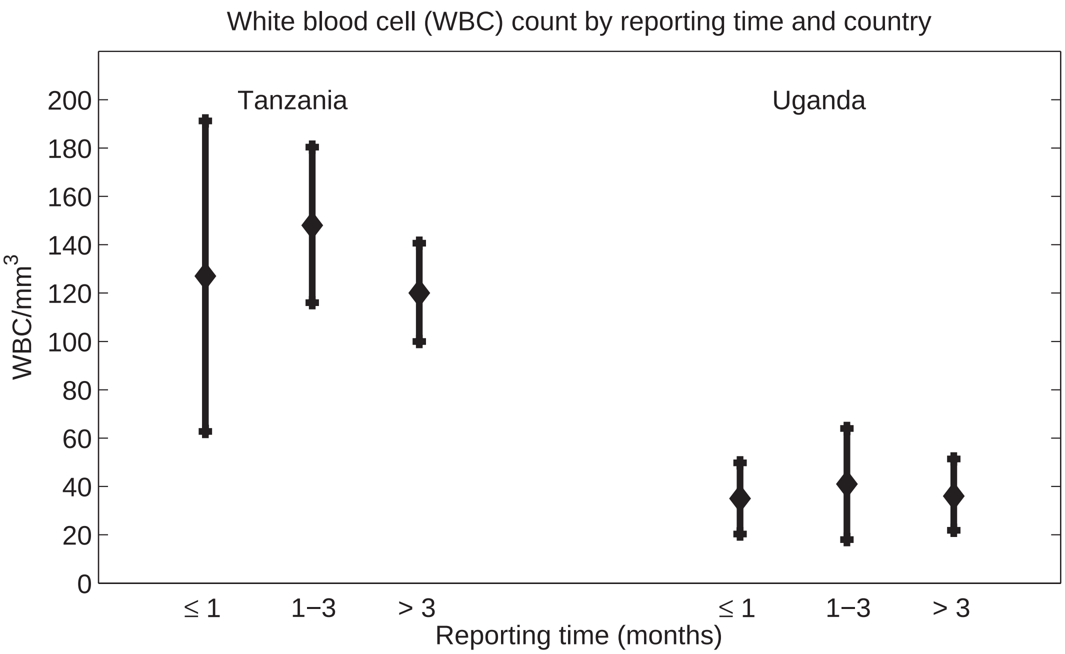
Note: [1]Body Mass Index, [2] CSF: cerebrospinal fluid, [3] WBC: white blood cell

Unspecific signs of infection (lymphadenopathy, hepatomegaly, splenomegaly) and pruritus were more frequent in Uganda than in Tanzania. Also diarrhea, dyspnoe and cough were more frequent in Uganda than in Tanzania. General body pain and oedema were more common in Tanzania than in Uganda as well as most of the characteristic signs and symptoms related to CNS involvement: tremor, abnormal movement, sleeping disorders at day and night time, inactivity and aggressiveness. No difference was observed for strange behaviour, walking difficulties, disturbed appetite and speech impairment.

Clinical suspicion for cardiac insufficiency was found in both centres: indication for left heart insufficiency (combination of cough and dyspnoe) and right heart insufficiency (combination of odemea and hepatomegaly) was seen for each in 3.6% of the patients.

To draw conclusions on changes in clinical presentation and diagnostic findings over time, the study population was categorized in 3 groups according the self-reported time of illness. 29% reported to the health centre within one month of illness, 38% reported after 2-3 months and 33% after more that 3 months of illness (see table 3). We analyzed the differences in distributions of clinical signs and symptoms and diagnostic findings among those groups. Significant differences were found for tremor (p=0.0026), abnormal movements (p=0.0378), walking difficulties (p=0.0331) and aggressiveness (p=0.0033). No difference was found for cough (p=0.8716), dyspnoe (p=0.7403), oedema (p=0.0854), hepatomegaly (p=0.2321), splenomegaly (p=0.8439), daytime sleep (p=0.0626) and night time sleep (p=0.2244) and unusual behaviour (p=0.7824). There was no significant change over time for the presence of trypanosomes in blood (p=0.7259) and CSF (p=0.8020). As shown in figure 1 also the WBC count did not change in relation to the reporting time (p=0.4549).

Figure 1: Mean and 95% confidence interval for white blood cell (WBC) count in the central nervous system (CNS) by country and reporting time.



Discussion

In this paper, clinical symptoms and signs of patients with second stage HAT due to *T.b. rhodesiense* are described for a cohort of 138 patients treated in a prospective study in Tanzania and Uganda. The only specific neurological signs in the Ugandan patients were sleeping disorders and walking difficulties whereas in Tanzanian patients the neuro-psychiatric signs such as sleeping disorders, aggressiveness, inactivity as well as abnormal movements and tremor dominated the clinical picture (see table 3). Headache and general body pain/joint pains were common in all patients whereas unspecific signs of infections such lymphadenopathy, hepatomegaly and splenomegaly was more frequently found in Ugandan patients.

Fever is a leading symptom in Ugandan first stage patients (97%) and in non-African patients with first and second stage HAT (close to 100%) (30). Fever was reported in a lower extend of second stage patients from Zambia (31-71%) and was only occasionally found in *T.b. gambiense* patients (16%) (22). We report fever in 30% of the patients. High fever (>38.5) was seen in 6.5% of the patients whereof three cases were children (33.3%). This is different to the findings of *T.b. gambiense* HAT where high fever was most frequently reported in children (2-14 years) (22). Fever seems to be a rather typical sign in the early stages of *T.b. rhodesiense* HAT but decreases over time.

Whereas the heart involvement is typical but rarely of clinical relevance in *T.b. gambiense* patients (31) we have limited knowledge in the role of cardiac involvement in *T.b. rhodesiense* patients. However, there is evidence that perimyocarditis seems to play an important part in the clinical course and fatal outcomes in *T.b. rhodesiense* HAT patients (32, 33). Based on our findings the symptoms oedema (swelling of legs) (29%), hepatomegaly (18%), dyspnoea (7%) and cough (20%) could be caused by a cardiac failure. Given the limitations for examination under field conditions this is only an assumption. More specific examinations such as auscultation of the lungs and interpretations of the congestion of the neck veins or a positive hepatojugular reflux would be needed. However, this could not be done under field conditions as the examination beds do not allow the assessment of the jugular veins at 45° and the medical workers were not trained for diagnosis of heart failure.

The percentage of neuro-psychiatric signs and symptoms is surprisingly high in the Tanzanian group and comparable to the clinical presentation of *T.b. gambiense* HAT. The clinical presentation of the Ugandan patients corresponds more to the clinical pattern of a first stage HAT. The literature described the CNS involvement on the basis of daytime sleep, night time sleep, abnormal coordination, abnormal speech and mental confusion (see table 1). The study on first stage patients in Uganda reported somnolence in 26.8% of the patients compared to 63.3% - 70.8% of the second stage patients from Zambia. In our study, daytime sleep in Tanzania was reported in 96% and in Uganda in 57% of the patients which corroborates the notion that daytime sleep is characteristic for second stage disease. Sleeping disorders at night were more frequent in Tanzania (92.8%) than in Uganda (34.8%). One study from the literature reported night time sleep in 28.3% of the patients which compares well with our findings in Uganda. Conclusively, sleep disorders correlate with the degree of CNS involvement. The literature reported abnormal speech in 38.5% of the patients whereas we report speech impairment only in 11.6% of the patients. No striking differences to the literature were seen for abnormal coordination (51%) that we compared to walking difficulties (54.3%) and mental confusion (17.4%) that we compared to strange behaviour (18.1%). None of the studies from the literature indicated tremor; either tremor was not present in those patients or it was outbalanced by other signs and symptoms.

Most of the literature described second stage HAT in patients from Zambia. The study from Mbulamberi *et al.* (34) describes the disease in first stage patients and so far, no information was available on the clinical presentation of second stage HAT in Tanzanian patients.

Our data confirm a wide spectrum of disease severity: a high variability of the symptoms and signs were observed between the two study populations as well as to the presented literature (see table 1).

Underlying causes could include an observation bias, concomitant infections, different cohorts of patients, and the admission of patients at different time points after infection. Differences in host or parasite genetics also need to be considered.

An observations bias cannot be ruled out. However, the same structured CRF and monitoring person and the variability as well for signs with a clear definition observed by the health personnel (e.g. lymphadenopathy, abnormal movements or tremor) as for subjective symptoms declared by the patient (e.g. insomnia, headache or inactivity) makes an observations bias less likely.

Malaria was a concomitant disease (80%) and could explain at least partially the presence of fever in the Tanzanian group. There were no observations of filariasis or scabies that could explain the differences in the frequency of pruritus in the two countries. No further concomitant diseases were reported. Based on the clinical assessment it was not possible to attribute the significant lower body mass index of Ugandan patients to cachexia or malnutrition. However, food security is very poor in this part of Uganda and most likely the reason for the poor nutritional status of the population. This could explain a weakness leading to inactivity and walking difficulties in the absence of neurological symptoms.

We consider differences in the patient collectives as rather unlikely. The male/female ratio from the literature (see table 1) was comparable to the ratio of 1.4 in the study population. Also, the children (18.8%) in the study population did not show differences for the incidence of fever nor for the incidence of neuro-psychiatric signs and symptoms.

The hypothesis of admission of the patients at different times of disease evolution could be possible and is supported by the fact that neurological symptoms correlate with the progression of the disease. However, the duration of self-reported symptoms was comparable in both groups. Further, sleeping disorders and presence of trypanosomes in blood and CSF and/or WBC counts did not correlated with the duration of symptoms.

Host genetics and eventually previous infections may be likely determinants for the severity of response to infection. There are speculations that apathogenic forms of the disease could influence the immune response to pathogenic infections (34, 35). Further, the clinical presentation is more acute in the white than in the black population (30, 36). Also high variability is seen among African populations (13) and might be related to their decent: people of Bantu descent, whose ancestors have been exposed to human trypanosomes for several thousand years, may have greater tolerance than people of Nilotic descent, who migrated into the East African region from tsetse-free areas during the past 2,000 years (14). However, this can not be confirmed by our data since in Tanzania the majority of the population is of Bantu origin and in Uganda the majority of the population is of nilotic origin. In summary, our data show a clear difference in the clinical presentation of the disease in Tanzania and Uganda but do not allow conclusive remarks on the influence of host factors.

Different parasite genotypes could be responsible for the observed spectrum of disease severity. This hypothesis has already been raised 60 years ago based on observation of epidemiological and clinical patterns (12, 13, 37). Different parasite genotypes were confirmed for trypanosomes isolates from Uganda and Malawi on the basis of the SRA gene polymorphism (15). The two spatially distinct Tororo and Soroti foci of *T.b. rhodesiense* in Uganda were shown to be genetically distinct *T.b. rhodesiense* parasites (38).

In an ancillary study to the molecular characterization of trypanosome DNA from all IMPAMEL III participants (see chapter 3) we analyzed the two trypanosomes populations by microsattellite analysis. For a first analysis we used the primers by MacLean *et.al.* (38) but the populations did not appear to be different. In a second analysis we used primers recently designed at the University of Glasgow (not yet published) but again the two populations did not appear to be significantly different. However, we don't consider these results as fully conclusive. Future attempts should be made designing different sets of primers in order to rule out potential limitations of the method. The assumption that the parasite belong to the same population contradicts recent findings on the phylogenetic relationship between different *T.b. rhodesiense* strains that showed that the high variability of the *T.b. rhodesiense* genome is attributed to multiple and independent evolutions from *T.b. brucei* (39).

The Ugandan picture of second stage *T.b. rhodesiense* HAT resembles more a first or an early second stage infection whereas in Tanzania it presents as an advanced second stage disease with predominance of neuro-psychiatric symptoms.

Biases caused by observation, co-infections and different patient cohorts appeared to be unlikely. Differences due to initiation of treatment at different time points of infections could be ruled out as the duration of self-reported symptoms was comparable in both groups.

Host factors such as genetic makeup or previous infections with apathogenic trypanosomes could contribute to the observed differences in the clinical presentation in patients from Tanzania and Uganda. However, the analyses of those factors were beyond the scope of our study.

Despite the evidence of different parasite genotypes in Uganda and Malawi we were not able to genetically distinguish the trypanosomes from Tanzania and Uganda with the currently available methods. However, limitations of the method can not be excluded and other sets of primers might allow differentiation.

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Chapter 5

Reflections on clinical research in sub-Saharan Africa

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Abstract

The urgent need for new, safe and sustainable interventions against diseases that disproportionally affect the poor is finally receiving global attention and the funding landscape for development projects has significantly improved during the past decade. For the development of new drug and vaccine candidates, clinical trials have become the most important tool to assess their safety and efficacy. Recently, there has been a seismic shift in the number of clinical trials conducted in resource-limited settings. We discuss the current framework of clinical research in sub-Saharan Africa, from building product pipelines to the capacities needed for the conduct of trials according the harmonized Good Clinical Practice (GCP) ICH E6 guideline. We place emphasis on clinical research in neglected tropical diseases which still frequently has to be conducted with limited financial, logistical and human resources. Given those short-comings we recommend minimum standards needed at the local, national and sponsor levels to provide GCP-compliant clinical research.

Keywords: Neglected tropical diseases; Sub-Saharan Africa; Research and development; Clinical research; Good clinical practice (GCP); Minimum standards

Introduction

Clinical studies and trials are the most important tools to assess the evidence of new medical interventions including drugs and vaccines. The correct and fair conduct of the investigations is essential and this was one of the key messages of the first International Clinical Trials Day of 20th May 2005 which was organized to boost clinical research worldwide (http://www.ecrin.org).

Almost 10 years ago, the term '10/90 gap' was coined, recognizing that in the preceding 30 years only 10% of global health research has been dedicated to diseases that accounted for 90% of the global disease burden (1). Fortunately, since 2000 the funding landscape for tropical disease research has significantly improved (2) and as a result, the number of clinical trials conducted in sub-Saharan Africa has multiplied. Today, a new challenge in certain fields is coordination of the efforts of numerous global initiatives and consortia contributing to new, sustainable interventions against diseases which disproportionately affect the poor.

During the same period, international rules for the conduct of trials have been advanced from guidelines to laws in the Western world and largely implemented. The harmonized Good Clinical Practice (GCP) ICH E6 guideline (3) set a quality standard, but has also added an unprecedented dimension of complexity to clinical research. Whereas many sub-Saharan drug authorities are still not in the position to impose fully GCP-compliant trials, most international funding agencies and sponsors do so and clinical research in resource-limited countries has to satisfy international laws and regulations. This has the advantage of increasing quality standards and credibility of the data produced, but may also lead to conflicts with cultural, political and socio-economic facts and values.

Trials on interventions against rare diseases or those which have a mainly rural distribution often have to be carried out with limited finances, logistics and human resources. It stands to reason that finding the equilibrium between those different realities remains a challenge. Those conditions and settings also stand in sharp contrast to the growing number of high standard research centers in sub-Saharan Africa.

In this article we review the current framework and the practical aspects of clinical research in sub-Saharan Africa with an emphasis on clinical trials for the most neglected diseases. Given the various challenges and restrictions of such research in collaboration, we attempt also to present minimum standards for the appropriate conduct of clinical trials and studies.

Creating and maintaining a pipeline of new interventions against neglected tropical diseases

Neglected tropical diseases

Respiratory infections and diarrheal diseases are the two main categories of infectious diseases responsible for a high burden of disease in resource-poor settings (http://www.who.int/mediacentre/factsheets/fs310_2008.pdf). In addition, the "Big Three" - HIV/AIDS, malaria and tuberculosis account for 5.6 million deaths and the annual loss of 166 million disability-adjusted life years (DALYs) (4). Premature mortality and high morbidity are also caused by another category of illnesses referred to as neglected tropical diseases, e.g. Human African Trypanosomiasis, Chagas disease, schistosomiasis, leishmaniasis, dengue fever and leprosy. Together those diseases cause approximately 534,000 deaths annually and are responsible for a very large number of years of life lost as a result of from premature disability (DALYs). Some estimates suggest that the neglected tropical diseases result in 57 million DALYs lost annually, a number that is almost as high as that of each of the 'Big Three' (4).

Neglected tropical diseases share three common characteristics. Firstly, they are only prevalent in resource-limited settings and hence the market incentives are far too low to trigger corporate investments for new interventions. Second, the target product profile of new diagnostics and chemotherapies for such diseases must allow their use under very difficult field conditions and be stable under extreme conditions of heat and humidity (5). Third, the interventions need to be cost-effective to have a fair chance to be delivered to the target population by the typically weak health systems or non-governmental organizations (NGOs).

Undoubtedly, we are significantly lacking the tools to properly diagnose and treat many neglected tropical diseases. The withdrawal of pharmaceutical companies from tropical disease research and development (R&D) in the 1970s left a very large gap in the development of new and affordable drugs (6). From 1975 to 1999, 1,393 new pharmaceuticals were marketed worldwide of which only 13 were against tropical diseases (7), mainly for malaria and tuberculosis.

The new landscape after the year 2000

The landscape for neglected disease R&D has dramatically changed since the beginning of the new millennium (2) and the reasons are manifold. An in-depth analysis performed in 2004 showed that 63 neglected disease drug projects were under way, including two new drugs in registration status and 18 new products in clinical trials, half of which were already in Phase III. With sufficient funding these projects were expected to deliver eight to nine new neglected-disease drugs until 2009, even if no further projects would have commenced after this (2).

Searching for new medical interventions against neglected tropical diseases

Due to the recognized need for new, rapid and sustainable interventions, and due to lengthy and expensive pharmaceutical development processes, short- and long-term strategies are combined in order to deliver new drugs and vaccines to this neglected market. One of the central pillars is the extensive screening of existing compound libraries. Rapid success and a comparatively low attrition rate can be expected from compounds which have already undergone pre-clinical and/or partial clinical development but were abandoned by industry due to economic reasons or inefficacy for the original indication. Unfortunately, such compounds are hard to come by, possibly due to potential embarrassment should the compound be successful and the fear of important trade secrets being passed on unintentionally. Other shorter-term strategies involve the development of follow-on drugs in the same class, fixed dose combinations of existing drugs, and of new pediatric formulations to make childhood treatment easier. Exciting results can also come from re-directing compounds developed for other diseases towards neglected diseases, also called “therapy-switching” or “piggy-backing” (8).

However, to create a sustainable pipeline of compounds, long-term strategies are important. There the focus is on “breakthrough” innovation; i.e. novel compounds with a novel mechanism of action against the pathogens (2). This approach is of primary importance to cope with the increasing problem of resistances in diseases where treatments exist (e.g. malaria and tuberculosis) but also to find acceptable therapies for those diseases where acceptable solutions are so far lacking (e.g. sleeping sickness, leishmaniasis). A recent example of drug resistance is the report of artemisinin-resistant malaria in western Cambodia (9).

On the positive side, a number of new screening centers tackling the above-mentioned tasks became operational during the past few years and the number of hits and lead compounds is increasing. However, a major challenge and persisting bottleneck appears to be the professional selection of the leads and particularly the advancement of the most promising compounds into funded professional pre-clinical programs.

Alternative business models for R&D in tropical diseases

Private sector investments in R&D are not triggered by commercial incentives, thus a system that not only shares the risk but also the cost of a highly expensive undertaking was established and became known as Public Private Partnerships (PPPs), now called Product Development Partnerships (PDPs). Examples of PPPs/PDPs such as the special program in research and training of tropical diseases (TDR) date back to 1975. Today, the development of new partnerships has accelerated and examples are numerous. The Initiative on Public Private Partnerships for Health lists over 90 such organizations in its database (http://www.globalforumhealth.org).

These organizations mostly operate like virtual drug companies and are largely responsible for the recent seismic shift in R&D for tropical diseases (6). At present the range of funding is mainly provided by philanthropic organizations led by the Bill & Melinda Gates Foundation (http://www.gatesfoundation.org). In 2005, philanthropic organizations contributed over 78% of the total funding of R&D partnerships for neglected diseases, whereas public funding was calculated at a mere 16%.

Public funding has a long history at the National Institutes of Health (NIH, USA) mainly in the form of competitive research grants, whereas other institutions such as the Institut Pasteur (France) or the Medical Research Council (MRC, United Kingdom) made targeted investments in satellite centers. Broad public funding is a major factor for sustainable tropical disease R&D, as only governments can guarantee a long-term commitment (10). The report of the World Health Organization's (WHO's) commission on public health, innovation and intellectual property rights released in April 2006, urged the WHO to develop a global plan of action to secure enhanced and sustainable funding for developing and making accessible products to address diseases that disproportionately affect developing countries (11).

An example of targeted public funding is the European & Developing Countries Clinical Trials Partnership (http://www.edctp.org) which was created in 2003. During the past year it has gained substantial momentum, funding drug and vaccine development in HIV/AIDS, malaria and tuberculosis.

Areas where governments can indirectly encourage corporate interest in rare and/or neglected diseases are indemnification strategies. Since 1983 the U.S. Food and Drug Administration (FDA) has offered tax incentives for clinical trials as well as 7 years of marketing exclusivity for drugs developed for rare diseases in the US through the Orphan Drug Act (http://www.fda.gov/orphan/oda.htm). In 2007, the U.S. Congress approved an amendment to the FDA Revitalization Act (http://www.fda.gov/oc/initiatives/HR3580.pdf), which created a transferable voucher to encourage treatments for tropical diseases. The sponsor of a newly approved drug which prevents or treats an eligible tropical or neglected disease will receive a priority review voucher, which can then be transferred to the submission of another human drug or sold to another company. Priority review reduces the time period for the review of the registration dossier from an average of 18 months to no longer than 6 months. Economists estimate that the priority review voucher used for a potential blockbuster drug could be worth more than US$ 300 million. In comparison, the average costs of developing a new chemical entity were estimated to be US$ 800 million in the year 2003 including capitalization. The voucher could thus enable a company to recoup a significant portion of the cost of developing a new drug (http://www.iavi.org/viewfile.cfm?fid=47963). This seemingly straight forward system awaits its first application, with some critical voices cautioning against potential misuse or a decreased quality of the review process (12).

In 2000 the European Union adopted the Orphan Medicinal Products legislation under which companies with an orphan designation for a medicinal product benefit from incentives such as protocol assistance (scientific advice during the product-development phase); marketing authorization (10-year marketing exclusivity); financial incentives (fee reductions or exemptions); and national incentives detailed in an inventory made available by the European Commission (http://www.emea.europa.eu/pdfs/human/comp/29007207en.pdf). The European Union pharmaceutical legislation may support authorities that lack regulatory capacity; those authorities can obtain scientific opinion from the European Medicines Evaluation Agency (EMEA) through WHO on products intended exclusively for markets outside the community (13).

Last but not least, the pharmaceutical industry has renewed its interest and engagement in tropical diseases. Examples for new industry sponsored research facilities are the Novartis Institute for Tropical Diseases (http://www.novartis.com/research/nitd), GlaxoSmithKline's Diseases of the Developing World Initiative (http://www.gsk.com /research/about/about_diseases.html), Sanofi-Aventis' Malaria Initiative (http://en.sanofi-aventis.com/sustainability/sustainability.asp) and Astra-Zeneca's (http://new.tballiance.org /newscenter/view-brief.php?id=52) effort in tuberculosis research. These institutions will have a major impact in coming years (6).

Persistent and emerging complexities - intellectual property rights

A key aspect of cooperative R&D is the role of intellectual property protection mechanisms (IPPM). Through adequate management of the resulting intellectual property (IP), the public sector can benefit from its R&D investments through the availability of the most modern products with conditions that are beneficial for the developing world, eliminating otherwise significant barriers to access (14). Joint research ventures such as partnerships among developing countries, the private sector, academic institutions and NGO's depend on IP management strategies. However, recent publications indicated that intellectual property rights negotiations are more complex in horizontal research joint ventures (same industry) and when universities are involved (15).

Patents are the most frequently used IPPM (15). For example, the Medicines for Malaria Venture (MMV) arrangement for the synthetic peroxide antimalarial project involved the assignment of a patent from a U.S. university. The patent included claims for treating cancer and schistosomiasis, which are retained by MMV. The compound was licensed to an Indian company, Ranbaxy, with a provision for reversion of the rights to MMV should Ranbaxy fail to meet certain milestones (such as meeting public sector demand in target developing countries at affordable prices). Likewise, further segmentation of the market into a "traveler's market" and worldwide private sector sales provides potential commercial incentives for Ranbaxy once they meet the criteria for public health interest (14). But innovative partnerships have also come up with non-patented drugs. The Drugs for Neglected Diseases Initiative (DND*i*) partnered with French pharmaceutical company Sanofi-Aventis in 2007 and brought fixed-dose artesunate-based combination therapies to the African market. This patent-free model is now also implemented for delivering anti-malarial treatment to South American patients (http://www.ip-watch.org/weblog/2008/04/17/innovative-partnership-to-create-another-patent-free-malaria-drug).

Capacity building

During the past two decades, numerous research centers have been developed in sub-Saharan Africa, where high quality research is conducted. The majority has strong links with a Northern partner and the number of regional and supra-regional networks of excellence is growing. In addition, many of the centers are linked to patient cohorts or even demographic surveillance systems (http://www.indepth-network.org). However, there exists a substantial regional imbalance with the majority of these centers being located in South and East Africa, a few in West Africa (http://www.africaclinicaltrials.org) and only limited activities exist in Central Africa. Furthermore, most of these centers are involved in research in the area of malaria and HIV/AIDS or tuberculosis, but almost never in neglected diseases.

Many of the research centers have evolved from being trial sites conducting epidemiological studies or single trials to project sites, and some have become research centers with all the capacities needed to maintain a portfolio of trials and to be involved in different fields of activity (see Table 1).

Table 1. Characteristics of the evolution from a trial site to a research centre (16)

	Trial site	Project site	Research centre
What is core?	Informal alliance of projects and trial sites	Established infrastructure to projects/trials	Fully established entity that provides all science infrastructure
Core funding	Resources from projects fund the core	Small amount of core - funding and resources	Funding of core established and projects contribute to core
Portfolio	Single or small number of projects that drive core	Small number of projects - able to add different diseases or interventions	Different interventions and/or different diseases
Time focus	Short term	Mid term (3-5 years)	Long term (> 10 years)
Infrastructure	Very basic and dependent on project funding	Established basics that survive individual trials/projects	Full infrastructure maintained over time with projects paying share

The development of a trial site to a research centre is a long process in which well-planned and sustainable capacity building has a central function. A critical mass of local researchers needs to be trained and given a career perspective to avoid excessive “brain drain”; the institution must build up the leadership to not only deliver research excellence, but also provide adequate governance, administrative, financial and management functions (17); the respective infrastructures needs to be built; and the quality assurance systems necessary for the compliant conduct of the trials must be implemented and enforced. In the experience of our team, which is routinely involved in monitoring and auditing of a substantial number of trials and research centers, this latter aspect still needs attention. The level of detail requested today is considerable and the main findings include the confusion of document versions, incomplete tracking of processes and unwanted deviations from protocols or procedures. Clear and practical internal standard operating procedures at all levels and the installation of internal quality control help to avoid such verdicts.

The creation of such sustainable and comparatively independent research centers may not always be achievable. Many of the most neglected diseases (e.g. human African Trypanosomiasis, Buruli ulcer, trachoma) are characterized by a rural distribution and may be locally controlled or even eliminated through dedicated disease control activities. Conducting clinical trials in such regions requires the involvement of small treatment facilities with often very basic infrastructure and very limited human resources. The conduct of a single clinical trial often leads to a significant reduction in the relevant patient number in the catchment area, preventing follow-up projects. The switch to other diseases or projects is normally impossible due to the remoteness of the center and the mentioned limitations.

Particular attention has to be paid in such situations to the appropriate information about the intentions of the researchers, the adequate training of local staff and site improvement. The minimum obligation is to leave behind a team well-trained in the relevant areas and a laboratory with specific improvements for the daily routine.

The conduct of clinical trials in sub-Saharan Africa

Ethics

The goals of research are always secondary to the well-being of the participants. This requirement is made clear in the Declaration of Helsinki and is regarded as the fundamental guiding principle of research involving human subjects (18). Discussions on bioethics of clinical research in resource-limited settings are manifold and include the therapeutic areas, the quality and quantity of research as well as discussion on the use of placebo or the use of the best versus the locally available standard of care.

Guidelines on bioethics and clinical research are numerous and sometimes conflicting. Where the Declaration of Helsinki has established the guiding principles of research involving human subjects, it does not cover all relevant aspects for the conduct of clinical research. Currently, the correct overall reference for the conduct of clinical trials is being discussed. The guiding role of the Helsinki Declaration was contested by the U.S. FDA and it was decided to replace it by the standards described in the ICH GCP (19). On the other hand the ICH guideline itself had been seen as being too comprehensive and strict for the conduct of clinical trials in resource-limited countries (20). The increase in clinical trials conducted in such settings goes hand in hand with an increasing capacity and this discussion will continue with a yet unknown outcome.

In this article, we only discuss ethical aspects that have a direct and/or practical impact on the trial and the trial participants. In this respect, we take post-treatment access to successful interventions for the relevant populations for granted, although this may be a very challenging issue in specific cases.

Essential literature on research involving human subjects is listed in Table 2.

Table 2. Essential literature on research including human subjects.

Documents
International Conference on Harmonization / ICH Harmonized tripartite guideline: Guideline for Good Clinical Practice GCP E6 (3)
World Medical Association Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm)
Council for International Organizations of Medical Sciences / CIOMS: International ethical guidelines for biomedical research involving human subjects (in collaboration with WHO) (21)
Nuffield Council on Bioethics: The ethics of research related to healthcare in developing countries (22)
National Bioethics Advisory Commission / NBAC: Ethical and Policy Issues in International Research: Clinical Trials in Developing Countries (23)

Ethics committees

The ethical clearance by an independent ethics committee in the host as well as in the sponsoring country is one of the key requirements. The first African ethics committee was established in South Africa, 1967 (24). But in 2005, 36% of the WHO African Region countries still had no ethics committees. Capacity development in the area of research ethics was promoted (25) and the establishment of ethics committees and the provision of training and capacity building is today actively supported by, for example, the Pan-African Bioethics Initiative (PABIN) (http://www.pabin.org) as a member of the global Strategic Initiative for Developing Capacity in Ethical Review (SIDCER) and the South African Research Ethics Training Initiative (SARETI) funded by the Fogarty International Center of the US NIH (http://www.fic.nih.gov). However, many committees are not yet fully functional due to lack of funding, infrastructure, training, standard operating procedures and sometimes simply the lack of political commitment from the governments.

Besides national ethical clearance of the research project in the host country we strongly encourage ethical clearance by local ethics committees. Local ethics committees can best represent the cultural, political and economic values of the region and can act as a hinge between community needs and research priorities. The value of the implementation of a local ethics committee depends on the number of clinical research projects conducted in the particular area/region and will likely be linked to a well-established research center.

Today, the lack of capacity of National Regulatory Authorities (NRA) leads to the situation that ethics committees often fill the role of local drug regulators, too. However, not only for this reason, strengthening of NRA capacities has a very high priority. Efforts must include the authorization and monitoring of clinical trials, the evaluation of clinical data for product registration, and quality control and pharmacovigilance of medicinal products.

In view of this formidable task, systems supporting NRAs and optimizing the use of regional resources and expertise are needed. An example is the WHO African Vaccine Regulatory Forum (AVAREF) which can be considered as an "ad-hoc" scientific advisory body that can help regulators to make an informed regulatory decision with regards to authorizations of clinical trials, evaluation of registration dossiers or any other challenging issues regarding evaluation of vaccines (11).

Informed consent and assent

Patient information and written informed consent are two of the most sensitive and complex parts of clinical research. This is true for all clinical research conducted but even more so in sub-Saharan Africa. In contrast to Western culture, communal consciousness and living is the norm in many African societies and for decision-making processes, the importance of community leaders and families cannot be eroded (26). Patient information forms which are long, complex and sometimes inappropriate in the cultural context where they are used, may confuse, rather than inform, participants and can be refused by the local Ethics committees (27). Balancing completeness versus simplicity is a real challenge in the preparation of patient information. An appropriate patient information and informed consent procedure is key in the prevention of the therapeutic misconception; when participants believe that they are receiving a new form of treatment rather than participating in research (28).

If children are involved, the parents or the legal guardian must give consent. However, children capable of making decisions (depending on the society and the disease of an age of 7 or older) should be asked for their assent which must be respected if the response is negative (29).

A further sensitive issue is the requirement for written informed consent (3). In certain cultures or situations, signing of a consent form has very negative connotations and may even be rejected (22). Whether written consent forms are adequate for use with illiterate patients has also been debated and the National Bioethics Advisory Commission has issued a recommendation to waive written consent in such situations (23) and to replace it with an alternative appropriate process which must be specified in the protocol (e.g. independent witnesses).

Finally, if the language in which the information is to be provided is different from the one used for the protocol, the text must be translated to a locally spoken language and should be back-translated to detect and rule out misunderstandings. This process may be particularly difficult and tedious in settings where indigenous languages with a limited modern word pool are spoken and where the translation and back translation process requires particular attention. If a language is not written, the information must be read and explained from a sheet written in an alternative official language. In this case, the presence of a knowledgeable witness seems to be appropriate.

Indemnities and undue inducement

Particular attention is required for the adequate indemnification of study participants. One has to be aware of the coercion or undue inducement (30) of overpaid indemnities and of exclusive payments linked to completion of the trial. It is clear that the patients in resource-poor settings need to receive a fair compensation for their efforts such as transportation or sometimes for the working time lost during hospitalization. The local representatives often opt for paying in kind, particularly in rural settings. However, there is a fine line and one should be aware that trial or project sites can comply with the research proposals because they bring work, salaries, technology transfer and materials to their locations.

Publication of results and trial registration

The publication of positive and negative trial results is an ethical requirement as stated in the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm). However, in reality the publication of results is frequently delayed or even omitted. Corporate involvement in clinical research may lead to the exclusive disclosure of results to regulators. The very long follow-up periods typical to a number of neglected diseases such as human African trypanosomiasis, contribute to extensive delays in the publication of data.

The increasing acceptance of the importance of public information and the reduction of selective reporting is reflected in the mandatory registration of clinical trials. In 2004, the International Committee of Medical Journal Editors published a respective joint editorial: before first patient enrolment any clinical trial must be registered in a public database to allow publication. There are various public registries for clinical trials but since 2005 the “WHO International Clinical Trial Registry Platform” (http://www.who.int/trialsearch/) has taken the lead. As another mechanism which might assist the timely publication of data on diseases requiring a long patient follow-up, we suggest discussion of the option to separately publish the safety and efficacy data in two linked publications. Table 3 summarizes the minimum ethical standards to be met at local, national and sponsor level.

Table 3. Minimum ethical standards for research involving human subjects.

LOCAL LEVEL	NATIONAL LEVEL	SPONSOR LEVEL
Staff understanding of the importance of a correct patient information and a correct informed consent process Respect of right for anonymity of trial participants Community information and involvement Fair information of all potential participants (complete, simple wording) Consent at relevant levels Individual consent of all participant Written consent as a standard option; alternatives with sufficient justification where necessary Fair compensation for trial participation	Timely ethical review by designated national or thematic Ethics committee Timely approbation of cleared trial by drug authorities or Ministry of Health and controlled importation of the investigational product	Study design respecting the local situation, capacities and cultural constraints Ethical review in sponsor (or CRO) home-country unless host country IEC is certified Trial registration

Implementation and conduct of trials in resource-limited environments

In the case that a clinical research project is carried out in collaboration with a rural trial site, the sponsor is confronted with challenges that do not exist in other settings. The absence of well educated health personnel, high staff turnover, poor infrastructure, lack of standard operating procedures and difficult accessibility are common. Often the situation is complicated by an unstable security situation created by political conflicts. Importantly, traditional beliefs and stigma have a strong impact on the behavior of the population and health staff, and may influence the research activities.

For the conduct of clinical trials in such situations, centre assessment and site selection are of paramount importance. The capacities and infrastructures very often must be built up and maintained on a continuous basis. Issues which are practically unknown in the implementation of trials in Western settings must be considered: the presence of an educated physician and other staff members, appropriate laboratories including (stable) energy supply, laboratory staff training and the installation of communication tools may be issues. The appropriate level of site improvements should be balanced between the requirements of the trial and the maximum capacities of the staff.

Quite often the questions asked in a protocol have to be adapted to the limited possibilities of the sites. It is wiser to focus on the key topics and to refrain from obtaining information on all details. The basic principle of GCP, that anything not documented does not exist, necessitates a thorough and clean recording of data and the creation of an appropriate and easy-to-monitor filing and recording system. Such consistent systems rarely exist in rural health facilities where records are usually kept in various books and booklets. Furthermore, long-term storage of the study and patient records must be assured, which can be problematic considering heat, humidity and the often precarious room situations.

Dedicated pharmacies and adequate clean and air-conditioned storage with limited access are often non-existent in such facilities and practical solutions must be found to protect the experimental product from misuse, to guarantee its quality and to account for its receipt, use and return or destruction.

A difficult and critical issue is the reporting of adverse and serious adverse events (SAEs). If not stressed, adverse events may not be recorded at all as they are not considered unusual. For SAEs it is important to (i) convey the difference between severeness (clinical term) and seriousness (technical and legal term) and (ii) to implement a reliable, monitorable communication system that allows timely reporting of SAEs to the sponsor/contract research organization and the Ethics committee.

Clinical data management (CDM) is a particularly delicate topic. Under the new regulations CDM has become a highly regulated field. Specialized software and tedious validation of the programs, systems, data transfer and trial setup are necessary. To avoid post-trial or even approval difficulties the CDM for multi-center, multi-country trials is usually concentrated at dedicated contract research companies. But centralized data collection and storage may conflict with the intentions and ambitions of the local investigators. In particular, sites with links to a demographic surveillance system or which routinely run large-scale epidemiological studies may have sophisticated equipment and statistical knowledge and opt for local CDM. In our experience with such situations, a pragmatic approach is advantageous, since inconsistent data quality and complicated trial structures cannot be in anyone's interest.

Final study reports to the regulatory authorities should be written by professional scientific writers but the sponsors should clearly recognize the needs and interests of their partners to publish results and their active involvement should already be defined in the study protocol.

For the control of certain diseases, e.g. human African trypanosomiasis and onchocerciasis, specialized mobile teams visiting the population at risk are necessary. Those teams have to be integrated in the work flow of the respective clinical trials and can be used for the information of the population, for pre-screening of potential patients and even enroll participants in a trial. To maintain a coherent technical standard, a strong presence of technical advisors, supervisors and specialized monitors familiar with the disease, the local situation and the language, and intensive external support in site management have to be warranted. Typically, this approach is only possible through a joint venture of the implementing organization with the responsible national and/or district authority. Such partnerships have to be built and preserved carefully, as the enrolment in trials on neglected diseases tends to be very slow. An example for this is given in the accounts of the Phase II trial for Moxidectin against onchocerciasis (31).

In summary, the conduct of clinical trials in rural settings is certainly not a low-cost option and entails particular challenges. Table 4 summarizes the minimum standards required to conduct GCP-compliant trials.

Table 4. Minimum standards for the conduct of clinical trials according to the International Conference on Harmonization/ Good Clinical Practice (3).

LOCAL LEVEL	NATIONAL LEVEL	SPONSOR LEVEL
The presence of educated medical staff and nursing care	Monitoring and surveillance of ongoing projects	Responsibility for quality assurance and quality control
Capacities for documentation and archiving		Qualified personnel for the trial design, trial management and medical expertise
Assurance of confidentiality		Trial registration
Accurate reporting and verification of the data		Insurance for participants
Adherence to the protocol		Financing
Version control for protocol and informed consent		Notification and submission to regulatory authorities
Correct, timely reporting and handling of adverse and serious adverse drug reactions		Information on investigational product (investigators brochure)
Infrastructures for correct storage and use of the investigational medicinal product		Manufacturing, packaging, labeling, (coding) and supply of investigational product
		Notification of end of study
		Reporting

Access and delivery

This topic, although of paramount importance for every intervention, goes beyond the scope of this paper and we will only summarily examine it.

Post marketing studies and pharmacovigilance

When a marketing application is filed for regulatory approval, the documentation for a new drug will typically comprise a few thousand patients with data mainly collected through Phase I to III trials (32). Although these data are considered to be sufficient to describe the safety and efficacy profile of a new compound, at this stage there is no data about the effectiveness of the drug in the real-life situation. Questions about unforeseen drug interaction, rare adverse events, effects of poor patient compliance and dynamics of resistances have not yet been addressed. This information is needed for the safe use of a drug, particularly in high risk groups such as pregnant and lactating women, children, malnourished and HIV-infected patients. In addition, the information is an important element in making a decision whether a new intervention should be added to the essential drug list of a country. At the global level, the WHO program for international drug monitoring at the Uppsala monitoring centre collates adverse drug reaction reports via the national pharmacovigilance centers of the 81 member countries (www.who-umc.org). Currently there are only six sub-Saharan African countries (South Africa, Zimbabwe, Tanzania, Mozambique, Nigeria and Ghana) that are full members of the program. In fact, less than 27% of lower middle income countries have national pharmacovigilance systems registered with the WHO program, compared with 96% of the high income countries in the Organization for Economic Co-operation and Development. The main reasons for this are lack of resources, infrastructure and expertise. Thus, although access to medicines is increasing in developing countries, there is a danger that their risk-benefit profiles in indigenous populations will not be fully monitored (33). To make up for this shortcoming of local data, at least substantial Phase IIIb or drug utilization trials should be conducted.

Access

The effectiveness of an intervention depends on many factors which may be linked to a product or be parameters of the health system, such as affordable market price, distribution channels and their accessibility, sustained availability of the product, quality control, information for health care professionals and the general public, and correct diagnosis. At each level, those involved may have conflicting interests, and poor populations are the first to suffer the effects of frail links in this long chain (34).

Recent experience indicates that the drugs developed in PDPs have a faster introduction to the market. This puts a lot of pressure on public health systems and currently, many projects fail because the health systems are not strong enough to deliver new interventions to the target populations. Also the Global Fund to fight AIDS, tuberculosis and malaria (http://www.theglobalfund.org) recognizes this need and supports the strengthening of health systems (35). An important aspect is health information. It is recognized that inadequate access to information is a significant factor in development, and particularly in health care development. Despite major global progress in access to information during the last decade, there is little evidence that health professionals, especially those working in rural primary health care, are better informed than they were 10 years ago (36). The unequal distribution of health care between developed and developing countries is matched by a similar unequal distribution of health information (37) and the 10/90 gap in health research probably translates into a 1/99 gap in health information (36).

The way forward

The development and provision of new interventions to prevent, diagnose, treat and control diseases that affect the poorest and most vulnerable populations finally is receiving global attention and support. Today, numerous initiatives, consortia and PDPs are engaged in those activities and the funding has reached unprecedented dimensions. This results in a large number of ongoing research projects and the proportion of clinical trials being truly performed according to GCP is increasing. This development has already led to tangible results. For instance, several new chemical entities for the treatment of malaria have been or will be registered shortly by first tier drug authorities. A number of new research centers in sub-Saharan Africa have emerged and are gaining momentum. A strong generation of scientists is developing and is reasonably well-supported through various competitive fellowship grants. This also opens the possibility that researchers will increasingly see career opportunities at home which may reduce the "brain drain" effect and with this, innovation and leadership will be strengthened in the long term.

So, can we lean back and wait for better days dawning? There are still serious gaps and short-comings which will require enormous effort to be overcome. It now seems critical to maintain impetus in the research area and to continue the processes started at all levels. This will not be achieved solely by continuing funding but also through genuine political interest and backing by the governments and institutional leaders of the developing countries. From the R&D point of view, there is a need for the technical strengthening of all divisions or drug regulatory authorities, participatory involvement of research scientists at institutional levels and the reduction of artificial logistical hurdles.

The treatment of many of the most neglected diseases is still dreadful and this will certainly still need increasing attention. However, the transition of health (i.e. the epidemiologic shift from infectious to chronic diseases particularly in urban areas) will incontestably create new challenges, but potentially also new opportunities for the research centers involved in R&D.

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Chapter 6

Discussion

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Access to patients

The two main bottlenecks for clinical research in *T.b. rhodesiense* endemic areas are the limited number of available patients and the difficulty of finding them. Populations at risk of contracting sleeping sickness live scattered in remote rural areas with limited access to health services. Patterns of health seeking behaviour and difficulties of the service provider to diagnose HAT lead to long delays in diagnosis and therefore in treatment.

Traditional beliefs, distance and cost of transport usually influence negatively health seeking and patients often attempt first to make use of local medicine. This is usually expensive and patients may be kept for weeks in the care of traditional healers. Family members bringing patients in a terminal disease stage to a health centre is unfortunately not a rare event, and often such patients die within a few hours after arrival.

Another difficulty is the lack of technical capacity for HAT diagnosis at the provider side. On one hand there is a limited sensitivity of the diagnostic tools in use, and on the other hand there are also major issues with the professional performance and disease awareness at provider level. Especially clinical officers and nurses should be aware of the signs and symptoms and the geographical distribution of the disease. They are in contact with patients whose condition is not improving despite repetitive antimalarial and antibiotic treatments. Also, they are in the position to request the laboratory to check for trypanosomes. It is therefore of central importance to inform health personnel working in HAT endemic regions regularly about the disease in order to improve case detection. In areas where the disease has newly emerged the competence of health services to response adequately is even further limited, and the capacity to identify and diagnose HAT should be built quickly.

Only 35% of the IMPAMEL III participants were diagnosed with HAT upon their first visit at the health centre. 44% had 2-3 and 21% had more than 3 contacts with health care providers before diagnosis was made. The delay in HAT diagnosis was significantly longer in the site in Uganda where patients had to visit a health facility in average 2.2 times (CI 2-2.5) compared to Tanzania where the diagnosis was made in average after 1.6 visits (CI 1.4-1.7). This is obviously problematic for remote rural populations for whom the cost of repeated trips to health centres is prohibitive. A study carried out on the patterns of health-seeking behaviour in an established *T.b. rhodesiense* focus in Uganda showed that the median total delay from onset of illness to diagnosis was 60 days. The median delay of the service provider to diagnose sleeping sickness among symptomatic individual was markedly longer (30 days) than the median delay of the patients (17 days) to present at the health facility (1). Further findings were that a large proportion of the patients were either referred to the sleeping sickness hospital by members of their community or presented there on their own initiative. Only few patients were referred by other tiers of the health system (1).

The WHO and the Foundation for Innovative and new Diagnostics (FIND) established a consortium for the research and development of new human African trypanosomiasis diagnostic tools appropriate for use in low-income countries in 2006 (2). In this framework WHO is currently establishing a HAT specimen bank by collecting reference materials from well characterized patients and controls. The specimen bank is physically located at the Institute Pasteur, Paris, France which is responsible for cryopreservation, and managing the distribution of specimens (3). The accessibility of the samples to public and private partners is controlled by a joint WHO/FIND committee. Hopefully these efforts will results in the delivery of new diagnostic tools for diagnosis and disease staging in the near future.

As of today, the loop-mediated isothermal amplification (LAMP) technique is the most promising tool for detection of *T.b. rhodesiense* HAT under field conditions (4, 5) and might be available commercially in the near future (http://www.finddiagnostics.org).

Control of *T.b. gambiense* sleeping sickness is largely based on active surveillance using serology (CATT) (6). However, for *T.b. rhodesiense* HAT this approach is of limited success due to the lack of a serological test with sufficient sensitivity (7). Diagnosis during active mass screening campaigns in *T.b. rhodesiense* affected areas has to rely on direct microscopy and if logistically possible, on the haematocrit centrifugation technique. Further, sick individuals often do not show up at community-based screenings a bottleneck of population screenings also described for in *T.b. gambiense* areas (8). The effectiveness of active population screenings in endemic *T.b. rhodesiense* settings is doubtful due to the low prevalence, the diagnostic constrains and the variability of the attendance rates. According WHO active screening should therefore be confined to areas where there is evidence of an outbreak (9). As an example, during an epidemic in Zambia (1980-1984) a total of 23'751 individuals were screened by mobile field teams and only 102 cases (0.43%) were diagnosed (10).

For the IMPAMEL III trials access to patients was increased by complementary activities to the standard passive case detection. In the catchment areas of the Kaliua Health Centre in Tanzania and the Lwala Hospital in Uganda we performed active case searches. On the basis of index cases mobile teams screened the local population using direct microscopy. Due to the low success rate in the first round of active case searches the index cases were followed up to household level at the second round but this did not yield better outcomes. Community sensitization was done through local councils, local leaders and churches. In Uganda, the missionary radio station informed the population about the IMPAMEL III program. Such advertisements were very effective in mobilizing people. However, mostly healthy people came for screening and the sick (weak and often unable to walk) were not seen. At district level, the officers for vector control and disease surveillance were informed, not only to support case identification but also the follow-up activities, which are known to be difficult. In the end, the most successful strategy to identify patients turned out to be visits to the surrounding health facilities and requesting blood slides for all inpatients.

Issues concerning the study design

Because of the existence of two forms of HAT the results of the IMPAMEL I&II programs could not be extrapolated to East African patients. The assessment of the abridged treatment schedule was regarded essential in order to provide access to the improved melarsoprol therapy also in *T.b. rhodesiense* affected areas and therefore, the IMPAMEL III trials were of high priority for the WHO and the affected countries. In addition, the alternative schedule may also provide the basis for future combination treatments and a harmonization of all East African treatment protocols. For the latter, it was important to assess as well the benefit of the suramin pre-treatment, which is only partially implemented in East Africa. For the IMPAMEL III program it was decided to use centre-specific suramin protocols. The heterogeneity of the protocols in use did not allow us to agree on a single one.

Concerns during the planning of Impamel III were the fear of unexpected toxicity because of the higher parasitaemia and reported higher incidence of encephalopathic syndromes (ES) in T.b. rhodesiense patients (11). However, the ES is most likely not an acute reaction to the disruption of trypanosomes (12) as it occurs in most cases after several days of melarsoprol treatment. Also it was shown in several instances that the development of ES is unrelated to either dosage or administration schedule (13, 14). The current hypothesis for the development of ES suggests an immune mechanism (15) which is corroborated by the efficacy of prednisolone in its prevention (15, 16) and the significantly increased risk of ES associated with a small number of alleles of the human leukocyte antigen (HLA) (17). Based on this evidence the 10-day melarsoprol schedule was not expected to trigger an increased incidence of ES in T.b. rhodesiense patients.

The efficacy of the 10-day melarsoprol schedule was expected to be similar to the efficacy of the national regimes in use. For these, the total exposure time of the parasite to melarsoprol was 9 days in Tanzania (3 series each for 3 days) and 12 days in Uganda (4 series each for 3 days). Each series was interrupted by resting periods of 5 to 7 days. Based on the pharmacokinetic properties of melarsoprol investigated in uninfected vervet monkeys and computer simulations it was shown that melarsoprol concentration in the CSF drops to sub curative levels during the resting periods (18). As maximum CSF concentrations of melarsoprol are reached about 10 hours after drug application (19) the parasites are exposed to a much higher drug pressure under the 10-day melarsoprol schedule. Therefore the efficacy of the 10-day melarsoprol schedule was considered unproblematic in comparison to the national regimens in use. Further, compared to the 10-day melarsoprol schedule patient hospitalization times were much longer in the national treatment schedules which negatively affected patient compliance and with this treatment efficacy.

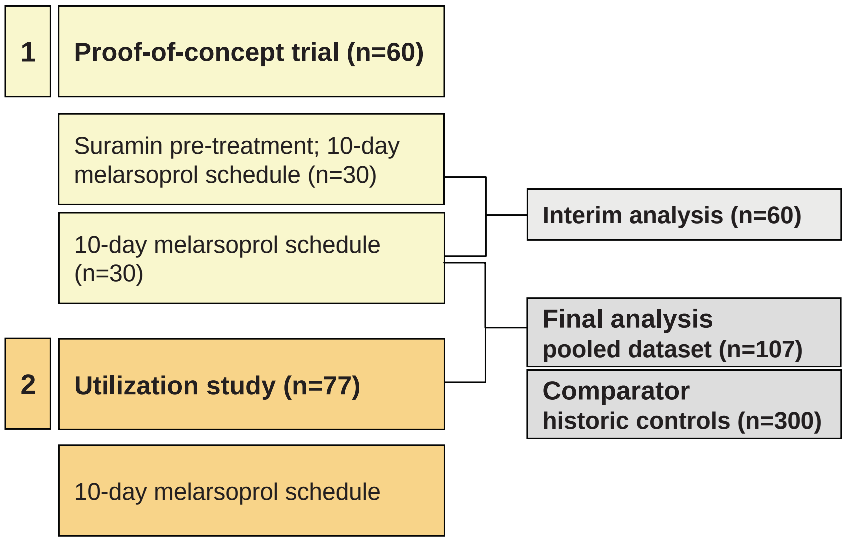
Given the high genetic heterogeneity of *T.b. rhodesiense* and the wide spectrum of disease severity (20) a multinational approach had to be considered in order to rule out significant differences in responses to treatment. In terms of reported number of cases per year, Uganda, Tanzania and Malawi were potential sites and centre assessments were done in the three countries. Finally, two were selected in Tanzania and Uganda. This decision was based on the experiences of the respective centre in HAT diagnosis and treatment, on the reported case numbers in the previous 6 months and on the accessibility of the centres.

IMPAMEL III – study design

The preferred design to test the effect size of any biomedical intervention is the randomized controlled trial. Our sample size calculations indicated that we would require a minimum of 400 patients (200 per arm) to show a difference of 10% between the 10-day melarsoprol schedule and the national regimens in use. The IMPAMEL I & II programs demonstrated the clinical non-inferiority of the 10-day melarsoprol schedule and demonstrating such non-inferiority would have required even a larger sample size. Hence, this approach was impossible given the number of expected patients.

As a result, we first performed a proof-of-concept trial to verify the safety of the 10-day melarsoprol schedule, collect preliminary efficacy data and assess the effect of the suramin pre-treatment. Based on that analysis, and after the decision had been taken that suramin might be omitted without pertinent risk of the patients, a utilization study was designed as an extension to the non-suramin arm of the proof-of-concept trial. This utilization study had a calculated sample size requirement of 100 patients. The sample size of 100 was chosen in order to have a precision of ±6% on the estimated endpoint. Since the pooling of the data of the 30 patients from the proof-of-concept trial with the data of the utilization study was planned to increase the total evaluable sample, the latter was carried out according the same eligibility criteria, endpoints and stopping rule. The design of the IMPAMEL III program is schematically shown in figure 1.

Figure 1: Study design IMPAMEL III



Safety

Based on the reported case fatality rates of the two trial centres, as well as literature, the cut-off point for case fatality under the 10-day melarsoprol schedule was set at ≥10%. Causes of death are difficult to determine under field conditions and in order to avoid difficulties in the causal relationship of death and the study drug, we used a composite endpoint of all-cause mortality. The censoring of the different risks attributable to the death was avoided and reduced bias. Such composite end-points of all-cause mortality are commonly used in toxicological studies and are a valuable endpoint also for melarsoprol trials.

Suramin pre-treatment

Our investigations on the risk-benefit ratio of the suramin pre-treatment were first based on the comparison of the two subgroups of the proof-of-concept trial (2x30) and in a second step on the comparison of all the patients who received suramin (n=30) and all the patients who were directly treated with melarsoprol (n=107).

The results of the proof-of-concept trial indicated a potential harm of the suramin pre-treatment; adverse events were significantly higher in the patient group that received the suramin pre-treatment (63.3%) than in patients who were directly treated with melarsoprol (23.3%, p=0.0018). Also more serious adverse events (SAEs) were reported in patients who received suramin; ES (4 vs. 3) and death (5 vs. 2) but this trend was not significant. The suramin pre-treatment was administered according to centre-specific guidelines; in Tanzania patients received a total dose of 25mg/kg and in Uganda 5mg/kg but no centre-specific differences in the frequency of adverse reactions were seen (p=0.7048).

However, comparing the 30 patients that received suramin and the 107 patients that were directly treated with melarsoprol we did not see statistically significant differences in the proportion of patients that experienced adverse events (63.3% vs. 61.7%; p=0.8692) and serious adverse events (26.6%, 16.8%; p=0.2243).

Overall, our data provided neither evidence of harm nor of benefit of the suramin pre-treatment. This confirms previous findings on the use of pre-treatments prior to melarsoprol: for *T.b. gambiense* HAT little or no value was found for a pentamidine pre-treatment (13, 21) and for *T.b. rhodesiense* HAT the suramin pre-treatment improved the condition of two patients (22) which triggered its use prior to melarsoprol. But in other patients it showed no effects (23).

Dose-escalating studies to determine the dose to be tested in Phase II trials are commonly designed according the classical 3+3 design: the dose-limiting toxicity is assessed in cohorts of 3 to 6 patients (24). Our study provided a larger sample size to assess suramin-related adverse and serious adverse events and we are confident that our results make an important contribution to this discussion.

Efficacy

The latest WHO technical report series recommends a 2-year patient follow-up of HAT patients in order to monitor the long-term efficacy of treatments. The clinical condition of the patient and the blood and cerebrospinal fluid (CSF) should be examined after 3, 6, 12, 18 and 24 months. The leukocyte number in the CSF should be determined though the interpretation is stated as difficult as the leukocyte number can be slow in returning to a normal level (9).

Based on the WHO recommendation of the informal consultation on issues for clinical product development for HAT (25), patients with no trypanosomes after treatment are classified as responders and should be followed up after 3 or 6, 12 and 18 months (25). The CSF WBC counts should not be taken into account to evaluate treatment efficacy at end of treatment as they may not have normalized at that time (26). A test of cure visit is recommended after 18 months: cure can be confirmed in case of no parasitological evidence of relapse and a WBC count $<20 cells/mm^3$. Patients in whom trypanosomes are detected in any body fluid are classified as relapses. Patients without parasitological evidence of relapse and who have WBC count$>20/mm^3$ that can not be explained by a disease other than HAT are classified as probable relapse (25). The threshold of 20 WBC is due to an additional disease stage in Gambian HAT which has recently been suggested and is known as the "early-late stage". The proposal is based on the observation that patients who had trypanosomes in the CSF but a WBC count below 20cells/mm^3 were successfully treated with the first stage drug pentamidine (27-29). However, this classification is debated; a retrospective analysis of data where the criterion for second stage disease set to a WBC count of >10cells/mm^3 showed that this threshold led to a higher risk of relapse compared to the figure of 5 cells/mm^3 (30). Intermediate disease stages were also described for *T.b. rhodesiense* HAT; e.g. if the CNS was only "slightly affected" suramin was said to possibly be curative. But for such cases a close follow-up with repeated lumbar punctures at relatively short intervals (at most two months) was made essential. If such surveillance was not possible melarsoprol should be given rather than suramin (31). The treatment of intermediate *T.b. rhodesiense* HAT stages with suramin never became operational in the field.

However, there is currently not sufficient data on the relationship of WBC counts and parasitological and clinical evidence of cure and relapse. Surrogate markers to determine the stage of the disease and to diagnose a relapse as early as possible is important in *T.b. gambiense* HAT since patients can be asymptomatic for long periods of time. Given the acuteness of *T.b. rhodesiense* HAT where the disease progresses to CNS involvement within 3 weeks to 1 month (32) we believe that surrogate markers are less important for the diagnosis of relapse and cure.

There are currently no guidelines on how to monitor treatment efficacy in *T.b. rhodesiense* patients. The WHO recommendation of the informal consultation on issues for clinical product development for HAT states that "while the data summarized deal with *T.b. gambiense* HAT, similar considerations apply to *T.b. rhodesiense* HAT" (25). This 79 pages document mentions "*T.b. rhodesiense*" a mere 6 times and not once in relation to second stage disease; a further indicator of how neglected *T.b. rhodesiense* HAT is.

It has been stated that *T.b. rhodesiense* patients should be monitored as in Gambian HAT except that the LP during the first year should be performed every 3 months (33). This approach is not feasible as no active patient follow-up in East Africa is implemented in practice. WHO stated that given the severity of the disease it is considered that patients would volunteer for treatment (9).

Under non-trial conditions the most reliable source of data on treatment efficacy in *T.b. rhodesiense* areas is the HATSENTINEL network, a sentinel surveillance network active in 9 sites; 7 sites are located in areas endemic for *T.b. gambiense* (Angola, Democratic Republic of the Congo, Sudan) and two sites are in areas endemic for *T.b. rhodesiense* (Uganda, United Republic of Tanzania) (25).

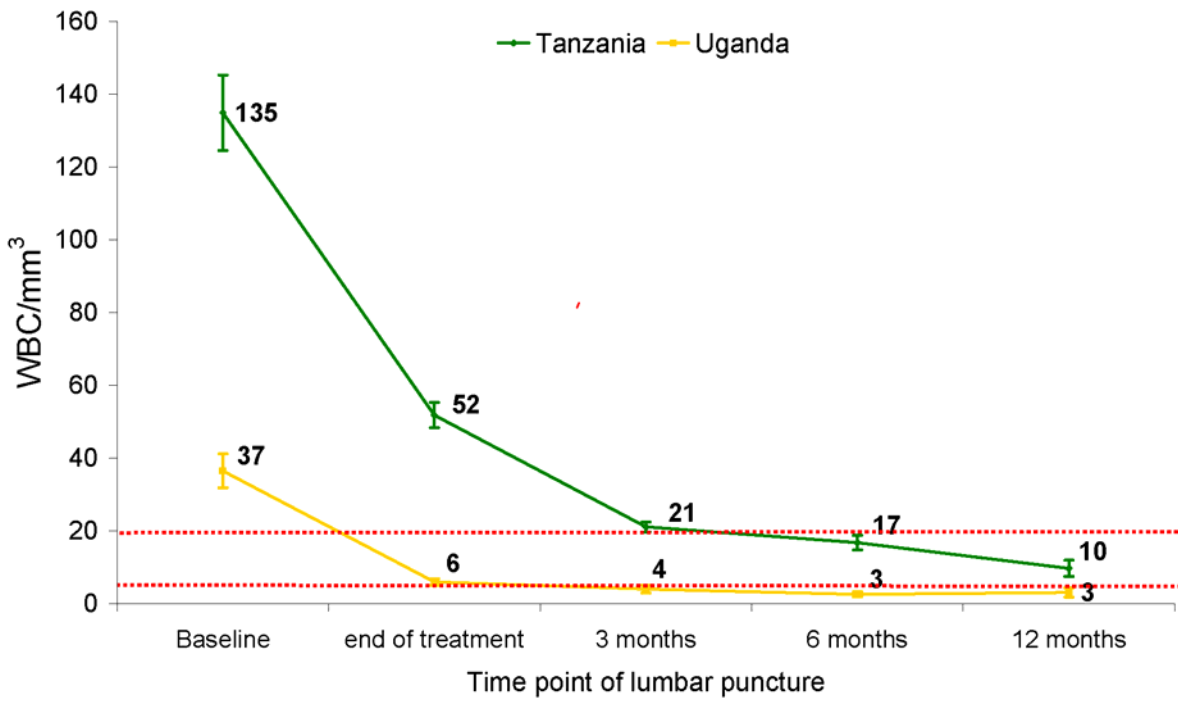
However, patient follow-up is mandatory for treatments in clinical development. The IMPAMEL III study protocol had two endpoints for efficacy; the primary endpoint (clinical and parasitological cure) at end of treatment in order to mitigate the problem of missing data. The secondary endpoint was during follow-up after 3, 6 and 12 months with a test of cure evaluation at the 12 months follow-up visit. Relapses were defined as the presence of trypanosomes in any compartment. Cure was defined as no parasitological and clinical evidence of relapse and a WBC count <5cells/mm^3. Patients that did not present for follow-up examinations or refused LP were considered clinically cured if they were in good general condition (see chapter 2).

Despite our efforts to emphasize the importance of the follow-up and reimbursing transport costs for patients as well as for one attendant we saw important losses to follow-up in the IMPAMEL III program. Common obstructions for follow-up activities in HAT are the fear of the painful lumbar puncture and lack of transport (34, 35) but also the perception on its importance, especially when patients are in good condition.

To obtain information on all patients that did not present at the centre for follow-up examinations we engaged community health workers and district officials. Through those channels we collected oral information on the general condition of the patients. Given the acuteness of *T.b. rhodesiense* HAT relapses or re-infections would not go undetected by the patients or the communities, but possibly unreported. A similar approach of engaging village health workers/sleeping sickness assistants for disease control was a successful control strategy in the past in Uganda (36). We consider this approach also as a satisfactory tool for monitoring long-term efficacy in patients not returning for follow-up visits.

The progression of WBC counts in the IMPAMEL III patients over time and per country are shown in figure 2. Thresholds for WBC counts (WBC=20cells/mm^3 and WBC=5cells/mm^3) are also shown.

Figure 2:WBC counts (±SE) during treatment and follow-up. And threshold levels of WBC counts for definition of cure.



In both countries a clear tendency of leukocyte decrease over time was observed. The WBC counts in Tanzania did not fall below 5cells/mm^3 during 12 months of follow-up. However, this was not surprising given the significantly higher WBC counts on admission.

Despite that the 12 months follow-up of the utilization study is currently ongoing we do not expect any significant changes in our findings. Data shown in figure 5 includes follow-up results of all IMPAMEL III patients up to the end of March 2009. At this point in time the 12 months follow-up was concluded in 74% of the patients form Tanzania and in 51% of the patients from Uganda.

According to the WHO definition of cure (no evidence of parasitological relapse and WBC count <20WBC/mm^3 at 18 months after discharge) (25) patients from Uganda were cured at end of treatment and patients from Tanzania were cured 6 months after discharge.

According the IMPAMEL III definition of cure (no evidence of parasitological relapse and WBC count <5cells/mm^3 at 12 months after discharge) patients from Uganda were cured after 3 months and patients from Tanzania could not be considered cured 12 months after discharge. Yet, we do not doubt the cure of patients in Tanzania as the trend in CSF WBC is more important than the absolute value; also many *T.b. gambiense* patients who were genuinely cured had slightly elevated

CSF WBC 6 months after treatment (11). Further, the significance of CSF WBC counts is inferior to clinical and parasitological evidence.

We consider a patient follow-up of 12 months as reasonable. Any follow-up examination beyond 12 months would be less appropriate given the fulminant progression of the disease. However, for the test of cure evaluation we found the 6 months follow-up visit provided better evidence than the 12 months follow-up. The attendance rate has decrease over time and at 12 months conclusions on treatment efficacy were mainly based on oral information on the general condition of the patients. From all patients enrolled into the proof-of-concept trial 51% presented at the centre for the 6 months and 34% for the 12 months follow-up. For patients enrolled into the utilization study the attendance rate at the 6 months follow-up visit was 70% and so far 17% for the 12 months follow-up. Follow-up attendance rates could probably be improved if patients would only have to come back once, but knowing that this single appointment can not be missed in order to confirm cure.

Given the acute nature of the disease we believe that most patients with a relapse / re-infection would report again to a health facility. Hence it would be reasonable to assume under non-trial conditions that most patients who are not seen again on follow-up at the health facility would likely to be cured. However, for clinical product development more specific guidelines for the assessment of long-term efficacy should be elaborated and a test of cure evaluation at 6 months after discharge could be considered.

For the assessment of treatment efficacy in *T.b. rhodesiense* HAT the risk of re-infection can not be disregarded. There is an increased human–fly contact due to the proximity of livestock and people (37) and certain patients returning to their sources of income are at higher risk of (re) infection such as hunters, fishermen, railway workers, honey gatherers and firewood collectors (38, 39).

Historic controls

The use of historic controls is generally considered as suboptimal due to the potential for selection bias. Clinical trial criteria for inclusion in the treatment group are usually more stringent than in historic controls that generally include all patients seen who meet the diagnostic criteria for the disease under study (40). However, we believe that in our case the trial population was comparable to the historic controls: the IMPAMEL III program clearly aimed at the inclusion of a patient population that reflects to large extends the reality in the *T.b. rhodesiense* endemic regions. All patients with confirmed second stage infection were eligible for participation;

excluded were children below the age of 6 years, pregnant women and unconscious or moribund patients. Also, the age distribution of HAT patients reflects the active adult population (80% of cases are adults) (41) and populations in endemic regions have similar sources of livelihood which makes them comparable for e.g. risk of infection, co-morbidities, nutritional status and health seeking behaviour.

Yet, many efforts were taken to minimize bias: we only considered patient files from the two trial centres and a maximum time period of two years prior to study initiation because ancillary care and referral patterns can differ between centres and change over time. A very thorough review process of all files was done. In comparison to the comprehensive trial documentation the historic files were poorly reported. We found that a large number of files were incomplete and some were missing completely. We selected only files that contained basic demographic data and valid information on treatment outcome. Fatal treatment outcomes were mostly clearly reported but information on clinical and diagnostic baseline findings, concomitant medications and adverse events were not reported in detail. We collected data on serious adverse events (SAEs) with extreme caution. The thorough review process and the excellent memory of the head nurses for most patient histories ameliorated the quality of the data. The final data set (n=300) was considered a robust source.

The mean age in the historic population was 29 years and the male/female ration was 1.4. The trial population was in average older with a mean age of 36 years and had the same male/female ratio of 1.4. As 7% of cases from the historic dataset were younger than 6 years the difference in the mean age of the two populations can be partially explained by the exclusion of this age group from the IMPAMEL III trials. There is little knowledge on HAT in children. We identified one study that compared the clinical presentation and treatment outcomes of *T.b. rhodesiense* HAT in children and adults. Similar to findings in *T.b. gambiense* HAT the symptomatology of HAT was described similar for children and adults, with the exception of the first years of life, where symptoms such as headache, sleeping disorder or motor weakness are difficult to evaluate (42, 43). However, data on treatment outcomes were not discussed due to co-infections of the children with measles (42).

In order to control for potential confounders we calculated the incidence of ES and death in the historic data once on the basis of all selected patient files and once by exclusion of all files of children younger than 6 years. However, no significant differences were found and for the comparison with the trial data the entire data set of historic controls was used (see chapter 2).

The incidence of ES and death reported in the historic data (13%; 9.3%) compared well with data on ES and death in *T.b. rhodesiense* patients from a systematic literature review on the ES during melarsoprol treatment (10.6%; 11.6%) (17) and provided confidence on the quality of the data.

We conclude that despite the controversies on historic controlled trials, the historic data generated in the framework of the IMPAMEL III program in Kaliua Health Centre and the Lwala Hospital were adequate controls.

Serious adverse events (SAEs) and case management

Surprising at the SAE profile of the 10-day melarsoprol schedule in *T.b. rhodesiense* patients was the high proportion (35%) of patients that had an event-free treatment course. Also the much lower rate of skin reactions (6.5%) compared to the IMPAMEL I & II programs that reported skin reactions in 28% of the patients (44, 45) was surprising. So far, the understanding of skin reaction was either a hypersensitivity reaction or the accumulation of heavy metal in the skin (44) and a dose relationship. Our results trigger thoughts on other causalities and possibly host or parasite genetics may influence the development of skin reactions.

The encephalopathic syndrome (ES) is the most severe SAE of melarsoprol therapy. Even though melarsoprol has been in use for more than 50 years, many aspects of the development of ES remain unclear. However, a rather specific aspect in the development of ES is the time to onset. In the IMPAMEL III trial population the median time to onset of ES was 7.5 days (range 3-10days) which was comparable to the median time of onset reported in the IMPAMEL II (median 9 days, range 1-28 days) (45). Thus, the suspected mechanism of a delayed immune reaction against immune complexes (46) seem to be similar for both forms of HAT.

However, the health personnel actually treating the patients would benefit from evidence on risk factors associated with a higher risk of ES and could either adopt preventive measures or monitor those patients extremely attentively. Several risk factors for ES were proposed and included selenium deficiency (47), seasonality, treatment with thiabendazole, bad general condition of the patient and alcohol intake during treatment (48). However, none of these were properly assessed or showed significant association. So far, the only risk factor for ES with useful implications in the field was shown in *T.b. gambiense* patients: the presence of trypanosomes in the CSF or WBC counts higher than 100 were associated with a higher risk of ES (49). The risk factor analysis of our data did so far not yield interesting results. We will extend the analysis with other statistical approaches which might enable us to describe risk factors for ES in *T.b. rhodesiense* patients. However, in order to investigate possible geographical patterns of ES we mapped the home villages of the patients and the villages of the patients who developed ES. In Uganda data was only available up to parish level.

In both countries no obvious clustering of ES events was found - see figure 3 for results in Tanzania and figure 4 for results in Uganda.

Figure 3: Location of home villages of patients with uncomplicated melarsoprol treatment (green) and patients who developed ES (red).

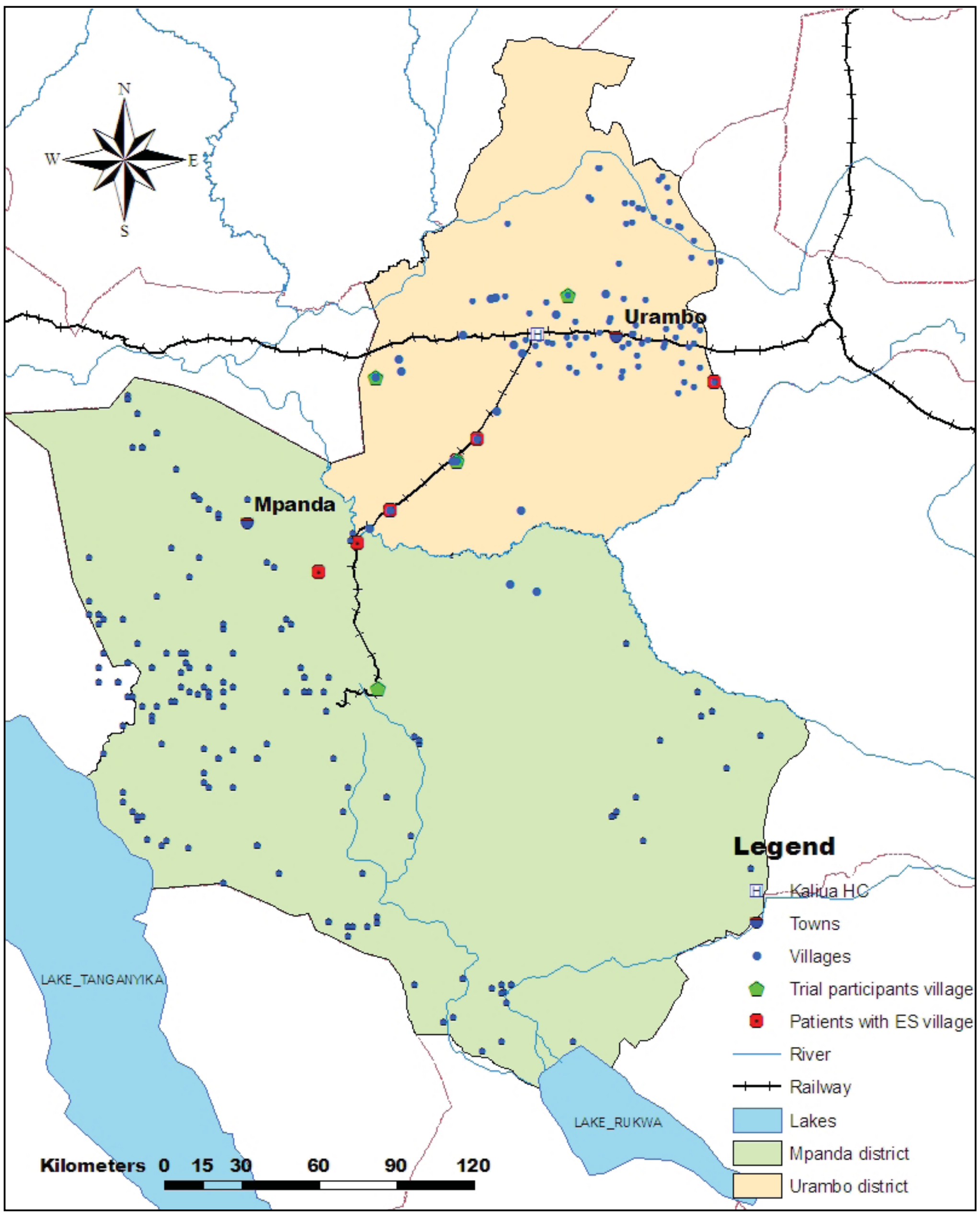
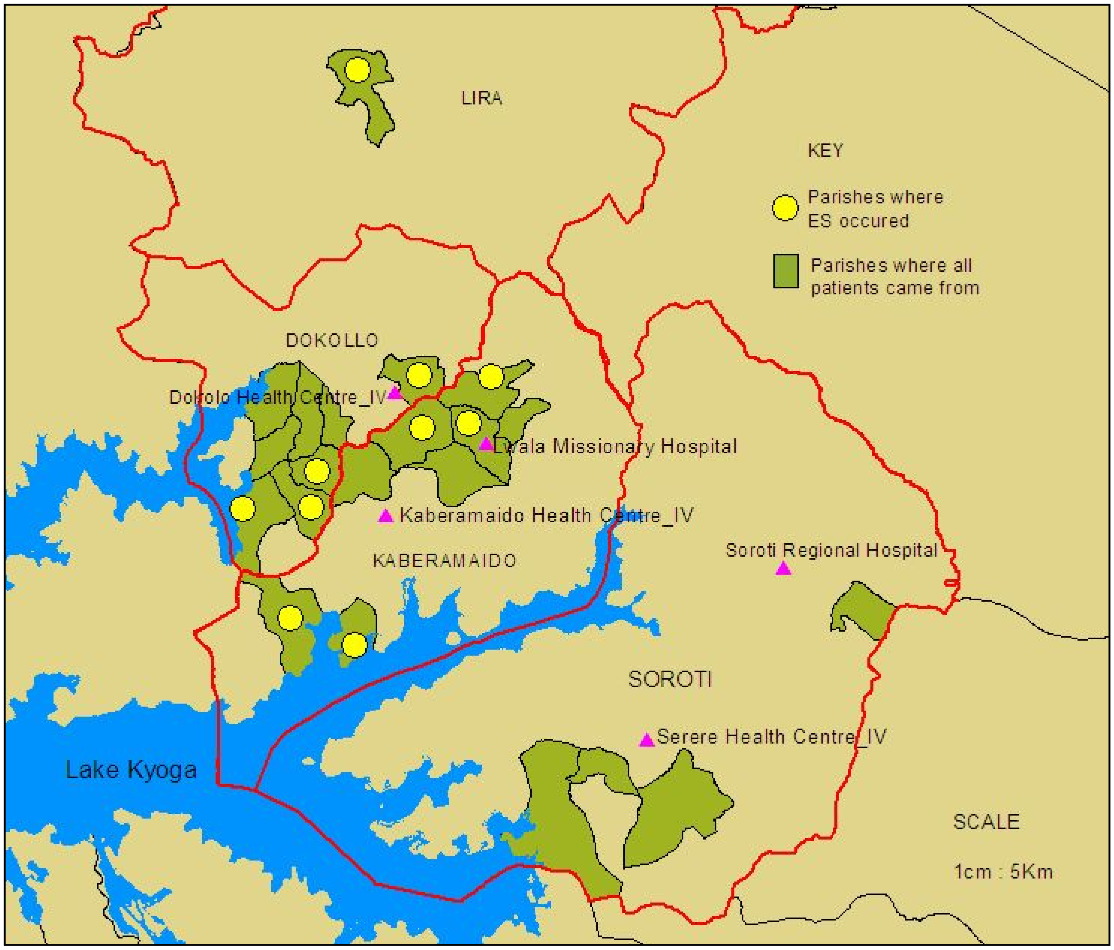


Figure 4: Parishes of patients with uncomplicated melarsoprol treatment and parishes with patients who developed ES.



Note: HAT patients from Soroti district are usually diagnosed and treated at the Serere Health centre. Only four patient of the IMPAMEL III trial came from Soroti district and none developed ES.

Case management

A much debated topic with regard to the treatment guidelines for sleeping sickness is the concomitant use of steroids (50). For *T.b. gambiense* HAT a randomized controlled trial conducted in 620 second stage patients showed a significant reduction in the incidence of ES and a non-significant but prominent reduction of the ES related mortality in the patient group that received prednisone (15).

We were able to identify three studies that investigated the concomitant use of steroids in *T.b. rhodesiense* HAT. One study reported no benefit of a concomitant use of dexamethasone. However, the study had three arms and compared two groups of 34 patients that received dexamethasone with one control group of 25 patients that did not receive dexamethasone. All three groups were treated with different treatment protocols for diminazene aceturate (Berenil®) and melarsoprol (51). Another study showed no reduction in the incidence of ES by the co-administration of corticosteroids (not specified which one). However, this was a retrospective study in two patient groups (n=200; n=183) that were treated according different melarsoprol schedules (52). One trial compared the use of prednisone in two patients groups (n=2x18) treated with the same melarsoprol schedule. But a reduction of the incidence of ES could not be shown (53).

The results of the IMPAMEL III program are clearly in favour of the concomitant use of steroids for the treatment of second stage *T.b. rhodesiense* HAT. During the proof-of-concept trial, steroids were used according to centre-specific guidelines: patients in Tanzania received 10mg of prednisolone daily. In Uganda steroids were given in case of adverse drug reactions. ES was observed at a higher incidence in Uganda (p=0.0444) and during the utilization study the use of steroids was adapted to the Tanzanian standard in both centres. The incidence rate of ES was subsequently similar between the two centres (p=0.5176).

A high variability of case fatality rates between different treatment centres has been reported (45) and certainly goes beyond the variability in the use of steroids. The treatment of patients with melarsoprol requires some experience and an early recognition of warning signs has an impact on the clinical outcome. Mental excitements, twitching movements, headache, dizziness, vomiting are predictor signs for ES and should lead to the immediate discontinuation of melarsoprol (54, 55). Further it is important to be vigilant for a possible malaria. Inexperienced staff would attribute drowsiness and fits during treatment to the severity of the disease and a diagnosis of severe malaria might be missed. Therefore, blood slides for malaria should be mandatory (42).

Inexperienced staff is often overwhelmed by the management of HAT patients, especially in the case of ES and this often results in an irrational use of drugs to cover all eventualities such as malaria, meningitis, shock, severe bacterial infections or raised intracranial pressure. Given the potential for adverse effects and interactions this is hazardous, especially in the case of a critically ill ES patient. The only realistic solution to this problem is to refer sleeping sickness patients to centres who have experience in the management of HAT and to provide staff in such centres with the best possible medical training. However, this solution can potentially aggravate the poor access to health services and could only me maintained by standard referral patterns.

Public health challenges in the control of *T.b. rhodesiense* HAT

While the incidence of reported *T.b. gambiense* HAT appeared to have decreased across Africa over the past five years (56), *T.b. rhodesiense* foci in Uganda have expanded with potential for a much larger number of cases (57).

The control of the two forms of sleeping sickness is fundamentally different. The cornerstone for the control of *T.b. gambiense* HAT is the early detection of cases as infected subjects remain asymptomatic and contagious for months before the disease develops. In contrast, *T.b. rhodesiense* HAT is mainly controlled by disease surveillance and vector control (11). The deployment of tsetse traps and screens has proven to be an efficient method of vector control. A highly successful example is the vector control project that was launched in the Busoga focus in Uganda to stop disease transmission. The tsetse population was reduced by more than 95% and some parishes even achieved elimination (58). Since the animal reservoir plays an important role in the epidemiology of *T.b. rhodesiense* HAT, tsetse control is crucial in order to reduce the risk of outbreaks (59-61). It was shown that tsetse were 5 times more likely to get infected from a blood meal on cattle than humans (62) and mass chemotherapy of animals has been advocated as an effective strategy for control of the spread of *T.b. rhodesiense* HAT (59). However, this approach can be hindered by the emergence of resistant trypanosomes which can be spread from humans to cattle and vice versa (63). In addition, many zoonotic diseases are not controlled effectively because adequate policies and funding are lacking (64). This also applies to the control of *T.b. rhodesiense* HAT. The Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) makes substantial efforts to control the vectors but there is still room for improvements to achieve a well coordinated control of animal and human trypanosomiasis. This would be economically beneficial with substantial benefits to livestock production as well as to the public health sector through reducing the burden on health services (57). Tsetse control as well as treatment of cattle falls under the Ministries of Agriculture in most governments of Africa and coordination with the Ministry of Health is a major limiting factor.

The most problematic factor in the control of *T.b. rhodesiense* HAT is the substantial underreporting of the disease. It has been estimated that for every deceased patient that is reported, another 12 die undetected. Approximately 85% of the unreported deaths do enter the health system at some stage, of which one-third die undiagnosed (65). The underreported cases account for 93% of the total DALY estimate of rhodesiense HAT (57). There are great benefits in reducing this hidden burden of disease and the cost for each DALY averted has been calculated at USD 10 (57). This is amongst the most cost-effective interventions in resource-limited countries (66).

The potential overlap of *T.b. gambiense* and *T.b. rhodesiense* in Uganda

The foci of *T.b. gambiense* and *T.b. rhodesiense* are moving towards each other in Uganda and an imminent overlap of the two disease forms is feared (67).We could not find any evidence for this in our study area (see chapter 3). However, this development has to be carefully monitored as such an overlap will have a major impact on the already strained health system and hamper proper diagnosis and treatment. The type of infection, as well as the stage of the disease, is of central importance for patient management. Currently, the type of infection is entirely dependent on the geographical location of the patient and implies the choice of treatment. Since the two parasites can only be distinguished by PCR analysis which is impossible under field conditions such an overlap would pose great problems.

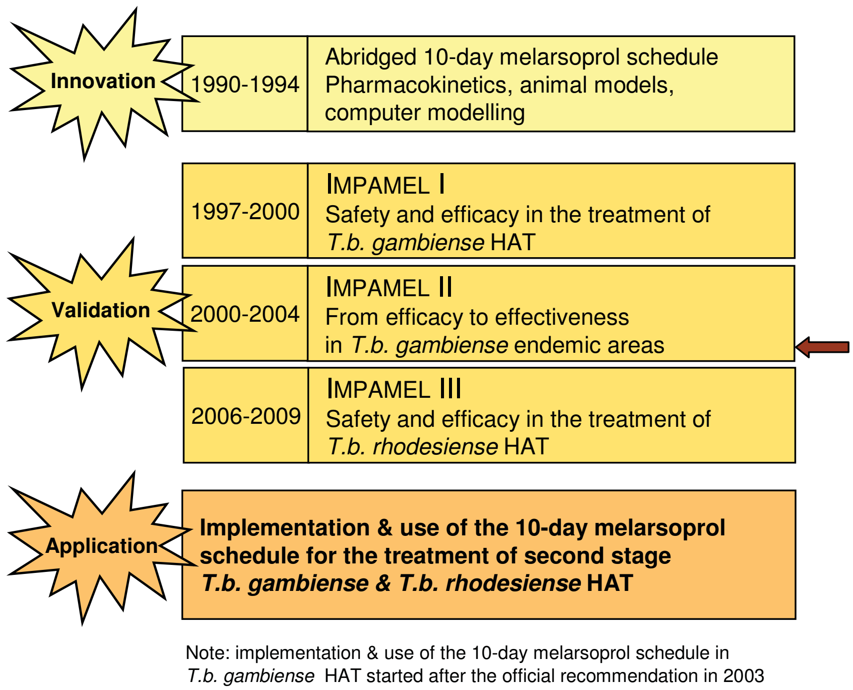
A potential disease overlap would also raise issues of mixed infections in human, animal and vector populations. Genetic recombination of trypanosome isolates from the same epidemic area has been shown under laboratory conditions (68). Also, an overlap of the two disease distributions is unlikely to be prevented by an incompatibility between vector populations and parasite (69) This would aggravate the problems of diagnosis and treatment that we face already.

IMPAMEL III: from innovation to validation to application

14 years ago the 10-day melarsoprol schedule was first suggested for the treatment of second stage *T.b. gambiense* HAT (70). A series of IMPAMEL programs validated the use of the 10-day melarsoprol schedule in second stage sleeping sickness patients in West and Central Africa (1997-2004) (44, 45, 71) and recently in East Africa (2006-2009). In both forms of the disease, a clinical non-inferiority of the abridged schedule over the standard regimens could be demonstrated. Given the major socio-economic benefits of the 10-day melarsoprol schedule this brought a great benefit to the patients as well as to the health care provider.

The development process of new and successful interventions can be divided in the three categories of innovation, validation and application. For the 10-day melarsoprol schedule these processes are schematically shown in Figure 5.

Figure 5: Innovation, validation and application of the 10-day melarsoprol schedule in HAT affected countries (arrow indicates official recommendation of 10-day melarsoprol schedule in *T.b. gambiense* areas at global level)



Only a successful translation of research findings into policy and practice at global, national and at local level will ensure the access of the target populations. Today, it is widely accepted that the traditional model of research dissemination through publication in peer reviewed journals has failed (72). In order to bridge the so-called "know-do gap", institutions and mechanisms that systematically promote interactions between researchers, policy makers and other stakeholders need to be strengthened (73). The WHO as well as national and international agencies take an active role in knowledge translation and emphasize that "research should lead to development, and development lead to more research relevant to development needs" (74). Further, the translation of generated knowledge and evidence into policy decisions and program management is vital for health system strengthening, scaling up health interventions and to tackle the health human resource crisis in developing countries (75).

New research findings have to first be disseminated at global level. For HAT, the biannual meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) is the main platform for discussion and dissemination of research findings at international level. The use of the 10-day melarsoprol schedule for all *T.b. gambiense* affected areas was officially recommended at the ISCTRC meeting in Pretoria in 2003. The same strategy is attempted for the findings of the IMPAMEL III program and the data will be presented during the 30th ISCTRC meeting in Entebbe, Uganda, 21-25 September 2009. It is expected that WHO and the *T.b. rhodesiense* affected countries will discuss the introduction of the 10-day melarsoprol schedule for *T.b. rhodesiense* according the data we have made available.

Knowledge translation at national levels can take some time, especially for neglected diseases due to their low priority setting on the health agendas. In case of an official recommendation for the use of the 10-day melarsoprol schedule, a successful implementation at national level could be supported by the Regional East African Community Health (REACH) Policy Initiative that mediates between policy makers and research communities (76). Another platform to be integrated in the dissemination of the IMPAMEL III results at national levels is the Eastern Africa Network for Trypanosomiasis / EANETT. Since 2001 the member countries (Sudan, Kenya, Uganda, Tanzania, Malawi, Switzerland) meet annually to strengthen the collaboration in research, training and control of trypanosomiasis in the East African region.

The third and last step is the implementation of the new treatment schedule in all treatment centres in a given country. Unfortunately, a successful translation from research into policy does not ensure a successful dissemination also at local level. Even though the 10-day schedule has been recommended for use in the treatment of second stage *T.b. gambiense* HAT six years ago, numerous centres in *T.b. gambiense* endemic areas still do not use this form of treatment. For the dissemination of the IMPAMEL III results at local level, a regional training should be organized inviting district medical officers, doctors, clinical officers, nurses, laboratory staff and a community representative of the HAT treating centres. Further, regular supervision should be done during the initial phase to monitor case fatality rates and cure rates at the individual centres.

All these processes would allow to reach the end line for this new intervention and ensure that a maximum number of patients benefit from this important advance in the treatment of HAT.

Conclusions

The IMPAMEL III program was the first clinical trial conducted in compliance with international standards of good clinical practice/GCP (ICH E6, GCP) in *T.b. rhodesiense* HAT and it allowed to generate representative data on clinical presentation and disease parameters.

The conduct of the program strengthened the local capacities for diagnosis and case management. In addition, the continuous mobilization of the affected communities and surrounding health centres have led to an increase in disease awareness, which is crucial for the timely detection of new cases.

Due to the limited number of *T.b. rhodesiense* patients and the difficulty to find patients it is almost impossible to conduct properly powered trials for this disease in the ordinary sequence of Phase II and Phase III. The sequential design of the IMPAMEL III program with the use of historic controls proved to be a good alternative design and provided conclusive results.

The findings of the IMPAMEL I & II programs could be confirmed in *T.b. rhodesiense* HAT. Patients treated with the 10-day melarsoprol schedule were not at higher risk of serious adverse drug reactions or death compared to patients treated according to previous national regimens. Also, the patients and the different levels of the health system favoured the abridged schedule due to the socio-economic benefits.

The suramin pre-treatment did not lead to a reduction of serious adverse reactions during melarsoprol therapy.

The short as well as long term efficacy of the 10-day melarsoprol schedule was very high.

Due to the acute nature of the disease we considered oral information on a patient in good health as a satisfactory tool to confirm long-term efficacy in patients not presenting at a health facility. The lack of specific guidelines on monitoring treatment efficacy in *T.b. rhodesiense* HAT should be addressed and our study could provide the basis for that.

The fear of a potential overlap in *T.b. rhodesiense* and *T.b. gambiense* disease distribution areas could not be confirmed in our study area. However, continuous monitoring of the situation is crucial.

Melarsoprol remains the only drug to treat second stage *T.b. rhodesiense* HAT. However, it is still highly unsatisfactory and there is an urgent need for new drugs which are equally effective but less toxic.

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Appendix 1

IMPAMEL III

Case Report Form (CRF) - Utilization Study

Swiss Tropical Institute
Institut Tropical Suisse
Schweizerisches Tropeninstitut
Swiss Centre for
International Health

IMPAMEL III

Assessment of an abridged Melarsoprol Treatment Schedule against late stage *T.b. rhodesiense* Sleeping Sickness

Multinational Utilization Study

CASE REPORT FORM

Protocol Number	P-001-05-01-03
Document Number:	CRF 001-05-01-03
Document Date:	12.07.2007
Study Coordinator:	Irène Kuepfer Swiss Tropical Institute Socinstrasse 57 CH-4002 Basel, Switzerland Tel +41 61 225 26 68 Fax +41 61 225 26 78 E-mail: Irene.Kuepfer@unibas.ch
Sponsor:	Swiss Tropical Institute CH-4002 Basel, Switzerland

1. Instructions for use of CRF's

➩ All entries must be clear, legible and made with a ball pen. All corrections must be made in a way which does not obscure the original entry (cross the original number and write adjacent to it, do not use correction fluid). Corrected data must be inserted with a justification, and the date and the initials of the investigator or authorized person

➩ The study patient number and centre number must be written in the fields on the top of each page

➩ Answer all questions or examinations

➩ The investigator must inspect in detail each CRF and certify by signature that the entries are complete and accurate

➩ The date format is day/month/year (Example: 14th of June 2006: 14.06.2006)

➩ The time format is 24 hours

1.1. Time point of data entry

Form 1-3:	Use at hospital admission and discharge of the patient
Form 4:	Use on a daily basis
Form 5:	Use during melarsoprol drug applications
Form 6:	Use whenever another drug is applied
Form 7:	Use at the end of treatment, insert information based on patients files; mention all events observed by your collaborators or reported by the patient and marked in the patient file; use additional pages if an event has occurred independently in more than one instance
Form 8:	Use in case of encephalopathy
Form 9:	Fill in whenever an event can not be appropriately documented on the CRF Forms 1 – 8 or if an explanation is necessary
Form 10:	At discharge of patient from hospital (to be done by Investigator!)

1.2. Remarks

Form 1

Patient Number in centre: Use the unique identifying file number of the treatment centre
Study Patient number: Number patients in sequence of enrolment to the trial; insert this number to the top of all pages of the CRF immediately. The name of the patient does not appear in the CRF, a list which relates the patient number to the patient name must be available confidentially and exclusively to the treating doctor, the responsible nurse and the head of laboratory

Form 2

Fill when laboratory results are available

Form 1/3

Fill in the forms directly during the entry examination and the exit examination

Form 4

A copy of Form 4 will be available to staff that performs the required measurements on the daily basis

Form 5

Note the exact time of drug administration. If an application can not be drawn the reason has to be stated in Form 9

Form 6

List all additional drugs applied with their doses and a justification. Include all drugs used during the application of melarsoprol. List everything until the day of discharge

Form 7

The form has to be completed at the end of treatment based on the patient files. All events observed by the doctor or nurse, or reported by a patient must be entered. Chose the most appropriate symptom or sign from the toxicity grading scale by WHO given in protocol Appendix 3 and grade accordingly. The maximum grade reached, the date of onset and the duration until normalization are entered. If there were multiple instances of the same symptom use additional pages

The grades have to be determined exactly following the definitions given

The field "Serious" will be filled in by the Local Investigator supervising the study

Form 8

In case of encephalopathy follow exactly the tests and instructions given in this form

Form 9

Fill in whenever an event can not be appropriately documented on the CRF Forms 1 – 8 or if an explanation is necessary; do not duplicate information from other CRFs. Keep to minimum, write in print letters

Form 10

The investigator completes this page when the trial participant is ready to be discharged

If questions on the use of any form arise, Irène Kuepfer / STI should be contacted. If the problem remains unsolved Dr. C. Burri, STI should be contacted without delay to prevent loss of adequate data

Study Patient Number └┴┴┴┴┴┘ Centre └──┘

CRF FORM 1: PATIENT INFORMATION

(Fill in the information asked └┴┴┴┘, tick appropriate box☒, or fill in the grade └─┘)

Patient number in centre └┴┴┴┴┴┴┴┴┘	**Patient Initials** └┴┘
Informed consent signed and dated yes ☐ (y) no ☐ (n)	Date of consent: └┴┘/└┴┘/└┴┘
All inclusion criteria fulfilled yes ☐ (y) no ☐ (n)	All exclusion criteria excluded yes ☐ (y) no ☐ (n)
Age └┴┘ (years)	Sex male ☐ (m) female ☐ (f)

Current residence	
District	└┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┘
Village /City	└┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┴┘

Previous treatment for trypanosomiasis	yes ☐ (y) no ☐ (n)	
How long ago (Best match)	1 month ago ☐ (m) 1 year ago ☐ (y)	6 months ago ☐ (s) 2 years or more ago ☐ (t)
What medication (Check boxes)	Suramin ☐ (s) Melarsoprol ☐ (m) Other ☐ (o) Specify ______________ Unknown ☐ (b)	Government centre ☐ (g) Non-Government centre ☐ (c) Was the treatment complete yes ☐ (y) no ☐ (n) Unknown☐ (b)

Health Seeking Behaviour	
How long ago were first signs observed └┴┘ (months)	
How many visits to health centre(s) before diagnosis was made	Once ☐ (1) 2-3 times ☐ (2) more than 3 times ☐ (3)
Any other diagnosis/treatment received	yes ☐ (y) no ☐ (n) Specify________________________
Visits to traditional healers before reporting to the health facility	yes ☐ (y) no ☐ (n)

Study Patient Number └─┴─┴─┴─┴─┘ Centre └──┘

Current treatment of trypanosomiasis			
Date of diagnosis / screen	└─┴─┘/└─┴─┘/└─┴─┘ (dd/mm/yy)	Patient examined by	└─┴─┘ (initials only)
Date of treatment start	└─┴─┘/└─┴─┘/└─┴─┘ (dd/mm/yy)	Date of treatment end	└─┴─┘/└─┴─┘/└─┴─┘ (dd/mm/yy)
Date of discharge	└─┴─┘/└─┴─┘/└─┴─┘ (dd/mm/yy)	Patient examined by	└─┴─┘ (initials only)

General status	Baseline (before treatment)	Study day 11 (after treatment)
Date of examination	└─┴─┘/└─┴─┘/└─┴─┘	└─┴─┘/└─┴─┘/└─┴─┘
Weight	└─┴─┘ (kg)	└─┴─┘ (kg)
Height	└─┴─┴─┘ (cm)	└─┴─┴─┘ (cm)
Consciousness (Glasgow coma scale)	└─┴─┘	└─┴─┘

For women only	Baseline (before treatment)	Study day 11 (after treatment)
Pregnancy Test	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)

Study Patient Number └┴┴┴┴┘ Centre └──┘

CRF FORM 2: DIAGNOSTIC LABORATORY EXAMINATIONS AT ADMISSION / DISCHARGE

	Baseline (before treatment)	Study day 11 (after treatment)
Date of examination	└┴┘/└┴┘/└┴┘ (dd/mm/yy)	└┴┘/└┴┘/└┴┘ (dd/mm/yy)

Diagnostic laboratory tests	Method	Result	Method	Result
Trypanosomes in blood (number and store slide)	Wet film	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Wet film	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)
	Thick blood smear	pos ☐ (p) neg· ☐ (n) n.d. ☐ (b)	Thick blood smear	pos ☐ (p) neg· ☐ (n) n.d. ☐ (b)
	Haematocrite centrifugation (WOO)	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Haematocrite centrifugation (WOO)	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)
Parasitaemia in blood	Microscopic	many ☐ (m) few ☐ (f) none ☐ (o)	Microscopic	many ☐ (m) few ☐ (f) none ☐ (o)

Blood in CSF	LP	yes ☐ (y) no ☐ (n)	LP	yes ☐ (y) no ☐ (n)
Trypanosomes in CSF	Direct, microscopic	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Direct, microscopic	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)
	Single mod. centrifugation	pos ☐ (p) neg ☐ (n)	Single mod. centrifugation	pos ☐ (p) neg ☐ (n)
White blood cells in CSF (n^o / mm^3) (counting chamber, average of 3 countings)	Microscopic	└┴┴┴┘	Microscopic	└┴┴┴┘
1st count	Microscopic	└┴┴┴┘	Microscopic	└┴┴┴┘
2nd count	Microscopic	└┴┴┴┘	Microscopic	└┴┴┴┘
3rd count	Microscopic	└┴┴┴┘	Microscopic	└┴┴┴┘

Haematocrit	Centrifugation	└┴┘ %		└┴┘ %
HGB	Centre specific	└┴┘ . └┘		└┴┘ . └┘

Concomitant infectious diseases		
Malaria; thick stained blood film	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Date: └┴┘/└┴┘/└┴┘
HIV; <u>only</u> if (I) patient agrees and (II) testing is routinely done and counselling to positive patients is regularly provided	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Date: └┴┘/└┴┘/└┴┘
Major filariae (incl. *Mansonella perstans*); microscopic	pos ☐ (p) neg ☐ (n) n.d. ☐ (b)	Specify if possible:

Protocol Number P-001-05-01-03, 12.07.2007

Study Patient Number └─┴─┴─┴─┴─┘ Centre └──┘

CRF FORM 3: CLINICAL EXAMINATIONS AT BASELINE AND AT DISCHARGE
GENERAL ASPECTS

(Enter grade observed or reported on day of clinical examination)

	Grade 0	Grade 1	Grade 2	Baseline (before treatment)	Study day 11 (after treatment)
Date of examination				└─┴─┘/└─┴─┘/└─┴─┘	└─┴─┘/└─┴─┘/└─┴─┘
Chancre	absent	present		└──┘	└──┘
Lymphadenopathy	absent	palpable (> 1 cm)		└──┘	└──┘
Malaise	absent	present	unbearable	└──┘	└──┘
General body pain	absent	present	unbearable	└──┘	└──┘
Joint pains	absent	present	unbearable	└──┘	└──┘
Headache	absent	present	unbearable	└──┘	└──┘
Body Temperature in ^{0}C (axillary)				└─┴─┘.└─┘	└─┴─┘.└─┘
Pruritus	absent	present	visible traces of scratching	└──┘	└──┘
Cough	absent	un-obtrusive	obtrusive	└──┘	└──┘
Swelling of legs	no swelling	swelling limited to foot	swelling whole leg	└──┘	└──┘
Dyspnoea	none or no change	on exertion	at rest	└──┘	└──┘
Heart rate	regular	non-regular		└──┘	└──┘
Diarrhea	absent	3 stools in the last 24 hours	more than 3 stools in the last 24 hours	└──┘	└──┘
Hepatomegaly	absent	present	severe	└──┘	└──┘
Splenomegaly	absent	present	severe	└──┘	└──┘

Study Patient Number └─┴─┴─┴─┴─┘ Centre └──┘

CRF FORM 3: CLINICAL EXAMINATIONS AT BASELINE AND AT DISCHARGE (CONTINUED)

SPECIFIC ASPECTS FOR SECOND STAGE

(Enter grades observed or reported on day of clinical examination)

	Grade 0	Grade 1	Grade 2	Baseline (before treatment)	Study day 11[(Ug)]/14[(Tz)] (after treatment)
Date of examination				└┴┘/└┴┘/└┴┘	└┴┘/└┴┘/└┴┘
Nutritional status	normal	suboptimal	malnourished	└─┘	└─┘
Daytime sleep	normal	repeatedly	continuously	└─┘	└─┘
Night time sleep	normal	few hours	rare	└─┘	└─┘
Tremor	absent	visible	severe	└─┘	└─┘
Speech impairment	absent	present	un-interpretable speech	└─┘	└─┘
Abnormal movements	absent	present	inability to perform daily tasks	└─┘	└─┘
Walking disability	absent	walking with difficulties	walking with help <u>or</u> inability to walk	└─┘	└─┘
General motor weakness	absent	ability to stand up from chair without use of hands	<u>no</u> ability to stand up from chair without use of hands	└─┘	└─┘
Unusual behaviour	absent	present	severe	└─┘	└─┘
Inactivity	absent	reduced workforce	inability to perform daily tasks	└─┘	└─┘
Aggressivity	absent	sporadic	severe, requires observation	└─┘	└─┘
Appetite	normal	disturbed	severely disturbed	└─┘	└─┘
Fertility (females only)	birth within last 9 months	no birth within last 2 years	no birth within the last 5 years or menopause	└─┘	└─┘
Breast-feeding (females only)	absent	present		└─┘	└─┘

Study Patient Number __ __ __ __ __   Centre __

CRF FORM 4: MONITORING OF VITAL SIGNS

REMARK: All measures have to be taken within half an hour before drug application and food intake

THE WARD COPY OF THIS PAGE IS CONSIDERED AS SOURCE DATA

Date (dd/mm/yy)	**Blood Pressure (mm/Hg)**	**Heart Rate (bpm)**	**Body Temp. (axillary, °C)**	**Done by (Initials)**	**Not done[1]**
[........../........../.........]			__ __ . __	__ __ __	☐ (b)
[........../........../.........]			__ __ . __	__ __ __	☐ (b)
[........../........../.........]			__ __ . __	__ __ __	☐ (b)
[........../........../.........]			__ __ . __	__ __ __	☐ (b)
[........../........../.........]			__ __ . __	__ __ __	☐ (b)
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[........../........../.........]			__ __ . __	__ __ __	☐ (b)
[........../........../.........]			__ __ . __	__ __ __	☐ (b)

[1] AN EXPLANATION HAS TO BE GIVEN IN THE OBSERVATIONS FORM 9 FOR EACH MEASUREMENT NOT TAKEN

Study Patient Number └┴┴┴┴┴┘ Centre └┴┴┘

CRF FORM 5: PREDNISONE & MELARSOPROL DRUG APPLICATION

REMARK: Prednisone has to be given to <u>all</u> patients half an hour before melarsoprol application. Time of melarsoprol application has to be registered exactly

Study Day	Date (dd/mm/yy)	Prednisone given	Prednisone Dosage (mg)	Melarsoprol (mg/kg)	Melarsoprol Dosage (ml)	Melarsoprol Application Time	Done by	Not done[1]
1	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
2	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
3	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
4	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
5	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
6	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
7	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
8	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
9	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)
10	[........./........./.........]	yes ☐ (y) no ☐ (n)	---------------- mg	2.2mg/kg	---------------- ml	[............h...........min]	└┴┴┘	☐ (b)

[1] AN EXPLANATION HAS TO BE GIVEN IN THE OBSERVATIONS FORM 9 FOR EACH DOSE NOT APPLIED

Study Patient Number └┴┴┴┴┘ Centre └┴┘

CRF FORM 6: ALL OTHER DRUGS APPLIED

Date medication started (dd/mm/yy)	Drug Name	Daily dose (Ex: 3x50mg)	Treatment duration	Justification of use (i.e. fever, headache, urticaria, allergy, encephalopathy etc.)
[........../............/........]			└┴┘	
[........../............/........]			└┴┘	
[........../............/........]			└┴┘	
[........../............/........]			└┴┘	
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IMPAMEL III, Utilization Study

Study Patient Number ______ Centre ____

CRF FORM 7: SAFETY AND TOLERABILITY (SELECTED CRITERIA AND OTHER ADVERSE EVENTS)

(Record Adverse Event from Study Day 1 to 11, based on original patient files; indicate maximum grade; in case of multiple occurrence use additional sheets)

Adverse event	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Duration	Grade	Relationship[2]	Date of onset (dd/mm/yy)	Serious[3]
Dizziness	Normal	Affecting normal daily activities	Inhibiting normal daily activities						__/__/__	yes ☐(y)
Nausea	None	Able to eat reasonable intake	Intake significantly decreased but can eat	No significant intake	--------				__/__/__	yes ☐(y)
Vomiting	None	1 episode in 24 hours	2-5 episodes in 24 hours	6-10 episodes in 24 hours	>10 episodes in 24 hours or requiring parenteral support				__/__/__	yes ☐(y)
Diarrhea	None	Increase of 2-3 stools over pre-Rx	Increase of 4-6 stools or nocturnal stools or moderate cramping	Increase of 7-9 stools, or incontinence or severe cramping	Increase of >10 stools, or grossly bloody or severe cramping				__/__/__	yes ☐(y)
Febrile reaction	None	37.1 – 38.0°C	38.1 – 40.0°C	>40°C for less than 24 hours	>40°C for more than 24 hours				__/__/__	yes ☐(y)
Headache	None	mild	moderate or severe but transient	unrelenting and severe	--------				__/__/__	yes ☐(y)
Neurological I Psychotic reactions[1]	Absent	Observed change of behaviour, not requiring medical intervention or restraint	Change of behaviour requiring medical intervention	Change of behaviour requiring medical intervention and restraint	--------				__/__/__	yes ☐(y)
Neurological II Convulsions[1]	Absent	One isolated seizure only	Several isolated seizures	Repetitive seizures or convulsive status	--------				__/__/__	yes ☐(y)
Neurological III Coma / perturbed consciousness[1]	Normal	Coma scale ≥ 7, < 10 for less than six hours	Coma scale ≥ 7, < 10 for more than six hours	Coma scale < 7 For less than six hours	Coma scale < 7 for more than six hours				__/__/__	yes ☐(y)
Other:		Use Grading in Toxicity Table							__/__/__	yes ☐(y)
Other:		Use Grading in Toxicity Table							__/__/__	yes ☐(y)

[1] IF ANY NEUROLOGIAL CONDITION IS CLASSIFIED AS GRADE 1 OR MORE, IMMEDIATELY FILL FORM 8

[2] RELATIONSHIP TO STUDY DRUG: **1**: NOT RELATED **2**: PROBABLY NOT RELATED **3**: POSSIBLY RELATED **4**: PROBABLY RELATED

[3] IF THE ADVERSE EVENT MEETS THE REGULATORY CRITERIA FOR A SERIOUS ADVERSE EVENT, THIS FIELD MUST BE FILLED BY THE RESPONSIBLE LOCAL INVESTIGATOR AS DEFINED ON THE ACTIVITY DELEGATION LOG. AN SAE REPORT NEEDS TO BE SENT TO STI WITHIN 24 HOURS OF THE EVENT.

Study Patient Number

Centre

CRF FORM 8: EXAMINATIONS AND LABORATORY TESTS IN CASE OF ENCEPHALOPATHY

Adverse effects	Grade 0	Grade 1	Grade 2	Maximum grade	Date occurred	Duration of max [days]
Headache (< 24 hours before onset)	Absent	Sporadic	Continuous			
Fever (< 24 hours before onset)	Absent	37.5 - 38.9°C	≥ 39.0°C			
Aggressivity	Absent or comatose	Verbal attack	Physical attack			
Confusion	Absent or comatose	Correct response on current place	Incorrect response			
Convulsions	Absent	Single	Repeated			
Consciousness	Fully conscious	Drowsy	Comatose			

Malaria	(Thick smear mandatory in all cases of encephalopathy!)	pos ☐ (p) neg ☐ (n)		
Glucose in blood	Method: ________________ ([mMol/l] if not done enter "b")			n.d. ☐ (b)

Study Patient Number └─┴─┴─┴─┴─┘ Centre └─┘

CRF FORM 9: OBSERVATIONS AND OTHER DIAGNOSIS

ONLY ENTER OBSERVATIONS WHICH HAVE NOT BEEN MADE ON ANOTHER PAGE OF THE CRF
ALL ENTRIES MUST BE WRITTEN CLEARLY READABLE

Date (dd/mm/yy)	Remark	Initials of Responsible
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘
[........../............/........]		└─┴─┴─┘

IMPAMEL III, Utilization Study Page 15 of 15

Study Patient Number └─┴─┴─┴─┴─┴─┘ Centre └──┘

CRF FORM 10: DISCHARGE CHECKLIST

Checklist at discharge	
Date of last contact with patient	└─┴─┘/└─┴─┘/└─┴─┘
Completed study	☐ (s)
Withdrawal for:	☐ (w)
▪ Consent withdrawn	☐ (c)
▪ Escape of the patient during treatment	☐ (e)
Specify______________________________	
▪ Discovery of pre-existing violation of entry criteria	☐ (v)
Specify______________________________	
▪ Protocol non-compliance	☐ (n)
Specify______________________________	
▪ Adverse signs and symptoms	☐ (a)
▪ Death	☐ (d)
Specify______________________________	
▪ Withdrawn for other reason	☐ (o)
Specify__________________________________	
Follow-up visits (calculate and enter the dates at discharge):	
Date of 1st follow-up after 3 months:	└─┴─┘/└─┴─┘/└─┴─┘
Date of 2nd follow-up after 6 months:	└─┴─┘/└─┴─┘/└─┴─┘
Date of 3rd follow-up after 12 months:	└─┴─┘/└─┴─┘/└─┴─┘

I have reviewed the case report forms for the above subject and certify that they are accurate and complete

Signature of Investigator Date

______________________________ Place, ______________________

Protocol Number P-001-05-01-03, 12.07.2007

Appendix 2

IMPAMEL III

Patient information and informed consent - Tanzania

English

Kaliua Health Centre, Urambo District, Tanzania

IMPAMEL III

Assessment of an abridged Melarsoprol Treatment Schedule against late stage *T.b. rhodesiense* Sleeping Sickness

Multinational Utilization Study

PATIENT INFORMATION & INFORMED CONSENT

Incorporating Amendment #2 (26.07.2007)

Protocol Number:	P-001-05-01-03
Document date:	26.07.2007
Study coordinator:	Irène Kuepfer Swiss Tropical Institute Socinstrasse 57 CH-4002 Basel, Switzerland Tel +41 61 225 26 68 Fax +41 61 225 26 78 E-mail: Irene.Kuepfer@unibas.ch
Sponsor contact:	Swiss Tropical Institute Socinstrasse 57, CH-4002 Basel, Switzerland

Informed consent IMPAMEL III, Version 03-01, Tanzania-English, 26.07.2007

Patient Information

Study title	IMPAMEL III - Assessment of an abridged Melarsoprol Treatment Schedule against late stage *T.b. rhodesiense* Sleeping Sickness / Multinational Utilization Study
Principal Investigator	Dr. Johannes Blum Swiss Tropical Institute Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Johannes.Blum@unibas.ch Tel +41 61 284 82 59 Fax +41 61 284 81 83
Local Principal Investigator	Dr. Lucas Matemba National Institute for Medical Research, Tabora, Tanzania
Study Director	PD Dr. Christian Burri Swiss Tropical Institute Swiss Center for International Health Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Christian.Burri@unibas.ch Tel +41 61 225 26 61 Fax +41 61 225 26 78
Study Coordinator	Irène Kuepfer Swiss Tropical Institute Swiss Center for International Health Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Irene.Kuepfer@unibas.ch Tel +41 61 225 26 68 Fax +41 61 225 26 78
Country Coordinator	Dr. Stafford Kibona, National Institute for Medical Research, Tabora, Tanzania
Sponsor	Swiss Tropical Institute Socinstrasse 57 P.O. Box CH-4002 Basel

INFORMED CONSENT FOR: ______________________________

General:

- The Kaliua Health Centre has agreed to support the Swiss Tropical Institute in the strive to improve the treatment of sleeping sickness. We collaborate in a clinical trial program to assess a new, abridged schedule for melarsoprol treatment against the late stage of this disease.
- We would like to inform you on this ongoing trial and would like to solicit your participation. Your decision to take part in this study is your choice and free of charge. Before you agree to participate in this study you have to understand all risks and benefits.

What is your disease?

You have sleeping sickness, a serious disease, which untreated progresses and leads to death.

All sleeping sickness patients undergo a lumbar puncture to determine how advanced their disease is. If parasites or more than five white blood cells can be found in the cerebrospinal fluid (CSF), the disease is considered late stage and such patients are treated with suramin & melarsoprol. The patients without parasites in their CSF and less than five white blood cells are considered early stage and are treated with suramin only.

What is the standard medication for late stage sleeping sickness?

Under standard treatment, all patients receive a pre-treatment with 2 injections of suramin over 5 days. Thereafter, a lumbar puncture is performed to determine the stage of the disease.

If no parasites can be found in the cerebrospinal fluid, or if the white blood cell count is below 5 cells, the treatment continues with suramin. Such a patient is not eligible for our study.

Patients with parasites and/or more than 5 white blood cells in the cerebrospinal fluid are considered late stage and will then receive 3 series of 3 melarsoprol injections. Each series of melarsoprol injections is spaced by 7 days. This adds up to a routine hospital stay of more than one month. If you fall into this group, you may participate in the study.

Which is the purpose of the study?

The standard treatment schedule for the late stage of sleeping sickness is very long and complicated, for you, for your accompanying family, but also for the hospital.

We want to improve this situation by shortening the treatment with melarsoprol. The drug used remains the same, but we will give it to you continuously for 10 days. This study will help us to further assess the safety, tolerability and efficacy of the short treatment schedule. This means you will have to be in hospital for less than two weeks compared to more than one month under the standard treatment.

Previous research on the short schedule

In East Africa, the short melarsoprol treatment schedule has been tested in 60 patients in Tanzania and Uganda (August 2006 until May 2007) so far. These 60 patients were divided into two groups: the first group received 2 suramin injections before the diagnostic lumbar puncture. In case that the late stage of the disease was confirmed, patients were treated with the 10-day melarsoprol schedule. The second group of patients did not receive suramin. Once the late stage of the disease was confirmed the patients were directly treated with the 10-day melarsoprol schedule. We have observed that the group that has only received melarsoprol, tolerated the treatment better. But before this new, short treatment schedule is made accessible to all late stage patients we have to confirm these findings. Therefore we have to study the short schedule in more patients.

Also, this short schedule has been successfully tested in 2800 sleeping sickness patients in West African countries (Angola, Democratic Republic of Congo etc). Sleeping sickness in West Africa is related to the one you suffer from, but it is not the same.

How does the study look like in practise?

It is planned that a minimum of 70 late stage sleeping sickness patients will participate in this research. The study will take place in the same two hospitals: here in Kaliua and in one hospital in Uganda. All of the patients participating will receive the 10-day melarsoprol treatment directly; no suramin is given before the diagnostic lumbar puncture.

Who can participate in the study?

Only patients with late stage sleeping sickness can participate in the study. In addition, you have to be older than 6 years of age and, if you are a women in the reproductive age, you can only participate if a pregnancy test confirms that you are not pregnant.

Which treatment will I receive?

If you participate in this study you will receive the 10-day melarsoprol treatment (10 injections).

You are completely free to participate in the study. If you decide **not** to participate in this research you will receive the same nursing care and be treated according to the National standard treatment: two suramin injections and 3x3 melarsoprol injections spaced by 7 days.

Study Procedures:

If you decide to participate in the study following tests have to be done:

First we have to confirm that you really suffer from late stage sleeping sickness.

1. To determine if you are in the early or in the late stage of the disease we will check your cerebrospinal fluid for parasites and the number of white blood cells (routine procedure for all sleeping sickness patients).
2. If you are a woman we will perform a pregnancy test.
3. In addition to the usual blood samples for diagnosis we will take one more blood sample (1 tea spoon) to determine the exact type (strain) of parasite that makes you sick and to determine the type of your immune system (HLA). From this we want to learn about the relation of the immune system and adverse drug reactions.

Every day of the study we will check how you are and measure your vital signs, i.e. blood pressure, pulse, temperature.

After treatment we will check your blood and CSF to confirm that you are free of parasites.

Follow-up:

Sleeping sickness patients need to be observed for relapses for a long time after treatment. You will be asked to come back three times for follow-up visits: after 3, 6 and 12 months, because we want to check if you are still healthy. For each follow-up visit we will pay you an indemnity of US $ 10 which will cover your transport costs to the hospital.

At each follow-up visit we will take a blood sample (1 tea spoon) and CSF sample to check that you are free of parasites. In case of a negative result you can go back home. In case of a positive result you will be re-treated with melarsoprol according the national schedule (3x3 injections). Also, we will take an additional blood sample (1 table spoon) to check if you were re-infected or if the old infection has never properly gone away.

Benefits	**Costs**
In participating in this study you will benefit from a significantly shorter hospital stay In addition you will receive 1. Free hospital stay & treatment 2. Organized transport or a refund of the costs 3. After the treatment you receive a mosquito net. 4. With this study we want to improve sleeping sickness treatment. But if you wish, we offer you a free HIV test (access to treatment will be provided via the National HIV/AIDS control program)	You don't have to pay anything to participate in this study. But after treatment you have to come back to the hospital 3 times: after 3, 6 and 12 months.

Risks of study participation:

Melarsoprol is unfortunately the only drug for treatment of late stage sleeping sickness. It is a toxic drug and causes a significant number of more or less serious drug reactions. Such reactions include headaches, nausea, fever, walking problems (neuropathies). In the worst case it may cause an affection of the brain called encephalopathic syndrome, which may even lead to the death of a patient. Your physician will do everything to prevent or minimize the impact of such events. In comparison to the standard treatment there is evidence that the 10-day treatment schedule leads to comparable frequency of adverse drug reactions.

Withdrawal from the study:

You always have to possibility to leave the study. You can decide this alone or you can ask the doctor to help you with the decision and information about the best backup treatment.

Medical care for injuries during the study:

In the unlikely case that you should be injured due to the research treatment (10-day abridged treatment schedule) you will be treated at the hospital at no cost to you. The institution organizing the research has taken insurance for this case. In this case you should contact Dr. Matemba.

Doctors and scientists from the Institution organising and coordinating the research (Swiss Tropical Institute) or from Health Authorities may review the results of this research, including your patient files. However, your personal information will not be disclosed to anybody else.

This document will be translated to the following local language:

Kiswahili

Consent Form
Signature Page

I agree to participate in the IMPAMEL III study that investigates a new melarsoprol short course treatment schedule against late stage sleeping sickness caused by *T.b. rhodesiense*. When I participate in this study, blood samples, lumbar punctures and urine tests (for women only) are involved. I also understand that I have to come back to the hospital 3 times, after 3, 6, and 12 months.

Name and signature of trial team member who read and explained the above text:

Name: ______________________ Signature: ______________________

Name and signature of participant or guardian:

Name: ______________________ Fingerprint: [ ]

Witness (Trial team member only applicable if the subject and/or his guardian are illiterate or relative if the participant is minor):

Name: ______________________ Signature: ______________________

Role:

Place and Date, ______________________ , ______ / ______ / ______

Trial patient number assigned after admission to trial: ______________

Appendix 3

IMPAMEL III

Patient information and informed consent - Tanzania

Kiswahili

Kaliua Health Centre, Urambo District, Tanzania

IMPAMEL III

Utafiti wa Kutathmini Matibabu Mapya ya Ugonjwa, wa Malale Katika Hatua ya pili Kwa Kutumia Melarsoprol

TAARIFA NA FOMU YA MAKUBALIANO KWA MGONJWA

Marekebisho #2 (26.07.2007)

Namba ya Rasimu:	P-001-05-01-03
Tarehe:	26.07.2007
Mratibu wa Mradi:	Irene Kuepfer Swiss Tropical Institute Socinstrasse 57 CH-4002 Basel, Switzerland
Mfadhili:	Swiss Tropical Institute Socinstrasse 57 CH-4002 Basel, Switzerland

Informed consent IMPAMEL III, Version 03-02, Tanzania-Kiswahili, 26.07.2007

TAARIFA KWA MGONJWA

Utafiti	Utafiti wa Kutathmini Matibabu Mapya ya Ugonjwa, wa Malale Katika Hatua ya pili Kwa Kutumia Melarsoprol
Mtafiti Mkuu	Dr. Johannes Blum Swiss Tropical Institute Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Johannes.Blum@unibas.ch Tel +41 61 284 82 59 Fax +41 61 284 81 83
Mtafiti Mkuu Mwananchi	Dr. Lucas Matemba National Institute for Medical Research, Tabora, Tanzania
Mkurugenzi wa Utafiti Huu	PD Dr. Christian Burri Swiss Tropical Institute Swiss Center for International Health Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Christian.Burri@unibas.ch Tel +41 61 225 26 61 Fax +41 61 225 26 78
Mratibu wa Utafiti Huu	Irène Kuepfer Swiss Tropical Institute Swiss Center for International Health Socinstrasse 57 P.O. Box CH-4002 Basel Switzerland e-mail: Irene.Kuepfer@unibas.ch Tel +41 61 225 26 68 Fax +41 61 225 26 78
Mratibu Mwananchi	Dr. Stafford Kibona, Director National Institute for Medical Research, Tabora, Tanzania
Mfadhili	Swiss Tropical Institute Socinstrasse 57 P.O. Box CH-4002 Basel

FOMU YA MAKUBALIANO KWA ………………………………………..

Kwa ujumla

- Kituo cha Afya, Kaliua kimekubali kushirikiana na Taasisi ya magonjwa ya nchi za joto ya Uswiss (Swiss Tropical Institute) kuboresha matibabu ya ugonjwa wa malale. Tunashirikiana kutafiti kiwango kipya cha utoaji wa dawa ya Melarsoprol kutibu ugonjwa katika ngazi ya pili.
- Tunapenda kukufahamisha kuhusu zoezi linaloendelea na tunaomba ushirikiano wako.Uamuzi wako wa kushiriki katika jaribio hili ni wa hiari na hauna gharama. Kabla hujakubali kushiriki katika zoezi hili, utambue kuna faida na madhara yake.

Unasumbuliwa na ugonjwa?

Una ugonjwa wa malale, ugonjwa hatari, kama usipopatiwa tiba husababisha kifo. Wagonjwa wote wa malale lazima wachukuliwe maji ya uti wa mgongo ili kujua mgonjwa yuko katika hatua ipi ya ugonjwa. Kama vimelea vy ugonjwa au chembechembe nyeupe za damu zaidi ya tano zikipatikana katika maji ya uti wa mgongo wa mgonjwa, ugonjwa utakuwa katika hatua ya pili na wagonjwa hawa hutibiwa na dawa aina ya Suramin na Arsobal. Na wagonjwa ambao hawana vimelea katika maji ya uti wa mgongo (CSF) na ambao chembechembe nyeupe za damu pungufu ya tano, huchukuliwa kwamba wako kwenye hatua ya kwanza ya ugonjwa ambao hutibiwa kwa dawa aina ya Suramin tu.

Ni nani anashiriki katika Utafiti huu?

Wagonjwa wote walio na umri zaidi ya miaka 6, walio katika hatua ya pili ya ugonjwa wanaweza kushiriki utafiti huu, katika hatua hii ni vigumu kuthibitisha kuwa wapo katika hatua ya kwanza au ya pili ya ugonjwa. Katika hali hii kwa kawaida uchukuaji wa maji ya uti wa mgongo ni muhimu.

Hivyo tunapenda kukutaarifu juu ya utafiti huu na kupata ridhaa/utashi wako katika kushiriki, ukiwa kama mmoja wa washiriki hutapata matibabu ya dawa ya Suramin kabla ya kuchukuliwa maji ya uti wa mgongo.

Kiwango gani cha matibabu ya kawida katika hatua ya pili ya ugonjwa?

Kwa kawaida wagonjwa wote hupata sindano mbili za Suramin kabla ya uchunguzi wa maji ya uti wa mgongo. Kama vimelea havikupatikana katika maji ya uti wa mgongo au kama chembechembe nyeupe za damu ni pungufu chini ya tano, matibabu yanayofuatia ni Suramin. Mgonjwa kama huyu hahusiki katika utafiti huu.

Wagonjwa wenye vimelea na chembechembe nyeupe za damu zaidi ya 5 (tano) katika maji ya uti wa mgongo, huwa katika hatua ya pili na hutibiwa na 3 x 3 kwa dawa ya sindano Arsobal.(Melarsoprol), katika mfululizo wa matibabu haya baada ya siku 7. Kwa hiyo mgonjwa hulazwa zaidi ya mwezi mmoja. Na kama uko katika kundi hili unaweza kushiriki katika utafiti huu.

Lengo la zoezi hili ni nini?

Matibabu yaliyopo sasa kwa ajili ya hatua ya pili ya ugonjwa ni marefu na maigumu zaidi kwako, familia husika pia kwa hospitali. Tunataka kuboresha hali ya wagonjwa kwa kufupisha kiwango cha matibabu kwa kutumia dawa aina ya Arsobal ambayo utapatiwa kwa siku kumi (10 mfululizo. Hii inaamanisha kuwa utalazwa hospitali chini ya wiki mbili ukilinganisha na matibabu yaliyokuwa zaidi ya mwezi mmoja.

Lengo kuu la utafiti hu ni kutathimini usalama, uhimili na ubora wa matibabu mafupi.

Utafiti wa awali wa matibabu haya mafupi

Utafiti wa kufupisha matibabu haya kwanza ulifanyika kwa wagonjwa 60 hapa Tanzania na Uganda (August 2006 mpaka May 2007) .Kila nchi walitibiwa wagonjwa 30. Wagonjwa 15 wa kwanza walio katika hatua ya pili ya ugonjwa walitibiwa kwa Suramin na Arsobal na wagonjwa 15 wliofuata walitibiwa kwa Arsobal pekee Kwa hiyo waliotibiwa kwa dawa aina ya Suramin na Arsobal walipata sindano 12 (2 x Suramin, 10 x Arsobal). Kwa wale wa Arsobal peke yake walitibiwa kwa sindano 10. Utafiti huu wa awali umeonyesha kuwa wale waliopata tiba ya Arsobal peke yake walionyesha kupona kwa haraka na bila madhala zaidi ya waliopata Suramin na Arsobal

Lakini kabla ya kuanza kutumia tiba hii fupi inatulazimu tuhakikishe tena kwa kushirikisha wagonjwa zaidi.

Tiba hii fupi imeonyesha mafanikio kwa wagonjwa 2800 wa malale waliotibiwa Africa Magharibi (Angola na Congo). Ugonjwa wa malale wa Africa Magharibi unafanana na huu unaougua wewe bali ni tofauti kidogo kwa dalili zake

Utapata matibabu gani?

Wagonjwa 70 wa daraja la pili la ugojnwa wa malale watashiriki katika utafiti huu Vituo viwili vile vile vitahusika na utafiti huu ambavyo ni Kaliua Tanzania na kimoja nchini Uganda. Wagonjwa wote watapata dawa ya melarsoprol kwa siku kumi bila kutanguliwa na Suramin.

Nani Mshiriki wa Utafiti huu

Wale wagonjwa ambao wako katika daraja la pili la ugonwa ndio watashirikishwa katika utafiti huu na pia ni lazima uwe na umri wa miaka 6 na zaidi na kwa mwanamke ni yule ambaye hatapatikana na ujauzito.

Matibabu gain utapata?

Ukiwa mashiriki wa utafiti huu utapata sindano jumla ya 10 za melarsoprol (kila siku sidano moja kwa muda wa siku 10)

Uko huru kuamua kushiriki au kutokushiriki katika utaffti huu na uamuzi wako hautaathiri huduma za matibabu unazopata kama kawaida

Taratibu za utafiti

Kama utaamua kushiriki katika utafiti, majaribio yafuatayo yatafanyika:-

Kwanza tutahakikisha kuwa unaugua ugonjwa wa malale hatua ya pili.

(1) Tutachunguza vimelea katika maji ya uti wa mgongo na namba ya chembechembe nyeupe za damu (utaratibu kwa wagonjwa wote wa malale).

(2) Kama ni mwanamke tutakupima ujauzito.

(3) Kwa nyongeza katika utaratibu wa kawaida wa kuchukua sampuli ya damu kwa uchunguzi, tutachukua sampuli tatu (3) za damu. Mbili (2) kati ya hizo tatu ili kufahamu aina halisi ya vimelea vinavyofanya uugue. Na sampuli nyingine kuelewa aina ya mfumo wa Kinga ya Mwili (HLA). Kwa hiyo tunataka kujua uhusiano wa mfumo wa kinga ya mwili na madhara mabaya ya dawa.

Kila siku ya utafiti tutachunguza maendeleo yako yalivyo. Hii itajumuisha upimaji wa vitu vifuatavyo.

1. Dalili za muhimu - shinikizo la damu, mapigo ya moyo na joto la mwili.

2. Kupima kiwango cha sukari katika damu (Blood for sugar). Siku zitakazoteuliwa tutafanya uchunguzi zaidi.

3. Tutapima mkojo ili kubaini kama figo zinafanya kazi vizuri.

4. Matibabu yakiisha/kamilika tutapima damu na maji ya uti wa mgongo kuthibitisha hamna vimelea vya malale.

Ufuatiliaji

Wagonjwa wa malale huhitaji ufuatiliaji wa kuangalia hali ya mgonjwa kwa kipindi cha muda mrefu baada ya matibabu. Unatakiwa kurudi tena mara tatu kwa ufuatiliaji baada ya miezi 3, 6 na 12. Ili kufahamu maendeleo ya afya yako. Utalipwa Dola za kimarekani 10 (USD 10) kwa kila mahudhurio a ufuatiliaji kulipia gharama zako za usafiri.

Zaidi ya utaratibu wa kuchukua sampuli ya damu na maji ya uti wa mgongo, tutachukua sampuli ya damu katika kila kipindi cha ufuatiliaji. Kama tutakuta vimelea utatibiwa tena na pia tutachukua sampuli 3 za damu. Tunataka kulinganisha vimelea vya mara ya kwanza ulivyokuja navyo na vya mara ya pili. Hivyo tunaweza kujua umeambukizwa tena au maambukizi ya zamani hayakwisha.

FAIDA	HASARA
Kwa kushiriki katika zoezi hili utamufaika kukaa muda mfupi hospitalini na zaidi utapata:- 1. Kukaa hospitali bila gharama na matibabu bure. 2. Usafiri na kurudishiwa gharama za matumizi. 3. Baada ya matibabu utapata chandarua cha kuzuia mbu. 4 Kwa zoezi hili tunataka kuboresha matibabu ya ugonjwa wa malale. Na kama ukipenda tutakufanyia upimaji wa bure wa virusi vya ukimwi (VVU)(Utweza kupata matibabu kupitia mpango wa Taifa wa Kuzuia Virusi vya Ukimwi (VVU na UKIMWI)	Haulipi chochote kushiriki katika zoezi hili. Lakini baada ya matibabu utarudi tena hospitali mara 3: baada ya miezi 3, 6 na 12.

Athari za kushiriki katika utafiti huu

Kwa ujumla kuna ushahidi katika tafiti zilizopita kwamba, kwa siku 10 za matibabu husababisha mfululizo wa madhara mabaya ya dawa kama ilivyo katika viwango vya kawaida vya matibabu. Isipokuwa matatizo ya magonjwa ya ngozi (kama kuwashwa n.k). Madhara ambayo yametokea kwa kurudia rudia katika majaribio yaliyopita. Hivyo daktari anayo fursa ya kutoa dawa kuondoa matukio kama hayo.

Arsobal ni dawa pekee ya kutibu ugonjwa wa malale katika hatua ya pili. Arsobal ni dawa yenye sumu inayosababisha madhara na hata katika kiwango kilichopo sasa cha matibabu, madhara yanayoweza kutokea ni kama kuumwa kichwa, kichefuchefu, ', matatizo ya kutembea na kama hali ni mbaya sana husababisha madhara kwenye ubongo (encephalopatie syndrome) ambayo inaweza kusababisha kifo kwa mgonjwa. Daktari atafanya kila liwezekanalo kuzuia au kupunguza matukio kama haya.

Kujitoa katika zoezi

Wakati wowote unawezeka wa kujitoa katika utafiti huu. Unaweza kuamua mwenyewe au kumuuliza daktari kuhusu uamuzi na taarifa kuhusu tiba bora zaidi.

Huduma ya afya ukiumia wakati wa zoezi

Ikitokea umeumia au kudhurika wakati wa utafiti wa matibabu (siku 10 za mpango wa matibabu) utatibiwa bila gharama yoyote. Taasisi inayohusika na utafiti tayari imetayarisha Bima ya afya kwa tukio kama hilo. Kwa suala hili wasiliana na Dr. L. Matemba.

Madaktari na wanasayansi kutoka taasisi zinazohusika na kuratibu utafiti (Swiss Tropical Institute) au Mamlaka ya Afya wanaweza kupitia matokeo ya utafiti ikijumuisha majarada ya wagonjwa. Hata hivyo taarifa zako hazitatolewa kwa mtu yeyote. Tutakuarifu kama taarifa mpya za utafiti wa matibabu DB 289 wakati ukishiriki katika zoezi.

FOMU YA MAKUBALIANO

UKURASA WA SAHIHI

Nakubali kushiriki katika zoezi la **IMPAMEL III** linalotafiti matibabu ya Arsobal yanayochukua muda mfupi kutibu ugonjwa wa malale unaosababishwa na vimelea aina *T.b.rhodensiense* katika hatua ya pili. Ninaposhiriki katika utafiti huu uchukuaji wa sampuli za damu, mkojo na maji ya uti wa mgongo vitafanyika. Vilevile natambua kwamba nitarudi tena hospitali mara tatu baada miezi 3, 6 na 12.

Jina na sahihi la mtafiti ambaye anasoma na kuelezea taarifa za hapo juu.

Jina……………………………………………… **Sahihi………………………**

Jina na sahihi ya mshiriki au mlezi

Jina…………………………………… **Alama za vidole** ☐

USHAHIDI (Mtafiti atahusika pale ambapo mhusika au mlezi hajui kusoma na kuandika au ndugu kama mshiriki ni mtoto mdogo).

Jina……………………………………………. Sahihi…………………………………

Uhusiano:

Sehemu na tarehe…………………………………………………………………….

Namba ya mgonjwa aliyeruhusiwa kushiriki katika zoezi …………………….