AMENDED VERSION LEAP 0104B MARCH 2009 CONFIDENTIAL

TITLE

A MULTICENTRE COMPARATIVE TRIAL OF EFFICACY AND SAFETY OF SODIUM STIBOGLUCONATE (SSG) VERSUS PAROMOMYCIN (PM) VERSUS COMBINATION OF SSG AND PM AS THE FIRST LINE TREATMENT FOR VISCERAL LEISHMANIASIS IN ETHIOPIA, KENYA AND SUDAN

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SUMMARY

Visceral leishmaniasis (VL) or Kala-azar is the most severe form of leishmaniasis. It is estimated that 500,000 new cases world wide of VL are diagnosed annually. 90% of VL cases occur in developing countries: India (especially Bihar), Bangladesh, Nepal, North Eastern Brazil and Sudan. For the past 100 years, antimony has been the first line of treatment for VL cases despite considerable toxicity and the requirement for 4 weeks hospitalization.

Resistance to antimony coupled with emergence of HIV associated with VL is on the increase. New and improved treatment options are urgently needed to replace or complement the few currently available drugs. The wide variety of epidemiological situations and clinical presentations of this disease further warrant a series of treatment options instead of one single treatment or control strategy for the affected populations.

During 2003, experts in VL together with representatives of regulatory authorities and health ministries from Kenya, Ethiopia and Sudan met (Nairobi, May 2003, Khartoum, August 2003) to discuss the development of new treatment options for this fatal but neglected disease.

This research proposal will be a multicentre, prospective, open label, parallel group, comparative trial to determine the efficacy and safety of sodium stibogluconate (SSG) 20mg/kg/day given for 30 days, Paromomycin (PM) 15mg/kg/day for 21 days, and a combination of SSG and PM, 20mg/kg/day, 15mg/kg/day respectively, given for 17days in the treatment of patients suffering from VL in Ethiopia, Kenya and Sudan. Primary endpoint will be cure rate at 6 months.

LITERATURE REVIEW

The leishmaniases are a group of diseases caused by Leishmania parasites, of which at least 20 different species can cause human disease. Leishmania infection is transmitted by the bite of female sandflies. The disease occurs in three forms: self-healing cutaneous leishmaniasis (CL), mutilating mucosal leishmaniasis (ML or MCL) and life-threatening visceral leishmaniasis (VL). Each form varies in degree of severity, with visceral leishmaniasis being by far the most devastating.

Today, of the estimated 350 million people at risk in 88 countries, 12 million people are thought to be affected by leishmaniasis in its different forms, with an estimated 1.5 -2 million new cases occurring annually (1-1.5 million cases of CL/MCL and 500,000 cases of VL) (WHO 2000). In the past decade, the number of leishmaniasis cases has risen (Desjeux 2001) due to increased human exposure to the sandfly vector as well as the spread of AIDS and other immunosuppressive conditions that have increased the risk of *Leishmania*-infected people developing the disease.

Visceral leishmaniasis (VL) or kala-azar is the most severe form of the disease. If untreated, VL has a mortality rate of almost 100%. In 1999, there were 57,000 (reported) deaths due to kala-azar. Ninety per cent of VL cases occur in five developing countries: India (especially Bihar), Bangladesh, Nepal, North Eastern Brazil, and Sudan.

Distribution of Visceral Leishmaniasis in Eastern Africa

In Eastern Africa, especially Sudan, Ethiopia and Kenya, visceral leishmaniasis is by far the most common form of the disease and is the cause of much death and disease.

VL in Ethiopia has been reported from over 40 localities in different parts of the country. The infection is either due to L. donovani, L. infantum or L. archbaldi. Most infections are acquired in north-west Ethiopia in the lowlands of Metema and Humera, south-west Ethiopia in the Segen, Woitu and Omo river basins, and in other isolated foci in the rift valley. The north-western Metema-Humera focus (which extends northwards to Eritrea and westwards into eastern Sudan) is a major VL focus which presently accounts for approximately 60% of the total disease burden in Ethiopia. This focus extends over a huge land mass in two regions, Region 1 (Tigray) and Region 3 (Amhara). In this focus MSF-H is actively involved in treatment of cases, with at least 2000 cases benefiting from treatment every year. The patients in this focus are mostly migrant laborers, and one would expect up to 40% of the cases to be HIV co-infected. VL foci in Segen, Woitu and Omo river basins represent typical endemicty. The VL cases from these foci, account for approximately 20% of the total burden in the country, and HIV co-infection is less than 2%. These foci are located in the Southern Nations, Nationalities and Peoples Regional Government (SNNPRG). Other foci are in Region 4 (Oromia), Region 5 (Somali), and Region 2 (Afar). Sporadic case reports are known from other smaller localities. For instance, in Moyale, at the borders with Kenya and in areas northeast of Lake Abaya. Members of the Ethiopian Army and Police Forces who acquire VL in the endemic areas are admitted in Addis Ababa referral hospitals. This is a special risk group and HIV co-infection could be expected to be more than 50%. (Hailu 2004, Ayele 2004)

In Eritrea, the Red Sea littoral (localities like Nakfa, Afabet, Algena, Keren) and the district of Teseney also in Eritrea (North of Humera) are endemic.

Eastern Sudan (Gedarif State), Upper Nile and Western-Upper Nile are known endemic areas for visceral leishmaniasis in the Sudan. VL is among the most important health problems in the Sudan with more than 24,660 cases and 1193 deaths that have been reported during 1996-2001. The number of reported cases is mainly a reflection of reporting rather than the actual disease transmission. Reports and published work from Sudan showed that the disease affects mainly children with few adult cases. The disease is reported to be more prevalent among poor people, malnourished, vagrant, farmers, laborers, water carrier, and those out of country, who have a very limited capacity to assume the costs of the disease (Sudan Manual 2004).

In Kenya, the endemic foci of VL include Baringo, Turkana, West Pokot, Kitui, Meru, and Machakos districts. The first 3 districts are in Rift Valley province while the latter are in Eastern province. Numerous outbreaks of VL were reported from these areas in the late nineteen seventies with over 2000 cases reported from Meru and Kitui districts only (WHO 1990)**.** All these areas are generally semi-arid, sparsely populated with low rainfall and high temperatures. Low agricultural and economic productivity has resulted in poor social economic status (SES) of the population in these areas.

Population displacements as a result of war, drought, famine, or rural-urban migration have exacerbated the spread of the disease. For instance, the epidemic in western Upper Nile, an area where VL was previously not endemic, caused an estimated 100,000 deaths between 1984 and 1992, or a population mortality of up to 36% (Seaman et al. 1996).

Clinical Aspects of Leishmaniasis in Eastern Africa

Visceral leishmaniasis, is a devastating illness, fatal if left untreated. Patients with VL present with fever, malaise, cough, abdominal pain, diarrhoea, epistaxis, splenomegaly, hepatomegaly, cachexia, anaemia, pancytopenia, lymhadenopathy and malnutrition.

Not all infected people develop clinical kala azar; some have a sub clinical infection that spontaneously resolves. The ratio of those with clinical disease to those with sub clinical disease varies remarkably from place to place, and during periods of epidemics. In eastern Sudan, the ratio of clinical cases to mild or sub clinical diseases was 1.6:1, and increased to 3.3:1 during a recent outbreak. The scenario is less critical in Brazil where, during an outbreak, the ratio was 1:8 or 1:16 (showing much less disease per infection); in Iran, it is 1:12 (MSFH 2003)

Malnutrition, anaemia and immune depression increase the likelihood that infection will progress to the disease. In Ethiopia, Kenya, and Sudan the problems of infected children are compounded by these very reasons, as well as opportunistic infections such as tuberculosis and pneumonia. Infected adults also bear the brunt of these problems – in Ethiopia HIV is found in association with kala-azar in approximately 35-50% of cases (Dr Asrat Hailu – personal communication).

The incubation period for VL varies widely and it is estimated to be between 2 – 6 months. Malnutrition, anaemia and immune depression increase the likelihood that infection will progress to the disease.

A complication of visceral leishmaniasis, especially prevalent in Sudan (and to a lesser extent Ethiopia, and Kenya) is post-kala-azar dermal leishmaniasis (PKDL) **(**Zijlstra et al 2003) occurring in people who have recovered from VL following treatment.

Main treatment options for visceral leishmaniasis.

Treatment of VL cases in Eastern Africa always presents with challenges such as patients coming late when they are extremely ill and may die during treatment due to the illness as well as toxicity of the drugs used. The other challenges include availability of drugs, drug resistance, and cost of treatment (drugs and hospitalization). In VL endemic areas, facilities may not be available for accurate diagnosis and follow up, and the increasing prevalence of HIV co-infection is an additional challenge, particularly in Ethiopia.

Table 1: Current treatment options for patients with visceral leishmaniasis

Drugs available for use	Associated problems
Pentavalent antimonials	Toxic, parasite resistance growing 30 day IV/IM treatment in hospital
Amphotericin B	Used in case of antimonial resistance but dose-limiting toxicity, 15-20 day IV treatment in hospital
Liposomal Amphotericin B	Less toxic but prohibitively expensive
Miltefosine	Teratogenic, only registered in India, and expensive

Need for new treatment options

In eastern Africa, the first line treatment today in most endemic areas is antimonial therapy for 4 weeks sodium stibugluconate,(Pentostam® from GSK in Kenya or generic SSG from Albert David in Sudan and Ethiopia) used at 20 mg/kg/day for 28-30 days). Although the efficacy of this treatment is not yet compromised by resistance in this region (in contrast to Bihar – India), the painful daily injections, the need for four weeks of hospitalisation, the toxicity when using longer treatments, the low efficacy in HIV co-infected patients and the risk of inevitable drug resistance, as observed in India, make alternative options a necessity. Second line treatments are either toxic or prohibitively expensive.

In 2002-3, a combination of SSG and PM given for 17 days was used in an epidemic situation in Southern Sudan, with an initial cure rate of 97% (personal communication from Koert Ritmeijer, Médecins Sans Frontières). These findings were in line with previous published experience in the same area (Seaman et al, 1993) and experience in Kenya (Chunge et al, 1990). The proposed study aims to confirm these results in a randomized prospective comparative study.

SSG

Despite the shortcomings listed in table 1, sodium stibogluconate (SSG) is still the most widely used drug for VL in Eastern Africa. SSG is known to cause cardiac,
muscle, joint and renal problems. Emergence of resistance as has occurred in the

Indian subcontinent (Bihar state) make investigating combination schedules a priority.

Paromomycin

Paromomycin (PM) is a broad-spectrum aminoglycoside antibiotic produced from culture filtrates of Streptomyces krestomyceticus and is identical to aminosidine (Shilling & Shaffner, 1961). PM is very poorly absorbed from the gut, an oral formulation is available for the

treatment of infections caused by bacteria, protozoa and worms from the intestinal lumen. For the treatment of systemic infections, for example VL, a parenteral formulation is required.
An injectable formulation of 500 mg of PM sulphate has been marketed in several countries for over 35 years for the treatment of bacterial and parasitic infections, however it has not been licensed specifically for the treatment of VL.

The anti-leishmanial activity of injectable paromomycin was first demonstrated in the 1960s and subsequently confirmed in vitro and in vivo. Since then, it has also been shown to be effective against visceral leishmaniasis (Chunge 1990, and others) and is affordable and well tolerated.
Efficacy of PM has also been shown in Bihar, India, the region with the greatest incidence of kala azar and the highest rates of antimony resistance (Thakur 2000)

Historical Product Profile – Farmitalia dossier
Summary data are available on a total of 2,397 patients treated with injectable paromomycin for various infectious diseases. Patient population ranged from newborn infants to the elderly. In most cases, adults received up to 2g/d for 30 days, although patients with skin infections were given up to 1.5g/d for 49 days.

Summary of results:
Paromomycin was well tolerated. Adverse events (AEs) involving hearing function were reported in 10 (0.4%); two patients had renal function decrease and one albuminuria; 21 additional patients had other AEs. The occurrence of AEs was not related to the age of patients. AEs involving hearing tended to occur in patients administered large dose of PM and/or multiple-drug regimens.

Safety Data - Historical Japanese Post Marketing Data:
Pre and post marketing safety surveillance safety data is available from 2220 patients. The incidence of adverse reactions is as follows: Pain at injection site 94 (4.2%), local rash 30 (1.4%), tinnitus 8 (0.4%), malaise 9 (0.4%), skin rash 5 (0.2%), nausea/vomiting 4 (0.2%), diarrhea 2 (0.1%). The major dose limiting toxicities of injectable paromomycin are the same as other drug in the aminoglycoside class (e.g. streptomycin, gentamycin) being oto- and renal toxicity. These toxicities are related total dose of the drug given and duration of therapy.

Pre-clinical toxicology
Mutagenicity/Genotoxicity: GLP Institute Pasteur Lille

- Mutagenicity test on bacteria using Ames technique
- Genotoxic activity using the micronucleus test
- Mutation assay at the TK locus in L5178Y Mouse lymphoma cells using a microtitre cloning technique
- Test for chromosomal aberrations by in vitro human lymphocyte metaphase analysis

Results: All tests were negative for mutagenicity/genotoxicity

Animal toxicology

Table 2: Animal toxicology studies – part 1

Study	Species	Route	Dose/Duration	Sponsor	GLP	Main results
Acute	Mouse, Rat	IV, IM, IP, IC, SC, PO	Mice- LD_{50} g/kg - IV 0.106- 0.110 - IP 0.750 - SC 0.70 - 1.06 - IC 0.023 - PO 15.0 –17.8 Rat – LD_{50} g/kg - IM 1.20 - SC 0.87 - PO 21.62	FCE	No	The LD_{50} is 8-10x greater than the therapeutic dose in humans
Chronic	Mice, Rats, Cats	IM	Mice mg/kg/day - 400 for 60 days Rats mg/kg/day - 264 for 82 days Cats mg/kg/day - 50 for 37 days	FCE	No	No mortality or vestibular damage seen
Nephrotoxicity	Mice, Rats, Cats	IM	Mice mg/kg/day - 400 for 60 days Rats mg/kg/day - 264 for 82 days Cats mg/kg/day - 50 for 37 days	FCE	No	Mice moderate renal damage Rats slight renal damage Cats moderate renal damage

Table 3: Animal toxicology studies – part 2

Study	Species	Route	Dose/Duration	Sponsor	GLP	Main results
Cochleo Vestibular	Rats, Guinea Pigs,	SC	Rats mg/kg/day - 200, 264 for 60 days G. Pigs mg/kg/day - 50, 100, 200, 400 for 30 days - 20 for 60 days - 200 for 28 days comparative trial with KM and DHSM	FCE	No	Rat dose related cumulative effect on acoustic sensitivity Guinea Pig dose related cumulative effect for oto-toxicity. In the comparative trial AM was less ototoxic than KM or DHSM
Reprotox	Mice, Rats, Rabbits	IM, SC	Teratogenesis Mice mg/kg/day - 100, 200, 300 IM for 7 days Rats mg/kg/day - 100, 200, 300 IM for 7 days Embryo-fetal Rats mg/kg/day - 100, 200 SC for 19 days Rabbits mg/kg/day - 12.5, 25 SC for 28 days	FCE	No	No teratogenic effect detected. No statistically significant embryo-fetal toxicity
Thirteen Week Chronic Toxicity	Dogs	IM	Dogs mg/kg/day 30, 100 for 13 weeks	SoloPak	Yes	Low dose dogs slight to minimal renal damage, and a frequency dependent hearing loss at high tones. High doses dogs severe chronic nephropathy, and renal tubular degeneration. Unable to detect audiometric hearing frequencies Swelling and chronic inflammation at injection site

Clinical Pharmacology

Pharmacokinetics

An HPLC assay was developed at the University of Illinois at Chicago under GLP conditions in order to be able to determine the concentration of paromomycin in biological fluids (e.g. urine and plasma)

Single Dose Intramuscular Pharmacokinetics in Healthy Normal Volunteers

Sixteen HNVs were given a single IM dose of paromomycin base either 12 or 15 mg /kg (8 per group)

Table 4: Pharmacokinetic parameters

Dose mg/kg /day	C_{max} (μg/ml)	T_{max} (h)	K_a (h^{-1})	T_{lag} (h)	AUC (μg h/ml)	CL/F (ml/min/1.73M^2)	Vβ/F (l/kg)	$t_{1/2}$ (h)
12	21.6	1.19	6.27	0.23	86.3	117.7	0.35	2.21
15	23.4	1.51	2.65	0.20	104.5	126.0	0.41	2.64

Dose finding Studies

1) Randomized phase II clinical study: Kala-azar Research Centre, Muzaffarpur, Bihar, India; T.K. Jha (Jha et al., 1998)

Group (mg/kg/d)	Enrolled	Treatment Completed	Treatment Failures	Relapses	Defaulters	Definitive Cure 180d (%)
PM 12 x 21d	30	30	2	5	0	23/30 (76.7)
PM 16 x 21d	30	30	0	1	1	28/29 (96.5)
PM 20 x 21d	30	30	0	1	0	29/30 (96.7)
SB 20 x 28d	30	30	8	3	0	19/30 (63.3)

2) Randomized phase II clinical study: Patna Medical College, Patna, Bihar, India; (Thakur *et al*, 2000: 94:)

Group (mg/kg/d)	Enrolled	Treatment Completed	Treatment Failures	Relapses	Defaulters	Definitive Cure 180d (%)
PM 12 x 21d	30	30	0	3	0	27/30 (90.0)
PM 16 x 21d	30	30	0	3	3	24/27 (88.9)
PM 20 x 21d	30	30	0	4	1	25/29 (86.2)
SB 20 x 28d	30	30	8	1	1	20/29 (69.0)

3) Randomized, comparative, open-label trial of the safety and efficacy of Paromomycin (PM) + sodium stibogluconate (SB) versus sodium stibogluconate alone for the treatment of visceral leishmaniasis: Patna Medical College, Patna, Bihar, India; (Thakur *et al*, 2000)

Group (mg/kg/d)	Enrolled	Treatment Completed	Treatment Failures	Relapses	Defaulters	Definitive Cure 180d (%)
PM12+SBx 21d	52	51	3	1	0	48/52 (92.3)
PM18+SBx 21d	48	46	2	1	0	45/48 (93.8)
SB20 x 28d	50	46	21	1	1	27/50 (54.0)

Clinical Experience with injectable paromomycin in the Treatment of VL

Previously, clinical trials with injectable PM either alone or in combination with SB for the treatment of VL have been conducted in Africa (Kenya and Sudan), India (Bihar), and in cases imported into the United Kingdom (Jha, et al., 1998, Hassan M, et al., 1995 Thakur et al., 1995; Seaman et al, 1993, Thakur et al, 1992, Scott et al, 1992, Chunge et al., 1990). In all the studies the investigators reported that PM, used as a single agent or combined with SB was highly efficacious and well tolerated in the treatment of VL caused by L. donovani or infantum.

Table 5: Summary of clinical studies using PM

Dose mg/kg/day	Single Agent No. Patients	Place	Combination Therapy with SSG No. Patients	Place
6			40	India (Thakur)
12	60	India (30 Jha, 30 Thakur)	120	India (96 Thakur, 24 Thakur)
14-16	19	Kenya (Chunge)	124	Kenya and Sudan (23 Chunge, 101 Seaman)
16	60	India (30 Jha, 30 Thakur)		
18			50	India (Thakur)
20	60	India (30 Jha, 30 Thakur)		
Total Patients	**199**		**384**	

TRIAL OBJECTIVES AND PURPOSE

Currently in the three countries, Sudan, Kenya and Ethiopia many of the patients present themselves in remote areas and need to be treated in relative resource poor settings. It is for this reason that standardised treatment with proven efficacy is much needed. A shorter course of treatment is not only advantageous for the patient but also reduces the overall case load in the clinics thus reducing the risk of disease outbreaks in already immuno-compromised kala-azar patients. Paromomycin, either alone or in combination with SSG would decrease the treatment duration substantially. An additional added value of combination therapy is that it is likely to reduce the chances of development of parasite resistance against the individual drugs.

Leishmaniasis experts in the three countries are in agreement that there are potential benefits of the combination treatment of SSG and PM and that its efficacy should be evaluated with the view to introduce this protocol if proven efficacious and safe. There is ample circumstantial evidence of the use of this combination therapy and its efficacy and tolerability as a standardized protocol. This can only be confirmed through a randomised controlled study with 6 months follow up.

HYPOTHESIS

The Null Hypothesis is that the efficacy of PM alone (21 days) is not less than 15% the efficacy of SSG alone (30 days) and the efficacy of the combination of PM and SSG (17 days) is no less than 10% the efficacy of SSG alone.

OBJECTIVES OF THE TRIAL

1) To assess the efficacy and safety of SSG 30 days alone in the treatment of patients with VL.

2) To assess the efficacy and safety of PM 21 days alone in the treatment of patients with VL.

3) To assess the efficacy and safety of SSG and PM as a combination course of 17 days in the treatment of patients with VL.

METHODOLOGY

STUDY DESIGN

This will be a multi-centre, prospective, open, parallel group, comparative trial of efficacy and safety of SSG alone given IM/IV (according to usual hospital practice) for 30 days versus paromomycin alone given IM for 21 days versus a combination of SSG and PM given for 17 days, in the treatment of patients suffering from VL in Ethiopia, Kenya and Sudan.

Patients who have clinical symptoms and a confirmed parasitological diagnosis of VL by splenic aspirate, lymph nodes aspirate or bone marrow aspirate (to be specified for each hospital site) and who have fulfilled the inclusion/exclusion criteria will be enrolled.

The primary endpoint will be cure rate at 6 months post treatment. Secondary endpoints will be cure rate at end of treatment (Day 31 for SSG, Day 22 for PM, Day 18 for PM + SSG) and at three months post treatment.

STUDY SITES

The study will be conducted at the following sites;

Ethiopia:

- Arba Minch hospital
- Gondar hospital

Kenya:

- Centre for Clinical Research (CCR), Kenya Medical Research Institute (KEMRI), Nairobi.
- **Kimalel Health Centre, Baringo**

Sudan:

- Kassab Hospital
- Um-El-Kher (MSFH treatment centre) **(site closed)**

INCLUSION CRITERIA

Patients who fulfill the following inclusion criteria will be enrolled into the study:-

1) Patients for whom written informed consent has been signed by the patients themselves (if aged 18 years and over) or by parents(s) or legal guardian for patients under 18 years of age.
2) Patients aged between 4 and 60 years (inclusive) who are able to comply with the protocol. It is justified to include children because they represent more than 50% of VL cases.
3) Patients with clinical signs and symptoms of VL and diagnosis confirmed by visualization of parasites in tissue samples (spleen, lymph node or bone marrow) on microscopy.

EXCLUSION CRITERIA

Patients with the following will be excluded from the study:

1) Patients who have received any anti-leishmanial drug in the last 6 months.
2) Patients with a negative splenic / lymph node / bone marrow smears.
3) Patients with a clinical contraindication to splenic/lymph node/ bone marrow aspirates.
4) Patients with severe protein and or caloric malnutrition (Kwashiokor or marasmus)
5) Patients with previous hypersensitivity reaction to SSG or aminoglycosides.
6) Patients suffering from a concomitant severe infection such as TB or any other serious underlying disease (cardiac, renal, hepatic) which would preclude evaluation of the patients response to study medication.
7) Patients suffering from other conditions associated with splenomegaly such as schistosomiasis.
8) Patients with previous history of cardiac arrhythmia or an abnormal ECG
9) Patients who are pregnant or lactating.
10) Patients with haemoglobin < 5gm/dl.
11) Patients with WBC < 1 x $10^3/mm^3$.
12) Patients with platelets < 40,000/mm^3.
13) Patients with liver function tests more than three times the normal range
14) Patients with serum creatinine outside the normal range for age and gender
15) Patients with pre-existing clinical hearing loss.

NB

Relevant tests will be done to exclude the above listed conditions.

HIV-STATUS AND VCT

All patients will be offered counseling and screening for HIV (voluntary counseling and testing programme (VCT). This may either be done at the same time as consent is obtained for inclusion in the trial or at a later date according to hospital practice. HIV positive patients will not be excluded from the clinical trial.

Subset analysis will be performed to assess any differences in response.

CRITERIA FOR PATIENT WITHDRAWAL

Patients will be considered to have completed the study if they satisfy all entry criteria, complete the course of treatment and attend the 6 month follow-up visit.

Patients will be considered to have withdrawn from the study if they had entered into the study (i.e. gave informed consent and received at least one day's treatment) but did not complete the treatment period and follow up period.

Treatment failure will be defined as no change or an increase in the patient's disease severity i.e. in signs and symptoms of VL, and parasitology, such that the patient is withdrawn from the study and alternative therapy given.

A patient may be withdrawn from the study at any stage if the investigator or the DSMB considers there is a serious risk to the patient from continuation in the protocol. Alternative therapy will be provided to the patient if needed, upon withdrawal from the study.

A patient may withdraw, or be withdrawn, from the study for one of the following reasons:

- Serious adverse events (drug related or not)
- Deviation from protocol (including non-compliance)
- Lost to follow-up
- Termination by the sponsor
- Withdrawal of consent

The reason for termination will be recorded on the CRF. Patients withdrawn from the study will be followed-up at 3 and 6 months for monitoring of adverse events wherever possible. Every effort will be made to follow up withdrawn patients in order to determine the final outcome. This information will be recorded in the CRF and these patients' data will be analysed as hose who failed to respond to treatment.

Randomization in the Clinical Trial (RCT)

The multi-country study adopted restricted randomization in a three-arm study per country. This approach prevents the potential pitfall/imbalance in study numbers that could have resulted if one used simple random sampling. In order to avoid manipulation of blocks of small sizes, blocks of size 15 will be used in randomization. This approach ensures that the study balances after the 15^{th} patient. In the allocation of the drugs to the patients concealment will be used in order to minimize selection bias. Opaque envelopes will be numbered sequentially and then sealed. This process will be carried out at the coordinating centre at KEMRI.

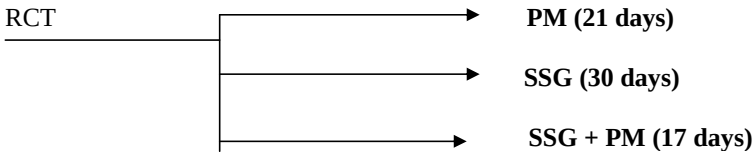


Fig 1: The structure of the RCT

Sample size Determination

The sample size will be used to test the statistical hypothesis as stipulated earlier:

Assumptions

- The efficacy data will be analysed overall, not by site
- The trial is not powered for by site analyses
- Interim and final analyses will be carried out only as specified
- Adjustments to the sample size have been made to power the study for a sub-group analysis in HIV negative patients if 10% of patients overall are known to be infected and also to allow for loss-to-follow-up (LTFU) of 10% at 6 months.
- Table 1 gives the numbers needed per arm after adjusting for HIV (10%) and LTFU at 6 months (10%) based on two-sided probabilities with 90% power.

Table 1. Number of patients required per regimen to detect a statistically significant difference, at the 5% level with 90% power, between the efficacies shown based on a two-sided test for ITT. Estimates are adjusted for 10% to allow for an adequate number of HIV negative patients in sub-group analyses and also for 10% loss-to-follow-up at 6 months.

	% cured in comparative regimen								
% cured SSG	0.5	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95
0.6	627								
0.65	274	2381							
0.7	150	576	2229						
0.75	93	245	531	2025					
0.8	62	131	223	474	1771				
0.85	43	79	117	195	404	1466			
0.9	31	50	68	99	161	322	1110		
0.95	22	34	43	56	79	121	226	703	
0.98	19	27	33	41	54	74	114	222	952

Table 1: Sample size per group
The total number of patients to be recruited to complete both analyses (SSG vs PM and SSG vs Combination) is 1003. As of 17 March 2008, there have been 710 patients recruited (440 from 0104B and 270 from 0104A). There are 293 patients still to be recruited to complete all analyses.

The current recruitment as of 17 mar 2008 and Amended maximum number of patients to be recruited into LEAP 0104B at each site will be:

Country	Patient recruited in 0104a & 0104b to 17 Mar 2008	Maximum number of patients recruited per site (maximum patients recruited in 0104B)
Sudan	440	up to 590 patients (500)
Kenya	120	up to 205 patients (175)
Ethiopia	150	up to 240 patients (90)
Uganda		up to 60 patients (60)
Total	710	1095 (825)

TREATMENT
Eligible patients for whom informed consent has been obtained will be randomized to either of the three treatment regimens using a computer generated randomization code provided.

Drug Administration:
SSG will be given IM or IV at a dosage of 20mg/kg/day* for 30 days.
PM will be given IM at a dosage of **20 mg/kg/day for 21 days.**
SSG + PM combination: SSG will be given IM or IV at a dosage of 20mg/kg/day* for 17 days and PM at a dosage of 15 mg/kg/day IM for 17days
***The maximum dosage of SSG per day for any patient is 850mg (8.5ml)**

Treatment will be given by the clinicians/nurse at the same time each day and a treatment sheet indicating time of dosing bearing the signature of the attending clinician/nurse will be kept.

Rescue medication
In the event of failure to respond to treatment, clinical deterioration or relapse at any time during the study, rescue treatment consisting of IV Ambisome® (a liposomal formulation of amphotericin B) at a dosage of 3 mg/kg/day for 5 days or according to local Ambisome® rescue protocol, the exact regimen used at each trial site to be documented in the case report form..

Prior and Concomitant Medications
No additional anti-leishmanial therapy will be permitted during the course of the study. If such therapy becomes necessary, the patient will be withdrawn from the study and considered a treatment failure.

Concomitant medication necessary for the health of the patient will be permitted during the course of the study. This will include the concomitant use of drugs such as paracetamol as an analgesic/antipyretic. Details of all concomitant medication taken during the study will be recorded in the CRF with indication, daily dose, route and dates of administration.

In the case of a patient presenting with co-infection, eg. pneumonia or malaria, these infections should be treated first. The patient should be re-assessed for suitability for inclusion in the trial after one week.

EFFICACY ASSESSMENT

Efficacy will be assessed using clinical, haematological, biochemical and parasitological responses.

- Clinical Assessment

The clinical evaluation will involve measuring the spleen size by palpation below the left coastal margin, temperature, blood pressure, body weight on days 0, 7, 14, 21 and end of treatment (18, 22, 31), 3 months and 6 months post treatment.
ECG and audiometry will be done at baseline, day 7, day 14, day 21, end of treatment 3 months and 6 months follow-up at the KEMRI study site.

Haematological and biochemical assessment

Blood will be analyzed for haemoglobin, WBC, platelets, urea, creatinine, and liver function tests on days 0, 7, 14, 21 and end of treatment (18, 22, 31), 3 months 6 months post-treatment.

- Uninalysis

Urinalysis will be performed on days 0, 7, 14, 21 and end of treatment (18,22,31) 3 months and 6 months post-treatment.

- Parasitological assessment

Parasitological assessment involves aspirating the spleen, lymph node or bone marrow at baseline, end of treatment (18, 22, 31 days depending on treatment arm) on, and at 3 months and 6 months follow up visits for all study patients. In addition, at selected sites aspirates will be cultured. Each patient will have a maximum of four aspirates. Patients who are clinically well with no signs or symptoms of VL and no palpable lymph nodes / spleen at the three months visit do not to have any aspirate at this visit. All patients will have an aspirate at 6 months post treatment.

The source of parasitological specimens should remain unchanged throughout the treatment and follow up periods, unless the spleen / lymph node initially chosen as the source of parasitological specimens becomes unpalpable, in which bone marrow aspirate should be performed.

Since bone marrow, lymph node and splenic aspirates are invasive procedures, these should only be performed at other times if clinically indicated.

PRIMARY EFFICACY ENDPOINT
The primary efficacy variable is parasitological clearance at 6 months post treatment by splenic, lymph node, or bone marrow smear.

SECONDARY EFFICACY ENDPOINT
The secondary efficacy endpoint will be parasitological clearance at the end of treatment (18, 22, 31 days depending on treatment arm – Test of cure (TOC)) and at 3 months post treatment.

SAFETY ASSESSMENTS
During treatment and at follow up, safety will be assessed by means of haematological, urinalysis and biochemical monitoring as above, and by ECG and audiometry at selected sites. In addition, patients will be asked at each visit if they have suffered any side-effects or other unexpected adverse events.

PHARMACOKINETIC ASSESSMENTS
All potentially eligible patients participating in the LEAP 0104 study in Kenya and Sudan will be asked to participate in this pharmacokinetic sub-study. A total of 72 adult patients, 36 in KEMRI and 36 from the Sudanese site in Khasab (12 in each treatment arm) with a body weight of 30kg or more will participate in the intensive pharmacokinetic sampling.

The dosing schedule will remain the same as that described in LEAP 0104 Protocol amendment dated 30th June 2006

The first twelve consenting patients in each treatment group, will have additional venous blood and urine sampling for pharmacokinetic evaluation as outlined in

Appendix 1

Inclusion and exclusion criteria are otherwise unchanged from those in the original protocol. (pages 18-19). An additional pharmacokinetic consent form will be used **Appendix 2.**

Patients who are HIV positive *will be excluded from participation in the Pharmacokinetic sub-study.*

Efficacy, Safety and rescue medication All Patients will undergo the same efficacy and safety evaluations and, if required, the same rescue therapy as in the original protocol LEAP 0104 of 31st July 2004 (page 26) and all relevant protocol amendments that have previously been approved by local ethics committees.

Plasma Sampling
Venous sampling for PK patients receiving all treatments (n=12 per treatment arm)

Day 1 and End of treatment (Day 21 for PM arm, Day 30 for SSG arm and Day 17 for SSG and PM combination arm):
At time 0, 0.5, 1.0, 3, 6, 10, and 24 hours after dosing

5 ml (SSG and PM alone) or 10 ml (SSG & PM Combination) of blood at each sampling point will be taken to ensure sufficient plasma is obtained for duplicate test analysis.
Total blood volume taken for PK on each sampling day is
Total blood volume for the Pharmacokinetic sampling during the treatment period will be 70ml for SSG and PM alone and 140 ml for SSG & PM Combination

Each sample will be collected in a heparinised tube. Samples will be centrifuged at 3000rpm for 10 minutes and plasma will be decanted off and stored in 3 aliquots at - 20°C.

All tubes will be labelled with the Protocol Number LEAP 0104, Centre and Patient number, date of sample and time point.

Urine sampling
All patients participating in the sub-study will also have 24 hour urine collections on PK venous sampling days, collected in aliquots 0-2h, 2-4h, 4-6h, 6-8h, 8-10 and 10-24h.

All tubes will be labelled with the Protocol Number LEAP 0104, Centre and Patient number, patient initials, date of sample and time point.

Safety assessments
Safety assessments (blood biochemistry, haematology, urinalysis, ECG and audiometry) will be performed as for other patients participating in the study.
Sample analysis
All samples will be shipped, with prior notice, to Prof Gilbert Kokwaro for analysis

Prof Gilbert Kokwaro,
KEMRI/Wellcome Trust Programme
Dept. of Clinical Pharmacology,
Next to National Public Health Laboratories,
On the Grounds of Kenyatta National Hospital,
PO Box 43640-00100, Nairobi, Kenya

ADVERSE EVENTS
An adverse event will be defined as any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory changes occurring in any phase of the clinical study, whether or not they are considered to be associated with the study drug. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction, or the significant worsening of the disease under investigation that is not recorded elsewhere in the CRF under specific efficacy

assessments. Anticipated day to day fluctuations of pre-existing conditions, including the disease under study, that does not represent a clinically significant exacerbation or worsening of the condition, will not to be considered adverse events.

All adverse events occurring after the start of the study (defined as when informed consent was obtained) are to be reported. This is regardless of whether or not they are considered to be drug related. Adverse event (AEs) elicited by the investigator asking the patient or the patient's parent or guardian a non-leading question such as "Do you/has your child felt different in any way since starting the new treatment/the last assessment?" If the response was "Yes", the nature of the event, the date and time (where appropriate) of onset, the duration, maximum intensity (see below) and relationship to treatment are to be established (see below). Details of any changes to the dosage schedule or any corrective treatment are to be recorded on the appropriate pages of the CRF.

Assessment of Intensity/Severity
The assessment of intensity/severity will be based on the investigator's clinical judgment. Maximum intensity/severity will be assigned to one of the following categories.

Mild: An adverse event, which is easily tolerated by the patient, causing minimal discomfort and not interfering with every day activities.

Moderate: An adverse event, which is sufficiently discomforting to interfere with normal everyday activities.

Severe: An adverse event, which prevents normal everyday activities.

Assessment of Causality
The investigator will use clinical judgment to determine the degree of certainty with which adverse event is attributed to drug treatment. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, etc are to be considered taking into account the known pharmacology of the drug, any previous reactions, literature reports and relationship to time of drug ingestion or recurrence on re challenge. Causality will be assessed using the following categories; not related, unlikely, suspected (reasonable possibility) or probable. Patients with adverse events will be followed-up until the event disappears or the condition stabilizes.

Serious Adverse Events
A serious adverse event will be defined as any event which is fatal, life threatening, disabling or incapacitating or results in hospitalization, prolonged hospital stay or is associated with congenital abnormality, cancer or overdose (either accidental or intentional). In addition, any experience which the investigator regards as serious or which suggests any significant hazard, contraindication, side effect or precaution that might be associated with the use of the drug will be reported as a serious event. Any serious adverse event occurring either during the study or within 30 days, or 5 half lives (whichever is longer), of receiving the last dose of study medication, is to be reported by telephone to the study monitor within 24 hours. This will be

followed by a full written summary containing relevant hospital case records and autopsy reports where applicable.

As treatment is by parenteral injection, over dosage is not anticipated. However, in the event of over dosage (error of dosage calculation or administration) will be communicated to the study coordinator, Dr. Monique Wasunna, within 24 hours or as soon as possible thereafter. Details of any signs or symptoms and their management will be recorded in the CRF including details of any antidote(s) administered. As there are no specific antidotes available for the medications to be used in this study, patients will receive all supportive care needed at discretion of the treating physician and after consultation with the study coordinator above.

DATA COLLECTION, STORAGE AND ANALYSIS

Data Management

In order to ensure data quality, a uniform hard copy i.e case report form (CRF) will be designed for use at all the sites. Data will then be sent to the coordinating sitefor data entry. It will be the responsibility of the investigator to ensure that the CRF is correctly completed to avoid unnecessary delays.

The software of choice will be EpiInfo 2003 which has an adequate electronic data capture (EDC) module especially for double entry. The data will be entered using pre-designed screens matching the data collection tool for ease of entry and validation. The entry program will also have in-built checks to minimize entry errors such as minimum and maximum, allowable values, legal values, jumps and values one must fill.

This exercise will be carried out by well-trained data entry personnel who will manage the data under the guidance of the biostatistician at the Centre for Clinical Research Centre of KEMRI.

ANALYSIS

- The trial will initially run with 3 arms: SSG, PM and Combination.
- The null hypothesis is that there is no difference across treatment arms / no difference between SSG and PM and no difference between SSG and Combination regimens
- A statistically significant p-value would provide evidence of a statistically significant difference between regimens.
- It is thought from LEAP0104 that the standard SSG regimen will clear parasites in approximately 85% of patients at 6 months
- It was considered that a case could be made for licensing PM (for use in combination therapy) if the difference in efficacy of SSG and PM was no more than 15%
- The trial will be powered to test for a statistically significant difference between SSG and PM if the efficacy of SSG is 85% and the efficacy of PM is 70% or less.
- This will require approximately 195 patients per arm for 90% power.
- Currently it is planned that Kenya will recruit up to 120 patients, Sudan 300 patients and Ethiopia 300 patients.

Revised Hypothesis
The Null Hypothesis is that the efficacy of PM alone (21 days) is not less than 15% the efficacy of SSG alone (30 days) and the efficacy of the combination of PM and SSG (17 days) is not less than 10% the efficacy of SSG alone.

1a) Interim Analysis PM: After recruitment into the trial of 100 patients per arm, an interim analysis will be carried out to compare the *end of treatment* efficacy (TOC) of SSG and PM.

- If the lower bound of the 95% CI of the difference in efficacy is *greater* than the maximum acceptable difference of 15% then there will be evidence to support decision to stop PM and question continuation of the trial. A decision will have to be made in such circumstances if it is worthwhile to continue with SSG and Combination arms only.
- If the lower bound of the 95% CI of the difference in efficacy is *less* than the maximum acceptable difference of 15% then continue recruitment to 195 patients per arm

1b) Efficacy Analysis PM: After recruitment of 195 patients per arm, stop recruiting into the PM arm and when all patients in that arm have reached 6 months follow-up compare the *6 months* efficacy (DC) of SSG and PM and test for a difference. If there is no evidence of significant difference, then conclude that there is no evidence to suggest that the efficacy of PM is more than 15% lower than the efficacy of SSG.

- Continue the trial with just 2 arms (SSG and Combination) and continue to recruit patients until there are 404 patients per arm

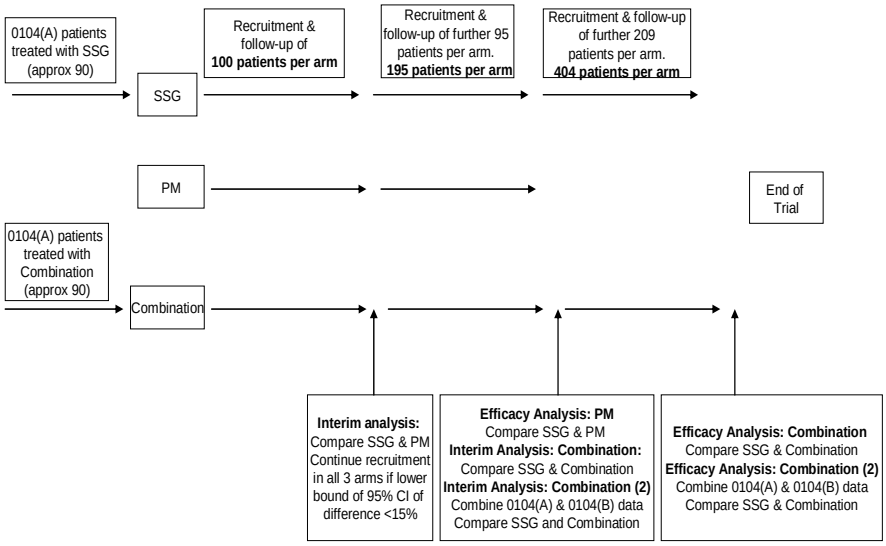
2a) Interim Analysis Combination: Compare the efficacy of SSG and Combination regimens *at 6 months* (DC) with 195 patients per arm followed-up for 6 months.

- Consider stopping the trial if the lower 95% confidence bound on the difference is greater than 10%.
- Combine the efficacy data from the SSG and Combination arms from 0104(A) and 0104(B) to include the historic data and repeat the interim analysis.
- Otherwise, continue until there are 404 patients per arm.

2b) Efficacy Analysis Combination: 404 patients per arm will provide 90% power to test for a statistically significant difference if the efficacy of SSG is 85% and the efficacy of the Combination regimen is 75% or less.

- Combine the efficacy data from the SSG and Combination arms from 0104(A) and 0104(B) to include the historic data and repeat the efficacy analysis.

Flow diagram of Recruitment and Analysis:
Following the end of the 1st phase 0104(A), patients will be randomised to receive one of 3 treatment regimens in the 2nd phase 0104(B)



Primary Endpoint and Efficacy Estimation

The definitive cure is measured at 6 months follow-up and the denominators for the efficacy analyses are the number of patients randomised to each treatment arm and entered 6 or more months previously. The numerators for the efficacy estimates are the number of patients who received no other VL treatment other than the randomised regimen and who were found to be

- Parasite free at end of treatment *or* parasite positive at end of treatment with at least 2 log reduction in parasite count (slow responder)

and

- parasite free at 6 months follow-up

Treatment Effect Estimation

The treatment effect will be the treatment difference plus the 95% confidence interval.

QUALITY CONTROL AND QUALITY ASSURANCE

All study sites and data generated during the study will be regularly monitored by GCP trained clinical monitors. Wherever possible, CRF data will be verified against hospital source data, for example patient notes or laboratory reports, etc.

ETHICAL CONSIDERATIONS

The study protocol together with patient information and consent forms will be submitted to the local scientific and ethics committee and any other regional or national regulatory authorities as required in the three countries, Kenya, Sudan and Ethiopia, before the study starts and any patient receives study medication.

The patients who participate in this study will be hospitalized and under close monitoring. The invasive diagnostic methods used in the study are those used in normal clinical practice when treating patients with VL. However, the frequency of testing might be increased depending on the patient's response to treatment.

Children will be included in this study because they represent more than 50% of VL cases in this region.

The effective treatment of VL benefits not only the individual patient but also the community by reducing the reservoir of infection for onward transmission by the sandfly vector. The evaluation of new and better treatments for VL, including shorter courses and combinations is anticipated to have a positive effect on development of parasite resistance and will reduce hospitalisation costs. If paromomycin is found to be efficacious and safe, it will be registered for the treatment of VL, providing a new alternative to treatments already available.

Patients will experience some pain while blood is drawn during venepuncture. The amount of blood to be drawn will be 10 mls before treatment and 7 mls at each subsequent visit, with a total of 42 mls (PM alone and a combination of PM and SSG), 49 mls (SSG alone) over the 6 months study period.

SSG has been extensively used in Sudan, Ethiopia and Kenya. Known adverse events include cardiac, muscle, joint and renal toxicity. PM has been used in clinical trials in Sudan and Kenya during the 1990's, and more recently in humanitarian emergency setting in Sudan. Known adverse events of the aminoglycosides include ototoxicity and renal toxicity.

Patients who are found to be HIV positive will be offered anti retroviral treatment at no cost in accordance with national guidelines for treatment.

A Data Safety and Monitoring Board (DSMB) will be set up to regularly review safety data.

Patients will be reimbursed for travel to and from the study site and will not receive any payment for trial participation. Any medication that is required during the trial period will be provided free of charge to the patient.

INSURANCE AND LIABILITY

DNDi is insured to indemnify the collaborating investigator for any injury or harm which occurs during the performance of the trial according to the protocol signed by the investigator. Furthermore, DNDi will in accordance with the declaration of Helsinki on Ethical principles for medical research involving human subjects, make all reasonable efforts to protect patients from any harm which may occur during the trial, and will wherever possible ensure that any patient that does suffer harm will receive the best possible treatment available in that country to alleviate their suffering.

TIME FRAME

The study is expected to start in October 2004 and will last until the expected sample size is attained.

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APPENDIX 1
PATIENT INFORMATION AND CONSENT

TITLE: A MULTICENTRE COMPARATIVE TRIAL OF EFFICACY AND SAFETY OF SODIUM STIBOGLUCONATE (SSG) VERSUS PAROMOMYCIN (PM) VERSUS COMBINATION OF SSG AND PM AS THE FIRST LINE TREATMENT FOR VISCERAL LEISHMANIASIS IN ETHIOPIA, KENYA AND SUDAN

PRINCIPAL INVESTIGATOR(S): ETHIOPIA Dr. Asrat Hailu
KENYA Dr. K.M. Wasunna
SUDAN Dr. Musa Amudawi
SUDAN Dr. Getahun Mengistu
MSFH Dr. Manica Balasegaram

SPONSOR: Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Introduction

We are studying kala-azar disease, which is common in our countries. The signs and symptoms you have suggest that you may be infected by a parasite called *Leishmania* that causes the illness kala-azar. We are studying new drug treatments for this disease, and would like you to participate in this trial to test a new drug called paromomycin. We wish to compare it with the usual treatment called SSG, and a combination of both drugs used together.

Paromomycin alone and the combination with SSG have been shown to be useful in small studies in some countries but we do not have sufficient evidence of safety and efficacy to enable us to get paromomycin registered in this country.

The study is expected to start in 2004 and will last until the expected sample size is attained. A total of 705 patients will participate.

We would like you to be included in this number, but your participation is voluntary.

Procedures during the trial

In this trial we shall test 2 drugs i.e. Sodium Stibogluconate (SSG) and Paramomycin (PM). There will be three arms to this trial i.e. SSG alone, SSG + PM and PM alone.

In the first trial we had, SSG alone was given at a dose of 20mg/kg/day for 30 days, PM was given at 15mg/kg/day for 21 days and SSG + PM for 17 days. In this combination, SSG was given at a dose of 20mg/kg/day and PM was given at a dose of 15mg/kg/day. After sometime it was found that PM at 15mg/kg for 21 days was not effective. A meeting was later held at which the dose of PM was increased to 20mg/kg/day for 21 days. No change was made with regard to other two arms of the trial.

Because we do not know which treatment is most effective, you will be allocated to one of the three treatment choices by a process called randomization, which means that the chances of you getting any of the three treatments is the same.
You will be admitted to the ward for the duration of the treatment. This is given as a daily injection into a vein or muscle for up to 30 days, depending on which treatment choice you are allocated.

Known side effects of these drugs include, pain at the injection site, skin rash, nausea and vomiting, diarrhea, feeling tired, ringing in the ears and rarely damage to the heart, kidneys or hearing. During the trial we will regularly assess your progress by means of blood tests, urine tests, heart tracings and hearing tests. A total of 10 mls of blood (two tea spoons) will be drawn at the beginning of the trial and 7 mls (one and a half tea spoons) at each subsequent weekly assessment during treatment and at follow up. We shall also need to repeat the test on your lymph nodes/spleen/bone marrow to make sure that the drugs are killing the kala- azar parasites. These tests are necessary but not without risk.

Occasionally splenic aspiration may result in internal bleeding. This is very unlikely but may occur as a complication in approximately 1 in 1,000 patients. The risks can be minimized in a number of ways. For instance, we will determine any bleeding problem you may have by a blood test. If this test indicates you are at risk of bleeding, we will use lymph node (LN) aspiration or bone marrow (BM) aspiration instead. If it is necessary to do a bone marrow test we will perform it under local anesthesia, to reduce the pain of this procedure. Special precautions will be taken in cases of children. The child must be calm and still during the procedure, so will be held gently

onto the bed by a nurse to avoid movement. Local anaesthetic and mild sedation will be given. In case bleeding occurs we will look after you until you are fully recovered.

On rare occasions there might be failure of treatment using the study drugs. In this case, you will receive treatment with another drug called liposomal amphotericin-B (AmBisome). This drug is known to be very effective and safe. It is not available in Ethiopia, Kenya and Sudan because it is very expensive, however, we shall make this drug available to you, at no cost, to make sure we can cure your disease.

We need to assess the long term effects of the drugs and therefore we shall need you to attend two follow up appointments at 3 months and 6 months after your treatment has finished. For school children, this will mean absence from school on those days.

Benefits

The main benefit of participation in the study is that you will be cured of the disease called kala azar. If the study is successful it means that an alternative shorter treatment will be available for this disease which will benefit your community and may reduce the likelihood of other people getting the disease.

Confidentiality

At the end of the study, we plan to write a report about the results of the study. The reports will not bear any information relating to you personally e.g. your name or identity. We assure you of the confidentiality of such information. Thus, we also need your permission to use the test results for writing a report.

In addition, clinical monitors of the sponsor (DNDi) or the regulatory authorities may wish to inspect your records.

Right to refuse or withdraw

You do not have to take part in this research, your participation is voluntary. If you do not wish to do so and this will not affect your treatment at this centre in any way. You will still have the benefit of treatment for your disease at this centre.

If you do decide to participate and then change your mind later, you may do so, at any time, without losing any of your rights as a patient.

It is also possible that we may decide to withdraw you from the study if we believe it is in your best interests, in which case you will continue to receive the usual treatment for kala-azar until you are better.

The sponsor (DNDi) may also decide to terminate the study. In this event we will continue to treat you until you are better.

In the event that you suffer an injury or illness related to participating in this trial, DND*i* will pay all costs relating to treatment of the injury or illness.

You will not receive any money for your participation in the study, however, we will pay your travel expenses to attend the hospital for treatment and hospital follow up visits at 3 and 6 months. In the rare event that you suffer complications due to study treatment, we will do everything possible to ensure you receive the necessary medical care and treatment for this complication.
If you agree to participate in the study, we will ask you to read and sign the consent form.

Do you have any questions?

Patient information for HIV testing
As we have explained to you, you have kala-azar infection and we are treating you with one of the three trial drug treatments. We are now asking you to be tested for another infection. It is a test for HIV infection. We have very important reasons to test you for HIV, which we would like you to understand.

If you are HIV positive you may not respond to treatment and we may need to give you additional treatment. In case you are HIV positive, it will be beneficial for you to know, both for your own well being, and also for your family, friends and other persons living with you.

We advise you to consider being tested. If you wish to be tested, a counselor will hold confidential discussions with you before and after the test. We will inform you of the test results. If you are HIV positive we will treat you for kala-azar first and then treat you for the HIV infection afterwards.
If you fulfill the national criteria for anti-retroviral therapy, we will provide you with anti-retroviral therapy for the duration of the project (18 months) or as required by national guidelines, at no cost to you.

If you do not wish to be tested for HIV, you will still benefit from the treatment for your kala-azar.

There is no obligation for you to accept the HIV test within this study, and if you refuse it, or do not wish to be informed of the results of your test, you will not be deprived of any other medical care that we offer you. You may wish to take time to think about being tested. If you change your mind later, and would like to be tested, we will do this for you at any time during this trial.

Consent Form:
I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with the recognition of my right to withdraw from the study if I change my mind.
I …….. do hereby give consent to Dr ……
to include me in the proposed research and the treatment. I have been given the necessary information and understand that there might be some risks involved in the treatment procedures. I have also been assured that I can withdraw my consent at any time without penalty or a loss of benefits. The proposal has been explained to me in the language I understand.
Name of patient: _____

Patient's Signature: _____

Name of Doctor: _____

Doctor's Signature:_____

Date: _____

Witness: _____Date:_____

CONSENT FOR MINORS (UNDER 18 YRS)

I Mr./Ms _____ being a person aged 18 years and over and being the Parent/Lawful guardian of master/miss_____

Hereby consent to Dr_____
to include Master/Miss _____ in the intended research as explained and understood by me.

I have understood the implications, risks and immediate benefits of the tests and treatment to Master/Miss _____.
I accept the tests and treatment to be carried out and the risks attached .

I understand that I have the right to withdraw
Master/Miss _____ from the research at any time, for any reason without penalty or harm. In case of withdrawal, I understand that the Physicians will continue to take care of Master/Miss _____ like any other patient.

All the above conditions have been explained to me in _____ language which I understand

_____ Guardian's full name

_____ Guardian's signature

Date:_____

_____ Child's full name

_____ Person obtaining consent

_____ Witness

Date:_____

Consent Form for HIV testing:

I, the undersigned, confirm that, as I give consent to HIV testing, it is with a clear understanding of the objectives of HIV testing in this study,
the availability of counseling services, the confidentiality of the test results
and in the case that I am positive for HIV, the possibility of receiving anti-retroviral therapy for the duration of the trial (18 months) should I fulfill the criteria set by the national guidelines
I …….. hereby give consent to Dr _____ to
perform this test.

I have been given the necessary information in a language that I understand.

Name of patient: _____

Patient's Signature: _____

Name of Doctor: _____

Doctor's Signature:_____

Date : _____

Witness: _____Date:_____

Contact persons (to be customized for each study site)

1. Name and address of study site investigator

2. Name and address of next of kin of study patient

3. Name and address of Ethics Committee Chair

Appendix 2
Consent form for Patients participating in PK sampling.

PATIENT INFORMATION AND CONSENT - Pharmacokinetic sub-study

TITLE: A MULTICENTRE COMPARATIVE TRIAL OF EFFICACY AND SAFETY OF SODIUM STIBOGLUCONATE (SSG) VERSUS PAROMOMYCIN (PM) VERSUS COMBINATION OF SSG AND PM AS THE FIRST LINE TREATMENT FOR VISCERAL LEISHMANIASIS IN ETHIOPIA, KENYA AND SUDAN

PRINCIPAL INVESTIGATOR(S): ETHIOPIA
KENYA
SUDAN

SPONSOR: Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Introduction

We are studying the disease, Kala-azar and new drug treatments for this disease. As well as the trial that you have agreed to take part in comparing paromomycin with, SSG, and a combination of both drugs used together, we are conducting a pharmacokinetic (PK) sub-study which looks at the way your body responds to the study drugs. We would like to ask you to participate in this research study.

Procedures during the sub-study

If you agree and you weigh more than 30Kg additional blood samples will be collected on the first day of treatment and again on the last day of treatment. A total of 7 samples will be taken at these times: before study drug, 0.5, 1.0, 3, 6, 10, and 24 hours after study drug.
Approximately 5 or 10 ml of blood (about 1or 2 teaspoons) will be taken at each sampling point. The volume will depend on the treatment you have been randomised too. The blood volume taken on one day will be approximately 35 or 70ml (about 3 or 5 tablespoons). A further 35 or 70ml of blood will be taken on the last day of treatment.

On the same day as the blood sampling, you will also have 24 hour urine collection. It is planned that this will be collected during the following times 0-2h, 2-4h, 4-6h, 6-8h, 8-10 and 10-24h.

At the site where blood was taken you may have some bruising and it may be painful for a short while.

Benefits

Your participation in this sub-study helps us to know more about your condition which could result in an improved treatment. There is no direct benefit to you but this new knowledge will benefit your community and may reduce the likelihood of other people getting the disease.

Confidentiality

At the end of the study, we plan to write a report about the results of the study. The reports will not bear any information relating to you personally e.g. your name or identity. We assure you of the confidentiality of such information. Thus, we also need your permission to use the test results for writing a report.

We also seek your permission to store any left over samples (blood, tissues) for future studies. We will seek permission from a relevant ethical review committee before any analysis not described in this document is carried out.

In addition, clinical monitors of the sponsor (DNDi) or the regulatory authorities may wish to inspect your records.

Right to refuse or withdraw

You do not have to take part in this research, if you do not wish to do so. This will not affect your treatment at this centre in any way. You will still have the benefit of treatment for your disease at this centre.

If you do decide to participate and then change your mind later, you may do so, at any time, without losing any of your rights as a patient.

In the event that you suffer an injury or illness related to participating in this trial, DNDi will pay all costs relating to treatment of the injury or illness.

If you agree to participate in the study, we will ask you to read and sign the consent form.

Do you have any questions?

Consent Form:

I, the undersigned, confirm that, I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with the recognition of my right to withdraw from the study if I change my mind.

I …….. do hereby give consent to Dr ……
to include me in the proposed research. I have been given the necessary information and understand that there might be some risks involved in the treatment procedures. I have also been assured that I can withdraw my consent at any time without penalty or a loss of benefits. The proposal has been explained to me in the language I understand.

Name of patient: _____

Patient's Signature: _____

Name of Doctor: _____

Doctor's Signature: _____

Date: _____

Witness: _____Date:_____

Appendix 3:

PROTOCOL AMENDMENT 8
Protocol Title: A multicentre, randomised, comparative trial of efficacy and safety of sodium stibogluconate (SSG) versus paromomycin (PM) versus a combination of SSG and PM as first line treatment for visceral leishmaniasis (VL) in Ethiopia, Kenya and Sudan.

This protocol amendment outlines a revision to the sample size and analysis plan for the study including the addition of interim analyses

Reason for Amendment
The following replaces the statistical section of the LEAP 0104 Protocol dated 31st Jul 2004 (Page 21 and 22)

Design:

- The trial will initially run with 3 arms: SSG, PM and Combination.
- The null hypothesis is that there is no difference across treatment arms / no difference between SSG and PM and no difference between SSG and Combination regimens
- A statistically significant p-value would provide evidence of a statistically significant difference between regimens.
- It is thought from LEAP0104 that the standard SSG regimen will clear parasites in approximately 85% of patients at 6 months
- It was considered that a case could be made for licensing PM (for use in combination therapy) if the difference in efficacy of SSG and PM was no more than 15%
- The trial will be powered to test for a statistically significant difference between SSG and PM if the efficacy of SSG is 85% and the efficacy of PM is 70% or less.
- This will require approximately 195 patients per arm for 90% power.

Revised Hypothesis
The Null Hypothesis is that the efficacy of PM alone (21 days) is no than 15%less than the efficacy of SSG alone (30 days) and the efficacy of the combination of PM and SSG (17 days) is no than 10% less than the efficacy of SSG alone.

1a) Interim Analysis PM: After recruitment into the trial of 100 patients per arm, an interim analysis will be carried out to compare the *end of treatment* efficacy (TOC) of SSG and PM.

- If the lower bound of the 95% CI of the difference in efficacy is *greater* than the maximum acceptable difference of 15% then there will be evidence to support decision to stop PM and question continuation of the trial. A decision will have to be made in such circumstances if it is worthwhile to continue with SSG and Combination arms only.
- If the lower bound of the 95% CI of the difference in efficacy is *less* than the maximum acceptable difference of 15% then continue recruitment to 195 patients per arm

1b) Efficacy Analysis PM: After recruitment of 195 patients per arm, stop recruiting into the PM arm and when all patients in that arm have reached 6 months follow-up compare the *6 months* efficacy (DC) of SSG and PM and test for a difference. If there is no evidence of significant difference, then conclude that there is no evidence to suggest that the efficacy of PM is more than 15% lower than the efficacy of SSG.

- Continue the trial with just 2 arms (SSG and Combination) and continue to recruit patients until there are 404 patients per arm

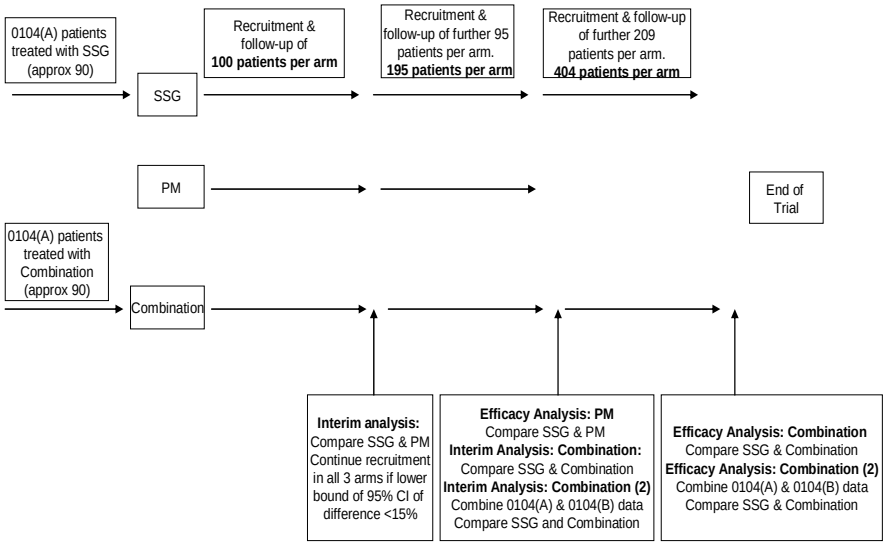
2a) Interim Analysis Combination: Compare the efficacy of SSG and Combination regimens *at 6 months* (DC) with 195 patients per arm followed-up for 6 months.

- Consider stopping the trial if the lower 95% confidence bound on the difference is greater than 10%.
- Combine the efficacy data from the SSG and Combination arms from 0104(A) and 0104(B) to include the historic data and repeat the interim analysis.
- Otherwise, continue until there are 404 patients per arm.

2b) Efficacy Analysis Combination: 404 patients per arm will provide 90% power to test for a statistically significant difference if the efficacy of SSG is 85% and the efficacy of the Combination regimen is 75% or less.

- Combine the efficacy data from the SSG and Combination arms from 0104(A) and 0104(B) to include the historic data and repeat the efficacy analysis.

Flow diagram of Recruitment and Analysis:
Following the end of the 1st phase 0104(A), patients will be randomized to receive one of 3 treatment regimens in the 2nd phase 0104(B)



Primary Endpoint and Efficacy Estimation

The definitive cure is measured at 6 months follow-up and the denominators for the efficacy analyses are the number of patients randomised to each treatment arm and entered 6 or more months previously. The numerators for the efficacy estimates are the number of patients who received no other VL treatment other than the randomised regimen and who were found to be

- Parasite free at end of treatment *or* parasite positive at end of treatment with at least 2 log reduction in parasite count (slow responder)

and

- parasite free at 6 months follow-up

Treatment Effect Estimation

The treatment effect will be the treatment difference plus the 95% confidence interval.

Assumptions

- The efficacy data will be analysed overall, not by site
- The trial is not powered for by site analyses

- Interim and final analyses will be carried out only as specified
- Adjustments to the sample size have been made to power the study for a sub-group analysis in HIV negative patients if 10% of patients overall are known to be infected and also to allow for loss-to-follow-up (LTFU) of 10% at 6 months.
- Table 1 gives the numbers needed per arm after adjusting for HIV (10%) and LTFU at 6 months (10%) based on two-sided probabilities with 90% power.

Table 1. Number of patients required per regimen to detect a statistically significant difference, at the 5% level with 90% power, between the efficacies shown based on a two-sided test for ITT. Estimates are adjusted for 10% to allow for an adequate number of HIV negative patients in sub-group analyses and also for 10% loss-to-follow-up at 6 months.

	% cured in comparative regimen								
% cured SSG	0.5	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95
0.6	627								
0.65	274	2381							
0.7	150	576	2229						
0.75	93	245	531	2025					
0.8	62	131	223	474	1771				
0.85	43	79	117	195	404	1466			
0.9	31	50	68	99	161	322	1110		
0.95	22	34	43	56	79	121	226	703	
0.98	19	27	33	41	54	74	114	222	952

	SIGNATURE	DATE
Dr Monique Wasunna Trial Medical Coordinator	……..	…….
Mr. Lawrence Muthami Trial Statistician	……..	…….

Appendix 4
Protocol Amendment No. 9

Protocol Title: A multicentre, randomised, comparative trial of efficacy and safety of sodium stibogluconate (SSG) versus paromomycin (PM) versus a combination of SSG and PM as first line treatment for visceral leishmaniasis (VL) in Ethiopia, Kenya and Sudan.

This protocol amendment outlines the Further pharmacokinetic (PK) evaluation of Paromomycin (PM), Sodium Stibogluconate (SSG), and the combination of PM and SSG in consenting patients of LEAP 0104 trial following preliminary analysis of initial Pharmacokinetic data presented to a meeting of the Principal and Site Investigators held in Nairobi, Kenya on 22nd Sep 2006.

This amendment applies to sites in Kenya and Sudan only

Addition of Intensive Pharmacokinetic evaluation in Kenya and Sudan

Reason for amendment
Initial results from 2 trial sites in Sudan (n = 90 Um el Kher, and n = 45 Kassab) in the LEAP 0104 trial indicated that paromomycin at a dosage of 15mg/kg/day for 21 days was less effective, for the treatment of acute, symptomatic visceral leishmaniasis.

The majority of cases of VL in the disease endemic area of the Horn of Africa occur in Sudan, therefore it was considered essential to find a dose which is effective in Sudan if paromomycin is to be a useful alternative therapy for VL in this region. Therefore, between September 2005 and March 2006, a total of 42 patients (21 in each treatment arm) were allocated to receive one of two Paromomycin treatment regimens. Results are shown in Table 1.

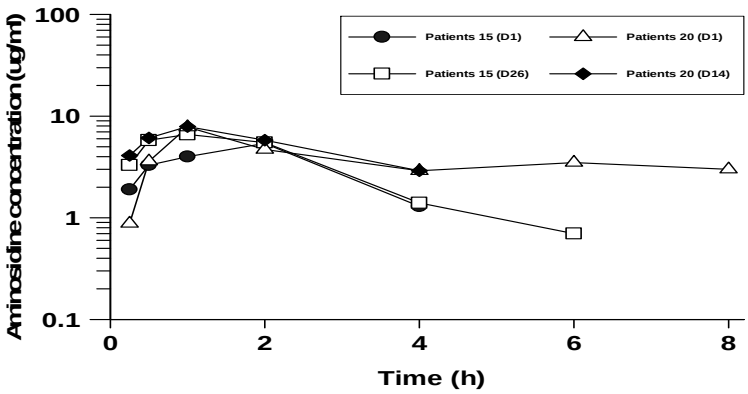
Table 1:

Regimen	Test of Cure (End of treatment)			3months	Definitive cure (6 month follow-up)
	Number of patients	Parasite -ve	Parasite +ve	Relapse	
15mg for 28 days	21	19	2	3	16/21 (76.2%)
20mg for 21 days	21	18	3	2	16/21 (76.2%)
Total	42	37	5	5	

There was no difference in efficacy either at end of treatment or at 3 and 6 month follow up, with an overall efficacy rate of 76% for both groups.

It was planned that the first six patients in each treatment group, with a body weight of 30kg or more, would have additional venous blood and urine sampling for pharmacokinetic evaluation. A total of 10 patients consented to participate in the PK sampling, 6 in the 15mg/kg/day arm and 4 in the 20mg/kg/day arm.

Figure 1 Mean aminosidine concentrations



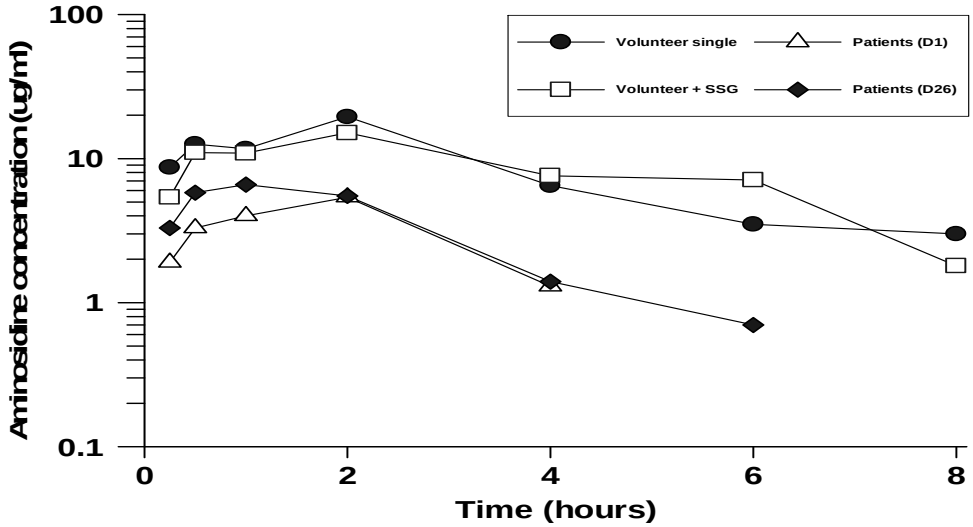
Footnote: Aminosidine is the name by which paromomycin was formerly known

The time to peak plasma concentration (t_{max}) was similar for both doses (1.5-2h). Fig 1 indicates that somewhat better concentrations were achieved with the 20mg dose, however patient numbers are small. Blood levels were undetectable after the 8 hour sampling point. Excretion in urine was rapid and complete at 24h.

Only limited published PK data for paromomycin in healthy volunteers are available, Kanyok et al 1997. These data indicate that American healthy volunteers achieved a C_{max} of 23.4ug/ml.

A small pharmacokinetic study (unpublished: Fig 2) was carried out in healthy Sudanese volunteers which demonstrated that they achieved similar maximum concentration to that of the published data and higher than that of the Sudanese VL patients.

Figure 2



Initial data from Indian VL patients treated with PM 15mg/kg/day demonstrated results (Mordenti et al 2006) similar to those previously published, Kanok et al.

There is also very limited published data on the pharmacokinetics of the combination of SSG and PM. P Ormas et al 1995 investigated the pharmacokinetics of Paromomycin and SSG alone and in combination in dogs.

Based on the evidence above the following protocol amendment is proposed to further elucidate the pharmacokinetics of PM, SSG and Combination of SSG and PM in a greater number of Kenyan, and Sudanese patients.

Amendment
All potentially eligible patients participating in the LEAP 0104 study in Kenya and Sudan will be asked to participate in this pharmacokinetic sub-study. A total of 72 adult patients, 36 in KEMRI and 36 from the Sudanese site in Khasab (12 in each treatment arm) with a body weight of 30kg or more will participate in the intensive pharmacokinetic sampling.

The dosing schedule will remain the same as that described in LEAP 0104 Protocol amendment dated 30th June 2006

The first twelve consenting patients in each treatment group, will have additional venous blood and urine sampling for pharmacokinetic evaluation as outlined in Appendix 1.

Inclusion and exclusion criteria are otherwise unchanged from those in the original protocol. (pages 18-19). An additional pharmacokinetic consent form will be used Appendix 2.

Patients who are HIV positive will be excluded from participation in the Pharmacokinetic sub-study.

Efficacy, Safety and rescue medication All Patients will undergo the same efficacy and safety evaluations and, if required, the same rescue therapy as in the original protocol LEAP 0104 of 31st July 2004 (page 26) and all relevant protocol amendments that have previously been approved by local ethics committees.

References

1. Kanyok, T.P., et al., *Pharmacokinetics of intramuscularly administered aminosidine in healthy subjects.* Antimicrob Agents Chemother, 1997. 41(5): p. 982-6.
2. Mordenti, J., et al, *Paromomycin: An old drug gives birth to a new treatment for Visceral Leishmaniasis* ICOPA 2006

	SIGNATURE	DATE
Dr Monique Wasunna Trial Medical Coordinator	…….	…….
<u>Principal Investigators</u>		
Dr. Musa A Mudawi	…….	…….
KENYA PI	…….	…….
SUDAN PI	…….	…….

***Appendix 1*: Pharmacokinetic sampling schedule for patients with a body weight of 30kg or more who consent to participate in the PK part of the study.**

Plasma Sampling
Venous sampling for PK patients receiving all treatments (n=12 per treatment arm)

Day 1 and End of treatment (Day 21 for PM arm, Day 30 for SSG arm and Day 17 for SSG and PM combination arm):
At time 0, 0.5, 1.0, 3, 6, 10, and 24 hours after dosing

5 ml (SSG and PM alone) or 10 ml (SSG & PM Combination) of blood at each sampling point will be taken to ensure sufficient plasma is obtained for duplicate test analysis.
Total blood volume taken for PK on each sampling day is
Total blood volume for the Pharmacokinetic sampling during the treatment period will be 70ml for SSG and PM alone and 140 ml for SSG & PM Combination

Each sample will be collected in a heparinised tube. Samples will be centrifuged at 3000rpm for 10 minutes and plasma will be decanted off and stored in 3 aliquots at - 20°C.

All tubes will be labelled with the Protocol Number LEAP 0104, Centre and Patient number, date of sample and time point.

Urine sampling
All patients participating in the sub-study will also have 24 hour urine collections on PK venous sampling days, collected in aliquots 0-2h, 2-4h, 4-6h, 6-8h, 8-10 and 10-24h.

All tubes will be labelled with the Protocol Number LEAP 0104, Centre and Patient number, patient initials, date of sample and time point.

Safety assessments
Safety assessments (blood biochemistry, haematology, urinalysis, ECG and audiometry) will be performed as for other patients participating in the study.

Sample analysis
All samples will be shipped, with prior notice, to Prof Gilbert Kokwaro for analysis

Prof Gilbert Kokwaro,
KEMRI/Wellcome Trust Programme
Dept. of Clinical Pharmacology,
Next to National Public Health Laboratories,
On the Grounds of Kenyatta National Hospital,
PO Box 43640-00100, Nairobi, Kenya

***Appendix 2*: Consent form for Patients participating in PK sampling.**

PATIENT INFORMATION AND CONSENT - Pharmacokinetic sub study

TITLE: A MULTICENTRE COMPARATIVE TRIAL OF EFFICACY AND SAFETY OF SODIUM STIBOGLUCONATE (SSG) VERSUS PAROMOMYCIN (PM) VERSUS COMBINATION OF SSG AND PM AS THE FIRST LINE TREATMENT FOR VISCERAL LEISHMANIASIS IN ETHIOPIA, KENYA AND SUDAN

PRINCIPAL INVESTIGATOR(S): ETHIOPIA
KENYA
SUDAN

SPONSOR: Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Introduction

We are studying the disease, Kala-azar and new drug treatments for this disease. As well as the trial that you have agreed to take part in comparing paromomycin with, SSG, and a combination of both drugs used together, we are conducting a pharmacokinetic (PK) sub-study which looks at the way your body responds to the study drugs. We would like to ask you to participate in this research study.

Procedures during the sub-study

If you agree and you weigh more than 30Kg additional blood samples will be collected on the first day of treatment and again on the last day of treatment. A total of 7 samples will be taken at these times: before study drug, 0.5, 1.0, 3, 6, 10, and 24 hours after study drug.

Approximately 5 or 10 ml of blood (about 1or 2 teaspoons) will be taken at each sampling point. The volume will depend on the treatment you have been randomised too. The blood volume taken on one day will be approximately 35 or 70ml (about 3 or 5 tablespoons). A further 35 or 70ml of blood will be taken on the last day of treatment.

On the same day as the blood sampling, you will also have 24 hour urine collection. It is planned that this will be collected during the following times 0-2h, 2-4h, 4-6h, 6-8h, 8-10 and 10-24h.

At the site where blood was taken you may have some bruising and it may be painful for a short while.

Benefits

Your participation in this sub-study helps us to know more about your condition which could result in an improved treatment. There is no direct benefit to you but this new knowledge will benefit your community and may reduce the likelihood of other people getting the disease.

Confidentiality

At the end of the study, we plan to write a report about the results of the study. The reports will not bear any information relating to you personally e.g. your name or identity. We assure you of the confidentiality of such information. Thus, we also need your permission to use the test results for writing a report.

We also seek your permission to store any left over samples (blood, tissues) for future studies. We will seek permission from a relevant ethical review committee before any analysis not described in this document is carried out.

In addition, clinical monitors of the sponsor (DNDi) or the regulatory authorities may wish to inspect your records.

Right to refuse or withdraw

You do not have to take part in this research, if you do not wish to do so. This will not affect your treatment at this centre in any way. You will still have the benefit of treatment for your disease at this centre.

If you do decide to participate and then change your mind later, you may do so, at any time, without losing any of your rights as a patient.

In the event that you suffer an injury or illness related to participating in this trial, DNDi will pay all costs relating to treatment of the injury or illness.

If you agree to participate in the study, we will ask you to read and sign the consent form.
Do you have any questions?

Consent Form:

I, the undersigned, confirm that, I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with the recognition of my right to withdraw from the study if I change my mind.

I …….. do hereby give consent to Dr ……
to include me in the proposed research. I have been given the necessary information and understand that there might be some risks involved in the treatment procedures. I have also been assured that I can withdraw my consent at any time without penalty or a loss of benefits. The proposal has been explained to me in the language I understand.

Name of patient: _____

Patient's Signature: _____

Name of Doctor: _____

Doctor's Signature: _____

Date: _____

Witness: _____Date:_____

Appendix 5

LEAP 0104 Final Protocol amendment Number 11: dated 20th March 2008

Protocol Title: A multicentre, randomised, comparative trial of efficacy and safety of sodium stibogluconate (SSG) versus paromomycin (PM) versus a combination of SSG and PM as first line treatment for visceral leishmaniasis (VL) in Ethiopia, Kenya, Sudan and Uganda.

This protocol amendment outlines a revision to the recruitment strategy of the protocol. The overall sample size remains unchanged and is as described in Protocol amendment number 8 dated 14 Dec 2006. The change will be to the number of patients that are recruited by each site.

Reason for Amendment
Due to the addition of a new site in Uganda and the length of the ethical review process in Ethiopia and in order to minimize the time taken to recruit the remaining patients into the clinical trial the recruitment strategy for the study will be revised. As of 17 March 2008 440 patients have been recruited under the LEAP 0104B protocol amendment

Details of the Protocol Amendment
The recruitment strategy will be revised and recruitment will continue in all countries, with the required ethical and regulatory approvals until the required sample size is achieved. The total sample size remains and is as described in Protocol amendment number 8 dated 14 Dec 2006.

SSG vs PM

- 195 Patients per arm for SSG (20mg/kg/day for 30 days) vs Paromomycin (20mg/kg/day for 21 days)
- Total patients to be recruited for SSG vs PM Analysis = 390 patients (195 per treatment arm)

SSG vs Combination

- 404 Patients for SSG arm vs 404 patients for Combination of SSG and Paromomycin arm
- Total patients to be recruited for SSG vs Combination of SSG + PM Analysis = 808 patients
- 808 patients comprise the following:
    - 538 out of 808 (269 per arm) are to be recruited in the current LEAP 0104B amendment no. 8 dated 14 December 2006.
    - 270 patients so far out of 808 (135 per arm (SSG and Combination only) have been recruited under the original protocol dated 31 July 2004)

The total number of patients to be recruited to complete both analyses (SSG vs PM and SSG vs Combination) is 1003. As of 17 March 2008, there have been 710 patients recruited (440 from 0104B and 270 from 0104A). There are 293 patients still to be recruited to complete all analyses.

The current recruitment as of 17 mar 2008 and Amended maximum number of patients to be recruited into LEAP 0104B at each site will be:

Country	Patient recruited in 0104a & 0104b to 17 Mar 2008	Maximum number of patients recruited per site (maximum patients recruited in 0104B)
Sudan	440	up to 590 patients (500)
Kenya	120	up to 205 patients (175)
Ethiopia	150	up to 240 patients (90)
Uganda		up to 60 patients (60)
Total	710	1095 (825)

	SIGNATURE	DATE
Dr Monique Wasunna Trial Medical Coordinator	……..	…….
Dr Musa A. Mudawi Principal Investigator	……..	…….