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Post-kala-azar Dermal Leishmaniasis (PKDL): a prospective observational study of the effectiveness and safety of an ambulatory short course treatment with AmBisome* 15 mg/kg total dose

***AmBisome is a liposomal formulation of 50 mg amphotericin B deoxycholate per vial, and produced by Gilead Sciences, USA**

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General objective
To evaluate the effectiveness and safety of PKDL treatment with AmBisome 15 mg/kg total dose, given over 15 days in 5 infusions (twice weekly) of 3mg/kg on an ambulatory basis in a primary health care setting.

Primary objective: to evaluate the effectiveness of AmBisome 15 mg/kg total dose at 12 M

Secondary objective:
- Evaluate the safety of AmBisome 3mg/kg x 5 infusions (twice weekly) (15 mg/kg total dose)
- Evaluate the occurrence of hypokalaemia
- Evaluate at which point in time lesions start to respond to treatment.

Patient population

Inclusion criteria
PKDL patients diagnosed clinically and supported by a positive rK39 rapid test, admitted and treated in the MSF clinic in Fulbaria.

Exclusion criteria
- Patients with PKDL and concurrent VL
- Patients who had prior treatment for PKDL

- Patients < 12 years
- Patients with known cardiac disease, hepatic impairment, or other chronic underlying disease
- Renal impairment as defined by a baseline serum creatinine > 1.3 mg/dL
- Patients with a known hypersensitivity to AmBisome
- Patients on long-term medication with a side effect profile overlapping with that of AmBisome
- Current or recent (< 1 month) pregnancy and lactating women
- Patients with serum potassium <3.5mmol/L
- Diarrhoea, vomiting, or other conditions resulting in significant body potassium losses

Trial end point
Primary endpoint is the healing of lesions, clinically assessed at 12 months. Patients will return for follow up visits at 1, 3, 6, and 12 months after treatment. Assessment of safety (the occurrence of AEs) takes place during treatment and until 1 month after treatment and is based on clinical adverse events, laboratory parameters during treatment and clinical assessment at 1 month after end of initial treatment. Assessment of hypokalaemia will be based on serum potassium, measured in blood samples taken at different time points during and after treatment.

1 INTRODUCTION AND BACKGROUND

Introduction: PKDL in Bangladesh
Post-kala-azar dermal leishmaniasis (PKDL) is a chronic skin condition that appears after cure of visceral leishmaniasis (VL). In Sudan, PKDL occurs in up to 60% of VL patients, usually within 6 months after VL onset; the majority of cases are mild and resolve without antileishmanial treatment in a year. In South Asia, PKDL is reported to occur in 5-15% of VL patients after 2-3 years, and it is generally assumed that treatment is required for PKDL resolution.

Mymensingh province in Bangladesh is a major focus of VL, and within Mymensingh province, Trishal and Fulbaria are the most affected upazilas. A recent field study by ICDDR'B (International Centre for Diarrhoeal Disease Research, Bangladesh) and CDC (Center Disease Control, Atlanta, US) in Fulbaria has shown that over the past few years the incidence of PKDL had increased to 10% in patients treated for VL [1].

PKDL in Bangladesh presents in over 95% of cases as multiple macular lesions characterised by mild to moderate hypopigmentation, usually starting in the face, and extending to extremities and trunk. Maculo-papular and nodular lesions are relatively rare. The lesions do not cause physical inconvenience, and patients generally feel well. Long delays in diagnosis and treatment are common.

It is widely accepted that PKDL patients harbour *Leishmania* parasites in the skin and, therefore, act as reservoirs of infection in VL transmission, especially during interepidemic periods. Untreated PKDL patients appear to remain infectious for years to decades. In anthroponotic VL, PKDL patients may constitute the sole reservoir of infection and therefore, from a disease control point of view, treatment is important, and plays a key role in eliminating the parasite reservoir. For this reason, active PKDL case detection and treatment is an important strategy of the program for the elimination of VL from the Indian subcontinent. However this strategy is currently not feasible in Bangladesh.

The standard PKDL treatment in Bangladesh consisted until recently of 120 injections of sodium stibogluconate; 6 courses of 20 days over 6 months. This regimen was very demanding and traumatic, and impractical, as patients would not finish such long hospitalizations. Moreover, the effectiveness of this SSG regimen has never been properly evaluated. A new National Protocol has just been established, which, in lack of better options, prescribes as first line treatment for PKDL a regimen of 12 weeks of daily miltefosine. This regimen is however problematic to roll out in a primary health care setting as compliance of such a long regimen is likely to be poor, especially seeing the high incidence of gastro-intestinal side effects of miltefosine, and it should therefore preferably be administered under direct supervision. Because of its teratogenicity, it cannot be used in women of child bearing age, and patients will need close monitoring for adverse events, as the safety of courses of miltefosine longer than 4 weeks has not been evaluated. In addition, treatment with 12 weeks of miltefosine is expensive (around 150 euro per adult treatment).
The cost of the short-course (15 mg/kg) AmBisome treatment will amount to USD 198 (144 EURO) per patient, based on the WHO negotiated preferential price for developing countries (18 USD per vial), which is comparable with the cost of Miltefosine treatment. However, since Bangladesh is a target country for the WHO/Gilead AmBisome donation programme, Bangladesh will have access to free AmBisome donations for at least five years (2012-2017), which will have a major impact on the cost effectiveness of AmBisome treatment for VL and PKDL.

Background

MSF's VL project in Fulbaria, Mymensingh

Médecins Sans Frontières-Holland (MSF) is a medical humanitarian agency that has been active in Bangladesh for the past 17 years. A project to address the high case load of VL and PKDL in Fulbaria was started in June 2010. A Kala Azar Treatment Centre clinic was built by MSF, neighbouring the Fulbaria Upazila Health Complex specifically for the treatment of VL and PKDL. Active case finding in villages combined with health education is performed by teams of outreach workers, and all VL and PKDL cases reporting to the clinic are treated. For each visit to the clinic, patients received full reimbursement of transport costs incurred. As one of the aims of the project was to contribute to the Elimination Program by demonstrating the feasibility and effectiveness of a short, safe and ambulatory treatment of PKDL, MSF decided to evaluate a new treatment regimen for PKDL, based on AmBisome. This choice was not evidence based, as no successful treatments for PKDL have been identified in clinical trials. Instead, the choice was made on compassionate grounds, considering the fact that PKDL is a non-fatal and non-debilitating disease, and that the treatment available at the time (6 months of SSG injections) was very demanding and traumatic and non-feasible for large scale implementation. Limited published data from small patient cohorts have demonstrated the efficacy of AmBisome in the treatment of PKDL [2,3]. AmBisome has proven to be a safe treatment and parasites were unlikely to become resistant due to its unspecific mechanism of action. Because of its very long half-life AmBisome can be given twice a week on an ambulatory basis, and thus has minimal impact on patient's lives and income-generating activities.
In agreement with the Ministry of Health, AmBisome with a total dose of 30 mg/kg, divided over 3 weeks, with 2 infusions of 5 mg/kg weekly, was used as first line treatment for PKDL and patients were actively followed up for a period of 12 months. An objective method for treatment response evaluation was introduced, including regular severity scoring of lesions and medical photographs at baseline and during 12 months follow-up.

Outcome PKDL treatment with AmBisome 30 mg/kg total dose

Effectiveness

Since the start of the project in June 2010 up to December 2012, over 1400 PKDL patients have been treated with AmBisome. Most patients (96%) presented with hypopigmented macular lesions. Loss to follow up was minimal and so far, 786 patients returned for a 12 month follow up visit. In 86.5% of these patients, a complete recovery of nodular and papular lesions, and a complete or very significant repigmentation of macular lesions was observed. Immediately after the 6 doses of AmBisome there was no significant clinical improvement of lesions, but significant reduction or even clearance of the lesions was observed in most patients after 1 to 3 months. 13.5% of cases with macular lesions failed to show significant repigmentation within 12 months. In most of these patients, further improvement of lesions was observed after 12 months. Only 17 patients required retreatment with a second course of AmBisome. Of these, 80% responded to treatment within 6 months. These observations confirm that repigmentation of macular lesions can be a very slow process. After parasite clearance, complete melanocyte regeneration can take months or years, and in some cases remains incomplete.

Safety

In January 2012 PKDL treatment was put on hold when patients that had received AmBisome treatment were identified with neuromuscular complaints suggestive of rhabdomyolysis. Laboratory follow up confirmed rhabdomyolysis post AmBisome in 6 patients treated for PKDL (serum-creatinine phosphokinase >5 times upper normal limit for sex and age). Patients still under treatment were closely monitored for the occurrence of hypokalaemia. Retrospective pharmacovigilance analysis of all patient data demonstrated that in another 19 PKDL cases documented neuromuscular complaints could probably or possibly be attributed to AmBisome treatment. Discourse was held with the producer of AmBisome (Gilead) and various other scientific advisors and a scientific manuscript is under preparation.

Treatment of PKDL patients was re-started in July 2012, with the use of a strict potassium monitoring and supplementation protocol for all baseline and treatment potassiums between 3.6-4.2. In September 2012 treatment was permanently stopped after it became clear that of a cohort of 110 PKDL patients treated between July and September, (6.5 % developed severe (<2.5mmol/L K^+) and 15.7% developed moderate (2.5-3.0 mmol/L K^+) hypokalaemia at one point during treatment. A high prevalence of side effects related to hypokalaemia (nausea, vomiting, weakness, muscle pain and anorexia) was observed.

Discussion

Hypokalaemia is a well-known side effect of AmBisome. However, severe (<2.5mmol/L K^+) hypokalaemia has not been reported during the use of AmBisome in varying doses for the treatment of VL [4]. The high proportion of severe hypokalaemia during or after treatment with a total dose of 30 mg/kg AmBisome, even while receiving high potassium food, was unexpected. Even more surprising was rhabdomyolysis as a side effect of this regimen. Rhabdomyolysis was not a known side effect of AmBisome until it was occasionally reported in post marketing surveillance, and remained very rare: in 21 years of experience only 8-9 cases have been reported from Japan (incidence 1:100,000). These cases all occurred in cancer patients with severe neutropenia that were receiving high dose regimen of AmBisome (verbal communication, Gilead). US FDA reports revealed 27 further cases, mostly in seriously ill patients that received very high doses of AmBisome. Gilead only included rhabdomyolysis as an infrequent adverse effect in the AmBisome package insert in 2012.

It remains unknown if predisposing factors existed that could have caused the high prevalence of rhabdomyolysis in the PKDL patient population in Fulbaria. Although the effectiveness of AmBisome 30 mg/kg in PKDL in Bangladesh has been demonstrated, the safety data obtained in our patient cohort are of concern. This regimen should not be given without strict potassium monitoring and supplementation, and therefore, this regimen is unsuitable for further roll out in Bangladesh.

WHO recently convened an expert meeting on the treatment of PKDL (July 2012) [5]. In the resulting new WHO PKDL guidelines, apart from the 30 mg/kg AmBisome regimen, only two other PKDL treatments options are mentioned. The first is miltefosine for 12 weeks (daily), the other is intermittent amphotericin B deoxycholate (20 days on, 20 days off) for up to 4 months. Both were proven effective in small patient cohorts. Both these regimen are however not suitable for roll out in a primary health care setting. Amphotericin B can only be administered in a hospital setting where clinical laboratory values can be monitored. Compliance to miltefosine is likely to be poor due to the long regimen. In addition, the high incidence of gastro-intestinal side effects suggests it should preferably be administered under direct supervision. Because of its teratogenicity, it cannot be used in women of child bearing age, and patients need close monitoring for adverse events, as the safety of courses of miltefosine longer than 4 weeks has not been evaluated.

As one of the aims of the MSF VL program in Bangladesh was to contribute to the Kala Azar Elimination Plan by identifying an effective short course, safe and affordable treatment for PKDL, it was decided to evaluate the safety and effectiveness of a new, lower dose AmBisome schedule. As PKDL does not cause any physical impairment and is not a life-threatening disease, the treatment regimen should not put a patient at risk unnecessarily and have a favorable risk/benefit ratio.

Alternative PKDL treatments and rationale for 15 mg/kg AmBisome total dose for PKDL

Seeing the high effectiveness of the 30 mg/kg total dose regimen, and the fact that hypokalaemia is known to be a dose-dependent side effect of AmBisome, it was deemed likely that lowering the dose of AmBisome will yield a regimen that is still effective but will have a better safety profile. A 15 mg/kg total dose regimen, in accordance with the National Guidelines for Kala-azar Elimination in Bangladesh (2008 and 2011), was used in over 1400 VL patients in the MSF project in Fulbaria without any notable side effects (analysis of routine data under publication). The minimally effective dose of AmBisome for PKDL is not known, but as a total dose of 15 mg/kg was observed to be effective in VL with few side effects, this was selected as the maximum dose. For safety reasons, it was decided to not use a total dose higher than 15 mg/kg. It is likely that the dosage and the total dose both have an influence on the occurrence of hypokalaemia. To increase the safety of this regimen, the dose of 15 mg/kg dose will be administered in implements of 3 mg/kg, with at least two days recovery time in between each dose. An improved potassium monitoring and supplementation protocol will be implemented.

2 EVALUATION PLAN

Patient population
All PKDL patients of Fulbaria Upazila, a densely populated, predominantly rural area. MSF manages VL and PKDL patients since May 2010 in a MSF clinic specifically constructed for this purpose, neighbouring the Fulbaria Upazila Health Complex (UHC). Additionally, PKDL patients will be recruited in Trishal.

Inclusion
PKDL patients diagnosed clinically and biologically supported by positive rK39 rapid test, admitted and treated in the MSF clinic in Fulbaria that have given informed consent to participate in the study. All PKDL patients meeting the inclusion criteria will be eligible to be included in the study, irrespective of prior treatment for VL (which may be SSG, Miltefosine, or AmBisome)

Exclusion
- Patients with PKDL and concurrent VL
- Patients who had prior treatment for PKDL
- Patients younger than 12 year, as 1) clinical signs and symptoms of hypokalaemia or rhabdomyolysis may be overlooked, because children may not be able to properly express physical complaints, 2) especially in young children, taking a series of venous blood samples for electrolyte monitoring can be a mentally traumatizing event, which is unlikely to be outweighed by the benefit of PKDL treatment
- Clinical symptoms of severe chronic underlying disease
- Patients on long-term medication with a side effect profile overlapping with that of AmBisome
- Known cardiac disease or hepatic impairment
- Renal impairment as defined by a baseline serum creatinine > 1.3 mg/dL
- Diarrhoea, vomiting, or other conditions resulting in significant body potassium losses
- Patients with serum potassium <3.5mmol/L
- Known hypersensitivity to AmBisome
- Current or recent (< 1 month) pregnancy and lactating women. Women within reproductive age will be assessed for pregnancy, and in case of doubt a pregnancy test will be performed. Women declining a pregnancy test will not be included in the study.

Assessment of effectiveness (at 12 M)
A treatment failure will be defined as
- A patient with no or little change in lesions (non responders) at 12 M
- Treatment not completed due to serious side effects (SAE's)
- A patient with lesions that worsened or presenting with new lesions at 12 M, as compared to baseline

Assessment of safety
Assessment of safety during treatment and follow-up is based on adverse events, laboratory parameters during treatment and clinical assessment at 1 month follow-up.

Sample size
In order to evaluate the effectiveness of the treatment we make the assumption that the 15 mg/kg regimen will only be slightly less effective than the 30 mg/kg AmBisome regimen: an estimated effectiveness of 80%, a precision of 5% and a significance level of 5% leads to a sample size of 250 patients. Based on previous follow up experiences we estimate a loss to follow up of maximum 10% (25 patients). The sample size will therefore be increased to 275 patients. This amount of patients can be treated in approximately 6 months (with the average of around 70 patient treated each month in Fulbaria).

3 DIAGNOSIS AND TREATMENT PLAN

Diagnosis and treatment will take place according to the MSF PKDL Treatment Protocol for Fulbaria, Bangladesh, valid at this time (version April 2013, see annex 1).

Initial diagnosis
Currently there is no agreed upon method for confirmation of the diagnosis of PKDL. The sensitivity of microscopic detection of *Leishmania* parasites in skin biopsies and skin slits is limited, and varies between 67-100% in nodular lesions to only 3-33% in biopsies of macular lesions [6,7,9]. The macular form of PKDL is the predominant form seen in Bangladesh (96%). PCR may increase the diagnostic sensitivity, but still varies widely between 43 and 95% [1,9]. PCR has not been validated as test-of-cure.
Diagnosis is therefore based on clinical suspicion of PKDL, based on visual assessment of skin lesions characteristic for PKDL (as defined in the Treatment Protocol, see annex 1) that have persisted at least one month and in combination with a reported history of treatment for VL, and a positive rK39 test result. Physicians will be trained in recognizing the typical clinical appearance of PKDL lesions, and differential diagnosis like leprosy, fungal dermatoses and vitiligo. Initial diagnoses will be counterchecked independently by a second physician. Cases with ambiguous lesions will, depending on the clinical picture, be advised to return to the clinic after 2 weeks, treated with anti-fungal or other dermatitis medication, or referred to a skin specialist. This is the diagnostic method that will take place once PKDL treatment will be rolled out in Upazila Health Centres in Bangladesh. In field settings where diagnostic methods are limited, PKDL diagnosis will often have to be based on clinical criteria [8]. This has been evaluated by counterchecking photographs of approximately 200 randomly chosen PKDL patients by a WHO designated PKDL expert. It was concluded that PKDL was highly likely to be the correct diagnosis in all cases. In another previously performed evaluation of PKDL treatment efficacy, PKDL diagnosis and assessment of treatment outcome was performed in a similar manner [2].

Data will be recorded using a predesigned form. At the same time, photographs will be taken from lesions in a standardized manner (according to a photography protocol).

Treatment
Drug: AmBisome (Gilead Sciences, USA) will be provided as a sterile lyophilised powder in a 15ml sterile type 1 clear glass vial containing a yellow powder with 50 mg of the active ingredient amphotericin B encapsulated in liposomes. The closure consists of a butyl rubber stopper and aluminium ring seal with a removable plastic cap. Vials are packed in cartons of 10, with 10 filters provided.
Storage: stored under 25 degrees Celsius or below; not to be frozen or exposed to light. Once reconstituted the product will be stored at 2-8 degrees Celsius and will be used within 24 hours.

Dose: 15 mg/kg given intravenously
Dosing schedule:3 mg/kg in 5 doses
A = 1st dose on Sunday (**Sunday**, Wednesday, **Sunday**, Wednesday, **Sunday**)
B = 1st dose on Monday (**Monday**, Thursday, **Monday**, Thursday, **Monday**)
C = 1st dose on Tuesday (**Tuesday**, Friday, **Tuesday**, Friday, **Tuesday**)
D = 1st dose on Wednesday (**Wednesday**, Saturday, **Wednesday**, Saturday, **Wednesday**)
E = 1st dose on Thursday (**Thursday**, Monday, **Thursday**, Monday, **Thursday)**
Administration: each vial is reconstituted with sterile water (12 ml a vial) and given as an intravenous infusion in 5% dextrose using a volume of at least 100 ml per vial. It should not be mixed with saline or other electrolyte solutions. The infusion is given over a period of 2 hours. Transfusion from the reconstituted vial into the infusion bag is done through a 5 micron filter provided to remove any particular matter. A test infusion of 1 mg is administered for about 10 minutes, after which the patient is observed carefully during half an hour. If no severe allergic reaction has occurred the infusion can be continued.

Safety precautions

Patients with renal impairment at baseline (serum creatinine > 1.3 mg/dL) will be excluded, in order to ensure adequate renal excretion of excess potassium in case of medical high dose potassium management for hypokalaemia.
Patients with diarrhoea, vomiting, or other conditions resulting in significant body potassium losses will be asked to come back for PKDL treatment once recovered.

A test infusion of 1 mg is administered for about 10 minutes, after which the patient is observed carefully during half an hour. If no severe allergic reaction has occurred the infusion can be continued. Adequate safety precautions are in place to mitigate effects of anaphylactic reaction to AmBisome infusion.

In order to prevent serious adverse effects of treatment all PKDL patients will be monitored for clinical signs of hypokalaemia (general weakness, nausea, muscle weakness). Clinical monitoring will be done at each time the patient receives a dose of AmBisome (during administration and for half a hour afterwards), and at the following follow up points: one week after the last dose, and one month after the last dose.

Patients will have laboratory assessments at different times before, during, and after treatment to monitor possible side effects:

- Baseline (before treatment): serum potassium, serum magnesium, serum creatinine
- Half-way treatment (before 3rd dose): serum potassium, serum magnesium
- End of treatment (before 5th dose): serum potassium, serum magnesium
- One week after last dose: serum potassium, serum magnesium, serum creatinine
- On indication: 2 weeks, 3 weeks, 4 weeks after 5th dose
- CPK (creatinine phosphokinase) is indicated at any time when serum potassium is below 3mmol/L
- ECG monitoring is indicated for patients with moderate (2.5 – 3.0 mmol/L) and severe (<2.5 mmol/L) hypokalaemia.

All PKDL patients will be counselled on intake of high-potassium food at home. In case laboratory results indicate the need, medical potassium supplementation will be started. Because AmBisome can also cause hypomagnesaemia, and because hypokalaemia is difficult to correct without simultaneous correction of hypomagnesaemia all patients indicated for

potassium supplementation (S-K <4.2mmol/L) will also receive magnesium supplementation during the potassium supplementation course. Supplementation will be given by oral magnesium oxide 300 mg/day.

All patients are asked to consent to inform the MSF doctor on the use of any concomitant medication which has not been prescribed by the MSF doctor (MSF will provide primary health care if needed). All patients will be clearly instructed to call the MSF doctor immediately and return to the clinic as soon as possible in case of any deterioration in their condition or occurrence of symptoms related to hypokalaemia: anorexia, nausea, general weakness, muscular complaints, and other signs. Patients will also be given a card with the telephone number of a contact person in the MSF clinic that they can call 7 days/week.

Discharge after infusion
Patient will be kept under observation for a minimum of 3 hours after starting the AmBisome infusion and then discharged unless additional observation is warranted. Discharge criteria are

- Generally well, ambulant and able to perform daily activities themselves
- No nausea, general weakness, muscle weakness, muscle pain and other signs which may be associated with hypokalaemia

Patients who do not fall into this category will be retained as an in-patient for further observation. They will be discharged once the site physician feels sufficient progress has been made.

Bednets
After enrolment into the study all patients will receive two Long-Lasting Insecticide-treated Bed Nets (LLIN) to prevent *Leishmania* transmission from the PKDL patient to other family members or close neighbours. Free LLIN distribution to all VL and PKDL patients is part of the National Kala-azar Elimination Programme in Bangladesh.

Follow up post treatment
After treatment, all patients will be requested to come to the MSF clinic for follow up visits at 1, 3, 6 and 12 months after discharge. In case patients do not show up at the agreed day, they will be visited by the outreach team within 2 weeks after the agreed date and encouraged to go to the clinic. Patients will be reimbursed for transport costs for any visit to the clinic. Loss to follow up is anticipated to be minimal. Loss to follow up will be defined as follows:

- One month follow up: any patient not reporting within 2 weeks before until 2 months after the agreed date
- Three month follow up: any patient not reporting within 1 month before until 1.5 months after the agreed date.
- Six and 12 month follow up: any patient not reporting within 1,5 month before until 3 months after the agreed date.

At each follow up visit, patients will be visually checked for PKDL signs and symptoms, using a predesigned form and the lesions will be photographed. At each follow up visit, lesions will be compared with the photographs taken at previous follow up visits in order to assess progress. Overall well being will be checked at each visit, and specific questions about side effects will be asked at the first follow up visit (one month after treatment).

Cure of PKDL (at 12 M)
As there is no validated test-of-cure, definite cure is defined on clinical grounds as complete resolution of nodular and papular lesions, and major or complete repigmentation of macular

lesions, as reported by the patient and assessed by the physician (each patient will be assessed by 2 physicians, independently). Considering that PKDL is not a life-threatening disease and PKDL patients are generally healthy, no rescue medication will be offered to those not cured at 12 M. Patients who have failed treatment at 12 months, but who are otherwise physically well, and who have not experienced any SAE's during the study, will be informed about the possibility of treatment with miltefosine according to the national guidelines, provided by the MoH in the Upazila Health Complex in Fulbaria or Trishal, or in the SK Hospital in Mymensingh. Patients will be counselled on risks and benefits of this treatment option. MSF will not bear the costs of referral or any other costs related to treatment at a MoH facility.

4 ADVERSE EVENT DEFINITIONS AND REPORTING

Adverse Event definition
An adverse event will be defined as any untoward medical occurrence (any unfavourable and unintended sign, symptom or disease, including an abnormal laboratory finding) in temporal association with the use of AmBisome treatment and may or may not be causally related to it. Abnormal laboratory results will be reported as adverse events if the abnormality occurs or worsens after starting AmBisome, and if they require clinical intervention or further investigation, unless they are associated with an already reported clinical event.

Serious Adverse Event
An adverse event will be defined as serious if it is

- fatal
- life-threatening
- requires or prolongs hospitalization
- results in persistent or significant disability
- is a congenital anomaly/birth defect
- results in an important medical event that may not be immediately life threatening or does not directly result in death or hospitalization, but which may jeopardize the patient or may require intervention to prevent the other outcomes listed above.

Any prolongation of the hospitalization that is not related to a medical event but related to any social and/or financial reasons will not be considered as an SAE.

Eliciting Adverse Event information
The Principal Investigator (PI) is required to report all directly observed adverse events and all adverse events spontaneously reported by the patient using concise medical terminology. In addition, each patient will be questioned about the occurrence of adverse events during each visit to the treatment centre during the treatment period, and 1 month after finishing treatment with a generic question such as "have you felt different in any way/ had any problems since starting the new treatment/the last assessment?" If the response is "Yes", the nature of the event, the date and time (where appropriate) of onset, the duration, maximum intensity (see below) and relationship to treatment will be established (see below). Details of any dosage/schedule modification or any corrective treatment will be recorded on the Adverse Event (AE) reporting forms.

Adverse Event reporting requirements

Information on adverse events must be evaluated by a physician. Each adverse event is to be classified by the Principal Investigator (PI) as serious or non-serious. This classification will determine the reporting procedure for the event.
All serious adverse events (SAE) are to be reported to the coordinating investigator (CI) within 24 hours of awareness of SAE by the PI, using the SAE report form. This includes a description of the event, onset date and type, duration, severity, relationship to study drug, outcome, measures taken and all other relevant clinical and laboratory data. The initial report is to be followed by submission of additional information (follow-up SAE form) as it becomes available. Any follow-up reports should be submitted as soon as possible and preferably within 7 working days.

Non-serious adverse events are to be reported on the AE reporting form. A given adverse event will be recorded only one time per patient, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the AE report form.

Grading of non-serious Adverse Event severity
Clinical adverse events and laboratory parameters will be graded by investigator using the Common Toxicity Criteria for Adverse Events (CTC AE) version 4 (May 2009). This specific adverse event severity grading scale is used in order to standardize reporting.
In the case that an AE is not listed, the assessment of intensity/severity will be based on the investigator's clinical judgment. The maximum intensity/severity experienced should be recorded and will be assigned to one of the following categories.

- **Mild:** an event easily tolerated with little discomfort. Treatment continued, no action needed.
- **Moderate**: an event that will cause treatment to be briefly stopped or necessitate close monitoring and possibly some supportive treatment.
- **Severe:** Treatment frequently discontinued and/or switched to another drug. Supportive treatment likely to be given.

It is to be noted there is a distinction between severity and seriousness of adverse events. A severe adverse event is not necessarily a serious event.

Adverse Event causality assessment
For both serious and non-serious adverse events, the PI is required to assess the possible relationship between the adverse event and AmBisome, i.e. to determine whether there exists a reasonable possibility that AmBisome caused or contributed to the adverse event. Causality will be listed as not related, unlikely, possible or probable.
To help investigators with the decision in the evaluation of causality, the PI is asked to consider the following before reaching a decision:

- Medical history
- Lack of effectiveness/worsening of existing condition
- Other medications (concomitant or previous)
- Withdrawal of study medication, especially following trial discontinuation / end of study medication
- Erroneous treatment with study medication (or concomitant)
- Protocol related procedure

The decision to suspend, and resume treatment or to permanently interrupt treatment due to an adverse event will be left to the clinician in charge.

Adverse event follow up
All adverse events should be followed until they are resolved or the PI assesses them as chronic or stable or the patient participation in the trial ends (i.e., until a final report is completed for that patient). In addition, all serious adverse events and those non-serious events assessed by the PI as possibly related to the investigational drug must continue to be followed even after the subject participation in the trial is over. Such events should be followed until they resolve or until the PI assesses them as "chronic" or "stable." In case of SAEs or severe AEs, close communication with patient and/or family about the event will be established and appropriate medical care, including referral hospital costs and long term care costs will be provided for.

5 CASE DEFINITION AND OUTCOME MEASURES

Case definition
All patients with a clinical diagnosis of PKDL, based on visual assessment of skin lesions characteristic for PKDL that have persisted at least one month and in combination with a reported history of treatment for VL, and a positive rK39 test result.

Outcome parameters

Main outcome
Treatment success: Complete resolution of nodular and papular lesions, and major or complete repigmentation of macular lesions at 12 M compared to baseline (with reference to medical photographs taken at start of treatment and at each follow up)

Treatment failure: Worsening or no change of lesions at 12 M compared to baseline (with reference to medical photographs taken at start of treatment and at each follow up)

Secondary outcome

Safety: Assessment of safety during treatment and follow-up based on clinical adverse events, laboratory parameters during treatment and 1 month (after end of treatment) follow-up.

Hypokalaemia: Assessment of laboratory parameters during treatment and 1 month (after end of treatment) follow-up.

Time for lesions to respond to treatment: Assessment of photo's and clinical scoring at each follow up (1,3,6 and 12 M)

6 DATA TO BE COLLECTED

Data will be collected via a predesigned patient form

At time of diagnosis: Name, age, sex, address, telephone number, previous treatment for VL, previous treatment for PKDL, other diagnosed infections, height, weight, rK39 result, pregnancy status, and location, type and severity of lesions. Photographs of lesions will be made.

Before treatment: A patient checklist will be used to see if all requirements are fulfilled before starting treatment (consent forms signed, inclusion and exclusion criteria, etc).

During treatment and at 1 month follow up: Any SAEs will be reported using a special form to the Coordinating Investigator (CI) within 24 hours (see annex 2 for SAE report form). SAE reports will be forwarded within 7 days to the following:

- All study group members
- Data Safety Monitoring Board (DSMB)
- National Research Ethics Committee (NREC)
- MSF Medical Director and Ethical Review Board
- and the manufacturer of AmBisome in a later stage.

Any other AEs will be reported using the AE report form (annex 3).

At 1, 3, 6 and 12 months follow up: location, type and severity of lesions and photographs of lesions

Study specific data will be entered into a Case Report Form (CRF). This form will contain all data regarding the patient's consent, diagnosis, treatment and progress during follow up, evaluation of photographs, side effects including SAE and AE forms, laboratory results, action taken, correspondence; in general it is a summary of all data collected by laboratory technicians, medical doctors, and nurses. All CRF data should be anonymised (identified by study patient number and initials only).

7 EVALUATION SCHEDULE

Results (effectiveness and adverse events) will be analyzed and evaluated every 3 months. With the expected average of 70 PKDL admissions per month, we expect a recruitment period of 4 months for the recruitment of 275 patients. A thorough analysis of 12 M FU data will therefore be carried out at approximately 17 months after starting patient recruitment.

In-between reports will be written and submitted to the MoH and BMRC at intervals of 6 months.

Reports with preliminary analysis of safety data will be sent to the Data Safety Monitoring Board (DSMB) every three months, or according to a frequency requirement defined by the DSMB.

8 QUALITY ASSURANCE

This evaluation will adhere to the principles of good clinical practice. Patients will be monitored and checked by experienced medical personnel, and will receive prompt and appropriate treatment of concurrent illnesses or complications. AEs possibly or probably caused by AmBisome will be treated as required and recorded.

Staff, Training, and Supervision

- **Staff**. There will be enough and clearly identified staff for proper assessments at admission, discharge and follow-up, for monitoring during the treatment phase and for performing the laboratory work.
- **Training**. Training of identified staff on aims and procedures of the study and role of staff will take place before the study starts.

- **Supervision**. The field-based PI will give training and supervise and monitor the study at all stages for the duration of the study.
- **Monitoring visits**. Monitoring visits to the trial site will be made every 2 to 3 months (or more frequent if deemed necessary by the study team) by study group representatives to ensure that all protocol requirements and SOP's are being followed. Source documents (laboratory and nurse records, patient forms, etc, will be reviewed for verification of consistency with data on CRFs. The progress of the study will be evaluated and the completeness, consistency and accuracy of the documentation such as consent forms, SAE and AE forms, CRF's, protocol and protocol amendments, etc, will be verified and it will be checked whether all adverse events were reported properly to all parties. Monitoring visits will be standardized using a pre-designed checklist. After each monitoring visit a visit report will be made available for the study team

Laboratory Quality Control

All rK39 tests will be stored at room temperature and kept for QC (blind checking). Appropriate QC will be maintained for the machines for serum creatinine, potassium, magnesium, and CPK testing

9 RESPONSIBILITIES

MSF-Holland is the sponsor of the study. Only on authority of the Medical Director of MSF-Holland should the evaluation be substantially altered or stopped. Earlier termination of the study will be jointly decided by the coordinating investigator and the sponsor, and will be due to safety reasons based on the recommendation of the DSMB.

The **coordinating investigator (CI)** (Dr. Koert Ritmeijer) of the program is accountable to MSF-Holland. He will be responsible for ensuring the quality of diagnosis, treatment, the completeness of CRF's, and follow up visits. He is also responsible for monitoring the study in order to ensure that harm is minimised and benefits maximised for the study subjects. The CI is responsible for reporting the occurrence of SAE's to the MSF Medical Director.

The **principal investigator (PI)** (Dr. Asish Kumar Das) based in the Fulbaria MSF clinic is the primary responsible person for the implementation of the study on a practical level. The PI is responsible for the training of all field staff involved, for supervising data collection by laboratory technicians, medical doctors, clinical officers and nurses and the informed consent procedure, and for monitoring the study. The PI will supervise clinical work by the medical staff who looking after the clinical care of the patients and the administration of the AmBisome infusions. He will be directly involved in the evaluation of treatment success of each patient at the trial end point (12 M), and of treatment failures occurring at any point. The PI is also responsible for the prompt (within 24 hours) reporting of SAE's to the CI, and in case of any other problem, for contacting the CI directly by phone or e-mail. The PI will ensure that the study protocol is strictly adhered to throughout, and that all data are collected and recorded correctly on the CRF. The PI will be responsible for reporting SAE's to the Bangladesh authorities.

Prof. Be-Nazir Ahmed will verify that systems and mechanisms are in place that will prevent any breaches of the ethical conduct, and verify their regular monitoring. This will be done through field visits and document review at any moment deemed necessary. He will liaise with the national drug regulatory and other relevant authorities to ensure that the study is in accordance to the national regulations. In his role of Director of the Centre of Disease

Control in the Ministry of Health and Family Welfare of Bangladesh, Prof. Be-Nazir Ahmed will play an important role in facilitating the adoption and implementation of the recommendations from the study in the National Kala-azar Elimination Program.

Data Safety Monitoring Board
Even if this is an open-label, case series study on treatment of PKDL, using a known anti-leishmanial agent, an independent Data Safety Monitoring Board (DSMB) will be set up prior to study initiation, for independent advice on the safety-aspects of the protocol. The DSMB will comprise of a minimum of 3 members from different constituencies, all of whom must be independent of the investigator and sponsor. The DSMB will define reporting requirements and clear stopping rules for the study, and will issue recommendations about the study.

10 DATA ANALYSIS AND STATISTICS

Patient records
Patient records and all other relevant study documentation will be permanently stored at MSF premises.

Data entry
Data will be double-entered in Excel at the study site. Absent data must also be recorded so that every field is filled. This will identify whether the data are *normal* (e.g. if in the 'PKDL' field no data are recorded, assume this is = "no PKDL"), *not done* (e.g. if weight is recorded but height is not, this is = "not done") or *the patient did not attend* (e.g. if there are no data for follow up, this is = "not known"). The database will also be used to prepare standard monthly reports where admissions, discharges, SAEs and deaths will be monitored.

Data analysis
Analysis will be done by MSF in the Netherlands. Analysis will be performed using two methods. A complete case analysis will only include patients who have attended 12 M follow up (per protocol analysis). A worst case scenario analysis will assume all lost to follow ups as failures (intention to treat analysis). Safety data will be summarised as the proportion of patients experiencing AE's and SAE's and at which dose they occurred. The evaluation of the occurrence of hypokalaemia will be analyzed separately.

Subject Disposition
The number of patients who were screened, failed screening, enrolled, completed the treatment period, completed the follow-up, lost-to-follow up and reasons for lost-to-follow up will be summarized by number of patients (n) and percentages (%).Compliance is not expected to be an issue though all incomplete treatments will be recorded and outcomes appropriately assessed.

Baseline data
The continuous variables of the baseline and demographic characteristics will be summarized using number of patients (n), mean, SD, median, minimum and maximum. The categorical variable gender will be summarized using number of patients (n) and percentage (%).

Reporting
An initial report will be written to inform involved actors / authorities of outcomes and recommendations made. Findings will be also directly communicated to the community, field

teams, and programme managers within MSF, and the Ministry of Health. Following this and depending on appropriateness, a scientific paper will be prepared for publication. The CI will be responsible for drafting the results of this study for publication in an appropriate medical scientific journal, for ensuring the collaboration and consent of all co-workers, and for submitting an agreed version.

Long term plans
Depending on results, a decision will be made with local partners on whether to continue the current protocol, change dosage, or consider further research. If implementation of 15 mg/kg total dose AmBisome for PKDL proves to be safe, effective and practically feasible, further roll-out of this protocol in Bangladesh will be advocated.

11 ETHICAL CONSIDERATIONS

The experimental protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and the ICH (International Committee for Harmonization) guidelines for Good Clinical Practice. MSF assures that it will comply with all applicable state, local and foreign laws for protecting the rights and welfare of human subjects.

Ethical aspects of subject inclusion and study procedures
The effective treatment of PKDL 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 PKDL is anticipated to minimise the development of parasite resistance and to reduce hospitalization and public health costs. Patients will experience some pain while blood is drawn during venipuncture. The total volume of blood drawn during and at one week after treatment is 8 ml (4 times 2 ml).

Patient costs
Patients will be reimbursed for travel to and from the study site but will not receive any payment for trial participation. Any investigations, medication or other interventions that are required for the appropriate management of Adverse Events, or management of other concomitant dermatological conditions during treatment and the 12 months follow up period, will be provided free of charge to the patient. Treatment is ambulatory, but if hospitalisation is deemed necessary, food will also be provided free of charge to the patient.

Benefits to the community
This evaluation will be part of the agreed plan for the Fulbaria MSF VL control program, one of whose aims is provision of affordable, safe and effective short course treatment for PKDL that can be implemented in a primary health care setting and can serve as an alternative to the currently recommended 12 week miltefosine regimen. From the patient's as well as the treatment provider’s perspective, a short course ambulatory regimen with AmBisome would be preferable over the 12-week miltefosine regimen, in terms of safety, tolerability and impact on patient’s lives and income-generating activities. Results of the implementation of AmBisome for PKDL will be communicated on a regular basis to the local and national health authorities in Bangladesh. The objective is to identify a short course, affordable treatment of PKDL that could be part of the National Protocol. An effective and practically feasible treatment plan for PKDL is seen as crucial for the success of the Kala Azar Elimination Programme in Bangladesh. Moreover, we expect that results can be extrapolated to other parts of the Indian subcontinent.

Potential risks
A potential risk of AmBisome is hypokalaemia, and the occurrence of other treatment-related side effects. The potassium monitoring system will allow for a prompt and adequate management of SAEs and hypokalaemia. The potassium supplementation protocol will minimize the risk of occurrence of severe hypokalaemia and severe clinical complications thereof, such as rhabdomyolysis or cardiac arrhythmias. A DSMB is instituted and will advise on action to be taken in case of safety events.

Informed consent
Inclusion in the study will occur only if written informed consent is given. All patients will be asked to give their informed consent to participate in the study, before undergoing any study-specific procedures. The Informed Consent Form (ICF) as well as the informed consent interview will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, the voluntary nature etc. The interviews will be conducted in the native language of the patients by a qualified person formally identified by the Investigator. Written information and consent forms in the local language will be provided to the patients or legally authorized representatives for their review. The written information and consent form is currently written in English, but it will be further translated in Bengali, If needed, the subject (or representative) will be given time to discuss the information received with members of the community or family before making a decision.
The subject will be asked to sign the consent. If the subject is illiterate, a literate witness must sign (this person should have no connection to the research team, and, if possible, should be selected by the subject). Patients will also be asked for their consent for data collection and the taking of medical photographs (see annex 4 and 5 for consent forms).
A person independent of the study team will monitor the consent procedures and will directly report to the MSF-Holland Project Coordinator (PC) in Fulbaria. The PC will directly inform the CI in case of observed irregularities in the implementation of the consent procedures.
A subject refusing consent will be informed about alternative PKDL treatment options provided in nearby MoH health facilities according to the national guidelines.

Confidentiality
All patient data and all consent forms will be kept confidential; anonymity of all data will be maintained during analysis publication. Identities will be protected from unauthorized parties. Authorized parties are medical personnel actively involved in the treatment of the patient and members of the Study Group. Patient data will be identified by numbers, all computer data containing patient information will be password protected and all files will be kept in locked cabinets.

Feedback of results
As there will be ongoing monitoring of the interim data, there will also be ongoing feedback. On a regular basis (every 6 months) feedback on treatment failures and occurrence of AEs will be provided to the medical authorities. Any SAEs will be reported using a special form to the CI within 24 hours of awareness of SAE by the PI.

Authorisation

The present plan will be submitted to the national authorities for review and approval, and ethical clearance will be sought through the Bangladesh Medical Research Council (BMRC) and the MSF Ethical Review Board.

12 EARLY TERMINATION

MSF reserves the right to terminate the study at any time prior to inclusion of the intended number of subjects, but they intend to exercise this right only for valid scientific or administrative reasons. In terminating the study, MSF will assure that adequate consideration is given to the protection of the patient's interest. Reasons for termination may be the occurrence of SAEs. Other reasons include a low enrolment rate, protocol violations, inaccurate or incomplete data, unsafe or unethical practices, questionable safety of the test article, suspected lack of efficacy of the test drug, lack of time and resources, or a recommendation of the DSMB, BMRC, or MSF Ethical Review Board to terminate the study.

In case the study is terminated, it will be ensured that all patients who had not yet reached a follow up time point are followed up with the necessary medical care and the reasons for this decision will be communicated to the BMRC.

13 FUNDING (budget)

As the patient group is the current one, the implementation will not incur any additional costs.

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ANNEXES

1 PKDL treatment protocol

2 SAE report form

3 Adverse Event report form

4 PKDL Patient Information and Consent Form

5 Medical Photography Consent Form