



## **Secondary prophylaxis of visceral leishmaniasis relapses in HIV co-infected patients using pentamidine as a prophylactic agent: a prospective cohort study**

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**Protocol ITMC0109 - NCT01360762**

**Secondary prophylaxis of visceral leishmaniasis relapses in HIV co-infected patients using pentamidine as a prophylactic agent: a prospective cohort study**

**Version 4**

**April 5, 2011**

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<b>Sponsor:</b>	Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium
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**List of modifications compared to the previous version**

All the modifications to the previous version of the study protocol, dated January 1<sup>st</sup>, 2011, have been done according to the comments and requests of the Ethiopian National Ethics Committee (NERC) and Food, Medicine and Health Care Administration and Control Authority (FMHACA).

- Page 14: The calendar of activities is revised
- Page 23-24: Procedures of pentamidine administration described in detail
- Page 28-32: Monthly follow up liver function tests and every fourth month amylase and ECG monitoring plan included
- Page 33-34: Detail description of the invasive procedures, splenic, bone marrow and lymph node aspiration added
- Page 45: Reasons and procedures in case of possible early study termination (under section 5, last paragraph) added
- Page 46: End of study procedures, reporting, product management (section 6: second paragraph) added
- Removed a duplicate information on pentamidine (older version) that was included as annex 4. Annex 3, the recent product information from the manufacturer is still there on pages 62-67

## **STUDY ACKNOWLEDGMENT/CONFIDENTIALITY**

### **By signing this protocol, the Principal Investigator(s) acknowledges and agrees:**

The protocol contains all necessary information for conducting the study. The Principal Investigators will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, current SOP's and other study-related documents provided by the Sponsor, and in compliance with applicable ethical, GCP and regulatory requirements.

The protocol and all relevant information on the study drugs relating to pre-clinical and prior clinical experience will be made available to all physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

This document contains information that is privileged or confidential. As such, it may not be disclosed unless specific permission is granted in writing by the Coordinating Investigators, or such disclosure is required by national or other laws or regulations. In any event, persons to whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied which is indicated as privileged or confidential.

The Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium will have access to any source documents from which Case Report Form information may have been generated. The conduct and results of this study will be kept confidential until all sites have completed the study, unless an interim publication or presentation is agreed upon. The results of this study will be published. The procedures to be followed for the publication of data are defined in the protocol.

<b>Approval</b>	<b>Signature</b>	<b>Date</b>
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Dr Johan van Griensven	_____ Author, Prince Leopold Institute of Tropical Medicine, Antwerp Coordinating Investigator - ITM	____/____/____
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Signing this document I commit to carry out and supervise the trial at my site, according to the protocol and to all the applicable GCP, ethical and regulatory requirements.

I also declare to have read the paragraph relevant to study acknowledgement and confidentiality and authorise the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium to record a copy of my data on a computerised archive containing all the data pertinent to the study.

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## ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-Retroviral Therapy
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CMV	Cytomegalovirus
CNS	Central Nervous System
CRF	Case Report Form
CTU	Clinical Trials Unit
DNDi	Drugs for Neglected Disease initiative
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
FBC	Full Blood Count
GCP	Good Clinical Practice
HAART	Highly Active Anti-Retroviral Treatment
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IF	Investigator file
IM	Intramuscular
IRB	Institutional Review Board
ITM	Institute of Tropical Medicine Prince Leopold
IV	Intravenous
LFT	Liver function tests
LRTC	Leishmaniasis Research & Treatment Centre
MSF-H	Médecins Sans Frontières Holland
OIs	Opportunistic Infections
PCP	<i>Pneumocystis carinii</i> Pneumonia

PI	Principal Investigator
PML	Progressive Multifocal leukoencephalopathy
PM	Pentamidine
PSP	Pentamidine Secondary Prophylaxis
RNA	Ribo-Nucleic Acid
SAE	Serious Adverse Event
SC	Scientific Committee
SNNP-RG	Southern Nations, Nationalities & Peoples – Regional Government
SOP	Standard Operating Procedures
SS	Sample Size
TB	Tuberculosis
TOC	Test-of-Cure
VL	Visceral Leishmaniasis
WHO	World Health Organization

## SUMMARY

**Title:** Secondary prophylaxis of visceral leishmaniasis relapses in HIV co-infected patients using pentamidine as a prophylactic agent: a prospective cohort study.

**Coordinating Investigator - ITM:** Johan van Griensven

**Coordinating Investigator - Ethiopia:** Ermias Diro

**Sponsor:** Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium

**Statistician:** Joris Menten, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium

**Trial management:** Clinical Trials Unit, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium

**Design:** prospective cohort study

### **Inclusion:**

Patients aged  $\geq 18$  years

**AND** confirmed HIV infection

### **AND ANY OF THE FOLLOWING**

1) Patients diagnosed with VL during the recruitment period that are:

a) treated for VL relapse and have a documented negative TOC

**OR**

b) treated for primary VL and have a documented CD4  $<200$  or WHO stage 4 disease during the recruitment period and have a documented negative TOC

2) Patients treated for VL in the past with

a) Documented CD4  $<200$  or WHO stage 4 disease during the recruitment period  
**AND**

b) Documented negative TOC after the latest VL treatment and currently asymptomatic OR currently negative diagnostic test (microscopy)

**AND** agreeing to start or continue ART (first or second line)

**AND** willing to provide written informed consent to participate in the study

### **Exclusion:**

- 1) Patients with known hypersensitivity to PM
- 2) Patients with known renal failure (defined as creatinine  $>2$  times the upper limit of normal for their age/gender - serum creatinine will be measured)
- 3) Patients with diabetes mellitus (type I or II); blood glucose will be measured (random or fasting depending on the patient). Diabetes is defined as a blood glucose  $\geq 11$

mmol/L (200mg/dl) at any time or fasting blood glucose  $\geq$  7mmol/L (126mg/dl) or already diagnosed patient and on treatment.

- 4) Patients unlikely to be able to attend follow up visits and comply with the study requirements
- 5) Pregnant and lactating women
- 6) Any other condition that, according to the study physician, could increase the risk of toxicity of PM to such an extent outweighing the expected benefit (eg severe cardiac dysfunction).

**Sample size and accrual:** The required sample size is at minimum 65 evaluable patients for the main effectiveness analysis. We plan to recruit an additional 7 (10%) patients for a total of 72 patients to account for patients who are lost to follow-up.

We expect to recruit the required sample size of 72 patients within 1 and a half year in the 3 study sites. The total study duration is 4 years.

**Treatment:** Pentamidine (Pentacarinat® provided by Sanofi-Aventis) 4 mg/kg IV as secondary prophylaxis

#### **Follow-up:**

Main study and treatment period: 12 months

Extended treatment period: 0 to 6 months depending on the CD4 cell count (for a total treatment period of 12 to 18 months)

Extended study period: 12 months follow-up subsequent after the extended treatment period

#### **Endpoints:**

##### Primary

- Efficacy: time to relapse or death (all causes) within 1 year of PSP initiation
- Safety: proportion of patients with SAEs which are possibly, probably or definitely drug-related following clinician's assessment or that lead to permanent drug discontinuations during the first year of PM administration
- Feasibility: proportion of patients that complete the first year of PSP following the protocol: i.e., completed 12 months of follow-up with at least 90% of planned doses (i.e., 11 out of 12) administered without experiencing relapse or drug-related SAEs)

##### Secondary

- Safety: during the first year of PSP administration
  - o any drug-related non-serious adverse events (with drug-related defined as possibly, probably or definitely related to primary therapy following physicians assessment)
  - o any serious adverse events (drug-related or not)
- Feasibility:
  - o the number of treatment discontinuations and interruptions
  - o the number of required additional clinical interventions/therapeutic procedures, as described in section 2.6.1.

Tertiary objectives: they are described in the main text (page 21)

**Calendar of activity**

YEAR	2010	2011				2012				2013				2014				2015				
QUARTERS		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
<b>Preparatory phase</b>																						
<i>Writing protocol</i>	X																					
<i>Ethical submission</i>	X		X																			
<i>Drugs procurements</i>			X																			
<i>Sites preparation</i>			X																			
<i>Pre-study assessments</i>			X																			
<i>Etc...</i>																						
<b>Implementation phase</b>																						
<i>Start of recruitment</i>				X																		
<i>End of recruitment</i>									X													
<i>End of the PSP period</i>													X									
<i>End of the extended PSP period</i>															X							
<i>End of the follow-up period</i>																					X	
<i>Database lock</i>																						X
<i>Study report</i>																						X
<i>Publication</i>																						X

# 1 BACKGROUND

## 1.1 HIV and VL in Ethiopia

VL in Ethiopia has been reported from several communities in different parts of the country. Most infections are acquired in northwestern Ethiopia in the lowlands of Metema and Humera; in the Segen, Woitu and Omo river basins of southwest Ethiopia; and in other isolated foci in the Rift Valley. The Metema-Humera focus (which extends northwards to Eritrea and westwards into eastern Sudan) is a major VL focus, and accounts for approximately 60% of the total disease burden in Ethiopia. This focus extends over a huge landmass in two regions, Region 1 (Tigray Regional State) and Region 3 (Amhara Regional State). Other foci are in Region 4 (Oromia Regional State) and Region 5 (Somali Regional State). Sporadic case reports are known from other smaller localities e.g., Region 2 (Afar Regional State).

VL cases particularly from region 1 and 3 are frequently associated with HIV co-infection (approximately 30% of cases). In Addis Ababa, among 291 soldiers and policemen hospitalized for VL treatment in army and police hospitals between 1992 and 2001, HIV co-infection rate was 48.5%; and has remained high ever since. In a clinical trial conducted in Humera Hospital, the rate of HIV co-infection among 145 VL cases was found to be 18.6% in 1998-99; and 29% among 375 tested in 2004. A retrospective analysis of data from patients treated in the same Hospital (Dec 1998 – May 2000) revealed HIV positive rate of 23% among 213 VL cases. A case series study in Gondar Hospital also revealed a co-infection rate of 41% among 212 VL cases diagnosed between January 1999 and July 2004. In Addis Zemen (northwest Ethiopia) and the surrounding rural areas, the current HIV co-infection rate in VL patients lies between 10 and 15% (unpublished observations). The ruralisation of HIV epidemic in VL endemic areas like Addis Zemen and surrounding settlements will hamper efforts to control visceral leishmaniasis. In sharp contrast, HIV co-infection of VL in southern regions remains low. In the foci located in the Southern Nations, Nationalities and Peoples - Regional Government (SNNP-RG), HIV co-infection is less than 2% (Unpublished observations).

Clinical experience in Ethiopia has shown that anti-leishmanial treatment in the absence of anti-retroviral therapy does not result in favourable outcomes. Poor prognosis, high mortality and relapse rates are characteristic of Ethiopian VL patients with HIV co-infection. Reported mortality rates in co-infected patients were 39.3% in Gondar and 14.3% in Humera. In effect the disease is nearly incurable, with inability to clear parasites and frequent episodes of relapse. While ART has improved outcomes, both issues remain a problem. The effective management of the initial VL episode, timely ART, and prevention of further VL episodes (relapse) should therefore be the cornerstones of effective management of HIV/VL co-infection.

Parasitological cure of VL in HIV co-infected patients cannot easily be established, and until cellular immunity returns with ART, the patient is at risk of relapses of VL. Crucially, such relapses carry several hazards:

- i) the risk of death or severe illness from VL.
- ii) the negative effect of VL on ART efficacy (persistent VL suppresses CD4 recovery) leading to other OIs.
- iii) relapses become increasingly unresponsive to VL treatment, with the potential emergence of drug-resistant parasites,
- iv) in areas of anthroponitic VL, this could possibly lead to transmission of drug-resistant *Leishmania donovani* to others.

## 1.2 Management of VL in patients with HIV co-infection

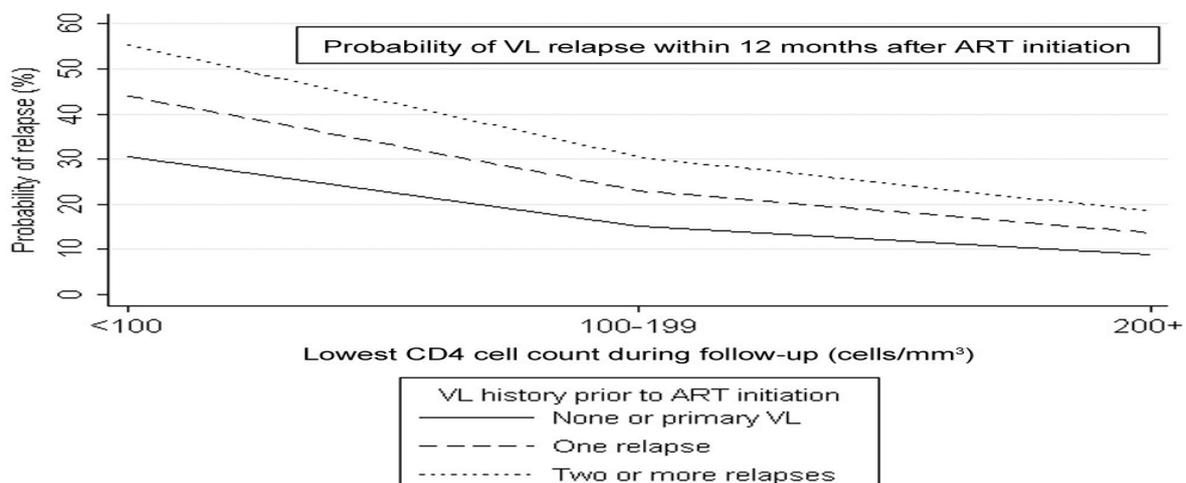
### 1.2.1 Rationale for secondary prophylaxis in VL patients with HIV co-infection

From recent published data, it is known that HAART reduces the risk of relapse/recurrence by ~50%. However, the type of anti-leishmanial treatment seems to have little effect on the risk of relapse. The most important factor for the primary treatment regimen is clearance of visible parasites – otherwise, if residual parasites are seen at the end of treatment, the relapse rate is 100%. It is therefore unlikely that secondary prophylaxis would benefit patients unless parasites are - at least visibly- cleared at the end of treatment.

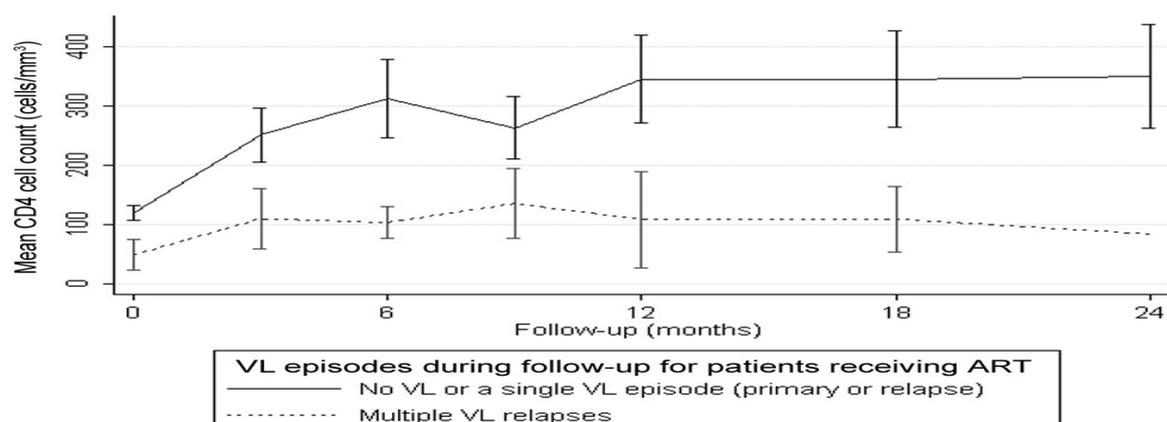
Drug toxicity and efficacy are the main factors to consider when choosing treatment, since relapse/recurrence is independent of drug choice. In HIV/VL, all drugs have lower response rates, and higher toxicity than in HIV-uninfected VL patients. Relapses become increasingly unresponsive to antileishmanial drugs.

### 1.2.2 Risk factors of VL relapse/recurrence

The risk of VL relapse/recurrence is related to at least two predictors, namely the number of previous VL episodes and the CD4 count. Figures 1 and 2 demonstrate the risks associated with each of these two factors. In addition tuberculosis (TB) adds a 2-fold increased risk of VL relapse/recurrence. This is likely to reflect the fact that such patients have had major OIs (opportunistic infections) other than VL. Therefore, vulnerable patients are 1) those with initial low CD4 cell count, 2) patients with previous VL episodes, and 3) patients with OIs.



**Figure 1: probability of relapse associated with CD4 cell count (post commencement of ART).** Source: ter Horst *et al*, 2008; *CID*, 46: 1702-9.



**Figure 2: CD4 cell count change over follow up associated with history of relapses**

Source: ter Horst *et al*, 2008; *CID*, 46: 1702-9.

### 1.2.3 Secondary prevention of VL relapses/recurrences

Limited studies in Europe show that HIV co-infected patients may benefit from maintenance treatment (secondary prevention), by significantly prolonging the relapse-free period (table 1 & 2). The drugs studied for secondary prophylaxis in Europe have been meglumine antimoniate, AmBisome, and pentamidine (PM). It is worth noting that both antimonials and AmBisome are part of mainstay treatment for VL in Ethiopia. The effect of such maintenance treatment has not been studied in African VL, but the poor outcomes without secondary prevention highlight a clear need to offer better care to patients at high risk of relapse. Indeed, secondary prophylaxis is generally recommended in Europe and the US. The 2009 CDC guidelines state that “secondary prophylaxis with an effective antileishmanial drug, administered at least every 2–4 weeks, is recommended, particularly for patients with visceral leishmaniasis and CD4+ counts <200 cells/ $\mu$ L” (AII level of evidence which implies that it is “should always be offered”).

The extent of relapse during secondary prophylaxis is one of the main study questions to be addressed. However, it could well be that the reduction in relapse with pentamidine secondary prophylaxis (PSP) is negated by a subsequent increase in events after discontinuation of secondary prophylaxis. For this reason, further follow-up within the study after discontinuation of secondary prophylaxis is of interest as well.

**Table 1: Outcomes & side effects of prophylaxis with pentamidine in co-infected patients (HIV/VL)**

	Country	Case (s)	Dose	Outcome of prophylaxis	Side effects
Durand 1998	France	Cutaneous leishmaniasis/HIV	4mg/ml, repeated after one week, then 4 mg/kg every 15 days for months	Parasitological clearance and clinical cure	No side effects noted
Fenske 1991	Germany	HIV/VL	4 mg/kg daily,	Cure and	No side effects

			then 3 mg/kg every 2 weeks	effective prophylaxis	noted
Perez-Molina 1996	Spain	6 x HIV/VL	4 mg/kg biweekly and monthly	No relapses 3-12 months	No side effects noted
Calza 2001	Spain	HIV/CL	4 mg/kg twice a week for 8 weeks, 4 mg/kg every 2 weeks for 10 months	No relapses	No side effects noted
Gazapo 1992	Spain	2x HIV/VL	4 mg/kg monthly	Effective 13 and 19 months	No side effects noted
Catorze 2006	Portugal	VL/HIV with cutaneous lesions	4 mg/kg /3 times a week for 2 months		No side effects noted
Laguna 1997	Spain	13 x HIV/VL	4 mg/ml monthly, 2-36 months	5 relapses	No side effects
Matheron 1992	Morocco	HIV/VL	3 mg/kg every 3 weeks	Free of infection for 16 months	No side effects noted

**Table 2: Side effects of treatment and prophylaxis with pentamidine in co-infected patients (HIV/PCP)**

	Country	Case (s)	Dose	Side effects
Aguado 1990	Spain	23 x HIV/PCP	Monthly 4 mg/kg, 3-13 months	2 mild nephrotoxicity
Lidman 1993	Sweden	42 x HIV/PCP	4 mg/kg every 2 weeks, one year	2 hypoglycaemia, 3 discontinued (hypoglycaemia, dermatitis, nephrotoxicity)
Schroeder 1994	London	8 x HIV/PCP	4 mg/kg monthly, 3-46 months	No side effects
Ena 1994	Spain	52 x HIV/PCP	4 mg/kg monthly, mean duration 11+12 months	2 hypotension, 2 hypoglycaemia, 2 hyperglycemia, all mild
Kim 2008	US	232 x HIV/PCP	4 mg/kg monthly	No serious side effects, no treatment discontinuation

#### 1.2.4 Choice of drug for secondary prevention in Ethiopia

If secondary prevention were to fail and relapse occurs, there is good evidence that the parasite could become wholly or partly resistant to the drug used for secondary prevention. In addition, drug-resistant parasites may be transmitted to others in areas within East Africa where man-to-man (anthroponotic) transmission is the dominant form of VL transmission. Thus drugs essential for treatment of VL should be avoided for secondary prevention. Antimonials (sodium stibogluconate and meglumine antimoniate), AmBisome, amphotericin B, and Miltefosine are essential for treatment of VL, and are thus not suitable drugs for secondary prevention.

We refer to the following WHO report with regards to the potential of drug resistance in anthroponotic VL:

WHO Report of the Fifth Consultative Meeting on *Leishmania*/HIV Co-infection [WHO/CDS/NTD/IDM/2007.5]:

*“In anthroponotic VL, the risk of resistance development means that HIV-co-infected patients may become an important reservoir of drug-resistant L. donovani. Further research is needed before maintenance therapy can be recommended for these patients.”*

and:

*“More studies are also needed on secondary prophylaxis in areas of anthroponotic transmission, taking into account the risk of drug resistance. In co-infected patients from anthroponotic foci, any drug could probably give rise to Leishmania resistance: drugs used to treat relapse should therefore be avoided for secondary prophylaxis.”*

For these reasons, pentamidine (PM) 4 mg/kg (IV, max 300 mg) every 3-4 weeks has been proposed (see also WHO report above) as secondary prophylaxis. This is being used in other countries (eg UK, Spain) and is currently being considered as an option for secondary prophylaxis in this study. We note that PM is currently not used for VL treatment in Africa. In this study, PM administration once every 4 weeks was chosen since visits at the ART clinic are monthly and consequently, this was considered most convenient for the patients if PM administration could coincide with the ART visits. We note that the monitoring of drug sensitivity is included in this project (see tertiary objectives).

### 1.2.5 Rationale of the study design

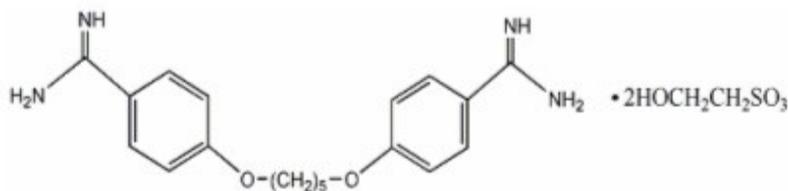
Pentamidine is registered in Ethiopia as an antileishmanial drug. Although, PSP has been studied in other settings, it is a new intervention within Africa/Ethiopia. Whereas all studies on PSP come from high income countries with strong health care systems the main challenge is to determine how to implement this effectively and safely in Ethiopia where health capacity and infrastructure is clearly different, and where a number of additional challenges like accessibility of care need to be taken into account. Consequently, this study aims to document the patient outcomes of this intervention from a feasibility perspective before it can be considered for more general use in Ethiopia.

A placebo group is not included, due to the clear advantages of the intervention to the patient population targeted herewith. As mentioned before, secondary prophylaxis is generally recommended in international guidelines for patients at risk for areas with zoonotic VL (AII level of evidence, CDC guidelines). Furthermore as other available VL treatments are used as main line treatments, they cannot be considered as alternative comparators, given the potential risk of rapid emergence of drug resistance and subsequent spread in areas of anthroponotic VL.

## 1.3 General information on pentamidine

Pentamidine belongs to the group of aromatic diamidines. Currently, it is already recommended in the Ethiopian National Drugs list for the treatment of visceral leishmaniasis.

Pentamidine isethionate structure



Although PM has been used for many years, the mechanism of its anti-parasitic and anti-protozoal action is still not exactly known. It is thought to act through interference with numerous cellular processes. Pentamidine was shown to inhibit the synthesis of DNA, RNA polymerase, and ribosomal proteins.

Pentamidine is commercially available as isethionate salt in 300 mg vials for injection (Pentacarinat, Aventis, Pentam 300). In 2001, Aventis made an agreement with WHO to donate parenteral PM in 200 mg vials) for the treatment of neglected tropical diseases in endemic countries for a period of 5 year. This agreement has been extended until 2011. In this study, the Pentacarinat® will be provided by Sanofi-Aventis.

### 1.3.1 Pharmacokinetics

After injection, PM is quickly and extensively taken-up by body tissues. Plasma levels measured after the first dose decreased 32% within the first 10 minutes after injection and after one day it was no longer detectable in plasma. The drug has a large volume of distribution, reported as a value of 821 L at steady-state after intravenous injection and a volume exceeding 100 L/kg in AIDS patients . Drug accumulation takes place mainly in kidney, liver and spleen tissues, but also in adrenal glands, lung tissue, pancreas and brain . In animal studies, PM concentrations were highest in the kidneys, half of that in the liver, and 5-10 times less in lungs, but PM was also found in abdominal and pelvic tissues, skin, brain and skeleton. After administration, approximately 70% of PM is bound by plasma proteins. Pentamidine also accumulates in red blood cells. The concentration in whole blood samples was found to be about 2 times higher than in plasma, except the first two hours after injection.

Pentamidine is very slowly released from body compartments. It was still detectable in patients one year after treatment . Excretion of the unchanged drug via urine is 2-29%. With daily dosing, PM was still found in urine 8 weeks after stopping therapy. Most of the drug is metabolised and then eliminated. Different elimination half lives have been reported, the latest were 11-12 days, meaning that a steady state will only be reached after that period . Therefore, PM plasma concentrations increase gradually during therapy. The mean plasma concentration measured after injection increased 5 fold over 10 doses in trypanosomiasis patients. In week 1, 2 and 3 plasma levels still increased in AIDS patients. It was shown that initial disposition of PM does not differ in AIDS and trypanosomiasis patients .

### 1.3.2 Route of administration: IM versus IV

Rapid IV infusion of PM has led to sudden drops in blood pressure, seizures and death, and hence intramuscular administration of PM became general practice. However, pain and abscesses were reported frequently. After 8-26 weeks of therapy, 5 out of 8 patients developed lumps and abscesses at the injection site that took many weeks to heal, with hardly any healthy tissue left for injection. In 82 patients treated for trypanosomiasis, 15 developed abscesses<sup>27</sup>. Eighteen (up to 67%) of patients experienced severe pain, dermal necrosis, sterile abscesses and local neuropathies. When in the 1980s, it was shown that hypotension is more common with IM injections compared to slow (over one hour) IV infusion in 100 ml 5% dextrose, IV administration was re-introduced . In trypanosomiasis, where IM injection is still

common, patients rest for one hour after injection to avoid hypotension. A kinetic study showed that IM administration can result in large plasma variations and delayed and erratic absorption.

### 1.3.3 Prophylactic regimen of pentamidine for HIV-VL co-infected patients

Secondary prophylaxis of VL in AIDS patients with a monthly or biweekly injection of PM 4 mg/kg was proposed by Perez-Molina in 1996 . In 6 patients, this prevented relapses during the monitoring time of 3-12 months. In another 13 co-infected patients, prophylaxis did not reduce the incidence of relapses compared to a control group, but patients on prophylaxis all had lower CD4 counts. Monthly prophylaxis with PM in AIDS patients for PCP also reduced the incidence of VL. Without PM prophylaxis, VL developed in 11 out of 138 patients and with prophylaxis in 2 out of 52 patients (one primary infection and one relapse). Side effects of the prophylactic regimen were mild and transient, and PCP was effectively prevented . Apart from case studies (see table 1 & 2), no additional experience with PM prophylaxis for VL has been documented. In these case studies, side effects of biweekly or monthly regimen were not recorded. Side effects of prophylaxis of PCP with a biweekly (n=42 AIDS patients) or monthly (n=84 AIDS patients) injection of PM led only to mild side effects, except in 3 cases in a biweekly regimen (see table 2).

Intravenous PM prophylaxis has been successfully administered in ambulatory care settings. Retrospective analysis over a period of 3 years in 35 clinically stable patients receiving biweekly prophylactic or curative regimen of PM, with a minimum of monitoring (routine blood tests were done monthly and in the treatment group biweekly), was safe; and the patients experienced only mild side effects. Extra fluid was given before and after injection, with the aim to prevent a drop in blood pressure.

### 1.3.4 Summary of information on pentamidine as secondary prophylaxis:

An extensive literature review on the safety of PM indicates that a monthly injection of PM 4 mg/kg is safe, and side effects are consistently shown to be mild. This data is summarised in tables 1 and 2 , . Monthly PM is also an effective prophylaxis against PCP, and has been used successfully as secondary prophylaxis against VL.

PM can be administered both IM and IV. However, IM administration can lead to painful abscesses, might be more difficult in HIV co-infected patients in case of malnutrition, and can lead to erratic absorption. For all these reasons, IV administration (slow infusion over 1 to 2 hours) is considered for this study. In addition, adequate hydration will be ensured by increased oral fluid intake before and during the infusion.

Important PM-associated anticipated adverse events, which may occur during/shortly after administration are as follows: hypoglycaemia and hyperglycaemia (diabetes); hypotension after administration, nephrotoxicity. Diabetes has predominantly been reported with the use of pentamidine for treatment. Although the risk of diabetes is expected to be very limited with the use of PM for prevention, the risk might theoretically be increased if PM is required for prolonged periods.

## **2 METHODOLOGY**

### **2.1 OBJECTIVES**

#### **2.1.1 General objective:**

To document the effectiveness, safety and feasibility of monthly PM secondary prophylaxis (PSP) in VL/HIV co-infected patients that have documented parasite clearance after VL treatment when used for prevention of VL relapse.

#### **2.1.2 Specific objectives of the primary study period**

##### **2.1.2.1 Primary objectives**

In VL/HIV co-infected patients that have documented parasite clearance after VL treatment:

- to assess the effectiveness of PSP in terms of preventing relapse and death;
- to assess the safety of PSP in terms of drug-related serious adverse events or permanent drug discontinuations due to adverse events;
- to assess the feasibility of PSP in terms of number of patients compliant to therapy during the first year of monthly PM secondary prophylaxis.

##### **2.1.2.2 Secondary objectives;**

In VL/HIV co-infected patients that have documented parasite clearance after VL treatment:

- to assess the safety of PSP in terms of:
  - drug-related non-serious adverse events
  - serious adverse events (drug-related or not)
- to assess the feasibility of PSP in terms of:
  - number of treatment interruptions/discontinuations,
  - number of therapeutic interventions needed to treat adverse drug reactions

##### **2.1.2.3 Tertiary objectives;**

To describe a cohort VL/HIV co-infected patients that have documented parasite clearance after VL treatment and receive monthly PM secondary prophylaxis in terms of

- mortality and causes of death
- number of patients lost to follow-up or who have withdrawn the informed consent-
- HIV related parameters: CD4-counts and opportunistic infections, ARV-related clinical events (as in the investigator's opinion)
- VL related parameters: diagnostic procedures performed, clinical, immunological and laboratory evolution
- number of patients by types of non-serious and serious drug-related adverse events
- parasitological, clinical, immunological and laboratory parameters in those who relapse, drug sensitivity testing
- relationship between patient characteristics at baseline and relapse/death

### 2.1.3 Specific objectives of the extended study period

- To assess the primary, secondary and tertiary objectives as described for the primary study period over the complete 12 to 18 months treatment period.
- To assess incidence of relapse and death in a 1 year follow-up period after discontinuation of PSP.

## 2.2 DESIGN

This will be a prospective cohort study of HIV positive VL patients managed in the routine care settings of Amhara and Tigray Regions, North Ethiopia.

The study will consist of 2 periods which will be analyzed separately: the initial 12 months of PM secondary prophylaxis (main study period) and an extended treatment period of 0 to 6 months (for a total treatment period of 12 to 18 months, depending on CD4 count evolution) and subsequent 1 year follow up period (extended study period).

## 2.3 STUDY SITES

The study will be conducted in VL treatment centres found in Amhara and Tigray Regional States, using in particular the facilities of Gondar University Hospital (i.e., the Leishmaniasis Research & Treatment Centre - LRTC), Abdurafi Health Center (supported by Médecins Sans Frontières), and Kahsay Abera Hospital in Humera.

## 2.4 INTERVENTION

HIV/VL co-infected patients with documented parasite clearance after VL treatment will be administered PSP

- Dose: PM, 4mg/kg (maximum 300mg)
- Frequency: once monthly
- Mode of administration:
  - o Intravenously: slow infusion in 100 ml 5% dextrose over a minimum of one, preferably two hours in supine position
  - o Deep intramuscular injection: after decision of the treating physician, in cases with difficult venous access
  - o Patients will be encouraged to take additional fluids before and during the infusion.
- Duration: Minimum of 12 months and maximum 18 months, guided by CD4 cell count evolution.

### Procedure of pentamidine administration

Pentamidine will be given to patients included in the study during their scheduled visits. A dose of 4mg/kg (maximum 300 mg) will be calculated. The product will be prepared according to the instructions of the manufacturer: a 300mg pentamidine isethionate will be dissolved with 10 ml of water for injection. The calculated dose for the specific patient will then be further diluted in 50 to 250 ml of 5% dextrose or 0.9% saline solution. The resulting solution will be infused intravenously slowly over 60 minutes with the patient lying supine and in a fasting state. The blood pressure of the patient will be checked before the start of the infusion and the every 30 minutes until one hour after the infusion is discontinued. In case

intravenous administration is not possible, the calculated dose from 300mg pentamidine dissolved in 10 ml water for injection will be given deep intramuscularly.

## 2.5 STUDY DRUG

Pentamidine will be donated by Sanofi-Aventis, the manufacturer of the innovator product (Pentacarinat®). The procedures related to storage and management of pentamidine will be detailed in the study procedures.

Antiretroviral treatment is part of the standard HIV care within the national program and will be provided through the public ART centers as part of the routine care.

## 2.6 INCLUSION AND EXCLUSION CRITERIA

Two kinds of patient populations will be eligible for the study. First, patients that are being treated for VL during the study recruitment period will be eligible if a) they are treated for relapse or b) they manifest risk factors for relapse: documented CD4 cell count <200 cells/ $\mu$ L or WHO stage 4 disease (other than VL). In both cases, they should have a negative TOC prior to PSP initiation.

The second patient group are patients that have been treated for VL in the past, but present with risk factors for relapse (CD4 <200 or WHO stage 4 disease (other than VL) during the recruitment period of the study. These patients should have a documented negative TOC after their latest VL treatment. For these patients, a TOC prior to PSP initiation will only be performed on clinical indication.

### **Inclusion:**

Patients aged  $\geq 18$  years

**AND** confirmed HIV infection

### **AND ANY OF THE FOLLOWING**

- 1) Patients diagnosed with VL during the recruitment period that
  - a) are treated for VL relapse and have a documented negative TOCOR
  - b) are treated for primary VL and have a documented CD4 <200 or WHO stage 4 disease during the recruitment period and have a documented negative TOC
- 2) Patients treated for VL in the past with
  - a) Documented CD4 <200 or WHO stage 4 disease during the recruitment periodAND
  - b) Documented negative TOC after the latest VL treatment and currently asymptomatic OR currently negative diagnostic test (microscopy)

**AND** agreeing to start or continue ART (first or second line)

**AND** willing to provide written informed consent to participate in the study

**Exclusion:**

- 1) Patients with known hypersensitivity to PM
- 2) Patients with known renal failure (defined as creatinine >2 times the upper limit of normal for their age/gender - serum creatinine will be measured)
- 3) Patients with diabetes mellitus (type I or II; blood glucose will be measured; random or fasting depending on the patient). Diabetes is defined as a blood glucose  $\geq 11$  mmol/L (200mg/dl) at any time or fasting blood glucose  $\geq 7$ mmol/L (126mg/dl) or already diagnosed patient and on treatment.
- 4) Patients unlikely to be able to attend follow up visits and comply with the study requirements
- 5) Pregnant and lactating women
- 6) Any other condition that, according to the physician, could increase the risk of toxicity of PM to such an extent outweighing the expected benefit (eg severe cardiac dysfunction)

**2.7 VARIABLES OF INTEREST**

**2.7.1 Primary study period: first 12 months of PSP administration**

**Primary variables of interest**

The study will consist of 2 periods which will be analyzed separately: the initial 12 months of PM secondary prophylaxis (main study period) and an extended treatment period of 0 to 6 months (for a total treatment period of 12 to 18 months, depending on CD4 count evolution) and subsequent 1 year follow up period (extended study period).

1/ Effectiveness

The primary outcome for effectiveness is the time to relapse or death (all causes) within 1 year of PSP initiation.

Relapse is defined as presence of parasites in aspirates from:

- the spleen or
- bone marrow or
- lymph nodes.

Aspirates will only be taken in case of clinical suspicion of relapse based on:

- recurrence of prolonged fever (>2 weeks)
- weight loss
- splenic or hepatic enlargement

2/ Safety

The primary outcome for safety is the proportion of patients with SAEs which are possibly, probably or definitely drug-related following clinician's assessment or that lead to permanent drug discontinuations during the first year of PM administration.

3/ Feasibility

The primary outcome for feasibility is the proportion of patients that complete the first year of PSP following the protocol: i.e., completed 12 months of follow-up with at least 90% of

planned doses (i.e., 11 out of 12) administered without experiencing relapse or drug-related SAEs).

## Secondary variables of interest

### 1/ Safety:

- the proportion of patients with:
  - any drug-related non-serious adverse events (with drug-related defined as possibly, probably or definitely related to primary therapy following physicians assessment)
  - any serious adverse events (drug-related or not)
- during the first year of PSP administration

### 2/ Feasibility:

- the number of treatment interruptions will be described as:
  - number of patients who interrupted (temporary discontinuation) treatment before 12 months of PSP treatment and reasons for interruption
  - number of patients who permanently discontinued treatment before 12 months of PSP treatment and reasons for discontinuation
  - number of patients who miss any of the monthly doses and number of doses missed
- the number of clinical interventions/therapeutic procedures needed to treat adverse drug will be described as:
  - number of patients who receive additional IV fluids and indication
    - number of patients who receive additional intravenous or oral glucose preparations for suspected or confirmed hypoglycaemia and indication
    - number of patients that receive additional drugs next to PSP for management of PM-related side-effects, type of drug and indication (antihistamines, antipyretics or anti-inflammatory drugs,...)
    - hospitalisations or prolonged observation periods related to the administration of PM
    - any other unplanned interventions/procedures related to study drug administration

## Tertiary variables of interest

Tertiary variables of interest are:

- mortality and causes of death (as described by treating physician); mortality will be categorized as
  - Death related to VL or HIV-related OIs

*Documented death during hospitalization:* will be attributed to HIV-related OIs if the patient was diagnosed with an active WHO stage 3 or 4 event at the time of hospitalization and if the causal relationship is confirmed by the treating physician

*Reported death by health care staff or family:* will be attributed to HIV-related OIs if the patient had an ongoing WHO stage 3 or 4 event during the last clinic visit

- Death unrelated to VL or HIV
- HIV related parameters:
  - CD4-counts
    - every six months after PSP initiation
  - opportunistic infections: stage II, III, IV OIs collected follow WHO-definitions;
  - ARV-related (as in the Investigator's opinion) clinical events
- number of patients by types of non-serious drug-related and serious adverse events: AEs will be summarized following MedDRA coding, using "preferred" terms and "body systems"
- VL related parameters
  - Number of diagnostic procedures performed
  - Number and type of VL-related events
  - Evolution of clinical, immunological and laboratory parameters
  - Parasitological parameters in those who relapse:
    - type of aspirate (spleen, bone marrow, lymph node)
    - parasite count (aspirate, blood)
    - clinical, immunological and laboratory characterisation

## 2.7.2 Extended study period

Extended study period: complete PSP administration (12 to 18 months) and 1 year of additional follow-up after PSP discontinuation.

Variables of interest for the extended study period are similar to those of the primary study period. Safety and feasibility parameters will be recorded during the complete treatment period (12 to 18 months); effectiveness parameters (death and relapse) are collected for the complete PSP administration + 1 year of additional follow-up.

## 2.8 PROCEDURES

### 2.8.1 Patient recruitment and follow-up

#### Routine management for visceral leishmaniasis in Ethiopia.

Diagnosis of visceral leishmaniasis relies on parasitological diagnosis of tissue aspirates, most commonly splenic aspiration, although in some places (field and primary health care settings) serological tests are used as well. First line treatment mainly consists of administration of sodium stibogluconate injections for a period of one month, for which patients are usually hospitalized. However, given the high toxicity of antimonials in VL-HIV co-infected patients liposomal amphotericin-B (Ambisome) is often preferred for VL-HIV co-infection and this is the current routine practice in all three study sites. Antimonials (SSG) will be reserved for unresponsive VL-HIV co-infected patients. Upon confirmation of the VL diagnosis, HIV-testing is routinely proposed for all, CD4 testing is done if HIV-positive. The diagnosis of visceral leishmaniasis is an indication for initiation of antiretroviral treatment, according to

national guidelines (stage 4 event). Antiretroviral treatment is available for free within the national program.

The national leishmaniasis treatment guidelines do not give strict limitations for use of spleen aspiration for diagnosis and test-of-cure (TOC) in VL patients. At the lowest level of the health care system, serological diagnosis and clinical follow-up of primary VL is often used, but for the better equipped facilities (district hospitals), spleen aspiration is routinely used to diagnose VL and to document VL treatment response. Spleen aspiration has been routinely performed in VL-HIV co-infected patients in the three study sites both for diagnosis and treatment monitoring for many years. The three study sites are the main treatment sites in Ethiopia where substantial numbers of VL-HIV co-infected patients are treated.

Recruitment of the patients for the study will proceed in two steps.

During the recruitment period, files of patients diagnosed at one of the three sites with HIV-VL co-infection will be reviewed by a member of the study team to determine potential eligible candidates. Patients will then be approached to inform them about the study and to confirm their potential eligibility in terms of HIV and VL history. The specific informed consent procedure is detailed in section 8.2.

In a first stage, general inclusion and exclusion criteria (eg CD4 count < 200 cells/ $\mu$ L) will be assessed. In a second stage, a test of cure (TOC, see section 2.8.2) will only be performed in those that remain eligible after verifying in- and exclusion criteria in step 1. Patients with documented tissue parasites (positive TOC) will be excluded from the study.

At every visit, clinical and laboratory data will be collected in the source document. A detailed CRF will be provided by the Sponsor for recording the data. The following data will be collected:

- Clinical
  - o Weight/height
  - o T, BP, pulse
  - o Spleen and liver size
  - o Functional/nutritional status
  - o General examination
- Laboratory (venipuncture)
  - o FBC
  - o Creatinine, liver function tests
  - o Glucose
  - o CD4 every 6 months
  - o On some visits only (as described later in this chapter): leishmania serology, urinary leishmania antigen, leishmanin skin testing, microscopy for parasites (aspirate (spleen or exceptionally bone marrow/lymph node), blood)), amylase, ECG
- Events
  - o Adverse events (all)
  - o Side effects
  - o VL-related events
  - o Other OI/ART-related events
- Interventions
  - o PSP
  - o Additional diagnostic/therapeutic interventions

○ ART-related

Patient recruitment and follow-up is summarized in the flowchart in Figure 3. The details of activities to be carried out at each specific visit are as follows:

2.8.1.1. Screening visit (it can be spread over several days, if the patient wants to go back and discuss the consent with family or others)

- Demographic data
- Collect history of VL and HIV and treatment
- Check for inclusion/exclusion criteria
- Introducing patient information
- Informed consent interview
- Recruitment into the study if patient agrees and signs the consent form. All the remaining procedures at this visit can only be undergone after consent has been given and confirmed in written.
- Laboratory (CD4 (once), leishmania serology (once) and urine antigen (weekly)) after treatment initiation (ie the treatment for VL – not the PSP) and at the end of treatment (FBC, leishmania serology and urine antigen, glucose, creatinine, leishmanin skin test, leucocytoconcentration, pregnancy test)
- Plan the date of the TOC, taking into account that it will only be performed in those patients who remain eligible after verifying all the other inclusion and exclusion criteria

2.8.1.2. Recruitment visit (it can be the same date of the screening visit, or later)

- Review TOC result. If the TOC is positive, the patient does not continue into the study. If the TOC is negative, the patient is recruited
- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status;
- Collection of information on previous medication (including ART) and any HIV/VL related event occurred before the study
- Give follow-up visit:
  - if not on ART: one month after ART initiation
  - if on ART: one month after VL treatment completion
  - for patients treated prior to the recruitment period, PSP can be started without delay

2.8.1.3. Active treatment visit 1

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status;
- Collection of information on previous medication (including ART) and any HIV/VL related event occurred before the study
- Laboratory (FBC, creatinine, liver enzymes, glucose, CD4) unless recent test results (< 1 month old) available; pregnancy test
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- First PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

2.8.1.4. Active treatment visit 2

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)

- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Second PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.5. Active treatment visit 3

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Third PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.6. Active treatment visit 4

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, amylase, glucose, pregnancy test, ECG)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Forth PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.7. Active treatment visit 5

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Fifth PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.8. Active treatment visit 6

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, , pregnancy test, leishmania serology and urine antigen, leishmanin skin testing, leukocytoconcentration) and CD4 level)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Sixth PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.9. Active treatment visit 7

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- - Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Seventh PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.10. Active treatment visit 8

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, amylase, glucose, pregnancy test, ECG)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Eighth PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

#### 2.8.1.11. Active treatment visit 9

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Ninth PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

2.8.1.12. Active treatment visit 10

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Tenth PSP Dose
- Collection on data on drug adverse reaction at administration

2.8.1.13. Active treatment visit 11

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- Eleventh PSP Dose
- Collection on data on drug adverse reaction at administration
- Plan next visit (one month later)

2.8.1.14. Active treatment visit 12

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, glucose, LFT, amylase, pregnancy test, ECG, leishmania serology and urine antigen, leishmanin skin testing, leucocytoconcentration) and CD4 count
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- TPSP Dose
- Data will be collected on drug adverse reaction at administration if patient requires PM
- Plan next visit (one month later)

2.8.1.15. Extended treatment follow-up: visit 1 (to be repeated for a maximum of six visits; if not applicable, the patient will start immediately the follow-up period as described in the next section)

- Clinical examination (general examination, weight/height, axillary temperature, systolic and diastolic blood pressure, pulse rate, spleen and liver size, functional status, nutritional status)
- Laboratory (FBC, creatinine, LFT, glucose, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse

- Review CD4 cell count of
  - If  $CD4 \leq 200$  cells/ $\mu$ l – PSP will be continued (extended treatment study follow up) for six more months with similar procedure as the first twelve months
  - If the CD4 count is  $> 200$  cells/ $\mu$ l, PSP will be stopped and the patient will be followed in the extended study period without monthly PM injection
- Data will be collected on drug adverse reaction at administration
- Plan next visit (one month later)
- CD4 count every sixth month

#### 2.8.1.16. Extended study follow up (12 months with visits every 3 months)

- clinical examination (general examination, weight, height, axillary temperature, systolic and diastolic blood pressure, pulse rate, the spleen and liver size, functional and nutritional status)
- Laboratory (FBC, blood glucose level, pregnancy test)
- Interview on any concomitant medication taken since the previous visit and on all adverse events experienced since previous visit (including but not limited to adverse drug reactions, VL-related, other OI/ART-related, additional diagnostic/therapeutic interventions and any other serious adverse events)
- Parasitological diagnosis (TOC, blood) if there is suspicion of VL relapse
- CD4 count, leishmania serology and urine antigen only at the six-month and twelve month visits; leishmanin skin testing only at the twelve month visit.

#### 2.8.1.17. End of study visit

- Patients will be advised to continue their ART follow up and on the need of strict adherence
- Those patients who might exceptionally need extended PSP will be referred to the relevant health institute at that point in time
- All patients will be advised to seek medical care during illnesses

#### 2.8.1.18. Unscheduled visits

- The date and the reason for the visit will be recorded
- Thorough physical examination will be done (general plus specific to any patients complaints).  
Relevant laboratory evaluations if applicable (Blood film, FBC, glucose, creatinine, liver enzymes, amylase, chest X ray, ECG)
- Leishmania serology and urine antigen, parasitological diagnosis (TOC, blood) if applicable
- Document all adverse events, OI, diagnostic and therapeutic intervention or other relevant event/condition

### 2.8.2 Tissue aspiration for diagnosis or TOC

Tissue aspirates from the spleen (preferable site), lymph node or bone marrow (alternative sites) will be done for TOC (test of cure; after VL treatment, before PSP) or if the patient is suspected of having a VL relapse. The platelet count should be checked and confirmed as adequate before splenic aspiration is undertaken. In the event of severe thrombocytopenia or clinical evidence of abnormal bleeding tendency, splenic aspirate will be deferred and bone marrow aspiration will be done. At the same time, a blood smear will also be examined for parasites (after leucoconcentration).

**Procedures of tissue aspiration** (detailed in the standard operating procedures)

**Splenic aspiration:** check for contraindications (bleeding tendency, platelet count less than 40,000, hemoglobin less than 3g/dl); explain the procedure to the patient and get consent; let the patient lie supine and palpate the spleen outlining the margin. Under aseptic condition (cleaning the site with iodine, using sterile glove and drape), puncture the skin with a 20gauge needle attached to a 5 cc syringe. Form 1cc vacuum by pulling on the plunger, push the needle about 3 cm into the spleen and withdraw in a quick in-and-out jerk. Handling the syringe during procedure is only with one hand. Culture and smear the aspirated tissue immediately. Close observation of the patient for the next 24 hours will be done.

**Bone marrow aspiration:** Explain the procedure and get consent from the patient. The patient should lie supine for sternal, and laterally for puncture from iliac bone. Under aseptic condition, the site of puncture should be infiltrated with local anaesthesia (lidocaine). Puncture into the bone to put the tip of the needle into the bone marrow and aspirate with a 10cc syringe. Culture and smear the aspirate immediately, clean and dress the puncture site.

**Lymph node aspiration:** Explain the procedure for the patient and get consent. Palpate the lymph node. Under aseptic condition, puncture at the centre with a 10 cc syringe and aspirate. Pull out the needle and drain the aspirate content for culture and smear.

### 2.8.3 ARV drug interactions

No specific drug interactions with ARVs are expected in patients on currently used first line ART regimens (Zidovudine or Stavudine / Lamivudine / Nevirapine or Efavirenz). However, the use of tenofovir is increasing in Ethiopia. Since both PM and tenofovir are potentially nephrotoxic, care should be taken when both drugs are given together. However, program data from Zambia indicate that nephrotoxicity related to the use of tenofovir is rare, and usually reversible after stopping tenofovir. Patients with significant baseline kidney dysfunction are excluded from this study. Finally, the kidney function will be routinely monitored in this study. Care should also be taken with the combination of didanosine (ddI) and PM for risks of pancreas toxicity (overlapping toxicity).

### 2.8.4 Concomitant Drug Therapies

Administration of ceftriaxone or ceftazidime using the infusion of the PM solution should be avoided as they are incompatible, but can be administered via other IV site. Patients should be strictly advised to avoid alcohol consumption as it will cause CNS depression and aggravate hypoglycemia.

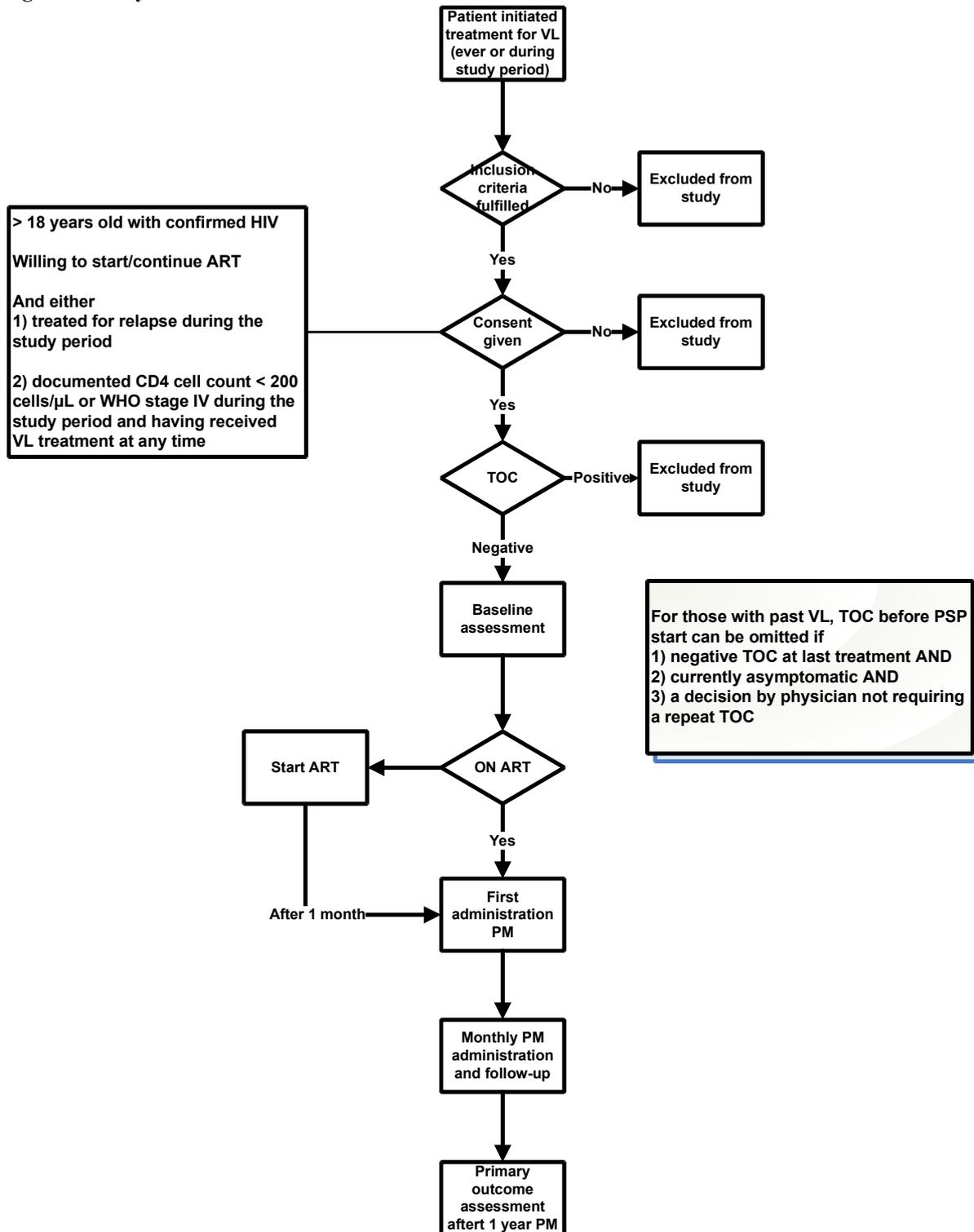
Patients with underlying cardiac disease or taking antiarrhythmic drugs (cisapride, sparfloxacin, gatifloxacin, moxifloxacin, pimozide, and type Ia and type III antiarrhythmics) should be closely followed with ECG monitoring for possible aggravation of QT prolongation. Most of these drugs are not used in Ethiopia. The management of patients taking any of these medications will be discussed on a case-by-case basis with the Field Coordinating Investigator prior to inclusion and during study follow-up.

### 2.8.5 Operational aspects and clinical management

All practical aspects and issues related to the trial management will be detailed in standard operating procedures, provided by the Sponsor. In addition, study-specific SOPs will be

written by the study team detailing the study clinical and laboratory procedures, including the administration of PM, the invasive diagnostic procedures (splenic aspiration), the laboratory procedures and the clinical measurements.

Figure 3. Study flow chart



**Table 3. Study visits for the primary outcome (within 1 year of PSP initiation)**

	Screening	Inclusion study+ start PSP	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M1 0	M1 1
<b>Consent</b>	X												
<b>Verification inclusion criteria</b>	X												
<b>TOC*</b>	X												
<b>Baseline assessment</b>		X											
<b>PM administration</b>		X	X	X	X	X	X	X	X	X	X	X	X
<b>Clinical investigation</b>		X	X	X	X	X	X	X	X	X	X	X	X
<b>Adverse event checklist</b>		X	X	X	X	X	X	X	X	X	X	X	X
<b>Laboratory investigation</b>	X	X	X	X	X	X	X	X	X	X	X	X	X
<b>CD4 cell count</b>	X							X					

\*Additional TOCs will be done on clinical indication (suspicion of relapse).

Additionally patients will also be assessed whenever they have new complains and come on unscheduled visits

## 2.9 Adverse events definitions and reporting

At each visit, the Investigator will ascertain the occurrence of any adverse events since the last visit. Any event must be documented in the source documents and entered in the CRF. An event is defined as an SAE when it meets one of the pre-defined outcomes.

### 2.9.1 Adverse Event general definition

An adverse event is any untoward medical occurrence (any unfavourable and unintended sign, symptom or disease, including an abnormal laboratory finding) in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of an AE **include**:

1. Significant or unexpected worsening or exacerbation of the condition under study.
2. Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
3. New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
4. Signs, symptoms, or the clinical sequelae of a suspected interaction.
5. Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).
6. Significant failure of expected pharmacological or biological action.

Examples of an AE **do NOT include** a/an:

1. Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
2. Anticipated day-to-day fluctuations of pre-existing chronic disease(s) or condition(s) present or detected at the start of the study that do not worsen.

### 2.9.2 Severity of event

The severity of a clinical adverse event is to be scored by the reporting Investigator according to the following scale:

- 1 Mild Awareness of sign or symptom, but easily tolerated
- 2 Moderate Discomfort enough to cause interference with usual activity
- 3 Severe Incapacitating with inability to work or perform usual activity
- 4 Life-threatening Patients at risk of death at the time of the event

### 2.9.3 Adverse Event causality assessment

The PI's will use clinical judgment to determine the degree of certainty with which the 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.

The relationship of an adverse event to study drug is to be assessed according to the following definitions:

1       Definitely unrelated

It should be reserved for those events which occur prior to test drug administration (e.g., washout or single-blind placebo) or for those events which cannot be even remotely related to study participation (e.g. injury caused by a third party).

2       Unlikely

There is no reasonable temporal association between the study drug and the suspected event and the event could have been produced by the subject's clinical state or other modes of therapy administered to the subject.

3       Possible

The suspected adverse event may or may not follow a reasonable temporal sequence from study drug administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the subject's clinical state or by other modes of therapy concomitantly administered to the subject.

4       Probable

The suspected adverse event follows a reasonable temporal sequence from study drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

5       Definitely related

It should be reserved for those events which have no uncertainty in their relationship to test drug administration: this means that a re-challenge was positive.

The outcome of each AE must be assessed according to the following classification:

- Completely recovered : The patient has fully recovered with no observable residual effects
- Not yet completely recovered : Improvement in the patient's condition has occurred, but the patient still has some residual effects
- Deterioration : The patient's overall condition has worsened
- Permanent damage : The AE has resulted in a permanent impairment
- Death : The patient died due to the AE
- Ongoing : The AE has not resolved and remains the same as at onset
- Unknown : The outcome of the AE is not known because the patient did not return for follow-up (lost to follow-up)

#### **2.9.4 Serious Adverse Events definition and reporting**

A serious adverse event (experience) (SAE) or reaction is any untoward medical occurrence that at any dose fulfils at least one of the following criteria:

- \* results in death.
- \* is life-threatening.
- \* requires hospitalization or prolongation of existing hospitalization.

*NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is an SAE. When in doubt as to whether "hospitalization" occurred or was necessary, the event should be considered an SAE. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE, nor hospitalization for non-medical reasons (e.g., the patient stays at the hospital overnight because (s)he lives too far and/or there is no transport).*

- \* results in disability/incapacity, or
- \* 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.

**All Serious Adverse Events, whether or not deemed drug-related, or expected, must be reported immediately or within 24 hours (one working day), using the Serious Adverse Event Notification Form attached to the CRF, by telefax or e-mail to:**

**Clinical Trials Unit  
Institute of Tropical Medicine  
Nationalestraat 155**

**B-2000 Antwerp, Belgium**

**Fax: + 32 3 247 66 47**

**Email: [pharmacovigilance@itg.be](mailto:pharmacovigilance@itg.be)**

The fax cover page and/or the e-mail title should state “**Urgent Serious Adverse Event ITMCO0109**”.

The sponsor is responsible to inform the ITM Institutional Review Board and the Ethics Committee of the Antwerp University Teaching Hospital by means of aggregated data on a quarterly basis.

Each Principal Investigator is responsible to report the SAE to the field coordinating investigator, who will inform the relevant Institutional Ethics Committees and the Ethiopian Regulatory Authorities immediately or according to the deadline established by each Ethics Committee.

SAEs will be recorded every three months after the last PSP dose, up to the end of the study.

The full reporting procedure is detailed in the CTU SOP on Reporting of Serious Adverse Events, which will be used throughout the study.

#### **2.9.5. Reporting of non-serious Adverse Events**

All other AE's not fulfilling the criteria of immediate reporting must be documented in the source documents and entered in the adverse event form in the Case Report Form. The following information will be recorded for all adverse events:

- 1) Diagnosis
- 2) Date of event onset
- 3) Date event resolved
- 4) Outcome of the event
- 5) Severity of the event
- 6) Possible relationship of the event to study medication
- 7) Possible relation of the event to VL
- 8) Is the event serious?
- 9) Action taken

The severity grading scale, based on toxicity grading scales developed by the WHO and the National Institutes of Health, Division of Microbiology and Infectious Diseases, will be used to grade severity of all symptoms, physical exam findings, and laboratory results.

Any new event, or an event present at baseline that is increasing in severity, will be considered as an adverse event.

AEs possibly related to PM will be recorded until 12 months after the last PM administration.

#### **2.9.6. Adverse Events expected in relation to pentamidine**

After each administration a checklist will be used to assess the possible relationship between pentamidine and adverse events:

- hypoglycaemia, defined as serum glucose < 55 mg/dL (< 3 umol/L)

- hypotension after administration defined as diastolic blood pressure < 60 mm Hg
- hyperglycaemia (diabetes) defined as random blood glucose  $\geq$  200 mg/dL ( $\geq$  11 umol/L) or fasting serum glucose  $\geq$  126 mg/dL ( $\geq$  7 umol/L).
- extravasation and tissue necrosis at IV infusion site
  
- Additional treatment procedures/therapeutic interventions during/after pentamidine administration are defined as any procedure/intervention occurring outside of the standard procedure. This includes:
  - IV perfusion initiated after PM administration (and indication/outcome)
    - Short term observation (< 6 hours) at the clinic/hospital (and indication/outcome)
    - Extra administration of intravenous or oral glucose preparations and indication/outcome
    - Slowing of the speed of infusion
      - Reason: due to subjective complaints or objective signs or other
      - Outcome
    - Administration of drugs (eg antihistamines, antipyretics or anti-inflammatory drugs, or other) and indication
    - Any other event/action occurring outside of the standard procedure

During follow-up visits or unscheduled visits:

- AE's will be recorded in the CRF.
- SAEs will be reported as described above
- Blood test results will be reviewed: abnormal laboratory (hematology and biochemistry) results will be reported as adverse events if the abnormality occurs or worsens after institution of the study treatment, and if they require clinical intervention or further investigation, unless they are associated with an already reported clinical event.

## 2.10 Discontinuation of PSP

Patients can be withdrawn from treatment on the decision of the Investigator, either due to failure of treatment (i.e. parasitological relapse) or lack of tolerability.

Pentamidine should be discontinued in particular if any of the following occurs:

- A relapse (signs and symptoms of VL with a positive tissue aspiration) of VL occurs during PSP.
- Once the CD4 count has reached at least 200 cells/ $\mu$ l for at least 6 months.
- Once the patients has been relapse-free for 18 months. However, at the discretion of the treating physician, PSP can be continued after 18 months on a case by case basis.”
- The patient has intolerance of PM and the physician or the patient request discontinuation
- In case of pregnancy, the study physician will decide on whether to continue or discontinue PM, weighing expected harm against expected benefit (further elaborated in the section on Ethics).

At the close out of the study, patients still in need of PSP will be referred to local health facilities for further follow-up

All patients who had to interrupt the treatment will receive follow-up according to the protocol even if withdrawn from therapy, unless they refuse any further participation in the study.

If a patient has an interval of >60 days between pentamidine doses on two occasions, or >90 days between doses on one occasion, patients should be carefully assessed to rule out relapse prior to restarting PSP, including the possibility of performing a TOC.

### **3 CLINICAL RECORD FORM AND DATA MANAGEMENT**

All data and observations initially documented in the source documents must be entered in the Case Report Form. For this study, a paper CRF will be designed as well as a database, where patients will be identified only by initials, date of birth and a study code. These activities will be carried out by the Coordinating Investigator-Ethiopia with the guidance and technical support of the Data Managers at the CTU of the ITM.

The data entry will be performed by a trained data entry clerk under the supervision of field Coordinating Investigator either at the central site at Gondar (with CRFs sent there) or at each recruiting site under the supervision of the PI. Datasets will be cleaned before the planned analyses, e.g. at the end of the first 12 months of PSP treatment and at the end of the study. Regular consistency checks will be done and queries will be produced to be answered by the local investigators. This will allow the rapid identification of potential problems. Besides the central management, monitors will periodically visits the sites and check the information entered in the CRF against the source documents available on site. The final database will be obtained after the resolution of all queries.

The multi-centre database will be managed and held at Gondar by the Coordinating Investigator-Ethiopia (with the guidance and technical support of the Data Managers at the CTU of the ITM). Data entry and review will be performed following the Data Entry Guidelines and the Data Management plan agreed with the Sponsor. The final database of each of the two study periods should be locked no later than three months after the last visit has been performed.

The investigators must ensure that subjects' anonymity will be maintained and that their identities are protected from unauthorised parties. Subjects should not be identified by their names, but by an identification code. The investigator should keep a subject identification log showing codes, names, and addresses, in a protected place. The investigator should maintain the study documents (particularly the subject's written consent forms), in strict confidence. The database will be pass-word protected and access restricted to the data management team.

## **4 DATA ANALYSIS**

### **4.1 Study Objectives and Hypothesis**

The aim of the study is to document the effectiveness, safety and feasibility of monthly PM secondary prophylaxis (PSP) in VL/HIV co-infected patients that have documented parasite

clearance after VL treatment. As a consequence, the study is descriptive and no statistical hypotheses will be tested. A specific list of objective is in 2.1.2.

## **4.2 Variables of interest**

All variables of interest are described in section 2.5.

## **4.3 Statistical Methods**

In general, all variables of interest will be described in term of standard summary statistics (mean, proportions, medians). Confidence intervals (two-sided, 95%) will be estimated for primary and secondary variables of interest, but not for tertiary variables of interest. In general, all analyses will be done for all 3 sites pooled. Some data may be described for each site separately, but no confidence intervals will be calculated within sites, nor will differences between sites be statistically tested.

The study consists of two study periods. The first study period, consisting of the first 12 months of PSP treatment will be analyzed separately and results of this period will be made available as soon as possible after completion of this study period for all patients. Results of the complete treatment period (12 to 18 months) and the 1 year follow-up period after PSP discontinuation will be described after completion of the complete study for all patients.

Similar methods will be used for analysis of primary and extended study periods.

Given the limited statistical complexity of this study it is not expected that a statistical analysis plan will be prepared. The statistical methods used will be detailed in the analysis report.

### **4.3.1. Description of study population**

The study population will be described using means and standard deviations, median and interquartile ranges, and counts and proportions. All patients who received at least 1 dose of PSP will be included in all analyses.

### **4.3.2. Description of effectiveness**

Effectiveness of PSP will be described in terms of proportion of patients free from relapse or death at 6, 12, 18, 24, and 36 months after starting prophylaxis (i.e., during and after PSP). In addition, in those successfully completing 12 to 18 months of PSP, the relapse and death rate at 3, 6, and 12 months after PSP discontinuation will be estimated.

All proportions and 95% confidence intervals will be estimated using survival (Kaplan-Meier) analysis (time to relapse, or death of any cause). Data from patients who discontinued early from study drug but remained in follow-up will be included in all analyses. If the proportion of patient who discontinue is substantial, this may however lead to over-estimating the PSP effectiveness or under-estimating the risks involved. To assess the impact of discontinuations, we will perform sensitivity analyses, for example excluding those who discontinue early from

the study or including those who discontinue as treatment failures. Details of this sensitivity analysis will be included in the data analysis plan.

For the purpose of statistical analysis patients were defined as lost to follow-up if they did not attend the clinic within the 3 months preceding the analysis time point (e.g., 1 year time point after start of PSP or 1 year after end PSP) and not known to have died, had experienced relapse, or been transferred out. Patients lost to follow-up for reasons known to be unrelated to treatment, leishmaniasis, or HIV (e.g. patients transferred or moved out of the area) will be considered censored at time of last contact. Patients lost-to-follow-up for reasons possibly related to treatment (e.g. patient refusal) or unknown reasons will be considered censored in the main study analysis, but will be included in those with events in a "worst-case" sensitivity analysis.

#### **4.3.3. Description of safety**

Safety will be described in terms of counts of patients with serious and/or drug-related adverse events or adverse events that lead to study drug discontinuation. Non-serious adverse events definitely or probably unrelated to study drug in the physicians opinion will not be presented. Proportions will be presented together with 95% confidence intervals estimated using Wilson's score method for the primary and secondary safety variables of interest. The denominator for all safety analyses will be the number of patients who received at least a single dose of study drug (all patients treated analysis).

#### **4.3.4. Description of feasibility**

Feasibility will be described in terms of proportions (and 95% confidence interval, estimated using Wilson's score method) of patients who complete the first year of treatment following protocol and in terms of numbers of patients with treatment interruptions/discontinuations or who need of clinical interventions or therapeutic procedures.

For the primary feasibility analysis, the denominator will be the number of patients who received at least a single dose of study drug and who were not transferred out of the study site during the first year of PSP. Patients who died due to reasons definitely unrelated to study drug, VL or HIV (e.g., accidental deaths) may also be excluded from this analysis.

#### **4.3.5. Other endpoints**

Other endpoints will be described in terms of proportions of patients and (arithmetic or geometric) means or medians (and IQR) as appropriate.

#### **4.3.6. Multiplicity adjustments and missing data**

As all analyses are descriptive no multiplicity adjustments will be done for repeated analyses (primary, extended study period) or multiple endpoints. No special procedures are planned to

correct for or impute missing data, apart from the handling of lost-to-follow-up described in the effectiveness analysis section above.

#### 4.4 Sample size

As this is an estimation study, the sample size is calculated for the required precision (expressed as the half-width of the 95% confidence interval or equivalently 2 times the standard error) of the estimates of the primary effectiveness, safety and tolerability parameters.

We required a precision of estimates of effectiveness (main analysis) of 10% or better, of the main safety parameters of 7.5% or better, and a precision of 12.5% or better for the "worst-case" (i.e., including lost-to-follow-up as failures) effectiveness and feasibility analysis.

We estimate:

- The failure rate (main analysis) to be 20%. We would require 65 patients to estimate this proportion to within 10%.
- No more than 10% of patients to experience drug-related SAEs or to discontinue PSP treatment due to AEs. We would require 67 patients to estimate this proportion to within 7.5%.
- The failure rate (worst-case analysis) to be 40%. We would require 62 patients to estimate this proportion to within 12.5%.
- At least 50% of patient to complete 1 year of PSP treatment following the protocol. We would require 64 patients to estimate this proportion to within 12.5%.

As a consequence, the required sample size is at minimum 65 evaluable patients for the main effectiveness analysis. As in this analysis patients who are lost-to-follow are censored, we will plan to recruit an additional 7 (10%) patients for a total of 72 patients.

## 5 STUDY DURATION

It is expected that the required sample size of 72 patients can be recruited within 1 and a half year in the 3 study sites. The treatment period is 12 to 18 months and additional follow up of 1 year. This brings the total study duration to 4 year. Primary study analysis can be performed after 1 year of treatment for all patients, i.e., after 2.5 years.

Patient recruitment updates will be sent on a monthly basis by each PI to the Field Coordinating Investigator and to the CTU, using standard Excel reporting forms. If recruitment rates are slower than expected additional sites may be included in the study, an on-site assessment of their suitability and after approval by the concerned EC and Regulatory Authorities. If the recruitment target of at least 80% of the required sample size cannot be reached, the data will be presented as a descriptive case series without statistical analysis.

Earlier termination of the study will be jointly decided by the coordinating investigators and the sponsor. It could be due to safety reasons based on the recommendation of the DSMB, or it could be decided in case an insufficient recruitment rate makes the study objective unattainable. In case of early termination all the recruited patients will be followed up by the study team according to their needs.

## **6 QUALITY ASSURANCE AND QUALITY CONTROL PROCEDURES**

The task of the Monitor, who is appointed by the sponsor, is to verify the conduct of the study through frequent contacts by phone and in person with the Principal Investigator and site staff, in accordance with applicable regulations, Good Clinical Practice requirements and study-specific Standard Operating Procedures. The objectives and specific tasks of the Monitor are described in the ICH Guidelines E6. The monitoring visits will enable the Monitor to maintain current, personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct of the study with the Investigator.

The monitoring will be detailed in a specific SOP. Each site will be visited by the clinical monitor before the study is started (“pre-study visit”), to assess the site suitability for the study. In addition, it will be visited at the beginning of the study (“site initiation visit”) and at least 3 times during the conduct of the trial, plus a “close-out visit” (or “end of trial visit”) after the last patient has completed the follow up and the database has been locked. The left over trial medication will be disposed according to the regulations in the presence of a representative from the hospital pharmacy or if needed from the regulatory authority (this will be checked at the close-out visit).

After completion of the trial, a final report will be prepared, signed and dated by the coordinating investigators. The report will be submitted to the relevant EC bodies and regulatory authorities before any publication.

The Principal Investigator must ensure that source documents are maintained for each patient in the study, consisting in case and visit notes (hospital or clinic medical records) containing the Investigator’s copy of the signed informed consent, demographic and medical information, laboratory data, and the results of any other tests or assessments, according to applicable GCP requirements. All information in the CRF must be traceable to these source documents in the patient’s file. The investigator must give the monitor, who is bound by a confidentiality agreement to protect patients’ confidentiality, access to all relevant source documents to confirm their consistency with the CRF entries. We refer to the relevant SOP for further details. Further adaptations to the specific context can be made after the pre-study visit.

The Principal Investigators - by signing this protocol - declare that he/she will permit trial-related monitoring, external audits, Independent Ethic Committee review, and regulatory inspections, providing direct access to source data/documents as well as any other trial related documentation.

The Principal Investigators agree to conduct the present study in full agreement with the principles of the “Declaration of Helsinki” as amended in Seoul in 2008 (see Appendix 3) and any subsequent versions.

## **7 PROTOCOL AMENDMENTS**

Once the final clinical protocol has been issued and signed by the Investigator and the authorised signatories, it cannot be informally altered. Clinical protocol amendments are alterations to a legal document (the clinical protocol) and have the same legal status and must pass through the appropriate steps before being implemented.

Any substantial change must be approved by all the Bodies and Ethics Committees that have approved the protocol (see next section for details) prior to be effective, unless it is due to

safety concerns (in which case immediate implementation can be needed for the sake of patient's protection).

Administrative changes need only notification to the Ethics Committees without approval.

The Investigators cannot change the clinical protocol without prior discussion with the Sponsor, which should give its approval, and before approval of the concerned Ethics Committees and Regulatory Authorities.

## **8 ETHICS**

### **8.1 Regulatory Authorities and Ethical Review Committee**

This protocol and any subsequent amendments will be submitted for approval to the Ethics Committee of Gondar University, to the Amhara Regional Health Bureau, Tigray Regional Health Bureau, the National Ethics Review Committee (NERC) of Ethiopia and to the Food, Medicine and Health care Administration and Control Authority (FMHACA) in Ethiopia. The study will be also submitted for approval to the ITM Institutional Review Board and to the Ethics Committee of the Antwerp University Teaching Hospital, and to the Ethical Review Board of MSF (for the MSF Operational Centre Amsterdam).

Patients can be screened and enrolled only after formal approval from all respective Ethics Committees and Regulatory Authorities has been obtained and documented.

Copy of the national Competent Health Authority and of the national IEC/IRB approvals will be transmitted from the Investigator to the Sponsor as soon as obtained and in any case before starting recruitment.

### **8.2 Informed Consent**

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, as described in the E6 ICH GCP Guidelines.

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 Amharic and Tigrigna, and back to English for the accuracy of proper translation, with certification after verifying the original and back-translated copy.

Individuals potentially eligible for the study (i.e. individuals diagnosed with VL and HIV co-infection) will be informed on the study by the PI or his/her research assistant. Additional information on the eligibility criteria will be obtained (e.g. number of relapses) if missing in the patient file. Once eligibility has been confirmed, the PI or research assistant (RA) will ask if the potential participant is ready to decide whether or not to join the trial. Patients must be given enough time to ask questions and to take a free decision (including a time frame of a few days to discuss their participation with family members). If the individual is willing to participate, he/she will be given the ICF and the content will be read by the PI or RA. Once checked the ICF is understood, the PI or RA will invite the participant to sign the form, add his/her name (or thumb-printing whenever they are illiterate) and countersign it.

If a patient (or guardian, if applicable) is unable to read or write, an independent witness must be present during the informed consent interview and the signature of the independent witness

will be obtained, in addition to the patients' thumbprint. For individuals incapable of providing informed consent for other reasons (e.g. mentally disabled), permission is obtained from a legally authorized representative in accordance with applicable law.

### **8.3 Other ethical issues**

#### Confidentiality

All patients' data will be anonymized in the CRF and database. The documents which can identify the patients, e.g. the ICF's, laboratory print-outs and medical record, will be accessible to monitors, auditors and inspectors only under confidentiality agreements.

#### Sample collection

Blood or tissue samples will be taken from patients at the time of diagnosis, on completion of primary treatment, during assessment of eligibility, during assessment/test of cure, any time clinical indications for treatment failure are noted, as described in this protocol; and in schedules set by the routine ART follow-up. These data will also be anonymized. The invasive diagnostic methods used in the study (spleen aspiration, TOC) are those used in normal clinical practice in the three sites when treating patients with VL. For this study, only exceptionally a spleen aspirate may be needed to demonstrate parasitological cure prior to PSP initiation, whereas this would not be done if PSP would not be offered (e.g. the exceptional HIV/VL patient having to interrupt (second line) VL treatment for drug-intolerance but being clinically asymptomatic might not have a TOC performed in routine care, but would need a TOC prior to PSP). For this reason, we still mentioned it as a study-related procedure, although we expect that the additional number of interventions needed due to the study to be very limited.

Samples will be stored in Ethiopia for the duration of the study for quality control purposes (internal and external). In addition, they might be used for additional tests, directly related to the research topic, that are currently not available in Ethiopia (like HIV viral load measurements) but might become available during the study. For any of these, an amendment will be submitted to all Ethics Review Boards involved. All samples will be destroyed one year after the end of the study.

Patients will experience some pain during splenic/bone marrow aspiration for parasitology and while blood is drawn during venipuncture. The amount of blood to be drawn will be 10 ml at the routine evaluation points (PM administration) and 20 ml at the start of the study and once every six months while receiving PM.

#### Reimbursement

Patients and the family member/caregiver accompanying them will be reimbursed for travel to and from the study site but there will be no payment for trial participation. Any medication for treatment of HIV and visceral leishmaniasis that is required during the trial period, hospitalisation during VL treatment and visits for intercurrent illnesses will be provided free of charge to the patient. Food during the in-patient treatment phase will also be provided free of charge.

### Risk-benefit

In terms of risk-benefit ratio, the following risk/discomfort has to be considered: 1) risks related to diagnostic procedures; 2) discomfort of blood sample collection and monthly visits for pentamidine administration; 3) potential side-effects related to pentamidine administration 4) possible negative interaction with the antiretroviral treatment.

Spleen aspiration may result in internal bleeding. This may occur as a complication in about 1 out of 1,000 patients and potentially be fatal. If, before tissue collection, blood tests indicate an increased risk of bleeding, a lymph node or bone marrow aspiration will be performed instead (using a local anaesthetic to reduce the pain of this procedure).

Persistent side-effects like diabetes are expected to be very rare. However, in the event of, we commit ourselves to direct patients to the relevant health care facilities for ongoing care after the end of the trial. Other potential side-effects have been detailed in the Background section of the protocol. Overall, the expected side-effects are well-defined. Based on experience from other countries with the monthly injections, as for this study, we expect that few patients will have had to interrupt the pentamidine injections because of side-effects (experience severe drug-related side-effects).

Although no specific drug interactions (pharmacokinetic or pharmacodynamic) of pentamidine and antiretrovirals that could result in reduced efficacy of antiretrovirals have been described in previous studies, these cannot be entirely ruled out, since this topic has not been extensively studied.

The following benefits should be considered: 1) possible reduction in relapse rate; 2) improved and close follow-up for VL care and treatment. As mentioned in the Background, VL-HIV infected patients have an overall poor treatment response and prognosis. International guidelines recommend secondary prophylaxis for VL-HIV co-infected patients, including with drugs like pentamidine, to prevent VL relapse. Compared to routine clinical care, the in-depth and accurate monitoring after leishmaniasis treatment by the participation in the study could also be considered a potential benefit. Overall, we consider the risk/benefit ratio to be favourable, with a high likelihood of improving patient outcomes with relatively limited risks.

### Other issues

The intervention consists of the administration of PM for a maximum duration of 18 months. However, if during the extended follow-up period, a patient might become eligible again (e.g. declining CD4 cell count), PSP can be reinitiated on a case by case bases, as judged in the interest of the patient by the study physician, within the study.

There is no evidence of the safety of pentamidine isethionate in any form of administration, during human pregnancy. Because the results of animal studies have suggested the occurrence of fetal toxicity, treatment with pentamidine isethionate during pregnancy and lactation should be avoided, unless it is considered essential. For this reason, this is an exclusion criterion for enrolment in the study. Women will be counselled at each visit on this topic and contraception offered.

If a women falls pregnant while receiving PSP, this would consequently be a reason to interrupt PSP. However, a number of other factors have to be taken into consideration including

- 1) the clinical condition of the patient also needs to be taken into consideration (eg if risk of relapse would be considered to be high)
- 2) the presumed benefit the patient is having from PSP
- 3) the actual tolerance of PSP
- 4) the phase of the pregnancy when detected (first or second trimester)
- 5) the perspective of the patient

For these reasons, discontinuation of PSP has to be decided on a case-by-case basis, with discontinuation of PSP unless the potential disadvantages of discontinuation outweigh the expected benefits. This should be based on discussions involving the patient (and family), the PI and the coordinating investigators. The latter can also decide to contact the DSMB for advice.

We note that, based on the previous experience of VL care in Ethiopia, we expect very few VL-HIV co-infected women to present for care. Pregnancy testing will be routinely done throughout the study.

Additional investigations outside of the planned routine monitoring (eg ECG, amylase) will be performed, as indicated by the clinical condition. If these tests are not available at the trial site, patients will be referred to the adequate health care facilities for these investigations. These tests will be paid for by the research project.

#### **8.4 Community participation:**

The study will be sponsored by the ITM and implemented as a collaborative effort of Ethiopian research institutes (Addis Ababa University and Gondar University), regional research institutes (Leishmania East Africa Platform (LEAP)), DNDi and MSF-Holland. The Ministry of Health at local and national level will be consulted prior to implementation, and the research findings will be discussed with relevant Ministry of Health bodies on completion of the study. The community of people living with HIV / AIDS (PLWHA) and VL co-infection will be informed of the study and its rationale. This will be done through open meetings with PLWHAs. The results will be shared with PLWHAs and local health professionals upon completion.

The study is expected to directly benefit the study community as pentamidine secondary prophylaxis may protect from relapse of VL and increase the effectiveness of HAART, and thus increase longevity in HIV/VL co-infected patients. Study results will contribute to improved guidelines for management of HIV/VL co-infection in Ethiopia.

## **9 INSURANCE AND LIABILITY**

A no-fault liability insurance will be taken by the Sponsor and will cover all trial sites, involved research staff as well as trial subjects with regard to the study related activities.

It will be a responsibility of the study team to inform the Sponsor when a patient wishes to contact the insurer and to ensure that the contact is efficient. Each PI may decide to delegate

officially this task to a qualified member of his/her staff or a community member. This is also an issue that will be addressed during the trainings for the study team prior to the study initiation, in the sense that PIs should be vigilant and pro-active in identifying study-related harm. Any indication of potential harm should be discussed with the coordinating investigators, who will communicate this to a dedicated contact at the sponsor. This will also be routinely addressed and re-emphasized during the visits of the study monitors.

## **10 REPORTING AND PUBLICATION**

The trial will be registered with a recognised clinical trial registry before the start of the trial.

It is anticipated that the results of this study will be of sufficient medical importance to warrant publication in an international peer-reviewed journal. Before publication, study results are considered confidential and should not be disseminated to third parties in any form (including abstracts or manuscripts for publication) by the investigators or members of the study team without an appropriate confidentiality agreement and prior to discussion with, and approval from the sponsor of the study.

The data will be analysed jointly with all the research partners, and the publication and communication strategy and content will also be jointly agreed upon by all research partners. The sponsor will ensure that all those who have significantly been involved in the study (according to ICMJE guidelines) will be included as co-authors in the main publication of the trial findings. Given his key role in this study and given the fact that this study is part of his PhD, the Coordinating Investigator-Ethiopia will be first author for the main study paper reporting on the treatment outcomes with PSP, under the condition that he continues to act as coordinating investigator during the study. This refers to the main paper on the study outcomes and not to possible other publications on other aspects of the study.

## **11 SCIENTIFIC COMMITTEE**

The scientific committee consists of the representatives of the main research partners (Ermias Diro (GU), Asrat Hailu (AAU/DNDi), Koert Ritmeijer (MSF) and Johan van Griensven (ITM) and will participate in decision-making on any strategical and scientific issues related to the protocol. The SC has responsibility for protecting the scientific conduct and integrity of the trial. Its functions include:

- Review of the protocol before ethical approval,
- Formulation of recommendation for any change in the design and operations of the trial during the course of the trial, when needed.

## **12 DATA SAFETY MONITORING BOARD**

Even if this is an open-label, case series study on secondary prophylaxis of VL, using a known anti-leishmanial agent, an independent Data Safety Monitoring Board (DSMB) will be set up, for independent advice on the safety-aspects of the protocol. The roles and responsibilities of the DSMB, which will comprise of three Ethiopian experts, will be defined in a DSMB Charter, which will be signed by the members of the DSMB.

### **13 ARCHIVING**

The relevant study documents are those documents which individually and collectively permit to assess the conduct of the trial, the quality of the data produced and the compliance with GCP standards and applicable regulatory requirements. The PI's file on site should at least contain all the essential documents as listed in the Sponsor's SOP "Set up and maintenance of the Investigator Trial File". A copy of all source data and CRF's must always be kept on site.

The PI is responsible for ensuring a secure and appropriate location for his investigator's file and any other trial related documentation present at site, as well as for ensuring that only site staff that is competent and delegated to work for the trial has got access to the files.

All the relevant study documentation present at all partners involved should be retained for a minimum of five years and according to the applicable National Legislation. The Sponsor should always be informed prior to destruction of the files.

After completion of the study, the IF will remain available for internal audits and/or inspections of regulatory authorities for a period of twenty years, unless differently requested by national authorities.

### **14 INVESTIGATOR RESPONSIBILITY**

Except where the Principal Investigator's signature is specifically required, it is understood that the term "Investigator" as used in this protocol and in the e-CRFs refers to the Principal Investigator or a physician, member of the staff that the Investigator designates to perform a certain duty under this protocol (co-Investigator). The Investigator is ultimately responsible for the proper conduct of all aspects of the study at his/her site. For all other relevant Investigator responsibilities see "CPMP/ICH/135/95 Topic E6 - Guideline for Good Clinical Practice", Chapter 4

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## **Annex 1: WHO CLINICAL STAGING OF HIV/AIDS AND HIV/AIDS CASE DEFINITIONS FOR SURVEILLANCE - AFRICAN REGION**

(<http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf>)

### **Adults: Clinical stage 4**

**Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations**

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)
- Oesophageal candidiasis
- Extrapulmonary TB
- Kaposi's sarcoma
- Central nervous system (CNS) toxoplasmosis
- HIV encephalopathy

**Conditions where confirmatory diagnostic testing is necessary:**

- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy (PML)
- Candida of trachea, bronchi or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
- Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
- Recurrent non-typhoidal salmonella septicaemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Visceral leishmaniasis

### **Children: Clinical Stage 4**

**Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations**

- Unexplained severe wasting or severe malnutrition not adequately responding to standard therapy
- Pneumocystis pneumonia
- Recurrent severe presumed bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)
- Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration)
- Extrapulmonary TB
- Kaposi's sarcoma
- Oesophageal candidiasis
- CNS toxoplasmosis (outside the neonatal period)
- HIV encephalopathy

**Conditions where confirmatory diagnostic testing is necessary**

- CMV infection (CMV retinitis or infection of organs other than liver, spleen or lymph nodes; onset at age one month or more)
- Extrapulmonary cryptococcosis including meningitis
- Any disseminated endemic mycosis (e.g. extrapulmonary histoplasmosis, coccidiomycosis, penicilliosis)
- Cryptosporidiosis
- Isosporiasis
- Disseminated non-tuberculous mycobacteria infection
- Candida of trachea, bronchi or lungs
- Visceral herpes simplex infection
- Acquired HIV associated rectal fistula
- Cerebral or B cell non-Hodgkin lymphoma
- Progressive multifocal leukoencephalopathy (PML)
- HIV-associated cardiomyopathy or HIV-associated nephropathy

## **ANNEX 2.**

# **WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI Ethical Principles for Medical Research Involving Human Subjects**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:  
29th WMA General Assembly, Tokyo, Japan, October 1975  
35th WMA General Assembly, Venice, Italy, October 1983  
41st WMA General Assembly, Hong Kong, September 1989  
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996  
52nd WMA General Assembly, Edinburgh, Scotland, October 2000  
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)  
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)  
59th WMA General Assembly, Seoul, October 2008

## **A. INTRODUCTION**

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

The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse

consent for themselves and those who may be vulnerable to coercion or undue influence.

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

## **B. PRINCIPLES FOR ALL MEDICAL RESEARCH**

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

- 18.** Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
- 19.** Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
- 20.** Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
- 21.** Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
- 22.** Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
- 23.** Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
- 24.** In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
- 25.** For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
- 26.** When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
- 27.** For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is

intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

### **C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE**

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
  - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
  - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it,

for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

- 34.** The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
- 35.** In the treatment of a patient where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

## ANNEX 3: DRUG INFORMATION 1 (From Sanofi-Aventis)

### SUMMARY OF PRODUCT CHARACTERISTICS

#### NAME OF THE MEDICINAL PRODUCT

**PENTACARINAT 300 mg, powder for aerosol and for parenteral use**

#### QUALITATIVE AND QUANTITATIVE COMPOSITION

Pentamidine isethionate 300 mg for one vial.

1 mg of pentamidine isethionate is equivalent to 0.57 mg of pentamidine base.

#### pharmaceutical form

Powder for aerosol and for parenteral use.

#### clinical particulars

##### Therapeutic indications

###### PENTACARINAT aerosol:

Prevention of infections due to *Pneumocystis carinii* in immunodepressed subjects, especially HIV patients at risk of *Pneumocystis carinii* pneumonia, with intolerance or contraindication to sulfamethoxazole-trimethoprim.

Sites of infection outside the respiratory tract are not prevented by aerosol treatment.

The reference nebuliser device is the Respigard II. Any other device with the same characteristics (see section 4.2) can be used on condition that the particle size has been validated using a pentamidine solution.

###### PENTACARINAT via the IM or IV routes:

- Treatment of pneumonia due to *Pneumocystis carinii* in patients with intolerance, or contraindications to sulfamethoxazole-trimethoprim.
- Visceral and/or cutaneous leishmaniasis.
- Sleeping sickness (treatment of the hemolympathic stage).

##### Posology and method of administration

###### Posology:

###### PENTACARINAT in an aerosol:

- Prophylaxis for pneumonia due to *Pneumocystis carinii*: 300 mg, once a month.

###### PENTACARINAT via the IM or IV routes

- Pneumonia due to *Pneumocystis carinii*: 4 mg/kg body weight, preferably as a slow IV infusion (longer than one hour), once daily for 14 days.
- Leishmaniasis:
  - visceral: 3 to 4 mg/kg body weight, every alternate day, up to a maximum of 10 injections; depending on the response, a second course of treatment may prove necessary.
  - East African diffuse cutaneous leishmaniasis and South American muco-cutaneous leishmaniasis (particularly due to *L. braziliensis* and *guyanensis*): 3 to 4 mg/kg body weight once daily ; 3 or 4 injections given every 2<sup>nd</sup> day are generally sufficient.
- Sleeping sickness at the hemolympathic stage: 4 mg/kg body weight once daily every 2<sup>nd</sup> day , with a maximum of 7 to 10 injections.

In the event of renal insufficiency, it is advisable to reduce the unit doses by 30 to 50%.

## **Method of administration:**

### **PENTACARINAT in an aerosol:**

Studies using this method of administration were performed and validated using the RESPIRGARD II (a disposable, pneumatic device that should never be reused), and this device currently serves as the reference.

It has the following characteristics:

- it is equipped with an inhalation mouthpiece and a filter for expiration,
- a mean particle mass diameter of 1.4 µm and a standard geometric deviation of 1.9 µm at the inhalation mouthpiece,
- the recycling of large particles in the nebuliser reservoir,
- the need to inject 6 ml water for injection into the vial and then shake, to obtain a clear solution.

Any other nebuliser producing particles with a median mass diameter between 1 and 2 microns at the mouthpiece can be used, provided its different characteristics are validated using a solution of pentamidine isethionate. If a reusable device is employed, it is necessary to sterilize or change the reservoir and the circuit.

Whatever device is used:

- All the pentamidine must be aerosolised and inhaled via the mouthpiece. Nebulisation is stopped when the aerosol is no longer delivered, usually after 15 to 30 minutes.
- Expiration must always be performed via the expiration circuit that includes a filter. If a session is suspended for any reason, the functioning of the device should also be halted.
- Compliance must always be ensured with the operating characteristics of the device (gas flow rate for pneumatic nebulisers, quartz vibration frequency for ultrasonic nebulisers).

### **PENTACARINAT via the parenteral route:**

- Injections must be administered to patients lying down and in a fasting state. Before use, dissolve the contents of a vial using 10 ml water for injection.
- Using the intramuscular route, administer the dose in a single injection.
- Using the intravenous route, dilute the reconstituted product in 50 to 250 ml 5% dextrose for injection. Administer as a slow, one-hour infusion.

## **Contraindications**

Hypersensitivity to one of the components.

## **Special warnings and precautions for use**

Use of this medicinal product is GENERALLY inadvisable:

- during pregnancy and breastfeeding,
- in combination with medicinal products that may cause torsades de pointes: class Ia antiarrhythmia agents (quinidine, hydroquinidine, disopyramide, etc.), class III antiarrhythmia agents (amiodarone, sotalol, dofetilide, ibutilide, etc.), some neuroleptics (thioridazine, chlorpromazine, levomepromazine, cyamemazine, sulpiride, sultopride, amisulpride, tiapride, pimozide, haloperidol, droperidol, etc.) and other medicinal products such as: bepridil, cisapride, diphemanil, IV erythromycin, halofantrine, mizolastine, moxifloxacin, IV spiramycin, IV vincamine (see section 4.5).

PENTACARINAT in an aerosol:

Inhaled or nebulised pentamidine isethionate may trigger bronchospasm or cough. The inhalation of a bronchodilator before or during the aerosol session can treat and/or prevent these side effects.

PENTACARINAT via the parenteral route:

- Monitor the renal function and blood sugar levels during treatment.
- Increased surveillance in the event of hepatic insufficiency, blood pressure disorders (hypertension or hypotension), glucose metabolism disorders, or pre-existing haematological disorders.

In the event of renal insufficiency, it is advisable to reduce the unit doses by 30 to 50%.

**Interactions with other medicinal products and other forms of interaction**

***Combinations to be avoided***

**+ Medicinal products that can cause torsades de pointes:**

- class Ia antiarrhythmia agents (quinidine, hydroquinidine, disopyramide, .....),
- class III antiarrhythmia agents (amiodarone, sotalol, dofetilide, ibutilide, .....),
- some neuroleptics of the phenothiazide (thioridazine, chlorpromazine, levomepromazine, cyamemazine), benzamide (sulpiride, sultopride, amisulpride, tiapride) or butyrophenone (haloperidol, droperidol) types, other neuroleptics (pimozide),
- other: bepridil, cisapride, diphemanil, IV erythromycin, halofantrine, mizolastine, moxifloxacin, IV spiramycin, IV vincamine.

Increased risk of ventricular arrhythmia, and notably of torsades de pointes ..

Whenever possible, discontinue the non-anti-infective medicinal product causing torsades de pointes. If the combination cannot be avoided, check QT interval before commencing, and perform ECG monitoring.

***Combinations requiring precautions for use***

**+ Didanosine:**

Increased risk of onset of pancreatitis because of overlapping side- effects.

Surveillance of serum amylase levels. Do not use the combination if serum amylase levels are at the upper normal limit.

**+ Foscarnet:**

Risk of severe hypocalcaemia. Monitoring of serum calcium levels, and supplementation if necessary.

**+ Stavudine, zalcitabine:**

Increased risk of onset of peripheral neuropathies because of overlapping side- effects  
Regular clinical monitoring.

**+ Bradycardic medication (bradycardic calcium antagonists: diltiazem, verapamil; beta-blockers (except sotalol); clonidine; guanfacine, digitalin; mefloquine; anticholinesterase agents: ambenonium, donepezil, galantamine, pyridostigmine, neostigmine, rivastigmine, tacrine, .....).**

Increased risk of ventricular arrhythmia, and notably of torsades de pointes

. Clinical and ECG monitoring.

**+ Hypokalemic medication (hypokalemic diuretics (alone or in combination; stimulant laxatives; amphotericin B (IV route); glucocorticosteroids (by mouth); tetracosactide).**

Increased risk of ventricular arrhythmia, and notably of torsades de pointes, (hypokalemia being a promoting factor). Clinical, electrolyte, and ECG monitoring.

### **Pregnancy and lactation**

Because the results of animal studies have suggested the occurrence of foetal toxicity, treatment with pentamidine isethionate should be avoided, unless it is absolutely essential.

### **Effects on ability to drive and use machines**

Not applicable.

### **Undesirable effects**

#### PENTACARINAT in an aerosol:

- Cases of pneumothorax have been reported, and the causal link with pentamidine cannot be ruled out.
- Local reactions in the upper respiratory tract may occur; these mainly consist of cough and breathlessness.
- Allergic reactions: asthmaticiform respiration, bronchospasm and exceptional cases of eosinophilic pneumonia have been reported.
- Other adverse effects may occur: rash, fever, anorexia, metallic taste in the mouth, fatigue and light-headedness, acute pancreatitis.

#### PENTACARINAT via the parenteral route:

- The injections are sometimes painful (with a local reaction) and may be accompanied by pallor, hypotension, lipothymia or nausea.
- Impaired renal function: elevation of blood urea and creatinine levels, reduction in creatinine clearance, elevation of blood potassium and protein levels, haematuria.
- Metabolic disorders: in some cases hypoglycaemia followed by secondary hyperglycaemia, hypocalcaemia, hypomagnesaemia.
- Cases of acute pancreatitis have been reported.
- Haematological disorders (leucopenia, anaemia, thrombocytopenia) and hepatic disorders (elevated transaminase levels).
- Prolongation of the QT interval on ECG, torsades de pointes.
- Elevated CPK levels, or, rarely, rhabdomyolysis, have been reported following intramuscular administration of pentamidine isethionate.

### **Overdose**

No cases of overdose have been reported to date with Pentacarinat.

In the event of an accidental overdose via the IV or IM route, or an infusion delivered too rapidly, it is reasonable to be concerned about an increase in adverse effects, and in particular: hypotension, hypoglycaemia, impaired renal function.

Treatment should be symptomatic (no antidote is available).

### **Pharmacological properties**

**ANTIPROTOZOAL AND TRYPANOCIDAL AGENT**, ATC Code: **P01CX01**.

#### **Pharmacodynamic properties**

Pentamidine isethionate is a synthetic organic derivative endowed with trypanocidal activity on *Trypanosoma gambiense* and *rhodesiense*. It is also active on leishmaniasis and on *Pneumocystis carinii*.

The mode of action of pentamidine is not fully elucidated. Its trypanocidal activity appears to be exerted either through the inhibition of DNA synthesis by blocking thymidine-synthetase, or by binding to transfer RNA.

## Pharmacokinetic properties

Following administration via the parenteral route of 4 mg/kg pentamidine isethionate:

*In subjects with normal renal function:*

- The peak plasma levels observed at the end of an IV infusion are approximately 500 ng/ml, and reach approximately 200 ng/ml less than an hour after an IM injection.
- The elimination half-life is longer following an IM injection (about 9.4 hours) than after an IV infusion (about 6.2 hours).
- The apparent distribution volume at the steady state is three times higher after an intravenous infusion (equal to about 2700 l) than after an IM injection (equal to about 820 l).

*In subjects with renal insufficiency:*

Because of the reduction in body clearance of the product, accumulation is observed, together with an increase in the half-life of  $C_{min}$  and the distribution volume.

After the aerosol administration of 4 mg/kg pentamidine isethionate:

The kinetic parameters of pentamidine display significant differences from those observed following parenteral administration.

- Plasma levels: peak plasma levels following aerosol administration are observed before the end of the first hour and reach  $14 \pm 12$  ng/ml, or approximately 10% and 5%, respectively, of the concentrations observed following administration via the IM or IV routes.
- After repeated daily treatments for 21 days, there is practically no plasma accumulation and peak levels reach  $20.2 \pm 21.4$  ng/ml, with the  $T_{max}$  occurring at around the fifth day.
- Concentration in bronchoalveolar lavage (BAL) fluid: Following aerosol administration, the levels found in BAL fluid are much higher than those observed following parenteral administration. There was a 10-fold increase in the supernatant ( $23.2 \pm 7.35$  versus  $2.64 \pm 0.73$ ) and 80-fold increase in the sediment ( $705 \pm 242$  versus  $9.34 \pm 1.74$ ). Data obtained in AIDS patients suggest that the half-life of pentamidine in BAL fluid is greater than 10 to 14 days, and may reach up to 30 days or more.
- No respiratory function parameters tend to worsen, even after long-term prophylactic treatment using pentamidine aerosols, whatever the administration rate and posology. The pulmonary diffusion capacity measured by DLCO is not changed.

## Preclinical safety data

Not applicable.

## PHARMACEUTICAL PARTICULARS

### List of excipients

Not applicable.

### Incompatibilities

Pentamidine isethionate does not precipitate in the presence of a normal sodium chloride solution (0.9%).

**Shelf life**

Before opening: 3 years.

After reconstitution: the reconstituted solution must be used within 24 hours and should be stored at a temperature of between +2°C and +8°C.

For intravenous administration, the reconstituted solution must be used immediately.

**Special precautions for storage**

Store at a temperature not exceeding 30°C.

**Nature and contents of container**

300 mg in a vial (glass)

**Special precautions for disposal and other handling**

**PRESENTATION and administrative identification number**

- 332 289-1: 300 mg in a vial (glass): 65% reimbursed by Social Security
- 331 464-4: 300 mg in a vial (glass), box of 5: not available.

**CLASSIFICATION FOR SUPPLY**

List I

**MARKETING AUTHORISATION HOLDER**

**Sanofi-aventis France**  
**1-13 bd Romain Rolland**  
**75014 Paris**  
**France**

**DATE OF REVISION OF THE TEXT**

**May 2008/v1**