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# Current challenges in treatment options for visceral leishmaniasis in India: a public health perspective

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## Abstract

Visceral leishmaniasis (VL) is a serious parasitic disease causing considerable mortality and major disability in the Indian subcontinent. It is most neglected tropical disease, particularly in terms of new drug development for the lack of financial returns. An elimination campaign has been running in India since 2005 that aim to reduce the incidence of VL to below 1 per 10,000 people at sub-district level. One of the major components in this endeavor is reducing transmission through early case detection followed by complete treatment. Substantial progress has been made during the recent years in the area of VL treatment, and the VL elimination initiatives have already saved many lives by deploying them effectively in the endemic areas. However, many challenges remain to be overcome including availability of drugs, cost of treatment (drugs and hospitalization), efficacy, adverse effects, and growing parasite resistance. Therefore, better emphasis on implementation research is urgently needed to determine how best to deliver existing interventions with available anti-leishmanial drugs. It is essential that the new treatment options become truly accessible, not simply available in endemic areas so that they may promote healing and save lives. In this review, we highlight the recent advancement and challenges in current treatment options for VL in disease endemic area, and discuss the possible strategies to improve the therapeutic outcome.

**Keywords:** Visceral leishmaniasis, Treatment, Drug resistance, Multidrug therapy

## Multilingual abstracts

Please see Additional file 1 for translations of the abstract into the six official working languages of the United Nations.

## Introduction

More than 1 billion people are affected by one or more neglected infectious diseases worldwide [1, 2], for which we lack effective, affordable, and easy to use drugs as well as other control methods. Visceral leishmaniasis (VL), also known as kala-azar, is one of the disorders in this group caused by a protozoan parasite, *L. donovani* and/or *L. infantum*, which is transmitted by the bite of an infected sand fly, *Phlebotomus argentipes* in the Indian subcontinent (ISC) [3]. VL results in prolonged fever, anemia, spleno-megaly, wasting; and is fatal when left untreated [4]. There are approximately 200–400 thousands new cases every year

occurring predominantly in just six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil [5]. More specifically, >10,000 cases occur in India alone every year and the state of Bihar accounts for majority of these cases. These figures, however, do not reflect the true social impact of this disease because VL has a focal distribution which is devastating to the affected communities. The cost of treatment is important when patients need to pay for treatment as ~75 % of the VL cases in Bihar live below the poverty threshold of less than US $ 1.0 a day, and this is similar in other endemic countries although exact data are scarce [6]. Poverty seriously affects the prognosis of VL because most of the patients and their families have to pay for diagnosis, drugs and hospital care, and this is often half or more of the annual household income [7]. As a result, families with a VL infected member descend deeper into poverty.

VL has never been featured as high priority for drug development programs funded by the pharmaceutical industries because it disproportionately affects the poor

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people in developing countries and are unlikely to yield good returns on R&D costs. In 2005, the governments of India, Bangladesh and Nepal signed a joint memorandum of understanding to eliminate VL with the aim to reduce the incidence to less than 1 per 10,000 people at sub-district level by the year 2015 [8] which has recently been extended to the year 2017 [9]. Because of the anthroponotic nature of the transmission of L.donovani in the ISC, the use of quality drugs is not only a therapeutic tool, but also a tool for VL control. Indeed, human beings are the only known reservoir of L.donovani, therefore, identification and proper treatment of parasite carriers will reduce the parasite biomass and prevents onward transmission and deaths (Fig. 1). These factors urge for search of new, effective, less toxic and simplified treatments to replace or complement the few currently available drugs. Unfortunately, no new antileishmanial drugs are expected in near future, because very few drugs are in the R&D pipeline at various stages of development [10]. Furthermore, resistance to first line treatment has long plagued effective treatment of VL in India, making second line treatments and extended hospitalization more common. In India, about 5–10 % of patients with VL, after recovery of acute illness, may develop a chronic cutaneous form called Post kala-azar dermal leishmaniasis (PKDL) that requires prolonged and expensive treatment [11]. The emergence of HIV and its association with VL results in a deadly synergy. A significant number of patients in Bihar are living with HIV-VL co-infection [12], and we expect to see more HIV-VL co-infections in near future which will further generate major therapeutic challenges [13]. In the following sections, we have briefly reviewed the current treatment of VL in endemic areas of Bihar, India, and discuss the challenges and its possible solutions.

## Treatment options for VL

There is no vaccine available for VL; hence control of VL exclusively depends on chemotherapy. Available treatment options for VL are limited and not up to satisfactory standards due to problem relating to efficacy, adverse effects, increasing drug resistance, high cost and need for hospitalization to complete the full dose of treatment. So far, many clinical trials have been done in India to optimize the therapeutic regimens and to protect the efficacy of limited number of available anti-leishmanials (Tables 1 and 2). A full list of completed, ongoing and proposed clinical trials for leishmaniasis in various parts of the world is available on clinical trial registry site (https://clinicaltrials.gov). Indeed, important recent advances have been made in the area of VL treatment that if implemented effectively could eliminate this disease from most endemic parts of the world. Table 3 lists the rank wise recommended dose of anti-leishmanial drugs for VL treatment.

### Pentavalent Antimonials

Past experiences have confirmed that response to a drug varies from region to region. For example, in hyperendemic regions of India and adjoining areas of Nepal, pentavalent antimonials (Sb$^v$ also known as sodium stibogluconate) has lost its efficacy with the result that about two thirds of patients in some of these areas are refractory to Sb$^v$ treatment [14]. However, in Bangladesh, the situation is different as no resistance to Sb$^v$ has been officially reported. Sb$^V$ was recommended and used in Bangladesh till 2009 as a first line drug [15]. Moreover, Sb$^v$ are still the
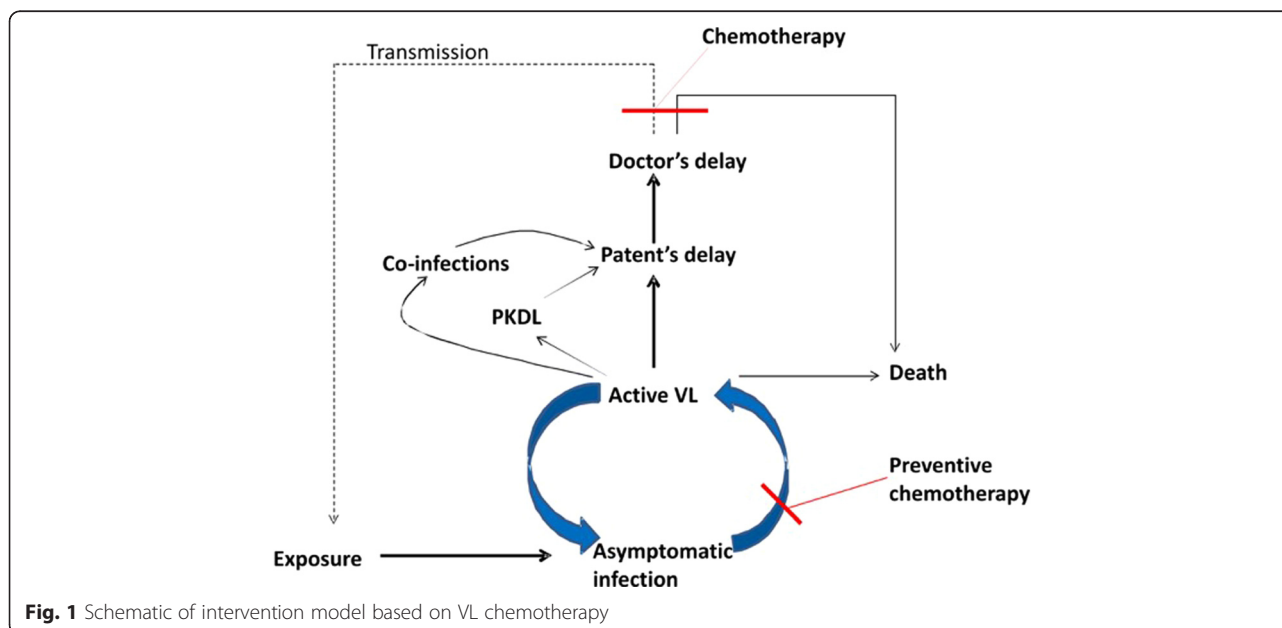


Fig. 1 Schematic of intervention model based on VL chemotherapy

**Table 1** Currently available anti-leishmanial drugs for treatment of VL: product name, cure rate, mode of action on parasite, advantages and limitations

| S.N. | Drugs | Marketing authotization and commercial product | Cure rate | Mechanism of action | Advantages | Limitations |
|---|---|---|---|---|---|---|
| 1 | Pentavalent antimonials | Albert David, India (generic SSG); Wellcome (Pentostam); Sanofi Aventis (Glucantime) | 39–95 % depending on geographical condition (50 % in Bihar) | Act as prodrug, inhibit trypanothione reductase, increase the ROS / Inhibit macromolecular biosynthesis in amastigotes | Low cost and easy available | Parasite resistance especially in India, cardiotoxic, 30 day iv/im treatment in hospital, painful injections |
| 2 | Amphotericin B | Life care, India (Fungitericin); Bristol – Myers (Fungisone) | >98 % in all regions | Form complexes with sterols mainly ergosterols of parasite membrane leading to increase permeability resulting in cell death | High efficacy, 1st line treatment in case of antimonial resistance | Dose-limiting renal toxicity, 15–30 day slow iv infusion treatment over 4–6h in hospital, hypokalaemia |
| 3 | Liposomal amphotericin B | Gilead (AmBisome); Lifecare Innovation (Fungisome); Bharat Serum and Vaccine (Amphomul); Sequus Pharmaceuticals (Amphocil); ENZON Pharmaceuticals (Abelcet); Lifecare Innovation (Kalsome) | >98 % | Targeted delivery of drug to infected macrophages and kill the parasites as AmB | Highest therapeutic index of available VL drugs, No need of hospitalization, substantially less nephro-toxic than AmB | Expensive, requirement of cool chain temperature maintainance |
| 4 | Miltefosine | Paladin Labs (Impavido) | 94–97 % | Modulate cell surface receptors and inositol metabolism of parasites, and cell death is mediated by apoptosis, Inhibit the cytochrome C oxidase | Oral drug, Safe in HIV-VL co-infection | Teratogenic in experimental animals, originally developed as an anti- cancer drug, expensive, GI toxicity, hepato- & renaltoxicity in phase-4 |
| 5 | Paromomycin | Gland Pharma/iOWH | 94 % (India) | Binds to 30S ribosomal subunit and interfere with protein biosynthesis, decrease the membrane potential of parasite | Cheapest drug | An aminoglycoside, therefore nephro- and ototoxicity possible, but have not been reported in VL patients, although reversible high tone audiometric shift may occasionally occur during treatment |
| 6 | Pentamidine | Sanofi Aventis (Pentacarinat) | 70–80 % | Inhibit mitochondrial topoisomerase II and inhibit the transcription process | Effective in combination therapy | Gastointestinal adverse effect, hypotension, diabetes mellitus |

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**Table 2** Summary of studies and clinical trials on monotherapy in treatment of visceral leishmaniasis in India

| Authors | Year | Drug | Type of study | Patients (N) | Treatment Scheme | Cure rate | Reference |
|---|---|---|---|---|---|---|---|
| Thakur et al. | 1988 | Pentavalent antimonials | Randomized dose finding trial of SSG | 371 | 20 vs. 10 mg Sbv+/kg/day for 28 days (i.m) | 60–100 % | [108] |
| Thakur et al. | 1991 | Pentavalent antimonials | Randomized trial | 312 | 20 mg Sbv+/kg/day for 20, 30 & 40 days (i.m) | 71–94 % | [109] |
| Mishra et al. | 1991 | Amphotericin B deoxycholate | Non-Comparative study | 15 | 0.5 mg/kg/day for 28 days (i.v) | 93 % | [110] |
| Mishra et al. | 1992 | Amphotericin B deoxycholate vs. Pentamidicine | Randomized comparative study in antimony unresponsive patients | 120 | AB: 0.5 mg/kg/on alternate day for 14 days (i.v)<br><br>Pentamidinine: 4 mg/kg on alternate days for 20 injections (i.m) | 77–98 % | [111] |
| Thakur et al. | 1993 | Amphotericin B deoxycholate | Non comparative study on SSG resistant patients | 300 | 1.0 mg/kg on alternate day, total dose 20 mg/kg (i.v) | 99 % | [19] |
| Thakur et al. | 1993 | Amphotericin B deoxycholate vs. Pentavalent antimonials | Randomized controlled comparative trial | 150 | AB: 1.0 mg/kg on alternate day, total dose 20 mg/kg (i.v)<br><br>SSG: 20 vs. 10 mg Sbv+/kg/day for 30 days (i.m) | 80–100 % | [112] |
| Mishra et al. | 1994 | Amphotericin B deoxycholate vs. Pentavalent antimonials | Randomized controlled comparative trial | 80 | AB: 0.5 mg/kg on alternate day for 14 days (i.v)<br><br>SSG: 20 mg Sbv+/kg/day for 40 days (i.m) | 62–100 % | [113] |
| Thakur et. al. | 1994 | Amphotericin B deoxycholate | Radomized dose finding study | 80 | 1.0 mg/kg on daily vs. alternate day, total dose 20 mg/kg (i.v) | 100 % | [114] |
| Thakur et. al. | 1994 | Amphotericin B deoxycholate | Dose finding study | 120 | 1.0 mg/kg/day on incremental increasing dose vs. contantdoase, total dose 20 mg/kg (i.v) | 100 % | [115] |
| Giri | 1994 | Amphotericin B deoxycholate | Non comparative study in pentamidinde relapse patients | 25 | 0.75 mg/kg on alternate days (i.v) total 15 infusions | 100 % | [116] |
| Giri& Singh | 1994 | Amphotericin B deoxycholate | Non comparative study in antimony relapse patients | 100 | 0.75 mg/kg on alternate days (i.v) total 15 infusions | 100 % | [117] |
| Jha et. al. | 1995 | Amphotericin B deoxycholate | Dose finding study in multidrug resistant patients | 34 | 1.0 mg/kg/day on alternate days (i.v) total 10–15 infusions | | [18] |
| Thakur et.al. | 1996 | Amphotericin B deoxycholate | Randomized dose finding study | 288 | 1.0 mg vs. 0.75 mg vs. 0.5 mg/kg/day for 20 days (i.v) | 79–99 % | [118] |
| Thakur et.al. | 1998 | Amphotericin B deoxycholate | Randomized dose finding study | 130 | 1.0 mg/kg/day for 20 days (i.v) vs. escalating dose for 5 days then 1.0 mg/kg/day for 43 days | 99 % | [119] |
| Thakur et.al. | 1999 | Amphotericin B deoxycholate | Non comparative dose finding study | 938 | 1.0 mg/kg/day for 20 days (i.v) | 99.2 % | [120] |
| Thakur & Ahmed | 2001 | Amphotericin B deoxycholate | Non comparative study | 309 | 1.0 mg/kg/day for 20 days (i.v) | 95.8 % | [22] |
| Thakur & Narayan | 2004 | Amphotericin B deoxycholate vs. SSG | Randomized comparative study | 60 | AB: 1.0 mg/kg/day for 20 days (i.v)<br><br>SSG: 20 vs. 10 mg Sbv+/kg/day for 28 days (i.m) | 46.6 and 100 % | [121] |
| Singh et.al. | 2010 | Amphotericin B deoxycholate | Randomized study in children's | 605 | 1.0 mg/kg/day for 15 days daily vs. alternate days (i.v) | 100 % | [122] |
| Thakur et.al. | 1996 | Liposomal Amphotericin B (LAB) | Randomized open study of different schedule | 30 | 2 mg/kg/day on day 1, 2, 3, 4, 5, 6 and 10 vs. days 1, 2, 3, 4 and 10 | 100 % | [123] |
| Thakur et.al. | 2001 | Liposomal Amphotericin B vs. | Randomized trial | 34 | LAB: 15 mg/kg single dose (i.v)<br><br>AB: 1.0 mg/kg/day for 20 days (i.v) | 100 % | [28] |

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**Table 2** Summary of studies and clinical trials on monotherapy in treatment of visceral leishmaniasis in India *(Continued)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Amphotericin B deoxycholate | | | | | |
| Sundar et.al. | 2001 | Liposomal Amphotericin B | Open label dose finding study | 91 | 5 mg/kg (single dose) vs. 1 mg/kg for 5 days (iv) | 91 and 93 % | [30] |
| Sundar et.al. | 2002 | Liposomal Amphotericin B | Randomized double-blind dose ranging multicentre trial | 84 | 0.75 mg/kg/days for 5 days (i.v) Vs 1.5 mg/kg/days for 5 days (i.v) Vs 3.0 mg/kg/days for 5 days (i.v) | 89, 93 and 96 % | [124] |
| Sundar et.al. | 2003 | Liposomal Amphotericin B | Open label non comparative study | 203 | 5.0 mg/kg/days for 5 days (i.v) | 90 % | [29] |
| Sundar et.al. | 2004 | Liposomal Amphotericin B vs. Amphotericin B deoxycholate | Randomized comparative study | 153 | AB: 1.0 mg/kg/day for 15 dose on alternate days (i.v); LAB: 2.0 mg/kg/day for 5 days (i.v) Vs. Amphotericin B lipid complex 2 mg/kg/day for 5 days (i.v) | 96, 96 and 92 % | [125] |
| Sinha et.al. | 2010 | Liposomal Amphotericin B | Observational cohort study | 251 | 5.0 mg/kg/day (i.v) on 0,1,4, and 9 | 98.8 % | [126] |
| Sundar et.al. | 2010 | Liposomal Amphotericin B vs. Amphotericin B deoxycholate | Open label randomized controlled non inferiority study | 412 | LAB: 10.0 mg/kg/day (i.v) single dose AB: 1.0 mg/kg/day for 15 alternate dose (i.v) | 95.7 and 96.3 % | [27] |
| Thakur et.al. | 1984 | Pentamidine | Non-comparative study in SSG unresponsive patients | 86 | 4 mg/kg/(i.m) for 15 dose (total dose 60 mg/kg) | 93.4 % | [127] |
| Thakur et.al. | 1991 | Pentamidine | Randomized controlled comparative study | 312 | Group1: 4 mg/kg (i.v) 3 times weekly Group2: 4 mg/kg (i.v) 3 times weekly + SSG 20 mgSbv+/kg (i.m) daily for 20 days Group3: 4 mg/kg (i.v) 3 times weekly until spleen were free from parasite + SSG 20 mgSbv +/kg (i.m) daily for 20 days | 78, 84 and 98 % respectively | [128] |
| Mishra et.al. | 1992 | Pentamidine | Randomized controlled comparative study | 120 | Pentamidine: 4 mg/kg (i.m) on alternate days (total 20 dose) AB: 0.5 mg.kg (i.v) on alternate days | 77 and 98 % | [111] |
| Das et.al. | 2001 | Pentamidine | Randomized controlled comparative study | 158 | Group1 : 2 mg/kg/day (i.m) on alternate days + oral allopurinol 15 mg/kg/day in 3 divided dose for 30 days Group 2 : 4 mg/kg/day (i.m) on alternate days for 30 days | 91.2 and 74.3 % | [67] |
| Das et.al. | 2009 | Pentamidine | Randomized controlled comparative study | 82 | Group 1: AB- 1 mg/kg/day alternate days for 15 days (i.v) Group 2: Pentamidine- 4 mg/kg/day alternate days (i.m) | 92 and 73 % | [129] |
| Jha et.al. | 1998 | Paromomycin | Randomized controlled comparative study | 120 | Group1: 12 mg/kg/day for 21 days (i.m) Group 2: 16 mg/kg/day for 21 days (i.m) Group 3: 20 mg/kg/day for 21 days (i.m) Group 4: SSG 20 mg Sbv+/kg/day for 30 days (i.m) | 76.7, 96.7, 96.7, and 63.3 % | [130] |
| Sunder et.al. | 2007 | Paromomycin | Randomized controlled phase III open label comparative study | 667 | Group 1 : Parmomycin 11 mg/kg/day for 21 days (i.m) Group 2 : AB 1 mg/kg/day for 30 days (i.v) | 94.6 and 98.8 % | [49] |
| Sunder et.al. | 2009 | Paromomycin | Randomized open label study | 329 | Group 1 : 11 mg/kg/day for 14 days (i.m) Group 2 : 11 mg/kg/day for 21 days (i.v) | 82–92.8 % | [131] |

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**Table 2** Summary of studies and clinical trials on monotherapy in treatment of visceral leishmaniasis in India *(Continued)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sinha et.al. | 2011 | Paromomycin | Phase IV open label study | 506 | 11 mg/kg/day for 21 days (i.m) | 94.2 % | [132] |
| Sundar et al. | 1998 | Miltefosine | Phase-I/II safety and efficacy trial | 30 | 50 mg-250 mg/day for 28 days (oral) | 20–100 % | [133] |
| Jha et al. | 1999 | Miltefosine | Phase II randomized open label, dose finding | 120 | 50 mg/day for 6 weeks; 50 mg/day for 1 week followed by 150 mg/day for 3 week; 100 mg/day for 4 week; 100 mg/day for 1 week followed by 150 mg/day for 3 week | 93–97 % | [134] |
| Sundar et al. | 1999 | Miltefosine | Phase II comparative clinical trial | 45 | 100-200 mg/day for 28 days | 94–100 % | [135] |
| Sundar et al. | 2000 | Miltefosine | Phase II, Comparative study | 54 | 100 mg/day for 14 days, 21 days or 28 days | 88–100 % | [136] |
| Sundar et al. | 2002 | Miltefosine | Randomized open label comparative study | 398 | Miltefosine: 50-100 mg/day for 28 days AmB:1 mg/kg/day (i.v) for 15 days | 97–100 % | [58] |
| Sundar et al. | 2003 | Miltefosine | Open label phase II dose ranging study in childrens | 39 | 1.5 or 2.5 mg/day for 28 days | 88–90 % | [137] |
| Bhataacharya et al. | 2004 | Miletfosine | Phase II trial in childrens | 80 | 2.5 mg/kg/day for 28 days | 94 % | [138] |
| Singh et al. | 2006 | Miltefosine | Prospective multicentric cross sectional study | 125 | Miltefosine: 2.5-100 mg/kg/day for 28 days AmB: 1 mg/kg/day (i.v) for 15 days | 91.3–93.2 % | [139] |
| Bhattacharya et al. | 2007 | Miltefosine | Phase IV open label single arm trial | 2109 | 2.5–100 mg/kg/day for 28 days | 93.6–96.6 % | [140] |
| Sundar et al. | 2012 | Miltefosine | Open label comparative study | 567 | 50–100 mg/kg/day for 28 days | 90.3 % | [61] |

first-line drugs in many countries worldwide for all clinical forms. Major side effects are cardiac arrhythmias, prolonged QT interval, ventricular premature beats, ventricular tachycardia and ventricular fibrillation [16]. There are accumulating evidence suggesting that $Sb^v$ has a dual mode of action, and acts on both the parasite and the infected macrophage. Upon contact with infected macrophages, $Sb^v$ stimulates the macrophages to kill the intracellular parasites and when reaching the parasite, $Sb^v$ is reduced to $Sb^{III}$, which can directly kill the parasite inside phagolysosome by inhibiting trypanothoine reductase (an enzyme that recycle oxidized trypanothione to keep the trypanothione in reducing state) [17]. Pentamidine, a diamidine compound, was the first drug to be used in $Sb^v$ refractory patients, and cured most patients initially, but after a decade its efficacy also declined from ~100 to 70 % in hyper-endemic areas of India.

**Amphotericin B Dexoycholate (AmB)**
Amphotericin B (AmB) was initially recommended in India for treatment of patient's refractory to $Sb^v$ [18, 19]. However, due to increasing unresponsiveness of $Sb^v$ in endemic areas, it is currently being used as first line drug for VL treatment. AmB formulated with sodium deoxycholate was the first parenteral amphotericin B preparation available commercially as Fungizone (Bristol-Meyer-Squibb). Several clinical trials have been conducted till date for treatment of VL involving AmB (reviewed in ref.[20, 21]) with excellent cure rate (~100 %) at dose of 0.75–1.0 mg/kg for 15–20 intravenous infusions [22, 23]. The drug has high safety and efficacy; however, prolonged hospitalization, adverse reactions like high fever with rigor and chills, and the need to close monitoring of renal functions and electrolyte levels are well-recognized drawbacks of AmB treatment (Table 2 enlists the studies in India with AmB). The mechanism of action of AmB is still not fully investigated but it is assumed that it interferes with the ergosterol in the cell membrane of *Leishmania* parasite, causing changes in the membrane permeability and leakage of intracellular components [24]. Cell death occurs in part because of these permeability changes, but other mechanisms may also contribute to AmB antifungal activity. AmB is not active *in vitro* against organisms that do not contain sterols in their cell membranes (e.g., bacteria). Binding to sterols in mammalian cells (e.g., certain kidney cells, erythrocytes) may be responsible for the toxicities associated with AmB (reviewed in ref. [25]).

**Lipid formulations of Amphotericin B (L-AmB)**
The advent of Liposome Technology in mid 1960s and subsequently its application for minimizing dose-limiting toxicity has added a new paradigm in AmB treatment, providing a highly effective and safe therapy for many forms of systemic mycosis. There are six lipid formulations of

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**Table 3** Recommended treatment regimens for VL in Indian subcontinent (ranked by preferences)

| Anthroponotic VL caused by *L.donovani* in India, Bangladesh, Bhutan and Nepal |
|---|
| 1. Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over 3–5-day period up to a total dose of 15 mg/kg (A) by infusion or 10 mg/kg as a single dose by infusion (A). |
| 2. Combinations (co-administered) (A)<br>• liposomal amphotericin B (5 mg/kg by infusion, single dose) plus miltefosine (daily for 7 days, as below)<br>• liposomal amphotericin B (5 mg/kg by infusion, single dose) plus paromomycin (daily for 10 days, as below)<br>• miltefosine plus paromomycin, both daily for 10 days, as below. |
| 3. Amphotericin B deoxycholate: 0.75–1.0 mg/kg per day by infusion, daily or on alternate days for 15–20 doses (A). |
| 4. Miltefosine: for children aged 2–11 years, 2.5 mg/kg per day; for people aged ≥12 years and <25 kg body weight, 50 mg/day; 25–50 kg body weight, 100 mg/day; >50 kg body weight, 150 mg/day; orally for 28 days (A) or Paromomycin: 15 mg (11 mg base) per kg body weight per day intramuscularly for 21 days (A). |
| 5. Pentavalentantimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30 days in areas where they remain effective: Bangladesh, Nepal and the Indian states of Jharkhand, West Bengal and Uttar Pradesh (A). |

Note: Amphotericin-B or Liposomal amphotericin B at higher dose should be used as rescue treatment in case of non-response
Source: WHO Technical Report Series (2010) Control of the leishmaniasis. WHO,Geneva [83]
Grade of evidence (A)- evidence based on at least one randomized controlled trial

amphotericin B available commercially that differ from each other with respect to dose, efficacy and toxicities. These are: i) liposomal amphotericin B (AmBisome®; Gilead Sciences); ii) Amphotericin B lipid complex (Abelcet®; ENZON Pharmaceuticals Inc.); iii) AmB cholesteryl sulfate complex, also called AmB colloidal dispersion [ABLC] (Amphocil; Sequus Pharmaceuticals); iv) FUNGISOME™ (Lifecare Innovation Pvt Ltd); v) AmB emulsion (Amphomul, Bharat Serum and Vaccines, India); and vi) amphiphilic L-AmB (KALSOME™10, Life care Innovation, Pvt. Ltd, India). Among these, Ambisome® is tested in most of the clinical trials and is probably the most efficacious of all anti-leishmanial drugs currently available [26]. Most of the clinical trials of L-AmB for the treatment of VL have taken place in India, where more than 10 different regimens have been tested (Table 2). Most have been open-label, dose-finding studies or randomized controlled comparisons. Indian experience has demonstrated that L-AmB caused substantially less toxicity than conventional AmB or amphotericin B lipid complex (ABLC), but high cost is the major drawback. Much of research has been focused to reduce the course of L-AmB whilst retaining its efficacy, to limit the cost to patients. Sundar et al. showed that 15 mg/kg of Ambisome® (3 mg/kg on each of 5 injections) cured 96 % patients [27]. Later on in separate study by Thakur *et al.* [28], and Sundar *et al.* [27, 29, 30] have demonstrated the efficacy and safety of Ambisome® achieving efficacy rates in excess of 90 % in single doses of 5–15 mg/kg (Table 2), making it an excellent treatment option for VL in the ISC. Low toxicity of L-AmB has made it best treatment option for HIV-VL co-infection patients. In a study by Sinha *et al.*, excellent long term survival and retention rate were obtained; however, relapse within 2 year remained frequent [31]. It can be given safely in doses as high as 30–40 mg/kg body weight in HIV-positive VL patients [32, 33]. It has been speculated that lipid formulations enhance uptake by macrophages (the site of parasite replication) which results in the localization of the drug in the macrophage abundant areas in the body. Ambisome® is currently being used as a first line drug for the treatment of VL in India, under the kala-azar elimination program.

Among other lipid formulations, Fungisome™ with the dose 7.5 mg/kg daily for 2 days showed 100 % cure rate (without any serious adverse effect) in an open label randomized study [34, 35]. Abelcet has shown cure rate 90–100 % at total dose of 10–15 mg/kg in Sb$^V$ resistant patients [36]. Amphocil was evaluated at three different doses (7.0, 10 and 15 mg/kg) which showed final cure rate up to 97 % [37]. Amphomul (single dose: 15 mg/kg body weight) was found highly effective and safe for treatment of VL [38]. KALSOME$^{TM}$ is still not tested on human VL, however, in murine model with 7.5 mg/kg double dose results in almost complete clearance of parasites from both liver and spleen [39].

## Paromomycin

Paromomycin is a broad-spectrum aminoglycoside antibiotic produced from culture filtrates of *Streptomyces krestomyceticus* and with activity against a variety of Gram-positive and negative organisms, mycobacteria, protozoa. The anti-leishmanial activity of paromomycin was first demonstrated in the 1960s [40, 41] and subsequently confirmed *in vitro* and *in vivo* [42]. This drug was first tested in Kenya in 1980s for treatment of human VL [43]. It was registered for treatment of patients with VL in India in 2006 by Gland Pharma Ltd., Hyderabad, India, who is now the sole manufacturer for intramuscular paromomycin worldwide [44]. Several clinical trials have been conducted in Kenya, Sudan and India [43, 45–48], and all these studies have reported that paromomycin, when used as a single agent or in combination with sodium stibogluconate was highly efficacious and well tolerated in the treatment of VL. High efficacy rates for paromomycin (i.m) injection (dose-11 mg/kg for 21 days) has been reported to 98.4 % with initial cure (defined as the initial response after complete treatment), and the final cure (defined as a complete response with no evidence of relapse up to 6 months after an initial cure of 21 days of treatment) was approximately 94.6 % [49]. Shortening the

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course of this drug from 21 to 14 days has subsequently shown inferior cure rate [50]. Pain at injection site, elevated liver function tests (LFTs); fever, proteinuria, vomiting, elevations in alkaline phosphatase and bilirubin values are the main adverse events associated with this drug. The mechanism of action of paromomycin is thought to be interference with protein synthesis in the ribosome of the target organism and inhibit the respiration [51].

### Miltefosine

Miltefosine is an alkyl phospholipid compound was the first effective oral anti-leishmanial agent in VL, and registered for the treatment of VL in India in 2002, Germany in 2004, Colombia in 2005 and Bangladesh in 2006 (reviewed in ref. [52]). Miltefosine was originally intended for breast cancer and other solid tumours [53]. However, due to dose limiting gastro-intestinal toxicity, it could not be developed as an oral agent in cancer [54]. Evidence of excellent anti-leishmanial activity both *in-vitro* and in experimental animal models [55–57] prompted the clinical assessment of oral miltefosine in human VL in1996 [53]. Miltefosine was licensed for use in VL patients in India in 2002 following a Phase III clinical trial in which 94 % long term cure rate was observed in a dose 50–100 mg/day for 28 days [58]. It was licensed in Europe for treatment of HIV-VL co-infected patients in 2005 [59, 60]. However, because of its teratogenic effect in animals and its long-term residual persistence shown in humans, there is some concern on unrestricted use of the drug in women of child-bearing-age. Being orally administrable, miltefosine has a big advantage of domiciliary treatment. However, it has the drawback of poor compliance due to its prolonged treatment regimen well beyond the period in which there is almost a complete physical recovery of these patients. Also, with a long half-life of seven days, the chances of parasites developing resistance are high. A recent studies showed that after a decade of this drug use in Bihar (India), 6.8 % of patients relapsed within 6 month of treatment [61]. These considerations suggest for alternative strategies to protect this drug from failure due to non-compliance or resistance and to prolong its clinically useful life. One such way is to combine a short course of miltefosine, to which compliance should be high, with a short course of another effective anti-leishmanial compound to obtain complete cure and to protect against single-agent resistance (Table 2).

### Sitamaquine

Sitamaquine is another oral drug after miltefosine developed by Walter Reed Army Institute of Research (WRAIR, USA) in collaboration with GlaxoSmithKline (UK). Clinical trials using this drug have been completed in India, Kenya and Brazil [62–65] with cure rate ranging from 27 to 87 %. A major side effect was nephrotoxicity. Exact mechanism

of this drug is not known but it is thought that it targets succinate dehydrogenate causing oxidative stress in *leishmania* parasites [66]. Further development of this molecule has been abandoned.

### Pentamidine

Pentamidine, an aromatic diamidine that emerged earlier in Bihar, India as a second line drug to circumvent the problems of $Sb^V$ resistant in VL patients. However, due to inferior cure rate to AmB and toxicity issues (cardiac, hypotension, diabetes mellitus, gastrointestinal), use of this drug as monotherapy has been abandoned in endemic areas [67]. It is commercially available as Pentacarinat® (Sanofi-Aventis). Pentamidine is currently recommended as secondary prophylaxis in HIV-VL co-infection. The mechanism of action of pentamidine in *Leishmania* and other kinetoplastids is the inhibition of active transport system and DNA-mitochondrial complex [68].

### Multidrug therapy

In VL, multidrug therapy has been advocated for several reasons: i) reduce the treatment time and cost; ii) slow the emergence of parasite resistance as mode of action of drugs will be different; iii) increase the efficacy rate even in the case of co-infection [20]. This strategy of multidrug treatment has been successfully used in the treatment of tuberculosis, malaria and leprosy. It also holds promises especially in complicated situations like HIV co-infection. Ideally, drugs used in combination therapy must be of synergistic and additive effect. One of the best approaches is to use one very active drug with a short half-life with second slow acting drug having a longer half-life to clear the remaining parasites. A comparative overview of different combination therapy studies for treatment of VL in India has been presented in Table 4, which suggests that multidrug therapies are safe and effective.

### Immune responses and immunomodulatory activity of anti-leishmanial drugs

One of the major hurdles for developing an effective vaccine for VL, as well as safer and more appropriate drugs and therapies, has been a limited understanding of the precise immune mechanisms required for controlling parasite growth (reviewed in ref. [69]). It has been thought that clinical efficacy of the disease treatment not only depends on direct effect of drugs alone, but an effective immune response also play critical role in final cure (Table 1). The use of biological molecules or compounds to stimulate or modulate innate and cell mediated immunity in order to achieve the therapeutic goal has been tested in both preclinical and clinical studies in treatment of leishmaniasis (reviewed in ref.[69]). For example, $Sb^V$ was not able to clear the parasites in T cell deficient

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**Table 4** Studies on combination therapy for VL in India

| Authors | Year | Drug | Type of study | Patients (N) | Treatment scheme | Definite cure (95 % CI) | Reference |
|---|---|---|---|---|---|---|---|
| Thakur et al. | 1991 | SSG and Pentamidine | Randomised controlled comparative trial | 312 | Group-1 : Pentamidine (i.v) 4 mg/kg/day three times weekly until parasitological cure was achieved | Group 1:78 % Group 2: 84 % Group 3: 98 % | [128] |
| | | | | | Group-2: Pentamidine (i.v) 4 mg/kg/day three times weekly + SSG (i.m) 20 mg/kg/day for 20 days | | |
| | | | | | Group-3: Pentamidine (i.v) 4 mg/kg/day three times weekly until parasitological cure was achieved + SSG (i.m) 20 mg/kg/day for 20 days | | |
| Thakur et al. | 1992 | Paramomycin and SSG | Non comparative study | 22 | Paramomycin (i.v) 12 mg/kg/day + | 81.8 % | [47] |
| | | | | | SSG (i.m) 20 mg/kg/day for 20 days | | |
| Thakur et al. | 2000 | SSG and Paramomycin | Randomized comparative study | 149 | Group 1: Paramomycin 12 mg/kg/day + SSG (i.m) 20 mg/kg/day for 21 days | Group 1:92.3 % Group 2: 93.8 %, Group 3: 53.1 % | [141] |
| | | | | | Group 2: Paramomycin 18 mg/kg/day + SSG (i.m) 20 mg/kg/day for 20 days | | |
| | | | | | Group 3: SSG (i.m) 20 mg/kg/day for 21 days | | |
| Das et al. | 2001 | Pentamidine and Allopurinol | Randomized controlled comparative trial | 158 | Group 1: Pentamidine (i.m) 2 mg/kg/day on alternate days + allopurinol (oral) 15 mg/kg/day for 30 days | Group 1: 91.2 %, Group 2: 74.3 % | [67] |
| | | | | | Group 2: : Pentamidine (i.m) 2 mg/kg/day on alternate days for 30 days | | |
| Sundar et al. | 2008 | L-AmB and Miltefosine | Randomized non-comparative, group sequential | 226 | Group1: L-AmB (i.v) 5 mg/kg single dose | Group 1: 91 % (78–97); | [142] |
| | | | | | Group 2: L-AmB (i.v) 5 mg/kg single dose + miltefosine 100 mg/day for 10 days | Group 2: 98 % (87–100); | |
| | | | | | Group3: L- AmB (i.v) 5 mg/kg single dose + miltefosine 100 mg/day for 14 days | Group 3: 96 % (84–99); | |
| | | | | | Group 4: L-AmB (i.v) 3.75 mg/kg single dose + miltefosine 100 mg/day for 14 days | Group 4: 96 % (84–99); | |
| | | | | | Group 5: L-AmB (i.v) 5 mg/kg single dose + miltefosine 100 mg/day for 7 days | Group 5: 98 % (87–100) | |
| Sundar et al. | 2010 | L-AmB, Miltefosine | Non-randomized multicentric trial | 135 | L-AmB (i.v) 5 mg/kg for single dose + miltefosine (oral) 2.5 mg/kg/day for 14 days | 91.9 % | [143] |
| Sundar et al. | 2011 | AmB, L-AmB, Paramomycin, Miltefosine | Open label non-inferiority random-ized control trial | 634 | Group 1: AmB (i.v) 1 mg/kg on alternate days for 30 days | Group 1: 93 % (88–96); | [144] |
| | | | | | Group 2: L-AmB (i.v) 5 mg/kg for single dose + miltefosine (oral) 50 mg/kg for 7 days | Group 2: 98 % (93–99) | |
| | | | | | Group 3: Paramomycin (i.m) 11 mg/kg/day for 10 days | Group 3: 98 % (93–99); | |
| | | | | | Group 4: Miltefosine (oral) 50 mg/day for 10 days + paramomycin (i.m) 11 mg/kg/day for 10 days | Group 4: 99 % (95–100); | |

BALB/c mice [70] as well as IFNγ and IL-12 gene knock-out mice [71, 72]. Treatment with exogenous IL-12 along with Sb$^v$ resulted in rapid clearance of L.donovani parasites (Table 1). Later on in subsequent separate study, it was reported that treatment with a single-dose anti-IL-10 receptor monoclonal antibody and daily low doses of Sb$^v$ dramatically enhance the therapeutic effects of Sb$^v$ in experimental mice model [73]. These findings strongly supported the idea that immune mechanisms play an important role in clinical outcome of disease. This was further demonstrated by studies on human VL, where stronger parasitological and clinical cure have been observed with recombinant human IFNγ along with SSG compared with the SSG drug alone from India, Brazil and Kenya [74–76]. Basu et al. reported that SAG induces generation of reactive oxygen species (ROS) and nitric oxide (NO) dependent parasite killing via phosphorylation of ERK1/2 and p38 MAPK [77]. Similar immunomodulatory activity is also reported with miltefosine which induces IFN-γ, TNF-α and IL-12 production from macrophages [78]. In PKDL, miltefosine induces significant increase in levels of pro-inflammatory cytokines with concomitant decrease of anti-inflammatory cytokines via up-regulation of activation markers CD16 and CD 86 and

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down regulation of CD14 in circulating monocytes [79]. AmB has been shown to induce the production of TNF-α [80], IL-6 [81, 82], IL1ß [83–85] and M-CSF [86] from macrophage and monocytes and simultaneous suppression of mRNA expression of IL-10 and TNF-ß [87]. Therefore, immune based therapies in combination with chemotherapy that enhance immune responses to fight VL are of significant clinical interest. Such novel approaches may be of very useful for therapies to stimulate the immune system where patients are immune-compromised, such as those with HIV-VL co-infection.

## Treatment failure and parasite drug resistance

Treatment of VL cases is complicated by patients' late presentation at an advanced stage of their illness; and treatment outcome mainly depends on the interaction between the drug, the parasite and the human host [88]. Treatment failure is well documented for $Sb^v$ but the mechanism is far from being completely understood. Most alarming reports came from Bihar (India), where 65 % of VL-patients were found to be unresponsive to $Sb^v$ treatment, while in Nepal, recent reports indicate an unresponsiveness rate of up to 24 % in one district [89, 90]. Recently, it was reported that multiple $Sb^v$ resistance mechanism are circulating in the Indian subcontinent [91] including the loss of metal reduction, over-expression of thiol metabolism enzymes, multi drug resistant transporter and reduced drug uptake due to decreased expression of aquaporins in different experimental models [92]. However, knowledge gained from transcriptional profiling studies and proteomic approaches emphasized the involvement of HSP's, histones, calpain-related proteins and MAPK [93]. While the metabolomics studies have identified many changes and variation in the lipid composition that alters the membrane fluidity [94] and amino acid composition [95]. These facts suggested for the adaptability of the parasite, and therefore, genome plasticity in *Leishmania* which has further been validated by Downing and colleagues, to prove the existence of different genetic background in drug resistant parasites within a single geographical area [96] thereby consolidating the idea for the existence of drug resistant phenotypes in the population. Many other reports for the drug resistance have been available attributing to the role of efflux transporters, aquaporins, and alterations in the intracellular thiol levels in drug resistant clinical isolates has been discussed ahead.

AmB resistance, though rare, has been known to result in changes in the sterol profile where the ergosterol is replaced by its precursor cholesta-5,7,24-trien-3-ol in the membrane of parasite thereby reducing its affinity towards the drug. Amplification in the extra chromosomal DNA which has been directly associated with the resistant phenotypes [97]. The mechanism of resistance has further been explored by the proteomic analysis that shed light on the involvement of energetic pathways which are up-regulated including the glycolytic and TCA cycle while documenting the role of reactive oxygen species (ROS) scavenging pathways and heat shock proteins as additional weapon for protection against the drug induced stress [98]. As mentioned above, the role for the efflux pump (MDR) remains to be the important factor driving the drug resistance together with the thiol machinery for better coping up the ROS induced oxidative stress [99].

Emerging resistance against miltefosine is a matter of serious concern as it is the only available oral anti-leishmanial drug. Incomplete treatment and long half-life of this drug in the circulation has been thought to be one of the factors for driving the parasite machinery for adaptability against the drug induced stress. The exact mechanism of which could be imputed to the allele specific mutations in P-glycoprotein-LdRos3 and LdMT [100] which are responsible for drug uptake in ideal conditions, but the mutation causes the gene inactivation and consequently decreasing the drug accumulation and drug translocation in parasite. Apart from above mentioned reasons increased drug efflux has been another threatening cause of drug resistance. Mode of parasite killing involves the induction of apoptosis by accumulation of ROS but the resistant phenotype has been known to alter the cellular machinery and thereby reducing ROS mediated apoptotic phenomenon. The resistant phenotypes have also been known to be armed for coping up the oxidative stress by up-regulating several important enzymes as superoxide dismutase and ascorbate peroxidase [101].

Paromomycin, another drug targeting the parasite mitochondrial protein synthesis machinery has led the parasite to emerge as a strong survivor with reduced drug binding to the surface, increased translational activities with up-regulation of glycolytic enzymes and intracellular proteins, high expression levels of ATP-Binding Cassette (ABC) transporters [51]. The developments in the field of omics technologies like DNA microarray, MALDI, SILAC has further provided newer insight into the underlying mechanisms for the changes in gene copy number either by gene deletion, amplification or gene rearrangements.

Emergence and spreading of drug resistance can dramatically jeopardize the VL control program relying on chemotherapy, as shown in malaria. It is thought that inadequate treatment, either regimen or treatment duration, is the main cause for failure of treatment and resulting in emergence of drug resistance [102]. Nothing is known about the dynamics of drug resistant *Leishmania* populations in the presence or absence of drug pressure. Therefore, monitoring of drug efficacy and early reporting are essential to bring corrective actions in drug policy. This requires tools, a standardized way to use them and a structure to implement them: in the mid-term, a network

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of sentinel sites could be established by the VL elimination program, like it was done in East Africa for malaria [103].

## What are the possible solutions?

Although considerable scientific progress has been made over the past decade in the broad domain of leishmaniasis, including the genome sequencing of various pathogens causing different form of leishmaniasis, these have not had any impact so far on the quality of clinical care for VL in the field due to very limited number of available anti-leishmanial drugs. It is very important to safeguard the effectiveness of these drugs in order to cure patients and to sustain the VL control. For this, the uninterrupted supply of quality drugs, the promotion of treatment adherence and the monitoring of treatment effectiveness as well as drug resistance will be pivotal. One of the major hurdles for identifying VL patients who are unlikely to respond adequately to chemotherapy, has been a limited understanding of the precise immune mechanisms required for controlling parasite growth, particularly the immune mechanisms that are generated following drug treatment. Anti-leishmanial drugs as monotherapy are high risk of emergence of resistant parasites [16], therefore multidrug therapy needs to be recommended. Another important problem is disease relapse following drug treatment, and at present there are no good prognostic markers to identify individuals that might fail drug treatment. Because the knowledge on mechanisms of emergence of drug resistance, its dynamics and the impact of the introduction of new drugs is poor, and validated methods to monitor treatment of effectiveness under routine conditions do not exist, it is therefore very urgent to develop new tools to allow monitoring treatment effectiveness and drug resistance in order to support the drug policy of the VL elimination program. Furthermore, treatment outcome (the end of treatment) is not definite and patients need to be followed up 6 months after treatment to assess cure. This makes monitoring treatment effectiveness in routine conditions difficult, as patients may not come back for the requested visit 6 months post-treatment. Hence there is a need to compare existing and develop new approaches to monitor treatment effectiveness at the program level. Several possibilities exist: to work with proxy indicators, to develop a method adapted from the retrospective cohort analysis used in TB programs, to give incentives as transport allowances to patients etc.

Importantly, patients with PKDL represent an important but largely neglected reservoir of infection that perpetuates anthroponotic *Leishmania donovani* disease in India, and focal VL outbreaks have been linked to an index case of PKDL [104]. Treatment of PKDL in India is widely regarded as unsatisfactory, and the low incidence of PKDL in India makes any prospective clinical study challenging. Lack of animal models for PKDL is another challenge for laboratory testing of new drugs. Therefore, more research on an intervention that can reduce the risk of developing PKDL; and characterization of parasite strain are needed to resolve the mystery of this disease.

The use of chemotherapy alone as control tool is limited by the fact that only sick people will be treated. There are asymptomatic carriers of the parasite (estimated 6 times more than VL patients) [105–107], and in the absence of chemoprophylaxis of leishmaniasis, these will obviously not be exposed to the drug [3]. Exact role of asymptomatic infection in disease transmission is unknown, but control programs should take them into consideration, hence role of asymptomatic should be quickly elucidated in context to VL transmission.

Last but not the least, not only VL treatment programs should be maintained and improved, but research should also be taken into consideration those parasite reservoirs in populations in order to reduce the risk of transmission. Programs based on management of vector control should be continued as a critical part of treatment strategy. Research on development of safe and effective vaccine have to be promoted that could make a significant impact on the re-emergence of VL cases and sustain the transmission level in endemic areas.

## Conclusion

In the absence of effective vaccine and vector control measures, control of VL and PKDL almost exclusively depends on chemotherapy. The available drugs are costly and may require hospitalization that needs monitoring which cause substantial loss of income for affected families. Emergence of drug resistance further complicates the treatment of disease. Multidrug regimens for VL hold much promise and, experiences with single dose L-AmB are excellent and this will have obvious benefits to the patients who will not require hospital care and loss of wages. However, more studies are required on treatment of PKDL and HIV-VL co-infections as they serve as silent reservoir in endemic areas, and as such will jeopardize the sustainability of VL elimination in the ISC. We have now entered in the VL elimination year, and it is best time to repeat the experience of smallpox and polio eradication in order to open a whole new public health era for next generations.

## Additional file

**Additional file 1: Multilingual abstracts in the six official working languages of the United Nations.** (PDF 277 kb)

### Abbreviations
AmB: Amphotericin B deoxycholate; CD: cluster of differentiation; ERK: extra cellular signal regulated kinases; i.v: intravenously; IL-10: interleukin-10; IM: intramuscularly; ISC: Indian subcontinent; L-AmB: Liposomal Amphotericin B; MAPK: mitogen activated protein kinases; M-CSF: macrophase colony stimulating factor; MDR: multi drug resistance; NO: nitric oxide; PKDL: post

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kala-azar dermal leishmaniasis; R&D: research and development; ROS: reactive oxygen species; SSG: sodium stibogluconate; TCA: tri cyclic acid cycle; TNF: Tumor necrosis factor; VL: visceral leishmaniasis.

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