Developments in the treatment of visceral leishmaniasis

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Background: Visceral leishmaniasis (VL) is one of the most neglected parasitic diseases causing large scale mortality and morbidity among the poorest of the poor in the Indian subcontinent and Africa. *Objective*: This review aims to describe the potential and the (lack of) current impact of newly developed treatments on the control of VL. It describes how the problem of an empty research pipeline is addressed, and discusses the emerging threat of incurable HIV/VL coinfection. *Methods*: The literature was searched for drugs used in VL. *Conclusion*: Research and development of VL drugs has received a financial boost but no new drugs are expected in the next 5 years. Only three new and highly effective treatments have been licensed in the past 10 years. These remain, however, largely inaccessible as VL control programs in the developing world are lacking. This is deserving of immediate and urgent attention, especially in the context of the rapidly expanding HIV/VL coinfection.

Keywords: AmBisome, amphotericin B, antimonials, kala-azar, miltefosine, paromomycin, pentamidine, sodium stibogluconate, visceral leishmaniasis

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1. Background

Visceral leishmaniasis (VL, also called kala-azar) is a lethal vector-borne protozoal infection caused by different species of the *Leishmania* parasite, and characterized by fever, weight loss, hepatosplenomegaly, anemia and a depression of the immune system. Death is caused by the consequences of pancytopenia and opportunistic infections leading to pneumonia and diarrhea. In 95% of cases, death can be avoided by timely treatment, even in basic field circumstances [1]. After subclinical or cured clinical infection, long-lasting immunity prevents recurrence of VL in most patients. Post-kala-azar dermal leishmaniasis is a cutaneous and sometimes mucosal manifestation that occurs after clinical cure in 55% of Sudanese patients [2], and until recently in 5 – 15% of Indian patients [3]. Post-kala-azar dermal leishmaniasis can occur long after cure and often goes undetected, especially in the Indian subcontinent, and because the skin lesions are infectious to sandflies, it may be a significant contributor to the spread of the infection [4].

Infection is transmitted by the bite of the phlebotomine sandfly, which has fed either on infected humans – the anthroponotic form of VL caused by *Leishmania donovani* and found in the Indian subcontinent and eastern Africa – or has fed on infected dogs, the zoonotic form of VL caused by *Leishmania infantum/Leishmania chagasi* in the Mediterranean basin, the Middle East, Central Asia and South America [5].

The second largest parasitic cause of death (after malaria), VL is prevalent in 47 countries, with ~ 200 million at risk [6], and an annual estimated incidence of ~ 500,000 cases and > ~ 50,000 deaths [7]. Overall, 90% of cases occur in India, Bangladesh, Nepal, Sudan and Brazil [6], and 60% in the Indian subcontinent alone [8]. Eastern Africa (Sudan, Ethiopia, Kenya, Uganda and Somalia) has the second largest number of cases (**Figure 1**) [9]. The real burden of VL may be far higher than the number of reported cases [7]. In Bihar, India, only one in eight

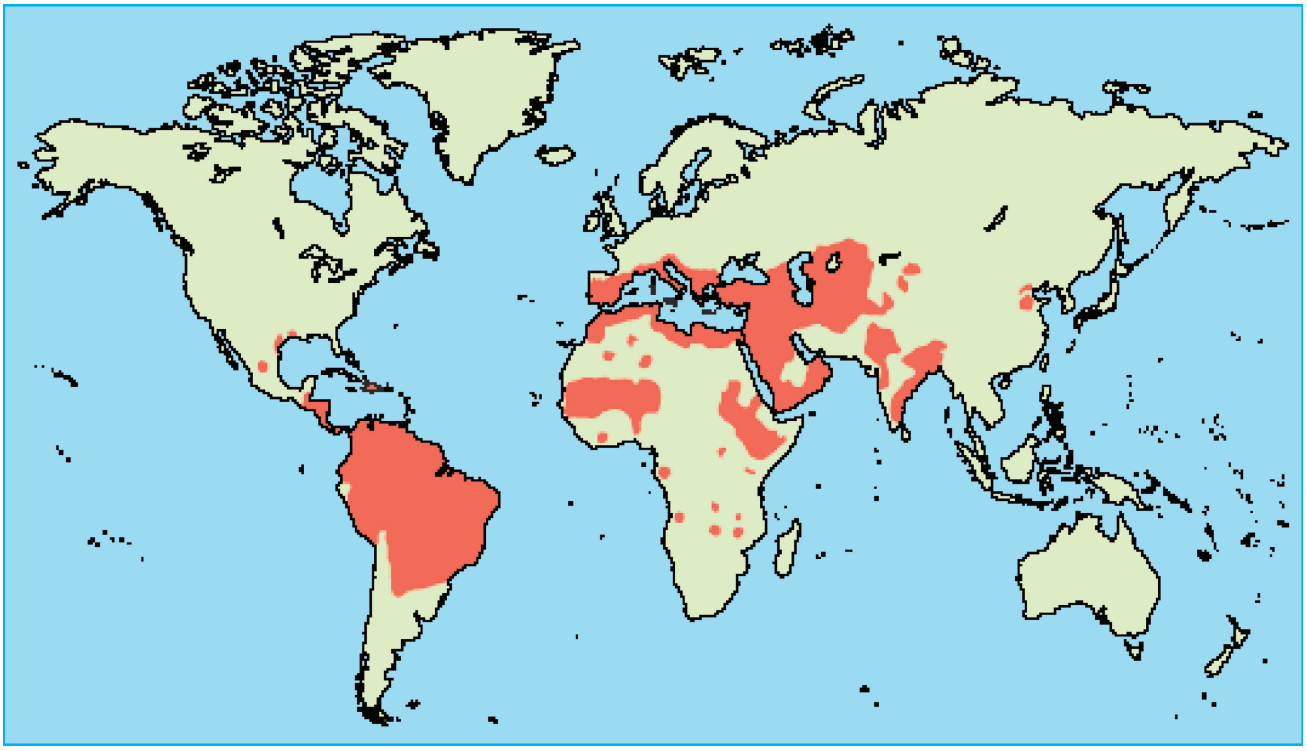

Figure 1. Distribution of visceral leishmaniasis.

cases is reported through official channels [10] and ~ 20% of cases end in death undiagnosed [11]. Retrospective mortality surveys in southern Sudan estimated that in an epidemic in Western Upper Nile province in the 1980s and 1990s, VL caused > 100,000 deaths in a population of 280,000 [12]. A later study estimated that only 50% of cases in Sudan can access treatment, resulting in ~ 90% of VL deaths going unreported [13].

Although also present in suburban settings, VL mostly affects the poorest of the poor, mainly in very remote rural regions, with malfunctioning or absent health systems [14]. In East Africa and particularly in Sudan, epidemics with a high mortality are frequent, especially where there is a lack of access to healthcare and widespread malnutrition [1,12,15,16]. In India, Nepal and Bangladesh, where the incidence is steadily rising, the disease is associated with extreme poverty and overcrowding in agricultural villages [9].

The HIV pandemic in South America, Asia and Africa is expanding into rural and remote VL endemic areas [17]. HIV/VL coinfection is a rapidly expanding problem, and makes a comprehensive approach for control of VL urgent and important. *Leishmania*/HIV coinfection is reported in 35 endemic countries [17]. According to a WHO coordinated monitoring system including 28 institutions worldwide, the number of new cases has declined in Europe since the end of the 1990s, mainly due to access to highly active antiretroviral therapy (HAART) [17]. In other parts of the world, however, where there is little access to HAART, the prevalence is steadily rising, especially in northern Ethiopia, where HIV infection increased from 19% in 1998/99 to 34% of those affected with VL in 2006/07 [17-19]. In Brazil, Sudan, India and Nepal, the estimated prevalence has so far remained below 10% [17] but is expected to dramatically rise, as long as there continues to be little access to HAART.

HIV and VL reinforce each other in a detrimental manner. HIV patients are much more likely to develop VL, whereas VL will negatively impact the response to HAART [17]. VL in coinfected patients cannot be cured, and those with $CD4^+$ counts < 200 cells/µl typically relapse more and more frequently until they become unresponsive to all drugs used [17,20]. They characteristically have very high parasite loads and thus contribute to an increased spread of the infection [21], which may include the spread of drug-resistant strains. In addition, these patients have been proven to be highly infective to sandflies [22]. In Africa, HAART is only partially effective in preventing VL relapse [23]. HIV/VL coinfection thus creates a significant threat to potentially large numbers of people and will form a considerable extra burden to healthcare systems, as management of these patients is difficult and expensive.

In terms of a lack of research and development (R&D) [24], a strong association with poverty, poor access to treatment, negligible funding for treatment and control programs, low

media coverage [25] and significant under-reporting [9], VL is undoubtedly one of the most neglected diseases. Recent developments indicate that this situation may improve in the near future. In May 2007, the WHO hosted the First Global Partners' Meeting on Neglected Tropical Diseases, leading to the establishment of the Neglected Tropical Diseases Scientific and Technical Advisory Group. Neglected diseases are increasingly linked to a human rights approach, including the right to health, education and housing, and WHO has published a comprehensive report on this topic [26]. Also in 2007, at the 60th World Health Assembly, a resolution on the control of leishmaniasis was accepted. As a consequence, a new WHO Technical Report on the Control of Leishmaniasis is in preparation. Meta-analyses on different aspects of the disease and country profiles are being developed to serve as background information for this report. WHO plans the establishment of a global program in 2010 [27].

These plans are very promising. However, any large scale implementation of VL diagnosis and treatment programs is now lacking, and an immediate solution for the many patients that do not receive treatment today is not apparent. Treatment is often unavailable or unaffordable. In India, most patients rely on partial and substandard treatment provided by the private market [28], which has led to the emergence of drug resistance. Many poor patients are fully dependent on non-governmental treatment programs, which only the international medical humanitarian non-governmental organization Médecins Sans Frontières (MSF) provides on a relatively large scale in Africa and India (Bihar).

Tropical disease experts are urging the Global Fund to Fight AIDS, Malaria and TB to include neglected diseases in its funding programs [29]. This would significantly increase access to treatment for VL and should be much encouraged. Treatment for VL as an opportunistic infection for HIV/AIDS can already be included in Global Fund programs, and this avenue for funding should be actively encouraged.

2. Medical need

Research and development in the field of VL is largely focused on the basic biological and immunological aspects of the *Leishmania* parasite. This has led to an abundance of publications and a thorough base of knowledge that includes significant progress in the cracking of their genetic code [27]. However, although these research efforts may lead to important public health benefits, such as the identification of new drug discovery targets, they are not directly linked to public health needs, and do not provide immediate benefits to the many patients in developing countries.

Research into much needed new drugs and their implementation has been stalled for many years, largely due to a lack of commercial interest and the absence of a public health oriented R&D agenda for neglected diseases. Between 1975 and 2004, only two of 1556 novel drug compounds were intended for VL [30], and both were not originally developed for this indication. Of these, liposomal amphotericin B (AmBisome®, Gilead, USA) was already in use for fungal infections before its high therapeutic index for VL was discovered [31]. Miltefosine (MF), an oral treatment, was originally marketed as an antineoplastic and only later developed for VL in cooperation with WHO's Special Programme for Research and Training in Tropical Diseases (TDR) [30,32].

Prospects for drug development have improved since the foundation of the Institute for One World Health (iOWH) in 2000 and the Drugs for Neglected Diseases initiative (DNDi) 3 years later. Both non-profit institutions focus on drug development for neglected diseases. For VL, this has so far resulted in the registration in India in 2006 of the low cost injectable drug paromomycin (PM), by iOWH [33,34]. However, both these organizations are mainly dependent on philanthropic funding. A sustainable and structural solution for R&D in VL and other neglected diseases still needs to be found. Governments of the developed world should take responsibility for creating and funding a R&D agenda focused on meeting global public health needs, including diseases so far neglected by industry and public research institutions [24,35].

None of the recently developed treatment options for VL (MF, AmBisome and PM) are freely accessible for patients in developing countries, although they present great advantages over the older treatments. Research was focused only on the clinical development phase, but not on ensuring that the drugs would reach the patients that need them after clinical development is finished. Now research is needed into the design of cost-effective intervention strategies in order to deliver innovation to patients, taking into account their day to day reality, and their poor access to diagnosis and treatment.

The ideal VL treatment should be feasible in the circumstances in which most VL patients live: in no proximity to hospitals, and in such poverty that travelling costs and prolonged absence from work are typically insurmountable obstacles to seeking treatment [13,36]. Such a setting also makes the follow-up of those that eventually reach the hospital very difficult. Any VL treatment should, therefore, preferably be a short course, easily administered in an out-patient setting, highly effective, with no known resistant strains, affordable and safe. None of the currently existing VL treatment options meet these criteria, and additionally their costs form a barrier (**Tables 1** and **2**). Treatment with pentavalent antimonials (Sb^V) is prolonged, potentially toxic, very painful and ineffective in parts of India due to resistant *L. donovani*. Yet, Sb^V has remained the mainstay of treatment in developing countries since the 1940s. AmBisome and MF are only affordable for VL patients in the developed world, while PM is not yet used outside Phase IV drug trials, except by MSF in South Sudan. Additionally, PM, MF and AmBisome are not yet registered by their respective manufacturers in all poor endemic countries (**Table 2b**). The efficacy and dose of VL drugs, except for Sb^V, have not been determined in Africa, where drug susceptibility of *L. donovani* may be different

Table 1. Prices of currently available drugs and rapid serologic tests for VL.

Compound	Company	Price	Dose used for calculation	Entered market in	Cost for patient of 35 kg* ($)‡
Sodium stibogluconate (generic)	Albert David, India (through IDA foundation§, Amsterdam)	€5.65/30 ml vial 100 mg/ml	20 MKD × 30 days	1940s. First used outside India in Africa by MSF in 1990s	51.00
Sodium stibogluconate (Pentostam®)	GSK	£66/100 ml vial 100 mg/ml	20 MKD × 30 days	1940s	198.80¶
Meglumine antimoniate (Glucantime®)	sanofi-aventis	*Price for developing countries* : $1.2/5 ml vial 85 mg/5 ml	20 MKD × 30 days	1940s	59.30
Paromomycin sulfate	Gland Pharma, India (single source)	2 ml vial 500 mg/ml. priced so that one adult treatment = $15	15 MKD × 21 days	2006	15.00
Amphotericin B deoxycholate	Combinopharm, Spain (also available from other generic manufacturers)	$1.9/50 mg vial	1 MKD alt days × 15 doses in 30 days	Not licensed for VL	20.00#
Liposomal amphotericin B (AmBisome®)	Gilead, USA (single source). Generic forms are in development by Cipla Ltd, India, and Bharat Serums and Vaccines, India	*WHO negotiated price for VL in developing countries:* $20/50 mg vial	Total dose of 20 mg/kg (more required in Africa and in HIV+)	1996	280.00
Miltefosine	Paladin Labs, Canada (single source)	*WHO negotiated price for VL in developing countries:* €54/56 capsules 50 mg [79]	100 mg × 28 days	2003	69.60
Rapid diagnostic test (rK39-dipstick)	Diamed, Switzerland and Inbios, USA	Inbios: $1.00/test DiaMed: $1.40/test			1.00 – 1.40

*Estimated average weight of an Indian/African VL patient is 35 kg.

‡Exchange rates through www.oanda.com (13 February 2009).

§International Dispensary Association, www.idafoundation.nl.

¶British National Formulary 56.

#Sources and Prices 2005 (http://www.who.int/medicinedocs/en/d/Js5414e/2.html).

IDA: International Dispensary Association; MKD: mg/kg/day; MSF: Médecins Sans Frontières.

Table 2a. Currently available treatments for VL.

Drugs	Pentavalent antimonials	Amphotericin B deoxycholate	Liposomal amphotericin B (AmBisome)	Miltefosine	Paromomycin
Regimen	20 mg/kg daily for 30 days	1 mg/kg on alternate days × 15 doses in 30 days	Total dose of 20 mg/kg split over several doses (more required in Africa and in HIV+)	1.5 – 2.5 mg/kg daily over 28 days (India only)	15 mg/kg for 21 days (India only)
Administration	I.v. or i.m.	I.v.	I.v.	Oral	I.m.
Clinical efficacy	35 – 95% (depending on geographic region)	> 97% all regions	Asia, Europe, Brazil: > 97%; India: single dose 91%; Africa: not clearly established	India: 94 – 97% Africa: 94% in immunocompetent VL South America: not established	India: 94% Africa: unacceptably low in Sudanese VL (in southern Ethiopia and Kenya efficacy of PM is good). South America; not established
Resistance	As high as 60% (Bihar, India)	Not documented	Not documented	Lab isolates	Lab isolates
Toxicity	+++ Arrhythmias, reversible pancreatitis, nephrotoxicity, hepatotoxicity, death especially in African HIV/VL	+++ Nephrotoxicity (in-patient care needed), infusion-related fever	+ Minor/no nephrotoxicity, mild infusion-related fever	+ Gastrointestinal (20 – 55% of patients, usually mild), nephrotoxicity, hepatotoxicity, possible teratogenicity	+ Minor/no nephrotoxicity, reversible ototoxicity, hepatotoxicity (all relatively rare)
Issues	Prolonged treatment; painful injection; high toxicity, especially in HIV/VL; resistance in India; recent drug quality problems	Prolonged treatment; need for slow i.v. infusion and nephrotoxicity requires hospitalization in relatively sophisticated setting; heat stability	Expensive; need for slow i.v. infusion requires hospitalization; heat stability (storage < 25°C); not registered in all endemic countries	Prolonged treatment so that compliance in out-patients may be low; expensive; teratogenicity and long half-life require long contraceptive use; potential for resistance; not registered in Africa and Asia apart from India and not on WHO EML	Efficacy very low in Sudanese VL and unknown in South America; potential for resistance; relatively long treatment; only registered in India
Advantages	Registered in all endemic regions and included in WHO EML; can be given as ambulatory care (daily injections); relatively cheap	Registered in all endemic regions except Bangladesh and included in WHO EML; no resistance despite wide use	Relatively short treatment and included in WHO EML; extremely safe, also in HIV/VL; highest therapeutic index of all VL drugs	Oral treatment, can partly be given on out-patient basis; safe in HIV/VL; few side effects	The cheapest VL treatment; few side effects; included in WHO EML for the Indian subcontinent; can be given as ambulatory care (daily i.m. injections)

+: Minor toxicity.

+++: Major toxicity.

EML: Essential medicines list; i.m.: Intramuscular; i.v.: Intravenous; PM: Paromomycin; VL: Visceral leishmaniasis.

Table 2b. Registration of VL drugs in VL endemic countries.

	Pentavalent antimonials	Amphotericin B	AmBisome	Miltefosine	Paromomycin
Bangladesh	Yes	No	No	No	No
Brazil	Yes	Yes	Yes	No	No
Ethiopia	Yes	Yes	No, but allowed for use on compassionate base	No	No
Kenya	Yes	Yes	No, but allowed for use on compassionate base	No	No
India	Yes	Yes	No, but MSF has permission for use as first-line treatment	Yes	Yes
Nepal	Yes	Yes	No	No	No
Sudan*	Yes	Yes	No, but allowed for use on compassionate base	No	No
Uganda	Yes	Yes	No	No	No

*South Sudan has no drug registration authority; MSF has obtained permission to use all available VL drugs on compassionate base.
MSF: Médecins Sans Frontières; VL: Visceral leishmaniasis.

to *L. donovani* in India and to *L. infantum/L. chagasi* in South America.

All drugs currently used for VL, except amphotericin B, are prone to the development of resistance, and *Leishmania* has already developed resistance to both Sb^V and pentamidine in India. Because of the paucity of agents, it is important to prevent the emergence of further resistant strains. A consensus has emerged that drugs in use for humans should not be used in canine leishmaniasis [37] and that anthroponotic VL (with its potential for developing drug resistance) should be treated with combination regimen of drugs, rather than with monotherapy. Combination therapy is expected to prevent the development of resistant strains, and shortens the duration of treatment, which will have a positive impact on compliance. As Sb^V resistance only developed after the drug was widely misused in incomplete treatment regimen, this will further reduce the risk of resistance.

Combination therapy will also increase efficacy, improve compliance, reduce side effects, be cheaper, and, therefore, allow for more cost-effective treatment programs. Preliminary results of an continuing cost-effectiveness analysis showed that of three possible combination regimen, AmBisome + PM is the most-cost effective combination ($133/death averted) [38]. No combinations have yet been used in treatment programs, except PM/sodium stibogluconate (SSG) [39,40].

There are only few clinical studies on the efficacy of treatments for HIV/VL coinfection, and most have been performed in Europe (*L. infantum* infections). None of the currently used drugs have proven effective in HIV/VL. Patients experience multiple relapses and become eventually unresponsive to all drugs used [17]. Enhanced toxicity of treatments is frequently experienced, and reduces treatment options further [17]. Possible overlapping toxicity, especially renal toxicity, of antiretroviral and antileishmanial treatments may cause further problems. Combination regimen should also be tested in coinfected patients, as they may improve treatment efficacy.

3. Existing treatments

3.1 Pentavalent antimonials

Pentavalent antimonials (Sb^V, SSG and meglumine antimoniate) are derivatives of stibonic acid in which antimony is joined to the carbon chains of glucose. Sb^V has been in use for all forms of leishmaniasis for over 70 years and are currently still the mainstream treatment for VL. Throughout Sudan, cure rates of 95% or higher have been consistently obtained with a standard 30 days regimen of Sb^V [1], but resistant strains have rendered Sb^V ineffective in > 60% of Indian patients in Bihar [41]. Despite this, Sb^V is still in use in India except when treatment is not constrained by cost or in pilot programs in which other drugs are in use.

SSG was originally developed in the 1940s for VL by Wellcome (now GlaxoSmithKline (GSK)) under the trade name Pentostam® [42]. A few years later, meglumine antimoniate (Glucantime®) was marketed and is currently produced by sanofi-aventis. Both were, however, priced beyond the reach of most patients in developing countries. In the 1990s, a cheaper generic form of SSG, produced by Albert David, India, was shown to be equally safe and effective [19,43,44]. This generic was then introduced in Africa by MSF and is now used in African VL treatment programs. sanofi-aventis has now reduced the price of Glucantime for developing countries, similar to the price of generic SSG (**Table 1**).

The recommended dose of Sb^V is 20 mg/kg/day, for 28 – 30 days, which is close to the maximally tolerated dose [45]. Sb^V is given either by intramuscular or by slow

intravenous administration. Intramuscular administration is painful, because it is an irritant and large volumes have to be given. The intravenous route is less painful but impractical when treating large numbers of patients in the field. Sb^V has a high incidence of side effects. Toxicity includes elevation of serum amylase and liver enzymes; arthralgia and myalgia; thrombocytopenia; leukopenia; anorexia and thrombophlebitis. Electrocardiograph changes are not uncommon; these develop gradually and are dose-dependent and reversible. Serious adverse events are rare, but in HIV positive patients Sb^V has an unacceptable toxicity and mortality [18].

3.2 PM

PM or aminosidine is an aminoglycoside that was originally licensed by Farmitalia Carlo Erba as a broad spectrum parenteral antibiotic against bacteria and by Parke-Davis as an oral agent against intestinal protozoa. It was first used successfully in human VL in Kenya in the 1980s [46]. WHO sponsored its development in India [47] but later ran out of funding [33]. After the International Dispensary Association (The Netherlands) brought it back into production, it was adopted by iOWH and a large Phase III trial showed that a regimen of 21 days of 15 mg/kg given as daily intramuscular injections was highly effective with an excellent safety profile in India [34]. No nephrotoxicity, < 1% reversible ototoxicity and < 5% minor hepatotoxicity were reported. PM was licensed for VL in India in 2006, with registration in other VL endemic Asian and African countries planned. A Phase IV study in India is currently undertaken by iOWH, and PM is being evaluated in mono-and combination therapy in East Africa by DNDi. Preliminary results indicate that monotherapy in Sudanese VL has unacceptably low efficacy, whereas in Ethiopian VL, results were much better. PM should, therefore, only be used in combination with other drugs in Sudanese VL. PM resistance is readily induced *in vitro* [48-50]. Secondary resistance has also been observed after 60 days of PM injections in cutaneous leishmaniasis [51]. PM's efficacy and safety in coinfected patients are still unknown.

3.3 Amphotericin B

Conventional amphotericin B, or amphotericin B deoxycholate, is a highly effective and relatively affordable treatment option for VL in all endemic regions. However, in-patient care in a relatively well-equipped hospital for 30 days is required because of the risk of potentially serious side effects (especially renal toxicity). This makes it unfeasible for the treatment of most patients. Lipid formulations of amphotericin B are much better tolerated and thus preferable to conventional amphotericin B. Of all lipid forms, AmBisome® (Gilead, Foster City, CA, USA), a liposomal formulation of amphotericin B, has been most extensively used and tested for VL. A very high therapeutic index, short treatment courses and the absence of side effects make AmBisome the most attractive existing treatment for VL [37]. However, AmBisome is currently also the most expensive treatment option for VL, and, therefore, inaccessible for most endemic countries, although the manufacturer has made the product available for a discounted price for the treatment of patients in low and middle income countries. In developed countries, where cost is not a limitation, AmBisome is the drug of choice for VL.

A regimen of 20 mg/kg (total dose) of AmBisome was recommended based on previous experience in different parts of the world in treating patients [37]. However, lower doses may be sufficient for the Indian subcontinent. In India, in a small study, a single dose of 5 mg/kg of AmBisome was effective in 91% of patients [52]. In MSF's African VL programs, in which AmBisome is used as second-line therapy, a regimen of 25 – 30 mg/kg total dose is often needed to achieve cure [53]. Data on AmBisome's efficacy are anecdotal, or based on small trials, and no dose-finding studies for AmBisome have taken place in any region. A dose finding study into the efficacy of a single dose is now in progress in East Africa by DNDi and partners.

Because of its safety, MSF is able to use AmBisome successfully in very basic field conditions in Sudan [54,55] and Ethiopia [56]. In India (Bihar), AmBisome is used as first-line regimen by MSF with an initial cure rate of 99% [57]. AmBisome is also in use by MSF as first-line treatment in Ethiopian HIV/VL coinfected patients in whom it was shown to be safe, but it lacks efficacy, with 18% parasitological failure in primary VL, and > 50% failure among relapsing HIV/VL patients [56].

Several other lipid forms have been evaluated in VL, such as Abelcet® (the Liposome Co., Princeton, NJ, USA), Amphocil® (Intermune, Brisbane, CA, USA), Fungisome® (Lifecare, India) and Amphomul® (Bharat Serums and Vaccines, India). None of these lipid formulations have yet been compared to AmBisome in a clinical trial. In a small study, safety and efficacy of Amphomul were similar to AmBisome [58] and if the price is competitive, it may form a future alternative to AmBisome. A promising oral form of amphotericin B is in an early stage of development by DNDi and partners.

AmBisome accumulates in tissues and is only slowly released and excreted [59]. This would theoretically increase the risk of resistant strains, but despite extensive use in VL, no *in vivo* resistance has yet been identified. This is likely to be due to the fact that parasites are killed very quickly by AmBisome, and thus get little opportunity to develop into resistant strains.

3.4 MF

MF (hexadeclyphosphocholine, Impavido®) is an alkylphosphocholine that was originally developed as an anticancer agent in the early 1980s. In the mid-1980s, its potential activity for VL was identified and registration was achieved in India in 2003 through collaboration between the manufacturer Zentaris, Research and Training in Tropical Diseases by TDR and the government. MF is registered in India, the EU and most Latin American countries, but not yet elsewhere in Asia or in Africa.

Table 3. Target profile for developing combinations from existing VL treatments (reproduced with the permission of DNDi).

	Target	Minimum acceptable
Leishmania species	All species	*Leishmania donovani* (anthroponotic endemic areas)
Distribution	All areas	One region (India, Africa or South America)
Target population	Immunocompetent and immunosuppressed, adults and children	Immunocompetent Primary VL Adults and children
Treatment regimen	10 days	14 days
Feasibility	Mostly given as out-patient (e.g., oral treatment)	Daily ambulatory care possible (e.g., daily i.m. injections)
Clinical efficacy	> 95% cure at 6 months	> 90% cure at 6 months or > 95% initial cure if follow-up incomplete
Resistance	Active against resistant strains	Active against resistant strains
Safety and tolerability	No AEs requiring in patient monitoring	Deaths during treatment < 1%
Contraindications	None	Pregnancy/lactating
Cost per treatment (2008 prices)	< $75/course	< $175/course (only if other cost saving possible through reduction in opportunity costs to patient and hospital care)

AE: Adverse event; DNDi: Drugs for Neglected Diseases initiative; i.m.: Intramuscular; VL: Visceral leishmaniasis.

A Phase IV trial in India in > 1100 patients reported an efficacy of between 82% (intention-to-treat) and 95% (per-protocol) [60]. Vomiting occurred in 8% and diarrhea in 5.7% of patients, but serious side effects requiring hospitalization occurred in < 1%. Mild renal impairment, probably as a consequence to dehydration caused by diarrhea and vomiting, occurred in ~ 15% and was severe in ~ 1%. Elevated liver enzymes occurred in ~ 30%, and hepatotoxicity was severe in ~ 1%.

A randomized-controlled trial in Ethiopia showed that MF was safe and effective in HIV-negative VL patients (94% initial cure), but lacked efficacy in HIV-positive patients (78% initial cure). Although Sb^V was more effective, MF was safer in this group of patients. Receiving Sb^V increased the risk of death by a factor of 6.53 (95% CI 2.5 – 16.9) [18].

Teratogenicity remains a concern and limitation of the drug. Whilst only demonstrated in three animal models, and whilst two women had apparently normal babies during the Phase IV trial [60], it remains imperative to use prolonged contraception when using MF in women of child-bearing age. Contraception has conventionally been given for 2 – 3 months post treatment, but a new finding of a long terminal half-life of over 30 days meant sub-therapeutic concentrations of MF continued to be detected beyond 5 months after treatment [61]. This suggests that contraception should be given for > 3 months, and when this is not feasible, or unlikely to be strictly practiced, MF should be avoided in women of child-bearing age.

Resistance was shown to relatively easily develop *in vitro* through a single point mutation [62]. The long terminal half-life of MF has the potential to increase the risk of the development of resistant strains, especially if it is used in incomplete courses and if relapses are not thoroughly re-treated. Although a compliance of 95.5% was reported in the Phase IV study, in a pilot roll out of MF in India 20 – 33% of patients discontinued treatment despite financial incentives (personal communication, S Sundar). Low adherence to therapy may promote resistance, and strategies to prevent this include administration only in settings in which directly observed treatment is feasible.

3.5 Pentamidine

Since the 1940s, pentamidine has been successfully used against different forms of leishmaniasis, but was later abandoned as first-line treatment for VL, due to the development of resistance in India and the availability of other drugs with less side effects [63]. It is currently used as secondary prophylaxis in monthly or biweekly administration in HIV/VL patients. Ideally, secondary prophylactic regimen should be effective, easy to administer, have minimal interactions with HAART, have minor toxicity in HIV positive patients and not be needed as treatment of relapses. Pentamidine is well-distributed in tissues and excreted very slowly [64,65]. Data on efficacy are scarce, but reduction of relapses and good tolerability of pentamidine monthly injections are reported [66-68]. These characteristics and its low toxicity in intermittent dosing regimen make it suitable for secondary prophylaxis. Pentamidine is donated for the treatment of neglected diseases in developing countries, and will thus be a cost-effective option in prophylactic regimen. Clinical trials on the prophylactic effect of pentamidine (and other drugs) in HIV/VL coinfected patients are needed. However, this should not delay immediate implementation of secondary prophylaxis on a compassionate

Table 4. Continuing and planned combination VL treatment studies.

Combination	Study location	Research performed by	Dose	Results expected
AmBisome + MF AmBisome + PM MF + PM	India, Bangladesh, Nepal	DNDi and partners	1 day AmBisome 5 MK + 7 days MF 1 day AmBisome 5 MK + 10 days PM 15 MK 10 days MF + 10 days PM 15 MKD	Study continuing. Phase III in India early 2010, Phase IV in all three countries in 2011
AmBisome + MF	East Africa	DNDi and partners	1 day AmBisome 10 MK + 10 days MF	Study planned. Registration of AmBisome and MF in 2011; results Phase III 2014
AmBisome + MF	India, planned for Bangladesh	WHO TDR/Paladin/ICMR	1 day AmBisome 5 MK + 14 days MF	March 2009 in India
AmBisome + Sb^V	East Africa	DNDi and partners	1 day AmBisome 10 MK +10 days Sb^V 20 MKD	Study planned; results Phase III 2014
Sb^V + PM	East Africa	DNDi and partners	17 days Sb^V 20 MKD + 17 days PM 15 MKD	Study continuing. PM registration 2010; results Phase IV 2012

DNDi: Drugs for Neglected Diseases initiative; ICMR: Indian Council of Medical Research; MF: Miltefosine; MK: mg/kg; MKD: mg/kg/day; PM: Paromomycin; Sb^v: Pentavalent antimonials; TDR: WHO's Special Programme for Research and Training in Tropical Diseases; VL: Visceral leishmaniasis.

basis for coinfected patients who are otherwise unlikely to survive their continuing VL relapses [23].

4. Current research goals

As there are no novel compounds expected to be approved in the next 5 years, efforts should be made to preserve the efficacy of currently available drugs, and combination regimen should be evaluated. Preferably, these regimens should be < 2 weeks duration to maximize compliance, matched pharmacokinetically to minimize the chance of development of resistance to a single component, affordable, so that roll out on a large scale is possible, and orally administered. **Table 3** gives an overview of the target and minimally acceptable criteria for combination regimen.

5. Scientific rationale

5.1 Combination regimen

Combination regimens have widely and successfully been applied for the treatment of infectious diseases such as tuberculosis and malaria, and this has formed the rationale for the same approach in VL. Combinations have a broader clinical efficacy, through the combined effect of compounds with different mechanisms of action, and also, ideally, through a synergistic effect. Combination regimens also reduce toxicity by allowing for lower doses of the individual drugs, and are expected to delay resistance.

Drug combinations should be tested *in vitro* to exclude antagonism, and should not have overlapping or additive toxicity. From their toxicity profiles, combinations of PM + MF, AmBisome + PM and AmBisome + MF are predicted to be as safe as the single drugs. Preclinical evaluations of combinations indicated potentiation of MF + amphotericin B; no significant potentiation was seen for Sb^V + MF. Multidrug regimen of interest were identified as AmBisome + MF, AmBisome + PM and PM + MF [69]. Combinations should ideally be matched pharmacokinetically to avoid the exposure of parasites to low concentrations of a single drug. PM, with an elimination half-life of 2.6 h will not optimally protect MF, which has a very long half-life and is still detectable in plasma of patients 5 – 6 months after treatment [61]. In contrast, AmBisome has a very long tissue half-life in spleen and liver, and is not prone to the development of resistance. A suitable regimen would be to start with a single dose of AmBisome, followed by another drug. An Indian dose-ranging study applied this principle with AmBisome 3.75 or 5 mg/kg + MF, and found cure rates > 90%, irrespective of the duration of days of MF (7, 10 or 14 days) [70]. In the Indian context, this is as expected considering the high efficacy (> 90%) of a single dose of AmBisome at 5 mg/kg [52]; however, the value of combination studies in preventing resistance will not be apparent from studies designed to evaluate efficacy only.

The combination of PM + SSG (a 17-day combination regimen of PM 15 mg/kg/day plus SSG 20 mg/kg/day) has been extensively used and this combination was shown in clinical trials to be more effective than monotherapy with either drug [40,46,71]. MSF started to use this regimen in 1991 to cope with a large VL epidemic in South Sudan. Retrospective data of > 3000 patients showed that this combination was safer than and as effective as SSG alone (> 97%) [39]. Compared to 1268 patients treated with SSG, combination therapy was associated with greatly reduced odds of death, and less diarrhea and bleeding during treatment. The combination is now being assessed in a clinical trial in Africa by DNDi and partners. Preliminary results suggest an efficacy of just over 90%.

Table 4 shows the continuing studies of different combination regimen in India and Africa (studies in South America will

be initiated in 2010 by DNDi). Combination therapy should be implemented widely as soon as results of these studies are known. In the interim period, monotherapy will have to be used. To minimize the risk of resistance, monotherapy should be carefully supervised to ensure adherence, initial and final cures, and effective treatment of relapses.

None of the combination regimens currently under study has the advantage of two oral drugs. A short course oral combination regimen is the preferred option. With an oral form of amphotericin B, this could become possible in the form of an oral amphotericin B + MF treatment regimen.

5.2 Novel drugs

In VL, the drug target is the amastigote that lives and multiplies intracellularly (in human macrophages). The exact mechanism of action of most VL drugs is unknown (**Table 5**). Despite the extensive knowledge about the biology and chemistry of the parasite, there is a lack of knowledge on drug-parasite interactions, and only few targets for drug development have been validated [72,73]. This is expected to improve once the genome of the *Leishmania* parasite is known.

Possible lead compounds that show *in vitro* activity are constantly being identified, but resources to bring them forward to the preclinical and clinical stage are missing. New compounds that have entered the clinical phase are either a result of alternative formulations of existing drugs (liposomal amphotericin B, lipid emulsion of amphotericin B) or use of known drugs (therapeutic switching) resulting in the discovery of VL activity of PM, amphotericin B and MF. In all cases, the preclinical work was already done. Other known drugs that were identified and brought forward to the preclinical phase are the antileishmanial biphosphonates and the antifungal azoles (fluconazole and other azoles); albeit, the former have not been demonstrated to be effective, except risedronate [74], and the latter had disappointing results [75].

Presented in **Figure 2** and **Table 5** are overviews of compounds in development. Oral amphotericin B is currently being assessed in Phase I as well as undergoing (animal) efficacy studies for VL. The drug could be considered for Phase II development by the end of 2009. Sitamaquine has undergone Phase II trials but issues remain about safety and relatively limited efficacy (under 90%). Even with an optimistic clinical development scenario, it is unlikely that either will be registered for VL before 2014. Another oral formulation of amphotericin B (iCo) and buparvaquone are currently in preclinical development. If successful, both are likely to be registered well after 2014.

To create a more robust pipeline, DNDi has invested in high-throughput *in vitro* screening of compounds in macrophage amastigote models (in partnership with Institut Pasteur, Korea). Druggable candidates will be further developed by a newly established drug discovery consortium (with Advinus Therapeutics, India and the Central Drug Research Institute, India, as main partners). At least one new candidate for clinical testing is expected to be delivered in 2011 – 12.

6. Competitive environment

See **Figure 2** and **Table 5**.

7. Potential development issues

See **Table 5**.

8. Conclusion

Current research for VL has mainly been focused on the biological aspects of the parasite, an approach that is not of benefit to the many patients who need treatment today and not targeted to identifying promising new drugs. However, with the foundation of iOWH and DNDi, prospects have been improving. Clinical trials testing the efficacy of combinations of existing drugs are in progress, while there are now several new compounds in the research pipeline. Drug screening programs for VL have received a financial boost; three highly effective new treatments for VL were identified through therapeutic switching and have been licensed in the past 10 years. However, translation of these results in VL control programs in the developing world is still painfully lacking and is an area deserving of immediate and urgent attention, especially in the context of the rapidly expanding HIV/VL coinfection.

9. Expert opinion

Now that the clinical development of PM and MF has been completed, and affordable, easy-to-use rapid diagnostic tests for VL are available (the rk39 immunochromatographic test) [76], the viability of large scale treatment programs is greatly increased. Based on this notion, in 2004, a memorandum of understanding was signed between Bangladesh, Nepal and India to reduce the incidence of VL to < 1 case/10,000 individuals in 2015 [77]. Elimination plans were drawn, combining mass diagnosis and treatment, vector management, an effective surveillance system, operational research and social mobilization. Funds for the Indian program have been committed by the Indian government and the World Bank. However, 5 years after initiation of the plans, roll out is only very slowly underway. Better control of VL is feasible with the currently available tools and the Asian elimination strategy should be implemented with much more urgency. The strategy should also be adapted and followed in other endemic regions. Plans are already being drawn in South America and the Middle Eastern regions, and the African region should be supported to develop a regional approach as well. At the same time, lessons need to be learnt from the leprosy eradication program in which the pressure to reach the target resulted in significant under-reporting of cases [78]. Next to elimination initiatives, there is a place for programs that intend to limit VL mortality today. In the African VL endemic region, where health systems are poorly

Table 5. Current and future drugs for VL.

Product	Company/PPP	Status	Mechanism of action	Comments
Pentavalent antimonials	GSK, sanofi-aventis and generic producers	Marketed and licensed for VL	Reduction to Sb (III) in amastigote or macrophage: exact mechanism unknown	
Paromomycin	Gland Pharma/ iOWH/DNDi	Marketed and licensed for VL (only in India). Phase IV in India, Bangladesh and Nepal underway Phase III in Africa underway	Binding to the 30S ribosomal subunit, impairing protein synthesis	
Miltefosine	Paladin, Canada	Marketed and licensed for VL (India, Europe)	Membrane integrity antagonist. The exact mechanism of action remains unclear	
Pentamidine	sanofi-aventis	Marketed and licensed for VL	Not known. Interference with numerous cellular synthetic processes	
AmBisome (liposomal amphotericin B)	Gilead	Marketed and licensed for VL in many countries. Dose finding study in Africa underway (DNDi)	Forms complexes with ergosterol and other sterols in cell membranes, causing leakage and subsequent cell death	
Amphomul (lipid emulsion of amphotericin B)	Bharat Sera India	Phase II, dose finding study finished [54]		
Oral amphotericin B	DNDi and BDSI	Preclinical		A very promising oral form of amphotericin B currently being assessed in Phase I as well as undergoing (animal) efficacy studies for VL. The drug could be considered for Phase II development by the end of 2009
Oral amphotericin B	iCo Therapeutics, USA and DNDi	Preclinical		Preclinical studies underway
Buparvaquone	DNDi and others	Preclinical	?	Buparvaquone is marketed as an injectable veterinary drug and active against *Leishmania* parasites *in vitro*. DNDi aims at finding an oral formulation
Sitamaquine	GSK	Phase II: < 90% effective as monotherapy	?	(8-aminoquinoline) Will require G6PD testing before use. It is < 90% effective as monotherapy and may have potential as combination therapy
Tafenoquine	GSK	Phase II	?	(8-aminoquinoline) Will require G6PD testing before use. Is currently not in development for VL but for malaria eradication. $t_{1/2}$ = 2 weeks

BDSI: BioDelivery Sciences International; DNDi: Drugs for Neglected Diseases initiative; iOWH: Institute for One World Health; PPP: Public Private Partnership; SSRI: Selective serotonin re-uptake inhibitor; VL: Visceral leishmaniasis.

Table 5. Current and future drugs for VL (continued).

Product	Company/PPP	Status	Mechanism of action	Comments
Risedronate (bisphospanate)	?	In clinical use as bone protection. VL preclinical	Probably targets farnesyl diphosphate synthase, involved in isoprenoid biosynthesis	RIS is a potent inhibitor of *Leishmania donovani* decreased ≥ 99% parasite burden in *L. donovani*-infected mice [74]
Quinolines (several)	?	Discovery research	?	Oral efficacy similar to MF in *L. donovani*-infected mice [80]
Sertraline (SSRIs)	?	Discovery research	Induces apoptosis through impaired oxygen metabolism	Decreased splenic (72%) and liver (70%) parasite burden in *L. donovani*-infected mice [81]
Tamoxifen	?	Discovery research	Alkalinization of parasitophorous vacuole?	Decreased > 95% spleen parasite burden in *Leishmania chagasi*-infected hamsters [82]
Niacin	?	Discovery research		Inhibited growth of *Leishmania infantum* in cultures [80]
Azithromycin	?	Discovery research	?	*In vitro* activity against *L. chagasi* [83]

BDSI: BioDelivery Sciences International; DNDi: Drugs for Neglected Diseases initiative; iOWH: Institute for One World Health; PPP: Public Private Partnership; SSRI: Selective serotonin re-uptake inhibitor; VL: Visceral leishmaniasis.

functional or non-existent, any elimination program will be difficult and slow to implement. Immediate interventions in outbreaks, with active case finding, diagnosis and treatment, are urgent and necessary. A more adequate outbreak response system should be developed to reduce mortality.

Any large scale treatment program should preferably make use of combination regimen. However, as these are not yet fully clinically evaluated, an interim strategy is needed, in which the best available evidence is used to guide therapy, and in which monotherapies will still have to feature. Clearly, different drugs and drug combinations will be needed in different settings. For the African region, where Sb^V still works, the SSG + PM (17 days) combination, of which the efficacy is already known, is preferred. PM should, therefore, be registered in this region as soon as possible. For the Indian subcontinent, MF monotherapy as first-line treatment is intended for the elimination program. However, to protect MF from resistance, its use in monotherapy should be restricted to programs in which adherence to therapy can be supervised. Roll out in the endemic areas of the Indian subcontinent before this can be guaranteed will create a real risk of 'losing' MF as a viable treatment option. AmBisome monotherapy, with its low risk of resistance and short duration of treatment, may be a more suitable interim regimen for the Asian elimination programs, especially if it proves to be effective in a single dose regimen, which would offer great practical advantages in terms of reduced hospital time and guaranteed compliance that should be weighed against the increased cost of treatment. AmBisome roll out requires resources (trained nurse) and a cold-chain, but despite this MSF is successfully implementing AmBisome as first-line therapy in India. PM roll out is being piloted by iOWH in India, and might prove to be another suitable choice, as long as compliance can be guaranteed. Prevention of resistance also needs to include monitoring of the effectiveness of treatment, relapse rates and cure rates of relapsed patients, and the establishment of a method for *in vitro* drug susceptibility testing.

When large scale treatment programs become a reality, it will be necessary for manufacturers to significantly upscale the production of VL drugs. Most of these are only produced by a single manufacturer (**Table 1**). At present, there is little demand for most VL drugs and, therefore, production is irregular and orders can face long delays. Manufacturers are not prepared for a significant increase in demand and will demand reliable forecasts before upgrading their production. These forecasts can only be made through a coordinated estimation of needs for different endemic regions. There is, therefore, a need for a central faculty in which estimations of needs are made, orders are pooled and from which all VL drugs are readily available. Pooled demands and reliable forecasts will make the timely manufacturing of batches possible, and drugs will be obtained at lower prices by this faculty as they can be bought in large quantities, which will generate considerable negotiation power. This faculty will be able to

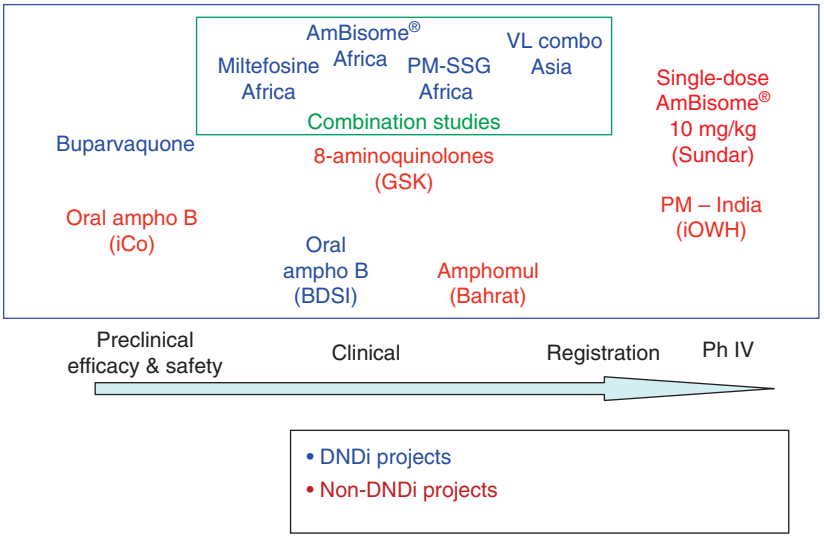

Figure 2. Drugs currently in preclinical and clinical development for VL.
Courtesy of Drugs for Neglected Diseases initiative.
VL: Visceral leishmaniasis.

supply sufficient quantities of drugs without delay in case of outbreaks, as well as the relatively small amounts that are needed for small scale treatment programs. Lack of access to drugs due to temporary quality problems or a lack of registration (**Table 2a**, **b**) is a recurring problem experienced in VL treatment programs. Such a faculty should also take the lead in tackling these access issues. WHO's role as coordinating agency should be emphasized and WHO should take the lead in establishing such a faculty.

The prices of most VL drugs are high and WHO should continue to negotiate with manufacturers, as they have successfully done recently. Generic production should be promoted to increase competition and remedy the vulnerable situation in which VL drugs are only produced by one manufacturer. To guard against unregulated use, the cash value of antileishmanial drugs needs to be minimized by the provision of free treatment to patients.

The problem of HIV/VL coinfection should be addressed with urgency. VL should be universally recognized as an opportunistic infection in HIV. As for now, only AmBisome and MF can safely be used for the treatment of HIV/VL coinfected patients, and access to these drugs in treatment programs is crucial. In Ethiopia, the country with the highest prevalence of HIV/*Leishmania* coinfections, MF is currently not registered and can, therefore, not be used. Testing and prevention of HIV should become a priority in any VL treatment programs. The scientific community should be mobilized and public attention should be focused on this emerging public health problem.

Declaration of interest

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