Combination therapy for visceral leishmaniasis

Johan van Griensven, Manica Balasegaram, Filip Meheus, Jorge Alvar, Lutgarde Lynen, Marleen Boelaert

Lancet Infect Dis 2010; 10: 184–94

Department of Clinical Sciences (J van Griensven MD, L Lynen MD) **and Department of Public Health** (F Meheus MSc, M Boelaert MD), **Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; Drugs for Neglected Diseases initiative, Geneva, Switzerland** (M Balasegaram MD); **and Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland** (J Alvar MD)

Correspondence to: Johan van Griensven, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium **jvangriensven@itg.be**

Combination therapy for the treatment of visceral leishmaniasis has increasingly been advocated as a way to increase treatment efficacy and tolerance, reduce treatment duration and cost, and limit the emergence of drug resistance. We reviewed the evidence and potential for combination therapy, and the criteria for the choice of drugs in such regimens. The first phase 2 results of combination regimens are promising, and have identified effective and safe regimens as short as 8 days. Several phase 3 trials are underway or planned in the Indian subcontinent and east Africa. The limited data available suggest that combination therapy is more cost-effective and reduces indirect costs for patients. Additional advantages are reduced treatment duration (8–17 days), with potentially better patient compliance and lesser burden on the health system. Only limited data are available on how best to prevent acquired resistance. Patients who are coinfected with visceral leishmaniasis and HIV could be a reservoir for development and spread of drug-resistant strains, calling for special precautions. The identification of a short, cheap, well-tolerated combination regimen that can be given in ambulatory care and needs minimal clinical monitoring will most likely have important public health implications. Effective monitoring systems and close regulations and policy will be needed to ensure effective implementation. Whether combination therapy could indeed help delay resistance, and how this is best achieved, will only be known in the long term.

Introduction

Visceral leishmaniasis, also known as kala-azar, is a disseminated protozoan infection caused by the *Leishmania donovani* complex and transmitted via phlebotomine sandflies.[1] The zoonotic form, for which dogs are the main reservoir, is present in the Mediterranean basin, China, the Middle East, and South America, and is caused by *Leishmania infantum* or *Leishmania chagasi*. The anthroponotic form (human reservoir) is caused by *L donovani* and is prevalent in east Africa and the Indian subcontinent.[1,2] Although the disease is endemic in more than 60 countries, with 200 million people at risk, 90% of the 500 000 cases every year happen in five countries: India, Bangladesh, Nepal, Sudan, and Brazil (figure 1).[1,3–5]

For most of the past 70 years, the therapeutic armoury for treatment of visceral leishmaniasis has been extremely limited.[6] Pentavalent antimonials were introduced in the 1940s, and include sodium stibogluconate and meglumine antimoniate. Use of amphotericin B followed after a few decades, and was later joined by paromomycin, a cheap and effective parenteral drug with an acceptable toxicity profile that can easily be given by intramuscular injection.[7] The development of miltefosine, the only drug at present that can be given orally for visceral-leishmaniasis treatment, has been a major breakthrough.[8,9] This drug is the mainstay of the recently launched visceral-leishmaniasis elimination plan in the Indian subcontinent,[10] and benefits from a preferential pricing scheme that puts it at the same price as generic pentavalent antimonials if large quantities are purchased. Finally, different lipid formulations of amphotericin B (ie, liposomal amphotericin B) have been developed, which are similar to amphotericin B in efficacy but with fewer toxic effects (table 1).[11] Although these formulations were initially prohibitively expensive, the preferential price now offered to governments of endemic countries, WHO, and non-governmental organisations make them an option for low-income and middle-income countries. Although other compounds are being developed, these drugs are likely to constitute the main therapeutic options for visceral leishmaniasis in the years to come.[6,12] These drugs belong to chemically unrelated classes and are thought to have distinct targets. All of them have several important disadvantages (table 1).

There are several reasons why consensus has grown over the past few years towards the use of combination regimens in visceral leishmaniasis.[6,13–17] First, combining drugs from different chemical classes could reduce treatment duration or total drug doses, resulting in fewer toxic effects, higher compliance, and less burden on the health system. This could also reduce the overall costs (direct and indirect) and provide a more cost-effective option. Increasing reports of treatment failure with pentavalent antimonials from the Indian subcontinent have raised the issue of acquired drug resistance.[14,18,19] This concern now extends to miltefosine, because of its long half-life and susceptibility to develop resistance with a single point mutation.[20–22] Combination therapy might help to delay the emergence of resistance and increase the therapeutic lifespan of the respective drugs, as has been seen for diseases like malaria, tuberculosis, and HIV.[13] Finally, combination therapy could improve treatment efficacy for complicated cases, such as patients coinfected with HIV, for whom treatment outcomes with monotherapy have been consistently poor.[23]

We review the evidence and explore the potential of combination therapy for visceral leishmaniasis in areas of anthroponotic transmission—in particular, the Indian subcontinent and east Africa. Given the anthroponotic pattern, these areas have the highest threat of drug resistance and bear the highest burden of visceral leishmaniasis. We will discuss the evidence and the criteria for a rational design of such combination regimens that focus on the parallel or sequential administration of separate drugs (co-administration), and

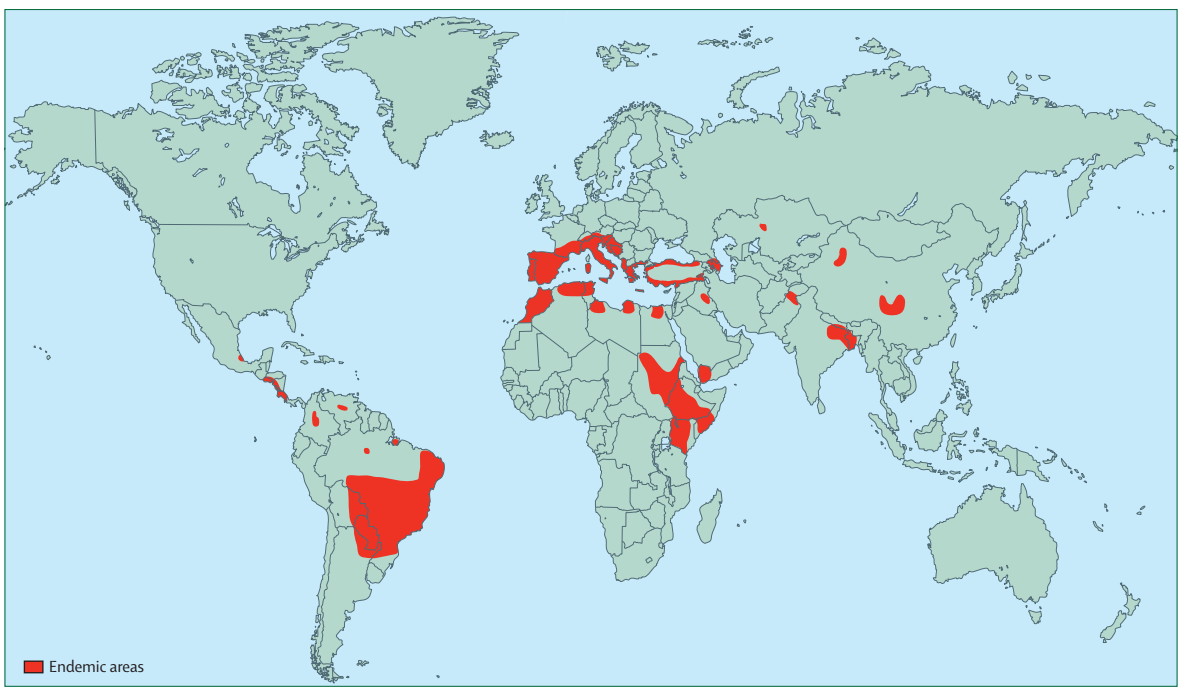

Figure 1: **Geographical distribution of visceral leishmaniasis**
Anthroponotic visceral leishmaniasis happens mainly in east Africa and the Indian subcontinent. The zoonotic form is prevalent in South America, the Mediterranean basin, China, and the Middle East. More than 90% of cases happen in five countries: India, Nepal, Bangladesh, Sudan, and Brazil. Reprinted from Desjeux,[5] with permission from Macmillan Publishers Ltd.

are not co-formulations as used for tuberculosis or malaria.

Efficacy and safety

Preclinical data

Few preclinical data on the efficacy and safety of combination therapies for visceral leishmaniasis are available. An early study looked at interactions between sodium stibogluconate and paromomycin.[24] Whereas a marked potentiation was reported against *L donovani* in vitro, a less-pronounced, additive effect of the antimonial drug was noted in mice.[24] Another study specifically focused on interactions in efficacy between miltefosine and sodium stibogluconate, amphotericin B, paromomycin, and sitamaquine (an oral amino-quinoline).[25] In vivo, the highest enhancement of miltefosine activity was seen with amphotericin B, which preceded paromomycin. No activity enhancement was seen with miltefosine combined with sodium stibogluconate. Whereas the combination of miltefosine and amphotericin B could theoretically have some advantages over the other combinations, its clinical relevance remains unknown. More recent findings have also shown a synergistic interaction between amphotericin B and paromomycin.[26] Preclinical studies on several drug combinations have been done, with no major safety concerns identified (R Don, Drugs for Neglected Diseases initiative, personal communication, Jan 15, 2010).

Clinical data

The combination of pentavalent antimonials and paromomycin was the first regimen to be studied in India, at a time when clinical failure with pentavalent antimonials was increasingly being reported.[27] Overall, these studies showed that 21-day regimen of paromomycin as monotherapy or combined with pentavalent antimonials were efficacious for visceral leishmaniasis. Subsequently, promising data became available on (shortened) monotherapy regimens. A phase 2 study showed that even with liposomal amphotericin B given as a single dose (5 mg/kg), a high proportion of patients could be cured (about 90%).[28,29] Equally high proportions could be achieved with 14 days of miltefosine.[30] These observations provided the rationale for a phase 2, non-comparative randomised trial in India, which assessed different combinations of a single dose of liposomal amphotericin B followed by miltefosine for 7–14 days (table 2).[31] All combinations were highly efficacious (more than 95% of patients cured) and well tolerated, irrespective of the duration of miltefosine treatment. A phase 2 trial studying the combination of liposomal amphotericin B (5 mg/kg) with miltefosine for 14 days is underway in India and planned in Bangladesh (B Arana, WHO Special Programme for Research and Training in Tropical Diseases, personal communication, Nov 3, 2009).[32] Several short combinations are being studied in a large non-inferiority phase 3 trial in India (table 2),[33] which has now moved into its second stage adding children into the study. In 2009, similar studies

	Manufacturer* (trade name of drug)	Regimen	Clinical efficacy	Resistance	Toxicity	Cost of drug course (US$)	Disadvantages
Pentavalent antimonials (sodium stibogluconate, meglumine antimoniate)	Sodium stibogluconate: Albert David (SSG), GlaxoSmithKline (Pentostam). Meglumine antimoniate: Sanofi Aventis (Glucantime)	20 mg antimony per kg bodyweight daily for 20–30 days (depending on geographic area), intravenous or intramuscular	35–95% (depending on geographic area)	Treatment failure up to 60% (Bihar, India)	Moderately toxic: cardiac effects, pancreatitis, nephrotoxicity, hepatotoxicity	53 (generic) to 198 (branded SSG)	Quality control; length of treatment; painful injection; toxicity; resistance in India
Amphotericin B	Bristol Meyers Squibb (Fungizone); generic companies	1 mg/kg every other day for up to 30 days (15 mg/kg total dose), intravenous	>97% for all regions	Not documented	Moderately toxic: nephrotoxicity (inpatient care required)	About 21 (generic)	Need for slow intravenous infusion; dose-limiting nephrotoxicity; heat instability
Liposomal amphotericin B	Gilead (AmBisome)	5–20 mg/kg total dose in 4–10 doses over 10–20 days, intravenous	Asia: >97%; India, single dose: 91%; Africa: not fully established	Not documented	Nephrotoxicity (limited)	280 (preferential) for 20 mg/kg dose; about 3000 (non-preferential)	Price; need for slow intravenous infusion; heat stability (needs to be stored below 25°C)
Miltefosine	Paladin, Montreal, Canada (Impavido)	2–2·5 mg/kg daily over 28 days (India only), oral	Asia: 94% (India); Africa: 60% (single field study), 93% in patients not infected with HIV and excluding those lost to follow up	Only in laboratory isolates	Gastrointestinal effects (20–55% of patients, usually mild), nephrotoxicity, hepatotoxicity, possibly teratogenic	About 74 (preferential), about 150 (non-preferential)	Price; possibly teratogenic; potential for resistance (half-life); poor patient compliance
Paromomycin sulphate	Institute for OneWorld Health;† Gland Pharma, Hyderabad, India	15 mg/kg daily for 21 days (India only), intramuscular	Asia: 94% (India); Africa: under evaluation	Only in laboratory isolates	Nephrotoxicity, ototoxicity, hepatotoxicity (all relatively rare)	About 15	Efficacy varies between and within regions; potential for resistance?

*Marketing authorisation holder. †Paromomycin was granted orphan drug status by the US Food and Drug Administration and the European Medicines Agency in 2005. Adapted with permission from the Drugs for Neglected Diseases initiative from data presented during Fourth World Congress on Leishmaniasis (Feb 3–7, 2009).

Table 1: **Drugs currently used for treatment of visceral leishmaniasis**

will be started in Bangladesh and Nepal; the first results from India are expected by 2010.

In Africa, combination therapy of sodium stibogluconate and paromomycin was studied in the late 1980s,[35] and was subsequently used by Médecins Sans Frontières, who needed a shorter treatment regimen when faced with large numbers of patients during an epidemic in Sudan.[36–39] Retrospective cohort data from more than 4000 patients showed that, relative to monotherapy with pentavalent antimonials (sodium stibogluconate), combination therapy was associated with clearly reduced mortality and fewer complications during treatment.[37] This experience formed the basis for the leishmaniasis in east Africa platform (LEAP) 0104 trial, which was started in 2004 (final results are expected in early 2010).[34,40] This phase 3 trial, which was done in Sudan, Ethiopia, Kenya, and Uganda, initially compared two monotherapy regimens—sodium stibogluconate (20 mg/kg for 30 days) and paromomycin sulphate (15 mg/kg for 21 days)—with the combination of both drugs at the same dose for 17 days (table 2).[34] In 2006, because of unexpectedly low efficacy with paromomycin monotherapy, the protocol was amended, and the dose of paromomycin was increased to 20 mg/kg in the second monotherapy group.[41] Whether the low efficacy related to drug resistance, differences in susceptibility, or pharmacokinetics is currently being investigated.

No large trials on other combination regimens have been done in Africa. Studies on miltefosine and amphotericin B as monotherapy are limited.[42–45] A phase 2 study, which is due to start recruiting patients early in 2010, will assess the use of miltefosine (including pharmacokinetics) and combinations of liposomal amphotericin B plus either miltefosine or pentavalent antimonial (sodium stibogluconate) in an HIV-negative population (our unpublished data, Drugs for Neglected Diseases initiative). This study will provide important data on the efficacy of miltefosine within a standard clinical trial in east Africa, intends to facilitate drug registration in the region, and will also provide the first data on combination therapy based on liposomal amphotericin B. Because pentavalent antimonials are still highly effective in east Africa and have the additional advantage of being cheap, they indeed deserve further exploration in combination regimens when given for shorter duration and at lower total dose.

Health-system issues

Access to and capacity of health systems

At present, major barriers exist in terms of access to diagnosis and care for visceral leishmaniasis, monitoring of treatment, and quality assurance of care. A recent study assessed the use of care by patients and delays in diagnosis and treatment in four endemic districts in

	Study design	ClinicalTrials.gov registration	Country	Study period	Patients enrolled	Drug combinations studied	Definitive cure (95% CI) at 9 months in intention-to-treat analysis
Completed trials							
Sundar et al[31]	Phase 2, randomised, non-comparative, group-sequential trial	NCT00370825	India	2008–08	181 adults: 45 each in groups A, C, D and E; 46 in group B	Group A: SD L-AmB 5 mg/kg alone; group B: SD L-AmB 5 mg/kg followed by miltefosine 100 mg for 14 days; group C: SD L-AmB 5 mg/kg followed by miltefosine 100 mg for 10 days; group D: SD L-AmB 3·75 mg/kg followed by miltefosine 100 mg for 14 days; group E*: SD L-AmB 5 mg/kg followed by miltefosine 100 mg for 7 days	Group A: 91% (78–97%); group B: 98% (87–100%); group C: 96% (84–99%); group D: 96% (84–99%); group E: 98% (87–100%)
Planned or ongoing trials							
DNDI (VLCOMBO-07 trial)[33]	Phase 3, randomised, open-label, non-inferiority trial	NCT00696969	India, Bangladesh, and Nepal	2008–09 (India), 2009–10 (Bangladesh, Nepal)	624 adults and children†	Group 1: AmB 1 mg/kg every other day for 30 days; group 2: SD L-AmB 5 mg/kg followed by miltefosine 2·5 mg/kg for 7 days; group 3: SD L-AmB 5 mg/kg followed by paromomycin 15 mg/kg for 10 days; group 4: miltefosine 2·5 mg/kg plus paromomycin 15 mg/kg for 10 days	..
Banaras Hindu University[32]	Phase 2, non-randomised, open-label, historical control, safety/efficacy trial	NCT00371995	India	Ongoing	150 adults and children†	SD L-AmB 5 mg/kg followed by miltefosine 2·5 mg/kg for 14 days	..
TDR‡	Phase 2	..	Bangladesh	Planned	150†	SD L-AmB 5 mg/kg followed by miltefosine 2·5 mg/kg for 14 days	..
DNDi (LEAP0104A/B trial)[34]	Phase 3, randomised, open-label, active control, safety/efficacy trial	NCT00255567	Kenya, Ethiopia, Sudan, and Uganda	2004–09 (LEAP0104A), 2007–10 (LEAP0104B)	1100 adults and children†	Active comparator group 1: SSG 20 mg/kg for 30 days; group 2: paromomycin 15 mg/kg (LEAP0104A) or 20 mg/kg (LEAP0104B) for 21 days;§ group 3: SSG 20 mg/kg plus paromomycin 15 mg/kg for 17 days	..
DNDi (VLCOMBO trial)¶	Phase 2, exploratory, randomised, open-label trial	..	Sudan and Kenya	Planned for 2009–10	189 adults and children; 63 in each group	L-AmB followed by miltefosine; L-AmB followed by SSG; miltefosine 2·5 mg/kg for 30 days.	..

*Non-randomised group assigned when it had become apparent that all other regimens were effective. †Estimated. ‡B Arana, WHO Special Programme for Research and Training in Tropical Diseases, personal communication, Nov 3, 2009. §Due to poor outcomes on paromomycin 15 mg/kg in monotherapy in Sudan in LEAP0104A, the dose for paromomycin monotherapy was increased to 20 mg/kg in LEAP0104B. ¶Our unpublished data. AmB=amphotericin B. DNDi=Drugs for Neglected Diseases initiative. L-AmB=Liposomal amphotericin B. LEAP=leishmaniasis in east Africa platform. SD=single-dose. SSG=sodium stibogluconate. TDR=WHO Special Programme for Research and Training in Tropical Diseases. VLCOMBO=visceral leishmaniasis combination treatment.

Table 2: **Studies on combination therapy for visceral leishmaniasis in the Indian subcontinent and East Africa**

India, Nepal, and Bangladesh.[46] In India, even poor people prefer to see a private medical practitioner for treatment of visceral leishmaniasis. In Bangladesh and Nepal, most patients rely on the public-health-care sector, although use of local pharmacists was high in Nepal.[46] Long delays in diagnosis and treatment were also reported. Overall, 23% of patients interrupted their treatment, mainly because of a lack of resources (67%) or side-effects (16%).[46] In Africa, provision of visceral-leishmaniasis care has, until recently, mainly relied on non-governmental organisations. Diverse populations, ranging from semi-nomadic people to migrant workers, need to be reached in different contexts and environments (figure 2).[3,15,47]

Therefore, the identification of an effective, well-tolerated, short regimen that can be given at the primary health-care level, needing little monitoring, and that is affordable both from the patient's and society's perspective would have a major public health impact on these populations. However, combination therapy might bring additional complexity in terms of logistics, service delivery, and programme monitoring. If not carefully considered and planned, combination therapy might actually increase the burden on the health-care system.

The combination of miltefosine and paromomycin might be an interesting option, because it could be given entirely within ambulatory care, and might even be suitable for delivery at the primary-care level. The weak health-care infrastructure would seem to argue against more complex intravenous administrations (eg, amphotericin B or liposomal amphotericin B) and drugs that need more complicated laboratory and clinical monitoring (eg, amphotericin B). The need to transport liposomal amphotericin B below 25°C could be another barrier for national programmes, although technological solutions exist that can guarantee transport of drugs below 25°C for 4–5 days. Steps could be taken to strengthen the supply chain of liposomal amphotericin B,

François Chappuis

Figure 2: **Visceral leishmaniasis happens in diverse contexts**
Typical environment in Bihar, India (A). Seminomadic lifestyle in east Africa (B).

and a single dose given at presentation could substantially ease the treatment protocol if the remaining drug could be given on an outpatient basis. Of note, ambulatory treatment also needs sufficient capacity to assure good monitoring and follow-up. In cases in which miltefosine is used, an effective system to provide contraception also has to be considered because of the risk of teratogenicity. Despite extensive counselling on contraception, two cases of pregnancy were reported among the 227 women (≥12 years) treated in a phase 4 trial of miltefosine in India, with the conception date close to the exposure date.[48] Fortunately, no birth anomalies were reported.

Cost-effectiveness

Thought must also be given to aspects of cost and cost-effectiveness of the various treatments available. In addition to the public health perspective, it is also important to consider the viewpoint of the household affected by visceral leishmaniasis. Studies found that the median total expenditure by the patient on visceral-leishmaniasis treatment was 1·2 times the annual per head income in Bangladesh,[49] 1·3 times in India,[50] and 1·1 times in Nepal.[51] Only limited data are available on the cost and cost-effectiveness of visceral-leishmaniasis treatment, and studies have mainly focused on monotherapy.[50–52] Olliaro and Sunder[53] recently calculated the cost of drugs on the basis of international drug prices and anthropometric data from a single health-care facility in Bihar, India. They found that paromomycin was the cheapest option (US$7·4 per patient) and that liposomal amphotericin B was the most expensive ($162–229 per patient).[53] Private treatment with miltefosine cost $119 per patient, and $64–75 at the WHO-negotiated preferential price. However, these calculations did not include other direct or indirect costs, and might differ widely from country to country.

Combination therapies have the potential to reduce the cost to the public health system and patients by reducing the duration of treatment. This not only lowers the burden to the health system but also reduces the economic inactivity of patients. Preliminary findings on the cost-effectiveness of combination therapies in India, Nepal, and Bangladesh, showed combination therapies to be a viable alternative to monotherapies, with liposomal amphotericin B and paromomycin the best combination economically.[54] However, when we use the preferential drug price of miltefosine in the analysis, it seemed that miltefosine and paromomycin would be the preferred option from an economical perspective (our unpublished observations). No data exist on the cost-effectiveness of combination regimens in east Africa, but a study is due to start in Kenya, Ethiopia, and Sudan, with results expected in 2010 (Meheus F, unpublished).

Regulations and policy

Irrational drug use is a potential threat to the lifespan of any drug, and has probably contributed to the high level of treatment failure with pentavalent antimonials in Bihar, India.[19] The unrestricted availability of antimonials in India resulted in widespread misuse by unqualified practitioners, leading to incomplete treatment courses. According to a survey of drug resistance in India, only 26% of patients were treated according to WHO guidelines, and patients often stopped treatment on their own initiative.[55] The high reliance on the private sector and local pharmacists in the Indian subcontinent even today highlight the need for tightened regulations on the modalities of visceral-leishmaniasis treatment, and for treatment to be made available at no cost.[15,20,46] When policy makers opt for combination therapy, they should take measures to limit the use of monotherapy, particularly in incomplete courses. The fact that miltefosine is available in India without prescription or regulation on dispensed quantities is worrying, since this could facilitate the development of drug resistance.[20]

Prevention of drug resistance

The problem of drug resistance in visceral leishmaniasis has been extensively reviewed elsewhere.[14] Treatment failure can manifest as initial treatment failure (failure to clear parasites at the end of the treatment course) or relapse (reappearance of parasites after initial cure, usually within 6 months of follow-up). Although pentavalent antimonials have been successfully used throughout the world for decades, poor treatment response (mainly due to initial treatment failure) has increasingly been reported since the 1980s from Bihar, India, with geographical and temporal clustering in several hyperendemic districts.[19,56] Although treatment outcomes could initially be improved with higher total doses, the improvement was only temporary.[57–59] In subsequent reports, therapy failed in up to 60% of patients that were newly diagnosed.[60–62] At the same time, misuse of the drugs was reported.[55] Increased treatment failure has also been reported in Nepal, in districts that neighbour Bihar.[63,64] Although treatment failure can have

several causes, including factors related to drug, host, and parasite, substantial evidence exists that acquired drug resistance is a key issue. Reduced drug sensitivity has been reported with *L donovani* strains from non-responsive cases in vitro.[18,65,66] Reduced susceptibility to pentavalent antimonials has also been reported with *L infantum* in both human beings and animals.[67–69] In these studies, post-treatment isolates had reduced sensitivity compared with pretreatment isolates, supporting the notion of acquired drug resistance. However, more recent studies have reported less clear associations of in-vitro susceptibility and clinical outcomes, underscoring the need of improved and standardised methods.[64] The limited understanding of the mechanism of resistance towards pentavalent antimonials, and the shortcomings of drug sensitivity assays, make it difficult to predict the risk of acquired resistance in other regions or drugs and to assess the need for combination therapy to help prevent resistance. However, on the basis of the evidence, acquired drug resistance should be thought to be a potentially serious threat to visceral-leishmaniasis control, and comprehensive strategies should be developed, including the use of combination therapy.[13,14,19,70]

Rationale

For individual drugs, the ease with which resistance develops will mainly depend on the parasite burden, the probability of spontaneous development of resistance mutations, and the fitness cost associated with those mutations.[71] The level and pattern of drug use in a population constitutes the selection force for the development of resistance, and intact host immunity is generally thought protective. The potency of the drug, therapeutic index, and pharmacokinetic properties of the drug also play a part.[71] Combination therapy could delay resistance if two drugs with different modes of action and mechanisms of resistance are used. The combination of synergistic drugs is preferred, because if more effective replication can be inhibited, resistance is less likely during treatment.

For resistance prevention, both drugs should ideally have similar pharmacokinetics. If parasites always confront both drugs, the probability of the emergence of double-resistant parasites would be expected to be extremely rare (ie, the product of their individual per-parasite probabilities). A rapid elimination phase minimises the duration of subtherapeutic drug concentrations, which could provide an opportunity for amplification or selection of resistant parasites.[13,71,72] In studies of malaria, the combination of one very active drug with a short half-life with a slower acting drug with a longer half-life to clear the remaining parasites has been explored as a way to shorten treatment duration and improve treatment compliance.[73] However, recent studies have focused on the terminal elimination phase of the second drug, which can act as a selective filter for resistant malaria parasites.[74,75] Artemisinin resistance has recently been reported in Cambodia, but underlying reasons remain to be established.[76–78] Finally, drugs can be combined to target different biological stages of the infectious agent. This has been done for tuberculosis and malaria, although the drugs are essentially targeted at preventing relapse and would only indirectly prevent or delay resistance.

Pharmacological considerations for combination therapy

Although the mechanisms of action and resistance remain poorly understood for all anti-leishmanial drugs in use (except amphotericin B), they are all thought to act on different targets.[6] Recent findings from India suggest that field isolates from areas with high-level resistance to pentavalent antimonials show reduced sensitivity towards other anti-leishmanial drugs such as amphotericin B and miltefosine.[79,80] However, true cross-resistance between the various drugs has not been reported so far. Several combinations have shown activity enhancement in animal experiments.[25,26]

Clear differences in pharmacokinetics exist.[13] Miltefosine might be particularly vulnerable to the emergence of resistance, because of its narrow therapeutic index and long half-life, which has been estimated at around 7 days.[21,81] Recent data from patients with cutaneous leishmaniasis suggested a terminal half-life of 31 days, with miltefosine still detectable 5–6 months after the end of treatment.[82] Resistant strains could be selected and amplified during this period because of subtherapeutic drug concentrations, either from relapsing patients, or from newly acquired infections.[21,81] If confirmed, this might have important repercussions on the risk of emerging resistance and on the duration of contraceptive measures.

Paromomycin has a short half-life (2–3 h in patients without visceral leishmaniasis), but has a low therapeutic index. Resistance can easily be induced in vitro,[83] clinical resistance has been noted with its antibacterial use,[13,36,84] and some have argued against its use in monotherapy.[15] Most of a pentavalent antimonial (about 99%) is eliminated within a few hours, followed by a slower elimination phase with a half-life of 76 h.[85] At least in east Africa, these drugs remain highly effective.

Amphotericin B could be thought less likely to induce resistance given its high efficacy and a relatively short half-life of 24 h.[13,14,86–88] Although resistance can been induced in vitro, clinical cases of amphotericin-B resistance have not been reported.[89–92] Liposomal amphotericin B has a bioavailability in tissues for several weeks despite a relatively short plasma half-life.[93,94] Given this long tissue half-life, a single dose of liposomal amphotericin B followed by daily administration of a second drug (eg, sodium stibogluconate, paromomycin, or miltefosine) would result in simultaneous exposure of the parasite to both drugs. The use of a single dose of liposomal

amphotericin B (10 mg/kg) in monotherapy is being explored in India.[95] Although this might be a simple and effective approach, concerns of resistance and cost should also be taken into account.

Compliance with treatment

Besides the intrinsic characteristics of the combination regimen, the use of and compliance to the regimen also affects the risk of drug resistance. All conventional monotherapies (apart from liposomal amphotericin B) need a long treatment duration (21–30 days), making compliance more challenging. This is of particular concern for treatment with miltefosine, the only oral drug, for which the risk of premature treatment interruption is high. Even in a phase 4 trial, 4·5% of patients were lost to follow-up before the end of treatment, and 14·5% were not available for assessment by 6 months after treatment.[48] High default rates (up to 30%) were noted in a pilot study of miltefosine in India in ten districts (our unpublished data). Shorter treatment duration, particularly if the drug is also more tolerable, might help to increase compliance, as has been the case for patients on combination regimens for malaria.[13]

The lower costs to patients associated with shortened combination therapy could also improve access to and acceptability of visceral-leishmaniasis treatment. Some have suggested that the directly observed treatment strategy, which has been successfully used for tuberculosis, might have a role in ensuring good compliance to miltefosine, although this will increase the indirect and direct costs.[14,16,21] The elimination programme for visceral leishmaniasis in south Asia has opted for miltefosine as first-line drug, but will need to engage in the monitoring of clinical treatment outcomes and pharmacovigilance to ensure effective management.[16]

Special populations: HIV coinfection

In east Africa, coinfection with HIV is a major challenge in the treatment of visceral leishmaniasis, with up to 30% of cases infected with HIV in some regions.[15,23,96] This problem also seems to be on the increase in the Indian subcontinent.[97,98] Because asymptomatic leishmania infections are thought to outnumber symptomatic infections,[1,4] the dramatically increased risk of progression to visceral leishmaniasis after infection with HIV could lead to increased disease burden,[23] as has also been seen with tuberculosis.[99] Additionally, coinfection with leishmania and HIV is associated with a high mortality and a high rate of treatment failure and relapse with all visceral-leishmaniasis drugs.[23,45,100,101] The efficacy of combination therapy for coinfection with leishmania and HIV has not yet been studied in a controlled trial.

Although widespread use of antiretroviral therapy has resulted in large reductions in the incidence of visceral-leishmaniasis–HIV coinfection in southern Europe, it seems to be only partly protective against relapses.[23,100] Patients with incurable disease (who present with relapse or post kala-azar dermal leishmaniasis) could serve as a reservoir for the development and spread of drug-resistant strains, particularly because such patients seem to be more infectious.[102] To increase efficacy, combination therapy might be particularly relevant for coinfected patients to prevent resistance, because repeated exposure to single anti-leishmanial drugs will facilitate the emergence of resistant parasites. Even if relapses cannot be prevented, combination therapy might preserve drug sensitivity. Although one study reported decreased sensitivity towards amphotericin B after several treatment courses in individuals infected with HIV,[86] this has not been confirmed.[87,88]

Given the overall poor treatment outcomes, patients infected with HIV are most likely to need a different first-line therapy from patients not infected with HIV, at least in terms of treatment duration. However, none of the planned or continuing phase 3 trials include patients infected with HIV. Whether secondary prophylaxis could prevent relapses remains unclear. WHO does not recommend secondary prophylaxis in foci of anthroponotic visceral leishmaniasis.[103]

Future perspectives

Although the principle of combination therapy is generally accepted, the rationale for the choice of the drugs in such combination regimens is still under debate.[13–15] Several combinations have been taken forward in clinical studies, which will most likely provide us with several efficacious treatment options in the near future. However, it remains to be defined what exactly we should expect from combination therapy, and which factors will be important in selecting a specific therapy within a given context.

The current or planned clinical trials on combination therapy for visceral leishmaniasis in Asia and Africa will essentially provide data on safety and efficacy of the different regimens. Whereas drug–drug interactions, both in terms of pharmacokinetics and toxicity, have been prime considerations in the development of combination therapy for other infectious diseases, only limited data on anti-leishmanial drugs are available. Since the leishmania parasite targets reticulo-endothelial cells within specific organs, factors such as tissue distribution and uptake into macrophages of the individual components within combination therapy might also be relevant.[25] Whereas reduced toxic effects with combination therapy could possibly improve drug tolerance, because of reduced total doses of the individual compounds, increased toxicity could also be possible. Although the available data from animal and human studies seem reassuring, more studies to address these issues should be done. At least some of the combination studies that have been planned by the Drugs for Neglected Disease initiative will include pharmacokinetic substudies (our unpublished data).

Forthcoming results of clinical trials will not address the future public health benefits of combination therapy nor how it should be applied in field settings. Particularly, data from east Africa on liposomal amphotericin B suggest that many patients present with advanced and complicated visceral leishmaniasis and that perhaps relatively intensive treatment might be needed for cure.[42,104–106] Because these patients are generally not included in phase 3 trials, these issues should be further addressed in field studies. Even in the recently reported phase 4 trial of miltefosine in India, 10% of patients were excluded.[48] More research on safety issues also needs to be done.

It will take several years before sufficient clinical data will be available and combination therapy will be effectively implemented in the field. In the meantime, care should be taken to minimise the development of resistance to the individual drugs. Compared with miltefosine, liposomal amphotericin B might be a more cautious option, because substantial resistance towards liposomal amphotericin B is unlikely to happen when used as large-scale monotherapy for a few years. Recent data from India on the use of standard dose of liposomal amphotericin B in operational settings have shown excellent treatment outcomes.[107]

Most likely, regional factors will play an important part in deciding on the best combination therapy for a particular area, region, or country. Regional differences in natural or acquired drug resistance or pharmacokinetics should be considered. Differences in nutritional status and prevalence of coinfections such as HIV and tuberculosis might also determine the choice of combination therapies. Finally, regional factors will also determine which combination could be made available with the largest coverage and compliance in a sustainable and stable matter, and which delivery model would be most cost-effective.

Key questions and challenges

Trial data of several combination therapies will become available over the next few years, and should address some of the issues that have been discussed. However, many questions and challenges remain (panel). Whereas combination therapy aims to increase the lifespan of available drugs, this could also lead to rapid loss of two therapeutic options, if not applied in a controlled and regulated way. Care should be taken to ensure that the increased complexity of the logistics of combination therapy does not hamper effective implementation. The evidence base for combination therapy should be regularly re-assessed within each specific context.

How an effective treatment strategy should be integrated within the private and public-health sectors and how this determines the choice of (combination) regimen are unclear. Without increased access to and capacity of health care, both in terms of diagnosis and treatment, the overall effect of any (combination) therapy will be small. Efforts to reduce transmission also need to be addressed. The factors that affect the acceptability of visceral-leishmaniasis care and how to improve it will also need to be taken into account.

Close monitoring of combination therapy will be important at several levels. Programme monitoring will allow assessment of the overall effect, to identify barriers, and to supervise the correct implementation and use of combination therapy. Systems should also be put in place to detect counterfeit drugs or illegal drug supply. In areas with high prevalence of HIV or tuberculosis, close links and integration with the national programmes will be pivotal. Although reliable programme data can show the effectiveness of treatment regimens in use, they do not allow emerging resistance to be traced at an early stage. Parasite drug susceptibility should be monitored within surveillance systems, as being deployed for tuberculosis, malaria, and HIV. Because in-vitro susceptibility assays used at present have substantial limitations, further optimisation and standardisation remain necessary.[108,109] The development of molecular markers of resistance, as pursued by several research groups, could be a way forward in the long run if also made available in endemic countries.[21] Moreover, visceral-leishmaniasis therapies carry a substantial risk of toxic effects, and there are is obviously no long-term experience with combination therapy. Particularly if miltefosine is to be used on a large scale, problems related to teratogenicity or other unknown side-effects should be traced at an early stage.

Whether combination therapy also has an important place in the prevention of drug resistance, and how this is best achieved, will only be known in the long run.

***Panel:* Key questions and challenges**

- To what extent are the available anti-leishmanial drugs threatened by the development of drug resistance when used in monotherapy?
- Will combination therapy effectively delay acquired drug resistance and how is this best achieved?
- How do we set up an effective surveillance system to detect the development of acquired drug resistance?
- What will be the field efficacy of combination therapy?
- How do we set up a pharmacovigilance system?
- How do we assure good compliance with ambulatory treatment?
- Is there a need for a (daily) directly observed therapy strategy for miltefosine?
- How do we effectively implement a combination therapy strategy and, at the same time, increase access to care and diagnostic and therapeutic capacity?
- What is most cost-effective and feasible delivery model for visceral-leishmaniasis care in a specific setting?
- How do we regulate and control prescription of combination therapy, and how do we detect or prevent extra-legal drug use?
- What is the place of the private health care in visceral-leishmaniasis treatment?
- Do public–private partnerships have a place in the provision of care and treatment for visceral leishmaniasis?

Search strategy and selection criteria

Articles cited in this Review were obtained through searches of PubMed or Medline for papers published up to June 1, 2009, with terms including, but not restricted to, the following combinations: "visceral leishmaniasis", "treatment", "combination therapy", "safety", "efficacy", "cost", "cost-effectiveness", "resistance", "compliance", "health system", and "human immunodeficiency virus". The search was limited to English. Reference lists of these articles were then searched to identify other relevant publications. Clinical trial websites were verified, and experts in clinical research in visceral leishmaniasis were contacted for information on planned or ongoing trials. Abstracts of recent international conferences on infectious diseases were also reviewed. We structured our Review around the following criteria: efficacy and safety, health-system aspects (feasibility, cost-effectiveness and regulations), potential for drug resistance, and use in special populations.

Aiming for a combination therapy that is highly effective, widely accessible, affordable, and with high rates of compliance should probably be the priority.

Contributors

JvG did the search of published work. JvG and MBo wrote the first draft. MBa, FM, JA, LL improved the intellectual content of the paper and reviewed the subsequent drafts. All authors read and approved the final draft.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

JvG is supported by the InBev-Baillet Latour Fund.

References

1 Chappuis F, Sundar S, Hailu A, et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat Rev Microbiol* 2007; **5:** 873–82.
2 Herwaldt BL. Leishmaniasis. *Lancet* 1999; **354:** 1191–99.
3 Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004; **27:** 305–18.
4 Guerin PJ, Olliaro P, Sundar S, et al. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect Dis* 2002; **2:** 494–501.
5 Desjeux P. Leishmaniasis. *Nat Rev Microbiol* 2004; **2:** 692–93.
6 Alvar J, Croft S, Olliaro P. Chemotherapy in the treatment and control of leishmaniasis. *Adv Parasitol* 2006; **61:** 223–74.
7 Sundar S, Chakravarty J. Paromomycin in the treatment of leishmaniasis. *Expert Opin Investig Drugs* 2008; **17:** 787–94.
8 Berman JD. Development of miltefosine for the leishmaniases. *Mini Rev Med Chem* 2006; **6:** 145–51.
9 Croft SL, Engel J. Miltefosine—discovery of the antileishmanial activity of phospholipid derivatives. *Trans R Soc Trop Med Hyg* 2006; **100** (suppl 1): S4–8.
10 WHO Special Programme for Research and Training in Tropical Diseases. Countries of the south-east Asia region plan to eliminate kala azar. TDR News, October 2004, page 8. http://www.who.int/tdr/publications/tdrnews/pdf/TDRnews-issue-73.pdf (accessed Jan 18, 2010).
11 Bern C, Adler-Moore J, Berenguer J, et al. Liposomal amphotericin B for the treatment of visceral leishmaniasis. *Clin Infect Dis* 2006; **43:** 917–24.
12 Maltezou HC. Visceral leishmaniasis: advances in treatment. *Recent Pat Antiinfect Drug Discov* 2008; **3:** 192–98.
13 Bryceson A. A policy for leishmaniasis with respect to the prevention and control of drug resistance. *Trop Med Int Health* 2001; **6:** 928–34.
14 Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin Microbiol Rev* 2006; **19:** 111–26.
15 Den Boer M, Davidson RN. Treatment options for visceral leishmaniasis. *Expert Rev Anti Infect Ther* 2006; **4:** 187–97.
16 Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. *Ther Clin Risk Manag* 2007; **3:** 733–40.
17 Singh RK, Pandey HP, Sundar S. Visceral leishmaniasis (kala-azar): challenges ahead. *Indian J Med Res* 2006; **123:** 331–44.
18 Lira R, Sundar S, Makharia A, et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. *J Infect Dis* 1999; **180:** 564–67.
19 Sundar S. Drug resistance in Indian visceral leishmaniasis. *Trop Med Int Health* 2001; **6:** 849–54.
20 Sundar S, Murray HW. Availability of miltefosine for the treatment of kala-azar in India. *Bull World Health Organ* 2005; **83:** 394–95.
21 Perez-Victoria FJ, Sánchez-Cañete MP, Seifert K, et al. Mechanisms of experimental resistance of *Leishmania* to miltefosine: implications for clinical use. *Drug Resist Updat* 2006; **9:** 26–39.
22 Seifert K, Perez-Victoria FJ, Stettler M, et al. Inactivation of the miltefosine transporter, LdMT, causes miltefosine resistance that is conferred to the amastigote stage of *Leishmania donovani* and persists in vivo. *Int J Antimicrob Agents* 2007; **30:** 229–35.
23 Alvar J, Aparicio P, Aseffa A, et al. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev* 2008; **21:** 334–59.
24 Neal RA, Allen S, McCoy N, Olliaro P, Croft SL. The sensitivity of *Leishmania* species to aminosidine. *J Antimicrob Chemother* 1995; **35:** 577–84.
25 Seifert K, Croft SL. In vitro and in vivo interactions between miltefosine and other antileishmanial drugs. *Antimicrob Agents Chemother* 2006; **50:** 73–79.
26 Seifert K, Syeda T, Munday J, Croft S. Drug combination studies in experimental leishmaniasis models—lessons learned and future directions. Proceeding of the 4th World Congress on Leishmaniasis (WorldLeish4), Lucknow, India; Feb 3–7, 2009. Abstract 423.
27 Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S. Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980–2004. *Lancet Infect Dis* 2005; **5:** 763–74.
28 Sundar S, Agrawal G, Rai M, Makharia MK, Murray HW. Treatment of Indian visceral leishmaniasis with single or daily infusions of low dose liposomal amphotericin B: randomised trial. *BMJ* 2001; **323:** 419–22.
29 Sundar S, Jha TK, Thakur CP, Mishra M, Singh VP, Buffels R. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin Infect Dis* 2003; **37:** 800–04.
30 Sundar S, Makharia A, More DK, et al. Short-course of oral miltefosine for treatment of visceral leishmaniasis. *Clin Infect Dis* 2000; **31:** 1110–13.
31 Sundar S, Rai M, Chakravarty J, et al. New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. *Clin Infect Dis* 2008; **47:** 1000–06.
32 ClinicalTrials.gov. Short course of miltefosine and liposomal amphotericin B for kala-azar. NCT00371995. http://clinicaltrials.gov/ct2/show/NCT00371995 (accessed Jan 23, 2010).
33 ClinicalTrials.gov. Safety and efficacy study to evaluate different combination treatment regimens for visceral leishmaniasis. NCT00696969. http://clinicaltrials.gov/ct2/show/NCT00696969 (accessed Jan 23, 2010).
34 ClinicalTrials.gov. Efficacy/safety of sodium stibogluconate (SSG) versus paromomycin (PM) and SSG/PM combination to treat V leishmaniasis. NCT00255567. http://clinicaltrials.gov/ct2/show/NCT00255567 (accessed Jan 23, 2010).
35 Chunge CN, Owate J, Pamba HO, Donno L. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Trans R Soc Trop Med Hyg* 1990; **84:** 221–25.

36 Davidson RN, Den Boer M, Ritmeijer K. Paromomycin. *Trans R Soc Trop Med Hyg* 2009; **103:** 653–60.

37 Melaku Y, Collin SM, Keus K, Gatluak F, Ritmeijer K, Davidson RN. Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. *Am J Trop Med Hyg* 2007; **77:** 89–94.

38 Seaman J, Mercer AJ, Sondorp HE, Herwaldt BL. Epidemic visceral leishmaniasis in southern Sudan: treatment of severely debilitated patients under wartime conditions and with limited resources. *Ann Intern Med* 1996; **124:** 664–72.

39 Seaman J, Pryce D, Sondorp HE, Moody A, Bryceson AD, Davidson RN. Epidemic visceral leishmaniasis in Sudan: a randomized trial of aminosidine plus sodium stibogluconate versus sodium stibogluconate alone. *J Infect Dis* 1993; **168:** 715–20.

40 Wakabi W. Hope for improved leishmaniasis treatment in Africa. *Lancet Infect Dis* 2007; **7:** 638.

41 Mudawi AM. New treatments for visceral leishmaniasis in east Africa: the story so far. Proceedings of the 4th World Congress on Leishmaniasis 2009 (WorldLeish4), Lucknow, India; Feb 3–7, 2009. http://www.worldleish4.org/program_details.php?controlId=symposia&&symp=dndi (accessed Jan 18, 2010).

42 Berman JD, Badaro R, Thakur CP, et al. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. *Bull World Health Organ* 1998; **76:** 25–32.

43 Mueller M, Balasegaram M, Koummuki Y, Ritmeijer K, Santana MR, Davidson R. A comparison of liposomal amphotericin B with sodium stibogluconate for the treatment of visceral leishmaniasis in pregnancy in Sudan. *J Antimicrob Chemother* 2006; **58:** 811–15.

44 Mueller Y, Nguimfack A, Cavailler P, et al. Safety and effectiveness of amphotericin B deoxycholate for the treatment of visceral leishmaniasis in Uganda. *Ann Trop Med Parasitol* 2008; **102:** 11–19.

45 Ritmeijer K, Dejenie A, Assefa Y, et al. A comparison of miltefosine and sodium stibogluconate for treatment of visceral leishmaniasis in an Ethiopian population with high prevalence of HIV infection. *Clin Infect Dis* 2006; **43:** 357–64.

46 Mondal D, Singh SP, Kumar N, et al. Visceral leishmaniasis elimination programme in India, bangladesh, and Nepal: reshaping the case finding/case management strategy. *PLoS Negl Trop Dis* 2009; **3:** e355.

47 Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001; **95:** 239–43.

48 Bhattacharya SK, Sinha PK, Sundar S, et al. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. *J Infect Dis* 2007; **196:** 591–98.

49 Anoopa SD, Bern C, Varghese B, et al. The economic impact of visceral leishmaniasis on households in Bangladesh. *Trop Med Int Health* 2006; **11:** 757–64.

50 Meheus F, Boelaert M, Baltussen R, Sundar S. Costs of patient management of visceral leishmaniasis in Muzaffarpur, Bihar, India. *Trop Med Int Health* 2006; **11:** 1715–24.

51 Rijal S, Koirala S, Van der Stuyft P, Boelaert M. The economic burden of visceral leishmaniasis for households in Nepal. *Trans R Soc Trop Med Hyg* 2006; **100:** 838–41.

52 Vanlerberghe V, Diap G, Guerin PJ, et al. Drug policy for visceral leishmaniasis: a cost-effectiveness analysis. *Trop Med Int Health* 2007; **12:** 274–83.

53 Olliaro P, Sundar S. Anthropometrically derived dosing and drug costing calculations for treating visceral leishmaniasis in Bihar, India. *Trop Med Int Health* 2009; **14:** 88–92.

54 Meheus F, Olliaro P, Rijal S, Sundar S, Faiz M, Boelaert M. Preliminary cost-effectiveness analysis of combination therapies to combat parasite resistance to antileishmanial drugs in the Indian subcontinent. Proceedings of the 4th World Congress on Leishmaniasis 2009 (WorldLeish4), Lucknow, India; Feb 3–7, 2009. Abstract 691.

55 Sundar S, Thakur BB, Tandon AK, et al. Clinicoepidemiological study of drug resistance in Indian kala-azar. *BMJ* 1994; **308:** 307.

56 Peters W. The treatment of kala-azar—new approaches to an old problem. *Indian J Med Res* 1981; **73** (suppl): 1–18.

57 Thakur CP, Kumar M, Singh SK, et al. Comparison of regimens of treatment with sodium stibogluconate in kala-azar. *BMJ* 1984; **288:** 895–97.

58 Thakur CP, Kumar M, Kumar P, Mishra BN, Pandey AK. Rationalisation of regimens of treatment of kala-azar with sodium stibogluconate in India: a randomised study. *BMJ* 1988; **296:** 1557–61.

59 Thakur CP, Kumar M, Pandey AK. Evaluation of efficacy of longer durations of therapy of fresh cases of kala-azar with sodium stibogluconate. *Indian J Med Res* 1991; **93:** 103–10.

60 Sundar S, Rosenkaimer F, Lesser ML, Murray HW. Immunochemotherapy for a systemic intracellular infection: accelerated response using interferon-gamma in visceral leishmaniasis. *J Infect Dis* 1995; **171:** 992–96.

61 Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis* 1997; **176:** 1117–19.

62 Sundar S, More DK, Singh MK, et al. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis* 2000; **31:** 1104–07.

63 Rijal S, Chappuis F, Singh R, et al. Treatment of visceral leishmaniasis in south-eastern Nepal: decreasing efficacy of sodium stibogluconate and need for a policy to limit further decline. *Trans R Soc Trop Med Hyg* 2003; **97:** 350–54.

64 Rijal S, Bhandari S, Koirala S, et al. Clinical risk factors for therapeutic failure in kala-azar patients treated with pentavalent antimonials in Nepal. *Trans R Soc Trop Med Hyg* 2009; published online Aug 31. DOI: 10.1016/j.trstmh.2009.08.002.

65 Laurent T, Rijal S, Yardley V, et al. Epidemiological dynamics of antimonial resistance in *Leishmania donovani*: genotyping reveals a polyclonal population structure among naturally-resistant clinical isolates from Nepal. *Infect Genet Evol* 2007; **7:** 206–12.

66 Dube A, Singh N, Sundar S, Singh N. Refractoriness to the treatment of sodium stibogluconate in Indian kala-azar field isolates persist in in vitro and in vivo experimental models. *Parasitol Res* 2005; **96:** 216–23.

67 Carrio J, Portus M. In vitro susceptibility to pentavalent antimony in *Leishmania infantum* strains is not modified during in vitro or in vivo passages but is modified after host treatment with meglumine antimoniate. *BMC Pharmacol* 2002; **2:** 11.

68 Carrio J, Riera C, Gallego M, Ribera E, Portus M. In vitro susceptibility of *Leishmania infantum* to meglumine antimoniate in isolates from repeated leishmaniasis episodes in HIV-coinfected patients. *J Antimicrob Chemother* 2001; **47:** 120–21.

69 Faraut-Gambarelli F, Piarroux R, Deniau M, et al. In vitro and in vivo resistance of *Leishmania infantum* to meglumine antimoniate: a study of 37 strains collected from patients with visceral leishmaniasis. *Antimicrob Agents Chemother* 1997; **41:** 827–30.

70 den Boer ML, Alvar J, Davidson RN, Ritmeijer K, Balasegaram M. Developments in the treatment of visceral leishmaniasis. *Expert Opin Emerg Drugs* 2009; **14:** 395–410.

71 Hastings IM. Modelling parasite drug resistance: lessons for management and control strategies. *Trop Med Int Health* 2001; **6:** 883–90.

72 Stepniewska K, White NJ. Pharmacokinetic determinants of the window of selection for antimalarial drug resistance. *Antimicrob Agents Chemother* 2008; **52:** 1589–96.

73 Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. *Am J Trop Med Hyg* 2007; **77:** 181–92.

74 Pongtavornpinyo W, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. *Malar J* 2008; **7:** 229.

75 White NJ. Antimalarial drug resistance. *J Clin Invest* 2004; **113:** 1084–92.

76 Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med* 2008; **359:** 2619–20.

77 Noedl H, Socheat D, Satimai W. Artemisinin-resistant malaria in Asia. *N Engl J Med* 2009; **361:** 540–41.

78 Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009; **361:** 455–67.

79 Prajapati V. Intrinsic macrophage sensitivity of different field isolates in respect to antileishmanial drugs. Proceedings of the 4th World Congress on Leishmaniasis 2009 (WorldLeish4), Lucknow, India; Feb 3–7, 2009. Abstract 134.

80 Kumar D, Kulshrestha A, Singh R, Salotra P. In vitro susceptibility of field isolates of *Leishmania donovani* to miltefosine and amphotericin B: correlation with sodium antimony gluconate susceptibility and implications for treatment in areas of endemicity. *Antimicrob Agents Chemother* 2009; **53:** 835–38.

81 Berman J, Bryceson AD, Croft S, et al. Miltefosine: issues to be addressed in the future. *Trans R Soc Trop Med Hyg* 2006; **100** (suppl 1)**:** S41–44.

82 Dorlo TP, van Thiel PP, Huitema AD, et al. Pharmacokinetics of miltefosine in Old World cutaneous leishmaniasis patients. *Antimicrob Agents Chemother* 2008; **52:** 2855–60.

83 Maarouf M, Adeline MT, Solignac M, Vautrin D, Robert-Gero M. Development and characterization of paromomycin-resistant *Leishmania donovani* promastigotes. *Parasite* 1998; **5:** 167–73.

84 Teklemariam S, Hiwot AG, Frommel D, Miko TL, Ganlov G, Bryceson A. Aminosidine and its combination with sodium stibogluconate in the treatment of diffuse cutaneous leishmaniasis caused by *Leishmania aethiopica*. *Trans R Soc Trop Med Hyg* 1994; **88:** 334–39.

85 Chulay JD, Fleckenstein L, Smith DH. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. *Trans R Soc Trop Med Hyg* 1988; **82:** 69–72.

86 Di Giorgio C, Faraut-Gambarelli F, Imbert A, Minodier P, Gasquet M, Dumon H. Flow cytometric assessment of amphotericin B susceptibility in *Leishmania infantum* isolates from patients with visceral leishmaniasis. *J Antimicrob Chemother* 1999; **44:** 71–76.

87 Durand R, Paul M, Pratlong F, et al. *Leishmania infantum*: lack of parasite resistance to amphotericin B in a clinically resistant visceral leishmaniasis. *Antimicrob Agents Chemother* 1998; **42:** 2141–43.

88 Lachaud L, Bourgeois N, Plourde M, Leprohon P, Bastien P, Ouellette M. Parasite susceptibility to amphotericin B in failures of treatment for visceral leishmaniasis in patients coinfected with HIV type 1 and *Leishmania infantum*. *Clin Infect Dis* 2009; **48:** e16–22.

89 Singh AK, Papadopoulou B, Ouellette M. Gene amplification in amphotericin B-resistant *Leishmania tarentolae*. *Exp Parasitol* 2001; **99:** 141–47.

90 Papadopoulou B, Kundig C, Singh A, Ouellette M. Drug resistance in *Leishmania*: similarities and differences to other organisms. *Drug Resist Updat* 1998; **1:** 266–78.

91 Ouellette M, Drummelsmith J, Papadopoulou B. Leishmaniasis: drugs in the clinic, resistance and new developments. *Drug Resist Updat* 2004; **7:** 257–66.

92 Mbongo N, Loiseau PM, Billion MA, Robert-Gero M. Mechanism of amphotericin B resistance in *Leishmania donovani* promastigotes. *Antimicrob Agents Chemother* 1998; **42:** 352–57.

93 Gangneux JP, Sulahian A, Garin YJ, Farinotti R, Derouin F. Therapy of visceral leishmaniasis due to *Leishmania infantum*: experimental assessment of efficacy of AmBisome. *Antimicrob Agents Chemother* 1996; **40:** 1214–18.

94 Adler-Moore J, Proffitt RT. AmBisome: liposomal formulation, structure, mechanism of action and pre-clinical experience. *J Antimicrob Chemother* 2002; **49** (suppl 1)**:** 21–30.

95 ClinicalTrials.gov. Single infusion of liposomal amphotericin B in Indian visceral leishmaniasis. NCT00628719. http://clinicaltrials.gov/ct2/show/NCT00628719 (accessed Jan 25, 2010).

96 Lyons S, Veeken H, Long J. Visceral leishmaniasis and HIV in Tigray, Ethiopia. *Trop Med Int Health* 2003; **8:** 733–39.

97 Mathur P, Samantaray JC, Vajpayee M, Samanta P. Visceral leishmaniasis/human immunodeficiency virus co-infection in India: the focus of two epidemics. *J Med Microbiol* 2006; **55:** 919–22.

98 Redhu NS, Dey A, Balooni V, Singh S. *Leishmania*–HIV co-infection: an emerging problem in India. *AIDS* 2006; **20:** 1213–15.

99 Harries AD. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet* 1990; **335:** 387–90.

100 Ter Horst R, Collin SM, Ritmeijer K, Bogale A, Davidson RN. Concordant HIV infection and visceral leishmaniasis in Ethiopia: the influence of antiretroviral treatment and other factors on outcome. *Clin Infect Dis* 2008; **46:** 1702–09.

101 Ritmeijer K, Ter Horst R, Chane S, Davidson RN. Poor effectiveness of liposomal amphotericin-B (Ambisome) in HIV co-infected visceral leishmaniasis patients in Ethiopia. 2009. Proceedings of the 4th World Congress on Leishmaniasis 2009 (WorldLeish4), Lucknow, India; Feb 3–7, 2009. Abstract 159.

102 Molina R, Gradoni L, Alvar J. HIV and the transmission of *Leishmania*. *Ann Trop Med Parasitol* 2003; **97** (suppl 1)**:** 29–45.

103 WHO. Report of the fifth consultative meeting on *Leishmania*/HIV coinfection. Addis Ababa, Ethiopia, 20–22 March 2007. http://www.who.int/leishmaniasis/resources/Leishmaniasis_hiv_coinfection5.pdf (accessed Jan 20, 2010).

104 Collin S, Davidson R, Ritmeijer K, et al. Conflict and kala-azar: determinants of adverse outcomes of kala-azar among patients in southern Sudan. *Clin Infect Dis* 2004; **38:** 612–19.

105 Mueller M, Ritmeijer K, Balasegaram M, Koummuki Y, Santana MR, Davidson R. Unresponsiveness to AmBisome in some Sudanese patients with kala-azar. *Trans R Soc Trop Med Hyg* 2007; **101:** 19–24.

106 Seaman J, Boer C, Wilkinson R, et al. Liposomal amphotericin B (AmBisome) in the treatment of complicated kala-azar under field conditions. *Clin Infect Dis* 1995; **21:** 188–93.

107 Lima N. Experience of Médecins Sans Frontières after 1 year of using liposomal amphotericin B (AmBisome) treatment for Indian visceral leishmaniasis under routine programme conditions in Bihar, India. 2009. Proceedings of the 4th World Congress on Leishmaniasis 2009 (WorldLeish4), Lucknow, India; Feb 3–7, 2009. http://www.worldleish4.org/program_details.php?controlId=symposia&&symp=dndi (accessed Jan 18, 2010).

108 Croft SL. Monitoring drug resistance in leishmaniasis. *Trop Med Int Health* 2001; **6:** 899–905.

109 Rijal S, Yardley V, Chappuis F, et al. Antimonial treatment of visceral leishmaniasis: are current in vitro susceptibility assays adequate for prognosis of in vivo therapy outcome? *Microbes Infect* 2007; **9:** 529–35.