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. 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 anthropoanotic form (human reservoir) is caused by L. donovani and is prevalent in east Africa and the Indian subcontinent. 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).

For most of the past 70 years, the therapeutic armoury for treatment of visceral leishmaniasis has been extremely limited. 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. The development of miltefosine, the only drug at present that can be given orally for visceral leishmaniasis treatment, has been a major breakthrough.

This drug is the mainstay of the recently launched visceral-leishmaniasis elimination plan in the Indian subcontinent, 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). 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. These drugs belong to chemically unrelated classes and are likely to constitute the main therapeutic options for visceral leishmaniasis in the years to come. 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. 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.

We review the evidence and explore the potential of combination therapy for visceral leishmaniasis in areas of anthropoanotic transmission—in particular, the Indian subcontinent and east Africa. Given the anthropoanotic 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
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.22 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.26 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),32 which has now moved into its second stage adding children into the study. In 2009, similar studies

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**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.
Drugs currently used for treatment of visceral leishmaniasis

<table>
<thead>
<tr>
<th>Manufacturer* (trade name of drug)</th>
<th>Regimen</th>
<th>Clinical efficacy</th>
<th>Resistance</th>
<th>Toxicity</th>
<th>Cost of drug course (US$)</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentavalent antimonials (sodium stibogluconate, meglumine antimoniate)</td>
<td>Sodium stibogluconate: Albert David (SSG), GLaxoSmithKline (Pentostam); Meglumine antimoniate: Sanofi Aventis (Glucantime)</td>
<td>20 mg antimony per kg bodyweight daily for 20–30 days (depending on geographic area), intravenous or intramuscular</td>
<td>35–95% (depending on geographic area)</td>
<td>Treatment failure up to 60% (Bihar, India)</td>
<td>53 (generic) to 198 (branded SSG)</td>
<td>Quality control; length of treatment; painful injection; toxicity; resistance in India</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Bristol Meyers Squibb (Fungizone), generic companies</td>
<td>1 mg/kg every other day for up to 30 days (15 mg/kg total dose), intravenous</td>
<td>&gt;97% for all regions</td>
<td>Not documented</td>
<td>About 21 (generic)</td>
<td>Need for slow intravenous infusion; dose-limiting nephrotoxicity; heat instability</td>
</tr>
<tr>
<td>Liposomal amphotericin B</td>
<td>Gilead (AmBisome)</td>
<td>5–20 mg/kg total dose in 4–10 doses over 10–20 days, intravenous</td>
<td>Asia: &gt;97%; India, single dose: 91%; Africa: not fully established</td>
<td>Nephrotoxicity (limited)</td>
<td>280 (preferential) for 20 mg/kg dose; about 3000 (non-preferential)</td>
<td>Price; need for slow intravenous infusion; heat stability (needs to be stored below 25°C)</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>Paladin, Montreal, Canada (Impavido)</td>
<td>2–2.5 mg/kg daily over 28 days (India only), oral</td>
<td>Asia: 94% (India); Africa: 60% (single field study), 33% in patients not infected with HIV and excluding those lost to follow up</td>
<td>Gastrointestinal effects (20–55% of patients, usually mild), nephrotoxicity, hepatotoxicity, possibly teratogenic</td>
<td>About 74 (preferential), about 150 (non-preferential)</td>
<td>Price; possibly teratogenic; potential for resistance (half-life); poor patient compliance</td>
</tr>
<tr>
<td>Paromomycin sulphate</td>
<td>Institute for OneWorld Health;† Gland Pharma, Hyderabad, India</td>
<td>15 mg/kg daily for 21 days (India only), intramuscular</td>
<td>Asia: 94% (India); Africa: under evaluation</td>
<td>Nephrotoxicity, ototoxicity, hepatotoxicity (all relatively rare)</td>
<td>About 25</td>
<td>Efficacy varies between and within regions; potential for resistance?</td>
</tr>
</tbody>
</table>

*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

No large trials on other combination regimens have been done in Africa. Studies on miltefosine and amphotericin B as monotherapy are limited.42,43 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 from data presented during Fourth World Congress on Leishmaniasis (Feb 3–7, 2009)).

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

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.36–38 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).39 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).40 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.

...
perspective would have a major public health impact on affordable both from the patient’s and society’s tolerated, short regimen that can be given at the primary care (figure 2).3,15,47

need to be reached in different contexts and environments ranging from semi-nomadic people to migrant workers, non-governmental organisations. Diverse populations, leishmaniasis care has, until recently, mainly relied on treatment, mainly because of a lack of resources (67%) or reported. Overall, 23% of patients interrupted their Long delays in diagnosis and treatment were also

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

<table>
<thead>
<tr>
<th>Study design</th>
<th>ClinicalTrials.gov registration</th>
<th>Country</th>
<th>Study period</th>
<th>Patients enrolled</th>
<th>Drug combinations studied</th>
<th>Definitive cure (95% CI) at 9 months in intention-to-treat analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sundar et al³⁵</td>
<td>NCT00370825</td>
<td>India</td>
<td>2008–08</td>
<td>181 adults: 45 each in groups A, C, D and E; 46 in group B</td>
<td>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 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</td>
<td>Group A: 91% (78–97%); group B: 98% (87–100%); group C: 99% (84–99%); group D: 96% (84–99%); group E: 98% (87–100%)</td>
</tr>
<tr>
<td>Planned or ongoing trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNDI (VLCOMBO-07 trial)³³</td>
<td>NCT00696969</td>
<td>India, Bangladesh, and Nepal</td>
<td>2008–09 (India), 2009–10 (Bangladesh, Nepal)</td>
<td>624 adults and children†</td>
<td>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</td>
<td>–</td>
</tr>
<tr>
<td>Banaras Hindu University⁶</td>
<td>NCT00371995</td>
<td>India</td>
<td>Ongoing</td>
<td>150 adults and children†</td>
<td>SD L-AmB 5 mg/kg followed by miltefosine 2.5 mg/kg for 14 days</td>
<td>–</td>
</tr>
<tr>
<td>TDR†</td>
<td></td>
<td>Bangladesh</td>
<td>Planned</td>
<td>150†</td>
<td>SD L-AmB 5 mg/kg followed by miltefosine 2.5 mg/kg for 14 days</td>
<td>–</td>
</tr>
<tr>
<td>DNDI (LEAP0104A/B trial)³⁴</td>
<td>NCT00255567</td>
<td>Kenya, Ethiopia, Sudan, and Uganda</td>
<td>2004–09 (LEAP0104A), 2007–10 (LEAP0104B)</td>
<td>1100 adults and children†</td>
<td>Active comparator group 1: SSG 20 mg/kg for 30 days; group 2: paromomycin 15 mg/kg (LEAP0104A); group 3: SSG 20 mg/kg plus paromomycin 15 mg/kg for 17 days;</td>
<td>–</td>
</tr>
<tr>
<td>DNDI (VLCOMBO trial)¶</td>
<td></td>
<td>Sudan and Kenya</td>
<td>Planned for 2009–10</td>
<td>1899 adults and children: 63 in each group</td>
<td>L-AmB followed by miltefosine; L-AmB followed by SSG; miltefosine 2.5 mg/kg for 30 days</td>
<td>–</td>
</tr>
</tbody>
</table>

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. 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. 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%). 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).

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 23°C could be another barrier for national programmes, although technological solutions exist that can guarantee transport of drugs below 23°C for 4–5 days. Steps could be taken to strengthen the supply chain of liposomal amphotericin B.
Typical environment in Bihar, India (A). Seminomadic lifestyle in east Africa (B).
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. Reduced susceptibility to pentavalent antimonials has also been reported with L infantum in both human beings and animals. 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. 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.

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. 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. 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 development of double-resistant parasites would be expected to be extremely rare (ie, the product of their individual 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. 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. 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. Artemisinin resistance has recently been reported in Cambodia, but underlying reasons remain to be established. 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. 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. However, true cross-resistance between the various drugs has not been reported so far. Several combinations have shown activity enhancement in animal experiments. 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. 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. Resistant strains could be selected and amplified during this period because of subtherapeutic drug concentrations, either from relapsing patients, or from newly acquired infections. 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, and clinical resistance has been noted with its antibacterial use, and some have argued against its use in monotherapy. 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. 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. Although resistance can be induced in vitro, clinical cases of amphotericin-B resistance have not been reported. Liposomal amphotericin B has a bioavailability in tissues for several weeks despite a relatively short plasma half-life. 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.46 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.94–96 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.97

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.9,21,56 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,14 the dramatically increased risk of progression to visceral leishmaniasis after infection with HIV could lead to increased disease burden,11 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.21,90,98,100 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.21,100

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.98 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,99 this has not been confirmed.97–98

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.101

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.7 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.4,34–36 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 therapeutically 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.106,108 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.35 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 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?
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.

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