

Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis

Thomas P. C. Dorlo^{1,2*}, Manica Balasegaram³, Jos H. Beijnen² and Peter J. de Vries^{1†}

¹Center for Tropical Medicine and Travel Medicine, Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ²Department of Pharmacy and Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute, Amsterdam, The Netherlands; ³Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland

*Corresponding author. Division of Infectious Diseases, Room F4–217, Academic Medical Center, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands. Tel: +31-20-5664380; Fax: +31-20-6972286; E-mail: thomasdorlo@gmail.com

†Present address: Division of Internal Medicine, Tergooziekenhuizen, Hilversum, The Netherlands.

Miltefosine is an alkylphosphocholine drug with demonstrated activity against various parasite species and cancer cells as well as some pathogenic bacteria and fungi. For 10 years it has been licensed in India for the treatment of visceral leishmaniasis (VL), a fatal neglected parasitic disease. It is the first and still the only oral drug that can be used to treat VL and cutaneous leishmaniasis (CL). The standard 28 day miltefosine monotherapy regimen is well tolerated, except for mild gastrointestinal side effects, although its teratogenic potential severely hampers its general use in the clinic and roll-out in national elimination programmes. The pharmacokinetics of miltefosine are mainly characterized by its long residence time in the body, resulting in extensive drug accumulation during treatment and long elimination half-lives. At the moment, different combination therapy strategies encompassing miltefosine are being tested in multiple controlled clinical trials in various geographical areas of endemicity, both in South Asia and East Africa. We here review the most salient pre-clinical and clinical pharmacological aspects of miltefosine, its mechanism of action against *Leishmania* parasites and other pathogens, and provide a systematic overview of the efficacy and safety data from all clinical trials of miltefosine, either alone or in combination, in the treatment of VL and CL.

Keywords: clinical pharmacology, pre-clinical pharmacology, alkylphosphocholine, cutaneous leishmaniasis, visceral leishmaniasis, mechanism of action, pharmacokinetics, pharmacodynamics, antiparasitic agents, antiprotozoal agents, *Leishmania*

Overview of treatment options for leishmaniasis

Leishmaniasis is an infection caused by obligate intracellular protozoan *Leishmania* parasites transmitted by the bite of certain sandfly species.^{1,2} A multitude of *Leishmania* species specific to various geographical areas are able to cause disease in humans and can result in several diverse clinical manifestations. Visceral leishmaniasis (VL or kala-azar, which means black fever in Hindi), the most severe form of leishmaniasis, is typically caused by the *Leishmania donovani* species complex. It is characterized by disseminated visceral infection of the reticuloendothelial system and is inevitably fatal if left untreated.³ The ulcerated skin lesions typical for cutaneous leishmaniasis (CL) are mainly caused by *Leishmania major* and *Leishmania tropica* in the Old World (Europe, Africa, Central Asia and the Middle East) and by the *Leishmania braziliensis*, *Leishmania guyanensis* and *Leishmania mexicana* species complexes in the New World (Latin America), of which the former two species complexes can disseminate to the nasopharyngeal tissues and evolve into a more destructive mucosal form (mucocutaneous leishmaniasis).

Treatment of VL and CL is complicated by intrinsic species-specific differences in drug susceptibility,⁴ and also by differences in the apparent efficacy of the drugs between geographical areas,⁵ which implies that each new treatment and combination has to be reassessed in every distinct geographical location where VL or CL is endemic.

Since their discovery in the 1940s, the toxic parenteral pentavalent antimony (Sb^V) compounds have been the mainstay of treatment for either type of leishmaniasis, most notably intravenous- or intramuscular-injected sodium stibogluconate (Pentostam®, GlaxoSmithKline, UK, or the generic product from Albert David, India) and meglumine antimoniate (Glucantime®, Aventis, France).⁶ Despite the apparent, sometimes life-threatening, toxicity of these compounds, antimonials are still first-line treatment for both VL and CL in most areas. Only in Bihar state, the area where VL is most endemic in India, has increasing non-susceptibility of the parasites to antimonials led to widespread treatment failure and a shift to conventional amphotericin B.^{7,8} Currently, liposomal amphotericin B (AmBisome®, Gilead Sciences, CA, USA) is preferred over conventional amphotericin B because of its milder toxicity profile, although its use

remains very limited in resource-poor settings due to its very high costs.⁹ Recent studies have shown the efficacy of a single-dose treatment of liposomal amphotericin B for VL in India, although it is still unclear whether this is applicable to other geographical areas as well.^{10,11} Paromomycin (aminosidine, Gland Pharma, India), an aminoglycoside antibiotic, was rediscovered as an antileishmanial agent in the 1980s and has been used successfully as a parenteral agent in the treatment of VL and, with more variable success, as a topical agent for CL as well.^{12–15} Several treatment combinations using paromomycin and, generally, sodium stibogluconate were evaluated both in India and East Africa.^{5,16–20} Another historic agent still being used mainly in the treatment of New World CL is pentamidine (Pentam®, Abbott, IL, USA),²¹ which has gained renewed interest for its possible use as secondary prophylaxis in HIV-coinfected VL patients.^{21–23} All these available agents, of which most have already been in use for multiple decades, have to be administered parenterally. The need for a safe oral agent that does not require hospitalization was therefore great. Since its registration in 2002, miltefosine is the first and still the only oral agent that is used for the treatment of all types of leishmaniasis.

Historical perspective

The simultaneous but independent discovery of the antiprotozoal and antineoplastic activities of miltefosine and related alkylphosphocholine drugs occurred in the early 1980s.²⁴ Coincidentally, the compound was synthesized by two different research groups who were screening platelet-aggregating-factor analogues for their anti-inflammatory properties in the UK and similarly for their antitumour activity in Germany. Despite the excellent activity profile of miltefosine against trypanosomatid parasites, priority was given to the development of the compound as a local treatment for cutaneous metastases of breast cancer and eventually led to the approval of a topical formulation of miltefosine (Miltex®, Baxter, UK).²⁵ The application of miltefosine in an oral formulation in the treatment of solid tumours was also evaluated in several Phase II studies with different tumour types,^{26–28} but was eventually discontinued due to dose-limiting gastrointestinal side effects in these cancer patients.²⁹ Incited by encouraging *in vitro* findings on *Leishmania* parasites, apparent high bioavailability in previous pre-clinical studies and a clear need for an easy-to-administer oral treatment for VL, subsequent evaluation of oral miltefosine for VL in a mouse model demonstrated superior activity of oral miltefosine over standard intravenous sodium stibogluconate.³⁰ The first Phase II study of oral miltefosine in the treatment of human VL was conducted in India with very promising results.³¹ These observations led to the development of a unique public–private partnership collaboration between ASTA

Medica (later Zentaris GmbH), the WHO Special Programme for Research and Training in Tropical Diseases, and the Government of India.³² Eventually, several successful Phase II and III trials on VL in India led to the approval of miltefosine in 2002 as the first and still the only oral drug for the treatment of VL.³³ Since 2008, Paladin Labs (Montreal, Canada) is the licence holder for oral miltefosine for the indication leishmaniasis (for more information, see the Pharmaceutical products, drug licensing and availability section). The clinical development and licensing of the compound, either as monotherapy or as part of a combination therapy for VL, is still ongoing in various VL-endemic countries in collaboration with, amongst others, national governments, Médecins Sans Frontières and the Drugs for Neglected Diseases initiative (DNDi).

Methods

For systematic identification of published clinical trials of miltefosine in the treatment of leishmaniasis, the literature database PubMed was searched with the following terms: (*miltefosine*) AND [(*visceral leishmaniasis*) OR (*cutaneous leishmaniasis*) OR (*mucocutaneous leishmaniasis*)], with the restricting limits ‘Article type’ set to all clinical study or trial-related publications and ‘Species’ set to ‘Humans’; there were no further restrictions for ‘Publication dates’, ‘Ages’, ‘Languages’ or ‘Sex’. For the other sections in this review, no systematic approach was applied to identify publications.

Pharmacological class

Miltefosine belongs to the class of alkylphosphocholine drugs, which are phosphocholine esters of aliphatic long-chain alcohols. These alkylphosphocholine compounds are structurally related to the group of alkyl-lysophospholipids, which are synthetic analogues of lysophosphatidylcholines or lysolecithins, but lack their glycerol backbone. From a functional point of view, miltefosine is considered an inhibitor of Akt [otherwise known as protein kinase B (PKB)]. Akt/PKB is a crucial protein within the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) intracellular signalling pathway, which is involved in cell survival.³⁴

Physicochemical properties

The chemical name of miltefosine is hexadecyl 2-(trimethylazaniumyl)ethyl phosphate, also known as hexadecylphosphocholine (Figure 1). The empirical formula is C₂₁H₄₆NO₄P, yielding a molecular weight of 407.57 g/mol. Other alkylphosphocholine compounds that were tested for their antileishmanial or anticancer activity differ from miltefosine both in alkyl chain length and/or backbone, and distances between P and N.³⁵ Another closely related group of tested compounds are the

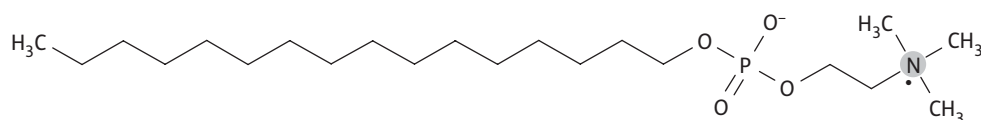


Figure 1. Structural formula of miltefosine.

alkylglycerophosphocholines, also known as ether lipids, which have only small structural modifications compared with miltefosine.³⁶ For example, edelfosine contains an ether-linked 2-methyl-glycerol backbone and has an octadecyl alkyl chain, while ilmofosine additionally contains a thioether group.^{37,38} Perifosine, another structurally related compound that has received considerable clinical attention, has an octadecyl alkyl chain and a piperidine head group instead of a choline head group.³⁹

Miltefosine is an amphiphilic and zwitterionic compound due to the positively charged quaternary amine group (permanently charged) and negatively charged phosphoryl group (pKa: ~2). The crystalline compound is a white to off-white hygroscopic powder and readily dissolvable in both aqueous and organic solvents. The solubility in ethanol and DMSO is 1.25 and 0.8 mg/mL, respectively, while in phosphate buffer solution (pH 7.2) or water this is ≥ 2.5 mg/mL.

Pharmaceutical products, drug licensing and availability

The following proprietary miltefosine products are available on the market: Impavido® (oral solid human pharmaceutical product), Miltex® (topical liquid human pharmaceutical product) and Milteforan® (oral liquid veterinary pharmaceutical product). Impavido®, the product that is licensed for the treatment of human VL, is available as 10 and 50 mg miltefosine capsules. This formulation contains, besides miltefosine, highly dispersed silicon dioxide, microcrystalline cellulose, lactose monohydrate, talc, magnesium stearate, gelatine, titanium dioxide, ferric oxide and purified water.⁴⁰ In India, a generic oral miltefosine product for the treatment of VL has been added to the list of registered drugs for human use.⁴¹ A locally procured poor-quality generic oral miltefosine product was found in Bangladesh, which contained no active pharmaceutical ingredient at all.^{42,43} Miltefosine (Impavido®) has been approved for the treatment of VL in Nepal, and both VL and CL in Argentina, Bangladesh, Bolivia, Colombia, Ecuador, Germany, Guatemala, Honduras, India, Mexico, Pakistan, Paraguay and Peru.³³ Furthermore, miltefosine was designated as an orphan drug product for the treatment of (visceral) leishmaniasis by both the European Medicines Agency in 2002 and by the US Food and Drug Administration (FDA) in 2006. Recently, the drug has also been added to the WHO Model List of Essential Medicines.⁴⁴ Oral miltefosine can be obtained in the USA from the manufacturer through an Investigational New Drug application to the FDA in conjunction with local institutional review board (IRB) approval,⁴⁵ whereas in European countries the drug can be imported from Germany on a named patient basis or through compassionate use programmes, depending on national legislation. In India, serious concerns have been raised about the unrestricted over-the-counter availability of miltefosine in the private sector in relation to non-compliance.^{46,47} Stricter regulation, free-of-charge supervised public distribution and directly observed therapy have therefore been advocated and implemented in the context of the national elimination programmes for VL in the Indian subcontinent; nevertheless, this drug availability issue remains of pivotal importance for the lifespan of miltefosine.

Drug dosage, costs and cost-effectiveness

The currently recommended dose for miltefosine as monotherapy for either CL or VL is 2.5 mg/kg/day for a total of 28 days. However, due to regular unavailability of the 10 mg capsule in clinical practice, other dosages are being administered. For example, the Indian government recommends 100 mg/day miltefosine for patients with a body weight ≥ 25 kg (corresponding to ~1.7–4 mg/kg/day) and 50 mg/day for body weights < 25 kg (corresponding to ~2–5.5 mg/kg/day). Recently, Dorlo *et al.*⁴⁸ demonstrated that children were relatively underexposed to miltefosine compared with adults when given the same mg/kg dosage. A new optimal miltefosine dosage was suggested that would lead to similar drug exposure.

For public use and control programmes in resource-poor countries 'where patients are being treated free of charge',³³ miltefosine is available at a preferential WHO-negotiated price, but only per 200 000 capsule batch order: depending on the size of the order, prices may vary between €45.28 and €54.92 for 56 capsules containing 50 mg of miltefosine, and between €34.36 and €39.30 for 56 capsules containing 10 mg of miltefosine. For a typical male VL patient from Bihar, India, weighing 39 kg,⁴⁹ this means that the drug cost for a standard monotherapy miltefosine regimen (28 days) has dropped on average from an initial US\$200⁴⁶ to a current cost of €50. For resource-rich countries the average drug cost for one complete miltefosine regimen (150 mg/day for 28 days) can amount up to €3000.

The cost-effectiveness of miltefosine, either alone or as part of a combination, has been investigated for the treatment of VL in the Indian subcontinent. Of the compared monotherapeutic options, miltefosine appeared to be the most cost-effective option in areas where there is a known non-susceptibility to antimony compounds.⁵⁰ When comparing monotherapies with a combination therapy of liposomal amphotericin B (single dose) and miltefosine (various durations) in the Indian context, the combination therapy is more cost-effective than most monotherapies, with US\$124–160 per averted death.⁵¹ In the case that also indirect costs (i.e. loss of productivity) are taken into account, the combination of miltefosine plus paromomycin was the most cost-effective, with US\$97 per averted death, although strategies employing liposomal amphotericin B were overall found to be the most effective.⁵²

Analytical assay

The structure of miltefosine lacks any chromophores, which makes ultraviolet or fluorescence detection very difficult. Only a single validated and sensitive bioanalytical method to quantify miltefosine in human matrices has been reported hitherto.⁵³ The method employed reversed-phase liquid chromatography coupled to tandem mass spectrometry to detect miltefosine in human EDTA plasma with pre-treatment by solid-phase extraction and had a lower limit of quantification of 4 ng/mL using a 250 μ L aliquot of plasma, which was sensitive enough to determine miltefosine concentrations up to 5 months after cessation of treatment (with a 28 day regimen).⁵⁴ Chromatography was performed on an alkaline-resistant C₁₈ column under isocratic and alkaline conditions. Several other methods have been reported for the bioanalysis of the structurally related compounds perifosine,^{55,56} edelfosine,⁵⁷ erucylphosphocholine⁵⁸

and CLR1401.⁵⁹ Recently, a platform of analytical techniques was presented for the determination and identification of miltefosine in pharmaceutical formulations, which can be used to distinguish poor-quality miltefosine-containing pharmaceutical products.⁴² Most notably, a simple and inexpensive colorimetric method was presented that can be used for the identification and semi-quantification of miltefosine in pharmaceutical formulations in the field.⁴²

Pharmacokinetics

The pre-clinical pharmacokinetics of miltefosine, investigated during the development of the drug, are summarized by Sindermann and Engel.²⁹ Little research was done on the clinical pharmacokinetics of miltefosine before the drug was available on the market. Scanty pharmacokinetic data on oral miltefosine in Indian VL patients are provided by the manufacturer and can be traced in registration documents.⁴⁰ After the approval of miltefosine, an extensive pharmacokinetic study was performed in Dutch soldiers treated with miltefosine for CL.⁵⁴ Currently, a pharmacokinetic study is ongoing as part of a randomized controlled clinical trial on the evaluation of miltefosine and combination therapy strategies for VL in East Africa.⁶⁰ Another study has recently been initiated as part of the evaluation of miltefosine in paediatric and adult CL patients in Colombia.⁶¹

Absorption

After oral administration, miltefosine showed a slow absorption process, with an absolute bioavailability of 82% in rats and 94% in dogs, with the time to reach the maximal concentration (T_{max}) between 4 and 48 h.²⁹ In humans, the absolute bioavailability has never been assessed due to possible haemolysis after intravenous administration,⁶² but the gastrointestinal absorption rate (half-life) estimated in a two-compartment population pharmacokinetic model was 0.416 h^{-1} (1.67 h).⁴⁸ The mechanism of absorption was investigated in more depth by Ménez *et al.*^{63,64} using *in vitro* permeability testing with Caco-2 cells. Below $50\text{ }\mu\text{M}$ ($20.4\text{ }\mu\text{g/mL}$), membrane translocation appeared to be mediated mainly through a non-saturable passive paracellular diffusion that was pH independent, while above this concentration the transport mechanism was found to be saturable and was probably an active carrier-mediated cellular transport.^{63,64} Most likely, both these transport mechanisms play a role in the gastrointestinal absorption of miltefosine. Indirect evidence suggested possible inhibition of the ATP-binding cassette (ABC) transporter P-glycoprotein in a Caco-2 cell line, which could imply possible drug–drug interactions.⁶⁵

Distribution

Distribution studies in rats following single oral administration of ^{14}C -radiolabelled miltefosine, and in mice following single oral and intravenous administration ^3H -radiolabelled miltefosine ($25\text{ }\mu\text{g}$ total dose) indicated a wide general distribution of miltefosine.^{29,66} These studies demonstrated that the uptake of radiolabelled miltefosine in rats and mice was extensive and in a range of tissues, with the highest accumulation of radioactivity in the liver, lungs, kidneys and spleen. This was confirmed in a subsequent study in rats in which a repeated steady-state oral

unlabelled miltefosine dose was administered, which demonstrated highest drug concentrations in the adrenal glands, kidneys, spleen and skin.⁶⁷ Steady-state concentrations could be achieved in all investigated organs and serum, except for the kidneys and brain. It remains unknown to what extent miltefosine penetrates the human brain; however, substantial miltefosine concentrations could be demonstrated in the CSF of patients treated for *Balamuthia* and *Naegleria* infections, although it was unclear how intact their blood–brain barrier was (T. P. C. Dorlo and G. S. Visvesvara, unpublished data). Placental distribution and transfer through the umbilical cord have not been investigated, but should be assumed given the results from reproductive toxicity studies in animals.²⁹ Plasma protein binding ranges between 96% and 98%, with no concentration dependence being observed. Miltefosine binds to both serum albumin and lipoproteins, with a preference for albumin (97% of the fraction bound) over low-density lipoprotein (3% of the fraction bound).⁶²

Metabolism

In pre-clinical *in vitro* studies, no oxidative metabolism by any of the investigated reconstituted cytochrome P450 (CYP) isoenzymes was observed.²⁹ The investigated isoenzymes included 1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7 and 4A1. Moreover, no induction of CYP3A isoenzymes by miltefosine could be demonstrated in both male and female rats.²⁹ Metabolic drug–drug interactions at the CYP level are therefore not expected, although evidence from human subjects on this topic is currently not available.

The main, and possibly only, metabolic pathway of miltefosine appears to be mediated by phospholipases. *In vitro* data suggested that miltefosine was a substrate only for reconstituted phospholipase D and not for phospholipase A, B or C.²⁹ However, other studies showed that both phospholipase C (*Bacillus cereus*) and phospholipase D (partially purified from cabbage) were able to hydrolyse miltefosine.^{42,66} Probably, the original source of the phospholipase enzymes from which they were purified determines the substrate specificity and can explain these different observations. The importance of the phospholipase-mediated metabolism was confirmed in mice in which the metabolic fate of ^3H -radiolabelled miltefosine in the liver was examined after intravenous administration.⁶⁶ After 24 h, the mainstay of the radioactivity could be characterized as unchanged miltefosine (63%), while radiolabelled choline (32%), phosphocholine (3%) and diacyllecithin (2%) were identified as metabolites of miltefosine in the liver of these mice. After 72 h, a decreased relative amount of radiolabelled miltefosine (37%) and more choline (53%) could be detected. *In vitro* studies with human hepatocytes showed consistently a slow release of choline after incubation with miltefosine. Phospholipase D enzymes generally cleave phosphocholines just after the phosphate before the choline group, resulting in the release of choline; phospholipase C enzymes have a preference for cleavage just before the phosphate group at the side of the alkyl chain, resulting in the release of an alcohol.⁶⁸ Both enzymes may play a role in the metabolic cleavage of miltefosine. The degradation products of miltefosine are endogenous compounds with physiological purposes and are therefore difficult to recover. Choline, the main metabolite found in animal studies and the product of phospholipase D cleavage, is likely used in the

physiological biosynthesis of cell membranes or as an important source for the synthesis of e.g. acetylcholine or lecithin. The long-chain alcohol that results from the phospholipase C cleavage of miltefosine can be oxidized into palmitic acid and subsequently used for the biosynthesis of other long-chain fatty acids.

Excretion

Miltefosine is almost completely eliminated by the metabolic mechanisms described above. Excretion in the urine appeared to be <0.2% of the administrated dose at day 23 of a 28 day treatment regimen.⁴⁰ Faecal excretion of miltefosine was not investigated clinically in humans, but is not expected based on its extremely long elimination half-life and high accumulation during treatment. However, in Beagle dogs, a slow faecal excretion was recently shown, where faecal clearance amounted to 10% ($\pm 4.86\%$) of the total miltefosine clearance.⁶⁹ Excretion into breast milk was not investigated due to the teratogenic potential of miltefosine, but must be expected.²⁹

Clinical pharmacokinetics

During the clinical development of oral miltefosine in general, little attention was paid to the clinical pharmacokinetics of this drug. No pharmacokinetic evaluation was performed in healthy individuals. Dose-finding studies were performed without extensive pharmacokinetic evaluations and only very sparse pharmacokinetic data from these studies, which were never published originally, can be traced in registration documents.⁴⁰ These data, obtained from Indian adults treated with 100 mg of miltefosine for 28 days, showed a median maximal concentration of

70 $\mu\text{g/mL}$ at day 23 of treatment and indicated an elimination half-life of between 150 and 200 h (~ 7 days). For Indian children, treated with 2.5 mg/kg, the reported mean pre-dose concentration between days 23 and 28 of treatment was 24 $\mu\text{g/mL}$, with an elimination half-life of 180 h.

After clinical development and marketing of the drug, several case reports describing miltefosine plasma concentrations in patients with CL⁷⁰ and with VL,^{71,72} and one larger and more detailed population pharmacokinetic study in CL patients⁵⁴ were published, which allowed a more extensive evaluation of its clinical pharmacokinetics. Absorption of miltefosine is slow, with an absorption rate of 0.36 h^{-1} in these CL patients.⁵⁴ Drug clearance and the volume of distribution are rather constant, as indicated by the estimated between-subject variation. Miltefosine keeps accumulating until the end of treatment (day 28) and, depending on the exact daily dosage and the individual's body weight, steady-state is reached in a subset of patients in the last week of treatment (see Figure 2). The extremely slow elimination of miltefosine is manifested by the long elimination half-lives estimated from a two-compartment pharmacokinetic model, with a primary elimination half-life of 7.05 days (range: 5.45–9.10 days) and a terminal half-life of 30.9 days (range: 30.8–31.2 days).⁵⁴ Various reported maximal or steady-state miltefosine concentrations, all taken around the last week of treatment, recorded 14.6–15.6 $\mu\text{g/mL}$ (100 mg/day, $n=1$),^{71,73} 11.9 $\mu\text{g/mL}$ (150 mg/day, $n=1$),⁷² 29–38 $\mu\text{g/mL}$ (150 mg/day, $n=2$)⁷⁰ and a median 30.8 $\mu\text{g/mL}$ (IQR: 25.2–33.4 $\mu\text{g/mL}$; 150 mg/day, $n=22$).^{54,74} Monitoring steady-state concentrations in the last week of treatment could be considered to assess treatment adherence, although this will only reveal individuals who missed a substantial part of their regimen.

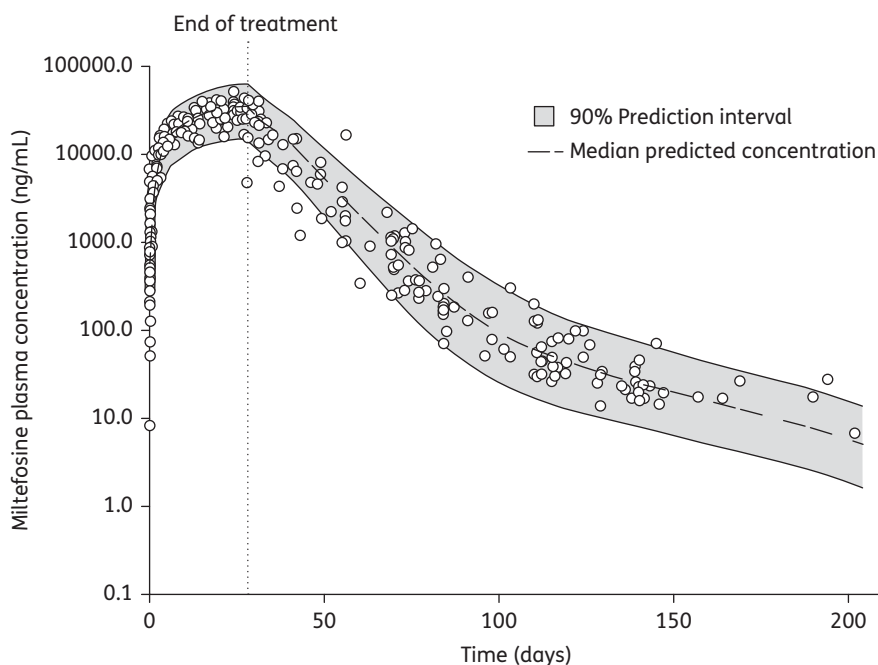


Figure 2. Visual predictive check of population pharmacokinetic model for miltefosine. Open circles represent observed data ($n=382$) from 31 CL (*L. major*) patients. All patients were treated with 150 mg/day miltefosine for 28 days. The grey area shows the 90% interval of the model predictions; the broken line displays the median predicted concentrations. From Dorlo et al.,⁵⁴ with permission.

Population pharmacokinetic modelling of the pooled original pharmacokinetic data collected during the paediatric Indian Phase II/III trial,⁷⁵ the adult Indian Phase II trial⁷⁶ and the adult European CL study,⁵⁴ yielding a pharmacokinetic dataset from a wide range of body weights, revealed that drug clearance for miltefosine is best scaled by allometric three-quarters scaling and not linear scaling, based on fat-free mass instead of total body weight.⁷⁷ The estimates of normalized pharmacokinetic parameters and variabilities from that pooled analysis can be found in Table 1.

Clinical pharmacodynamics

Besides the clinically observed effects of miltefosine, little is known about the clinical pharmacodynamics of this drug and other antileishmanial drugs in general, mainly because good quantitative markers of parasite load and treatment response remain lacking for leishmaniasis. A recent study looking at the parasite biomass in skin biopsies of CL lesions using quantitative real-time RT-PCR targeting the *Leishmania* 18S rRNA genome estimated a parasite clearance rate for miltefosine of ~1 log/week for an *L. major* infection.⁷⁰ A similar rate of decline of the parasite load was observed in blood of a VL patient (*Leishmania infantum*) treated with miltefosine (150 mg/day).⁷² Currently, trials are ongoing to evaluate the effect of different miltefosine regimens and combination therapies on the parasite biomass in peripheral blood using the same quantitative approach.⁶⁰

Activity and mechanism of action

Miltefosine has demonstrated activity against *Leishmania* parasites and neoplastic cells. Remarkably, very similar molecular modes of action of miltefosine were identified against both *Leishmania* parasites and human cancer cells, linking its activity mainly to (i) apoptosis and (ii) disturbance of lipid-dependent cell signalling pathways.

Antileishmanial activity and mechanism of action

The *in vitro* and *in vivo* antileishmanial activity of miltefosine was first described by Croft *et al.*⁷⁸ These results were replicated with the oral administration of miltefosine in experimentally VL-infected BALB/c mice.³⁰ Not all *Leishmania* species are

equally susceptible to miltefosine, and various pitfalls complicate the interpretation and comparison of *in vitro* results for the screening of antileishmanial drug activity. There is a general consensus that the most appropriate *in vitro* test model for *Leishmania* is the intracellular amastigote model.⁷⁹ However, interpretation of the results is complicated by variability in the (rate of) infectivity of the promastigotes for the macrophage host cells,⁸⁰ in drug activity dependent on the type of macrophage host cell used,⁸¹ but also in the intrinsic susceptibility of laboratory strains and clinical isolates.⁸² More standardization of *Leishmania* drug susceptibility testing is therefore needed. In a comparison between intracellular amastigotes of *L. donovani*, *Leishmania aethiopica*, *L. tropica*, *Leishmania panamensis*, *L. mexicana* and *L. major* laboratory strains, *L. donovani* was the most susceptible to miltefosine, with EC₅₀ values of 3.3–4.6 µM (corresponding to 1.3–1.9 µg/mL), while *L. major* was the least susceptible, with EC₅₀ values of 31.6–37.2 µM (corresponding to 12.9–15.2 µg/mL).⁸³ In general, intracellular *Leishmania* amastigotes are more susceptible to amphotericin B (~10-fold), but less susceptible to sodium stibogluconate (~0.1–0.3-fold) when compared with miltefosine. Interestingly, substantial intrinsic variability was found between clinical isolates. In a study by Yardley *et al.*, all *L. donovani* isolates from Nepal were very susceptible to miltefosine, with an EC₅₀ of 0.04–8.7 µg/mL, while the isolates from Peru showed remarkable variability, with EC₅₀ values between 8.4 and >30 µg/mL for *L. braziliensis* and *L. mexicana* (n=9) and between 1.9 and 3.4 µg/mL for *Leishmania lainsoni* (n=4).⁸² For comparison, a median miltefosine plasma concentration of 30.8 µg/mL in patients treated with 150 mg/day for 28 days was only achieved in the last week of treatment (see also the Clinical pharmacokinetics section).⁵⁴ There is thus a great natural variability of susceptibility to miltefosine among the various *Leishmania* (sub)species, certainly between the causative species of CL. This correlates with variability in the clinical response and is unrelated to the emergence of resistance (see the Clinical efficacy section).

Several potential hypotheses for the antileishmanial mechanism of action of miltefosine have emerged over recent years and are schematically depicted in Figure 3; however, no mechanism has been identified definitely. The multitude of proposed potential mechanisms and contradictory studies may indicate that miltefosine has more than one molecular site of action.

Table 1. Population pharmacokinetic estimates and derived parameters from a modelling study incorporating miltefosine pharmacokinetic data from Indian VL patients and European CL patients exhibiting a wide range of body weights; from Dorlo *et al.*,^{48,77} with permission

Parameter	Estimate [relative SE (%)]	Between-subject variability [relative SE (%)]
Absorption rate (k_a) (h^{-1})	0.416 (11.5)	18.2% (115.5)
Clearance (CL/F) (L/day)	3.99 (3.5) ^{a,b}	32.1% (18.4)
Volume of central compartment (V_2/F) (L)	40.1 (4.5) ^{a,b}	34.1% (27.3)
Intercompartmental clearance (Q/F) (L/day)	0.0347 (18.3)	not estimated
Volume of peripheral compartment (V_3/F) (L)	1.75 (8.2)	not estimated
Initial elimination half-life ($t_{1/2\alpha}$) (days)	6.13 (4.35–9.55) ^c	not estimated
Terminal elimination half-life ($t_{1/2\beta}$) (days)	35.6 (35.2–36.6) ^c	not estimated

^aEstimates are normalized to a fat-free mass of 53 kg and scaled allometrically with a power of 0.75 for CL/F and 1 for V_2/F .

^bBetween-subject variabilities in CL/F and V_2/F correlated with a correlation coefficient of 0.92.

^cRange.

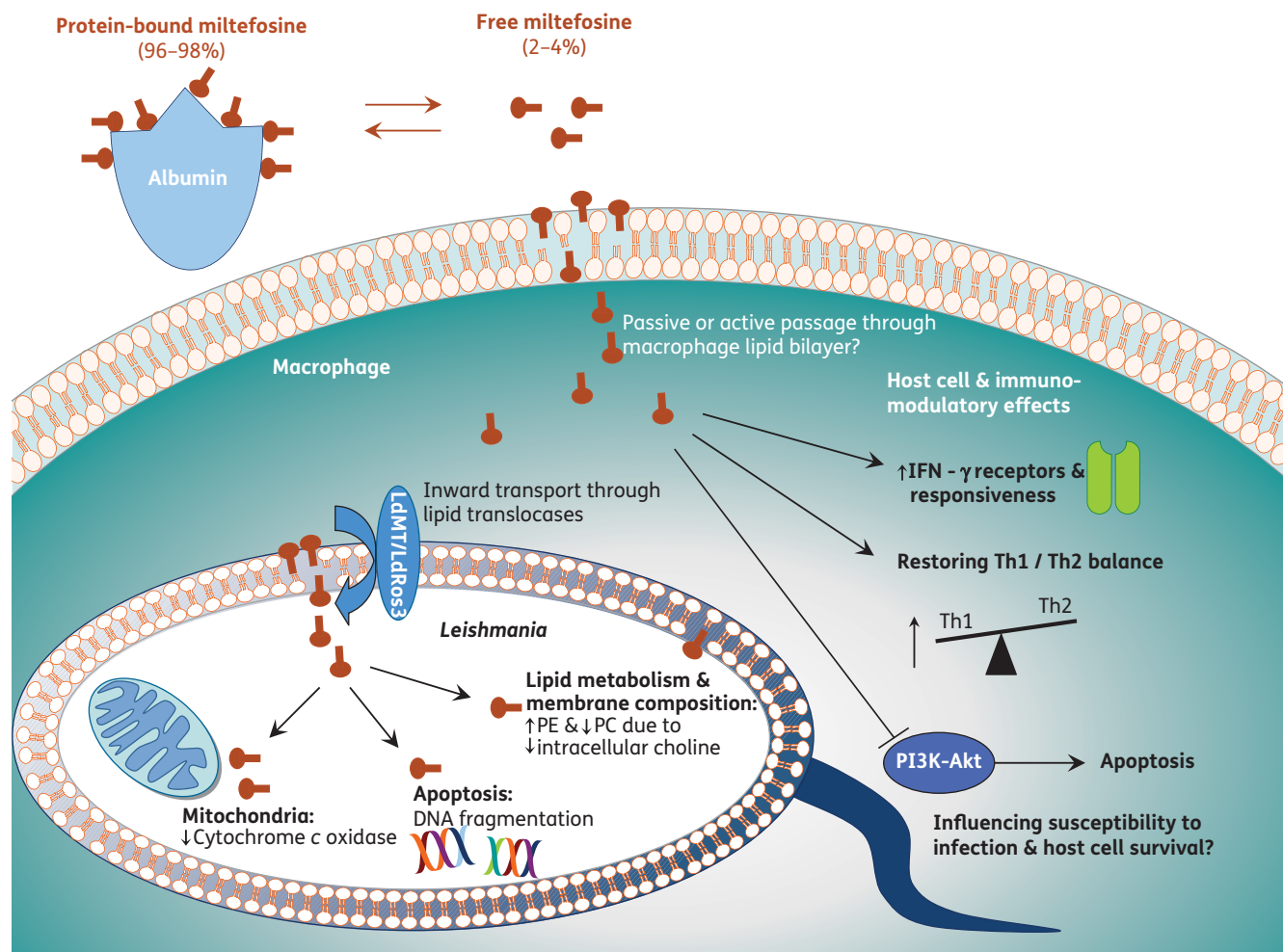


Figure 3. Antileishmanial mechanism of action of miltefosine. The various proposed mechanisms of action of miltefosine against the (intracellular) *Leishmania* parasite and the macrophage host cell during leishmaniasis infection. PC, phosphatidylcholine. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

Lipid metabolism

Preliminary studies in miltefosine-treated *L. mexicana* promastigotes showed an association between the efficacy of miltefosine and perturbation of ether-phospholipid metabolism (major components in the membrane), glycosylphosphatidylinositol (GPI) anchor biosynthesis (important parasite surface molecules implicated in virulence) and, more generally, signal transduction within the parasite.⁸⁴ However, the role of GPIs and phospholipids in the survival of *Leishmania* amastigotes was seriously questioned by the viability of an *L. major* knockout model overall lacking ether phospholipids and specific GPIs,⁸⁵ which makes this a less likely target for miltefosine. Later, the miltefosine-induced perturbation of ether phospholipid metabolism was specified to the inhibition of the glycosomal alkyl-specific acyl-CoA acyltransferase,⁸⁶ but this pathway also is probably not the primary target due to the high IC₅₀ value (50 µM) needed to inhibit this enzyme.³⁵ Nevertheless, promastigotes of a miltefosine-resistant strain of *L. donovani* showed changes in the length and level of unsaturation of fatty acids,

as well as a reduction in ergosterol levels, indicating that fatty-acid and sterol metabolism are probably targets for miltefosine.⁸⁷ Interestingly, transient treatment with miltefosine led to moderate effects on phospholipid metabolism and the parasite's membrane composition: a decrease in phosphatidylcholine by inhibiting its synthesis through the cytidine 5'-diphosphocholine pathway, but an increase in phosphatidylethanolamine (PE) by stimulation of cytidine triphosphate:PE cytidyltransferase activity and/or inhibition of PE-N-methyltransferase activity.⁸⁸ This observation might be related to the miltefosine-mediated inhibition of the inward transport of exogenous choline into the parasite.⁸⁹

Apoptosis-like cell death

Apoptosis-like cell death comparable to metazoan apoptosis has been demonstrated in *Leishmania* promastigotes following exposure to reactive oxygen species, resulting in e.g. nuclear condensation, DNA fragmentation and loss of cell volume.⁹⁰

Miltefosine is able to induce this programmed apoptosis-like cell death at its IC_{50} in promastigotes⁹¹ and intra-/extracellular amastigotes of *L. donovani*,⁹² and in *Leishmania amazonensis* and *L. infantum* promastigotes.^{93,94} This is corroborated by the recent finding that tolerance of programmed cell death in *Leishmania* is linked to emerging multidrug resistance within the parasite *in vitro*.⁹⁵

Mitochondrial effects

The involvement of mitochondrial dysfunction has also been investigated. In *L. panamensis* promastigotes, the mitochondrial membrane potential was substantially reduced after experimental treatment with miltefosine.⁹⁶ Within the mitochondria, cytochrome-c oxidase was inhibited by miltefosine in a dose-dependent manner and appeared a likely target for miltefosine in *Leishmania* promastigotes.⁹⁷ Recently, the inhibition of cytochrome c oxidase by miltefosine was linked to the apoptosis-like cell death induced in the yeast *Saccharomyces cerevisiae*.⁹⁸

Immunomodulatory effects

Miltefosine's immunomodulatory properties have been proposed as an additional contributory factor to its antileishmanial activity,^{99,100} despite the fact that miltefosine retains its activity against *Leishmania* infections in severe combined immunodeficient mice.¹⁰¹ The immunostimulatory properties of miltefosine were already shown in other pre-clinical models in which miltefosine induced cytokine release.^{39,99,102,103} In *Leishmania*, the intracellular antileishmanial activity of miltefosine was severely compromised in interferon- γ -deficient *L. donovani*-infected macrophages. Moreover, miltefosine enhanced interferon- γ receptors and thereby restored both the interferon- γ responsiveness in infected macrophages and the Th1/Th2 balance in infected macrophages by promoting the interleukin-12-dependent Th1 response.¹⁰⁰ However, the immunostimulatory contribution to miltefosine's antileishmanial activity is not without controversy. A recent study showed that miltefosine did not up-regulate major histocompatibility complex II or any costimulatory molecules that influence the maturation of dendritic cells, nor did it alter the release of the cytokines interleukin-10, interleukin-12 or tumour necrosis factor- α .¹⁰⁴ Related to this, miltefosine inhibits both the release of mediators from mast cells as well as the related mast cell activation.¹⁰⁵ In this role, the application of topical miltefosine for the treatment of skin lesions in mastocytosis patients is currently being investigated.¹⁰⁶

Recently, it was shown that host cells were less susceptible to infection by CL-causing parasite species after the inhibition of two PI3Ks (PI3K- γ and - δ).^{107,108} This supports that miltefosine, as a known human PI3K/Akt inhibitor, may influence the susceptibility of host cell infection also through this pathway.

Anticancer action

The antineoplastic mechanisms of action of alkylphosphocholines were recently reviewed by van Blitterswijk and Verheij.¹⁰⁹ The most prominent molecular targets for miltefosine's anticancer activity are related to the antileishmanial targets, and include the inhibition of phosphatidylcholine biosynthesis^{110–112} and the

induction of apoptosis by inhibition of the PI3K/Akt/PKB pathway.^{34,113} All these mechanisms lead to reduced cell survival or increased apoptosis, mediated either through inducing intracellular stress (reactive oxygen species), by blocking essential survival signals or by inducing various pro-apoptotic cell signalling pathways.¹⁰⁹

Other actions and activities of miltefosine

Besides its potent activity against *Leishmania* parasites, miltefosine has activity against other trypanosomatid parasites (*Trypanosoma* sp.), *Entamoeba* sp., *Acanthamoeba* sp., *Schistosoma* worms, pathogenic bacteria and various fungi.

Antitrypanosomal activity

African *Trypanosoma* parasites are less susceptible to alkylphosphocholines than other kinetoplastid parasites. Both *Trypanosoma brucei brucei* and *T. b. rhodesiense*, which cause sleeping sickness in animals or humans, demonstrated moderate EC_{50} values of miltefosine of 35.5 μ M (14.5 μ g/mL) and 47.0 μ M (19.2 μ g/mL), respectively,¹¹⁴ which was corroborated by limited life extension *in vivo* in infected mice.¹¹⁵ Against all phenotypes of *Trypanosoma cruzi*, the South American *Trypanosoma* species and the aetiological agent of Chagas' disease, the reported EC_{50} , $IC_{50}/72$ h and $IC_{50}/120$ h values of miltefosine were 0.5 μ M (0.2 μ g/mL), 0.7 μ M (0.3 μ g/mL) and 1 μ M (0.4 μ g/mL), respectively.^{114,116,117} Higher values were reported under different conditions.¹¹⁸ Suppression of *T. cruzi* infection in mice was only noted after five administrations of 30 mg/kg¹¹⁴ and 100% survival of infected BALB/c mice was achieved with a dose of 25 mg/kg/day for 20 days, comparable to benznidazole, the current drug of choice for Chagas' disease.¹¹⁶ The mechanism of action of miltefosine in *T. cruzi* seems to be specifically related to the inhibition of *de novo* phosphatidylcholine biosynthesis and phospholipid signalling pathways through the inhibition of phospholipase C.^{117,119}

Other antiprotozoal activity

Miltefosine possesses activity against various other protozoan parasites as well. Although less potent than against *Leishmania*, activity was demonstrated against *Entamoeba histolytica*, a protozoan parasite causing amoebic dysentery and liver abscesses. For example, the median EC_{50} after 48 h was 53 μ M (range 28–99 μ M) [corresponding to 22 μ g/mL (11–40 μ g/mL)] for the most susceptible *Entamoeba* strain, which was comparable to that of metronidazole.¹²⁰ Comparable amoebistatic activity was shown against free-living amoebae of the *Acanthamoeba* genus, causative species for both keratitis and granulomatous amoebic encephalitis, with complete cell death at 40 μ M (16 μ g/mL).¹²¹ The amoeba species displayed miltefosine-induced alterations of the membrane architecture.^{120,121} The anti-acanthamoebic activity of miltefosine was confirmed in an *Acanthamoeba* keratitis Syrian hamster model in which topically applied miltefosine [160 μ M (65 μ g/mL), 28 days] resulted in complete cure of the infection in 85% of the hamsters.¹²² Also, against *Trichomonas vaginalis*, the causative agent of trichomoniasis, miltefosine showed modest activities, most notably also against metronidazole-resistant strains, with EC_{50} values between 8 and 40 μ M (3.3 and

16.3 $\mu\text{g/mL}$).¹²³ Miltefosine is therefore also a potential new treatment for this common sexually transmitted disease. The activity of miltefosine against *Cryptosporidium parvum* was demonstrated *in vitro*,¹²⁴ but its clinical application seems to be limited in HIV-infected immunocompromised hosts.¹²⁵

Antischistosomal activity

Recent pre-clinical studies have shown activity of miltefosine against *Schistosoma mansoni*, the major cause of intestinal schistosomiasis. Its activity in *Schistosoma* seems to be related to both apoptosis and damaging of the tegumental outer surface and lipid bilayers of this flatworm.^{126,127} Eissa *et al.*¹²⁶ showed that administration of a high dosage (20 mg/kg/day) of oral miltefosine for 5 days to *S. mansoni*-infected mice is needed to induce a significant reduction of the worm burden, hepatic granulomata size and amelioration of hepatic pathology for different developmental stages of *S. mansoni*.

Antibacterial activity

Miltefosine has demonstrated significant bacteriocidal activity *in vitro* against pneumococcal bacteria. The determined MIC ranged between 5 and 6.25 μM (2–2.5 $\mu\text{g/mL}$) for *Streptococcus pneumoniae* strains, and from 10 to 20 μM (4–8 $\mu\text{g/mL}$) for other pathogenic streptococci.¹²⁸ Against methicillin-resistant *Staphylococcus aureus*, a lower MIC was demonstrated compared with susceptible *S. aureus*: 22 μM (9 $\mu\text{g/mL}$) versus 44 μM (18 $\mu\text{g/mL}$), respectively.¹²⁹ Miltefosine also had moderate activity against vancomycin-resistant *Enterococcus* [MIC: 44 μM (18 $\mu\text{g/mL}$)].¹²⁹ In a murine peritonitis/sepsis model, this activity could not be replicated *in vivo*, probably due to experimental intricacies.¹³⁰ Against Gram-negative bacteria, miltefosine's activity seems to be limited, since no activity could be observed *in vitro* on both *Escherichia coli* and *Pseudomonas aeruginosa*.¹²⁸

Antifungal activity

Miltefosine exhibits broad-spectrum antifungal activity. IC₉₀ values against *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Aspergillus fumigatus*, *Fusarium solani*, *Scedosporium prolificans*, *Scedosporium apiospermum*, *Candida tropicalis*, *Aspergillus terreus* and *Candida parapsilosis* were 2–8 $\mu\text{g/mL}$.^{131,132} Another study showed MICs of miltefosine against *C. neoformans* and *C. albicans* of 2.75 and 5.5 μM (1.12 and 2.2 $\mu\text{g/mL}$), respectively.¹²⁹ Fungicidal activity against *C. neoformans* was confirmed in a disseminated mouse model.¹³² In an *in vitro* test panel of 77 dermatophytes, including several *Microsporum* and *Trichophyton* species, miltefosine showed better activity (geometric mean MIC of 0.67 $\mu\text{g/mL}$) and broader specificity when compared with the standard drug itraconazole.¹³³

A proposed mechanism of fungicidal action is related to miltefosine's similarity to natural substrates (lysophospholipids) of phospholipase B, which is a major fungal virulence factor.^{129,132} However, high concentrations of miltefosine [e.g. 250 μM (102 $\mu\text{g/mL}$)] were needed to establish only minimal inhibition of phospholipase B and therefore it is unlikely that this is the main antifungal target of miltefosine or other alkylphosphocholines.¹²⁹

Antiviral activity

Recently, it was shown that the host PI3K/Akt pathway is exploited by the HIV-1 virus to establish a cytoprotective effect, which prolongs the lifespan of HIV-1-infected macrophages, thereby creating an important virus reservoir.¹³⁴ Miltefosine reverses this: it prevents activation of the PI3K/Akt pathways in HIV-1-infected macrophages and specifically inhibits Akt kinase.¹³⁵ Moreover, the drug induces cell death of HIV-1-infected macrophages upon exposure to stress and ultimately even terminates the production of viral particles.¹³⁴

Resistance in leishmaniasis

Miltefosine resistance, or rather drug non-susceptibility, could relatively easily be induced *in vitro*, although it has not been characterized *in vivo* yet. *L. donovani* promastigote clones that are resistant up to 40 μM (16.3 $\mu\text{g/mL}$) miltefosine have already been generated in the laboratory.¹³⁶ These clones appeared 15-fold less susceptible to miltefosine.¹³⁷ Both a defect in drug internalization into the parasite and increased drug efflux from the parasite were incriminated as possible mechanisms of resistance.

The transport of miltefosine over the parasite cell membrane is thought to be facilitated by a putative *L. donovani* miltefosine transporter (LdMT) and the protein LdRos3. It was shown that decreased miltefosine accumulation and defective inward translocation was the major determinant of decreased susceptibility,¹³⁷ which was demonstrated to be mediated through inactivation of LdMT and LdRos3 (Figure 3).^{138–140} LdMT is a novel inward-directed lipid translocase that belongs to the P4 subfamily of P-type ATPases and LdRos3 is a non-catalytic subunit of this membrane protein related to the Cdc50 family, which together play an important role in maintaining the phospholipid asymmetry of the parasite membrane.¹⁴¹ LdMT-associated miltefosine resistance could be transferred to the amastigote stage, with no apparent loss of infectivity, and even persists *in vivo*.¹⁴² In clinical isolates, low expression of the LdMT-LdRos3 complex was correlated to the natural non-susceptibility to miltefosine of *L. braziliensis* strains.¹⁴⁰

Increased efflux of miltefosine (and other endogenous phospholipid analogues) has also been implicated in miltefosine resistance, mediated through the overexpression of an ABC transporter: the *Leishmania* P-glycoprotein-like transporter (*Leishmania* ABCB1 or LtrMDR1).^{143,144} Probably, there are more *Leishmania*-specific ABC transporters implicated in phospholipid trafficking and reduction in miltefosine accumulation. The overexpression of two *Leishmania*-specific ABC subfamily G-like transporters (LiABCG6 and LiABCG4 half-transporters) conferred resistance to not only miltefosine *in vitro*, but also to aminoquinolines.^{145,146}

Whole genome sequencing recently revealed that miltefosine resistance in *L. major* mutants can be both genetically and phenotypically highly heterogeneous.¹⁴⁷ Two of the three identified markers of miltefosine resistance in this study were implicated in drug susceptibility: the previously described P-type ATPase and pyridoxal kinase.¹⁴⁷ Pyridoxal kinase plays a vital role in the formation of pyridoxal-5'-phosphate (active vitamin B6).

A clinical decrease in the susceptibility of parasites to miltefosine *in vivo*, a precursor of the emergence of drug resistance, has not yet been formally described, although several relapse

cases after initial successful primary miltefosine treatment in immunocompetent patients have been reported for both CL and VL.^{148–150} A recent evaluation of miltefosine for Indian VL, after a decade of availability of the drug in India, showed a 90.3% final cure rate at 6 month follow-up.¹⁵¹ This cure rate had decreased from the 94% final cure rate that was achieved in Indian Phase III trials a decade earlier (Table 2), but was still higher than the most recent reported cure rate in neighbouring Bangladesh, where miltefosine was only recently introduced (Table 2). The *in vitro* susceptibilities of clinical isolates from this recent Indian study remain to be reported. Another recent study reported that a gradual decrease of the miltefosine susceptibility of *L. infantum* isolates from a non-responsive HIV/VL patient was associated with the occurrence of a single nucleotide polymorphism in the *Ldmt* gene, L832F, which reverted back to the wild-type allele 3 years after withdrawal from miltefosine.¹⁵²

Safety

The main safety concerns for miltefosine relate to its effect on the mucosa of the gastrointestinal tract and its potential teratogenicity, as shown in pre-clinical reproductive toxicity studies in animals. The gastrointestinal side effects of miltefosine were already demonstrated in early studies in cancer patients, in which loss of appetite, nausea and vomiting were found to be dose-limiting side effects.^{26–28} Vomiting and/or diarrhoea were observed in every clinical trial performed with miltefosine (summarized in Tables 2 and 3), and were also the primary observed and most severe side effects in the large Phase IV trial in Indian VL patients ($n=1119$), especially in the first week of treatment (8.2% experienced one or more episodes).¹⁵³ During treatment, the severity of these effects decreased (3.2% in the last week of treatment).¹⁵³ The gastrointestinal side effects are most probably directly related to the oral intake of the drug and the detergent-like properties of miltefosine affecting the gastrointestinal lining. Intake of (fatty) food together or just before miltefosine intake drastically reduces the gastrointestinal side effects^{70,74} and probably has no effect on bioavailability.

Other frequently observed miltefosine-related toxicities are mainly associated with the kidneys and liver. Elevated serum creatinine levels during treatment are frequently observed (Tables 2 and 3), possibly related to occasional dehydration by severe vomiting/diarrhoea;²⁹ severe nephrotoxicity caused by miltefosine is, however, rare. Serum levels of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) tend to increase mildly in the first week of miltefosine treatment in VL patients, possibly due to immediate necrosis of pre-damaged hepatocytes.²⁹ This generally normalizes in the subsequent weeks together with the resolving infection. Ophthalmologic retinotoxic side effects have been reported in pre-clinical studies, but have so far not been observed in any VL or CL patients.²⁹

Pregnancy

Preclinical reproductive toxicity studies in animals showed both embryo- and fetotoxicity (in rabbits and rats) and teratogenic effects (in rats) of miltefosine at a lowest observed adverse effect level of 1.2 mg/kg for 10 days during gestation.²⁹ The use of miltefosine is therefore strictly contraindicated in pregnant females, and adequate contraceptive cover is mandatory for

females of child-bearing potential during and after miltefosine treatment. A recent study indicates that, based on translational animal-to-human pharmacokinetic modelling, 4 months of contraceptive cover after the end of treatment might be adequate for the standard 28 day miltefosine regimen, while for all shorter regimens (5, 7 or 10 days) 2 months may be considered adequate.⁷⁷ Based on the physicochemical properties of miltefosine, it may be assumed that miltefosine is transferred into breast milk.

Male reproduction

Preclinical animal studies additionally showed (reversible) testicular atrophy and impaired fertility in male rats at a dose of 8.25 mg/kg.²⁹ Spermiogram analyses in Colombian male patients as well as limited retrospective analyses of reproductive performance in Indian male patients suggested an absence of a clinically relevant effect on male fertility.^{153,154} Conversely, recently it was shown in a retrospective, observational study that a large proportion of miltefosine-treated males (1.3–2.1 mg/kg/day, 28 days) experienced a substantial treatment-related reversible reduction of ejaculate.⁷⁴ Although nothing is known about sperm count and quality in these patients, this finding does clearly point at effects of miltefosine on the male reproductive system.

Drug–drug interactions

Based on miltefosine's route of metabolism, no drug–drug interactions are to be expected at the level of CYP isoenzymes. Nevertheless, other interactions can be hypothesized, corresponding with e.g. its very high serum protein binding and its presumed (moderate) substrate affinity for the multidrug transporter P-glycoprotein.^{155,156} Theoretical relevant drug–drug interactions of miltefosine might therefore e.g. include ritonavir-boosted highly active antiretroviral treatment in VL patients coinfecting with HIV, possibly resulting in a decreased miltefosine bioavailability and/or intracellular accumulation. Clinical evidence pointing at either the presence or absence of these theoretical drug–drug interactions is still not available.

Clinical efficacy

VL

Several clinical studies have been conducted of miltefosine, both alone and in combination with other therapies, in the treatment of VL. The efficacy and toxicity data from these trials are summarized in Table 2. The role of miltefosine in the treatment of VL has been well established, however, it should be noted that almost all clinical trials of miltefosine for VL have been performed in a single area of VL endemicity, namely the Indian subcontinent, and from this region all studies except one originate from the state of Bihar, India (Table 2). The other important VL foci, in South America (Brazil), East Africa (Sudan, Ethiopia, Kenya and Uganda) and other South Asian countries (Nepal and Bangladesh), have been largely ignored in the evaluation of miltefosine, with the exception of a study conducted by Ritmeijer *et al.*¹⁵⁷ in northern Ethiopia, a study by Rahman *et al.*¹⁵⁸ in Bangladesh, and a Phase II trial in adults and children in Brazil sponsored by the AB Foundation,¹⁵⁹ which was terminated early and

Table 2. Efficacy and tolerance of oral miltefosine in trials with VL patients and HIV/VL-coinfected patients

Reference	Country	Study design	Patients enrolled	Treatments studied	Definite cure (95% CI) ^a	Tolerance
<i>Miltefosine monotherapy for VL</i>						
Sundar et al. ³¹	India	Phase I, randomized, dose finding	30 adults ^b : 5 in each arm	28 days of: (1) 50 mg MLF qod; (2) 100 mg MLF qod; (3) 100 mg/day MLF; (4) 150 mg/day MLF; (5) 200 mg/day MLF; (6) 250 mg/day MLF	(1) 40%; (2) 20%; (3) 100%; (4) 80%; (5) 100%; (6) 80%	dose-limiting GI toxicity in (5) and (6)
Jha et al. ⁷⁶	India	Phase II, randomized, open label, dose finding	120 adults ^b : 30 in each arm	(1) 50 mg/day MLF for 6 weeks; (2) 50 mg/day MLF for 1 week+100 mg/day MLF for 3 weeks; (3) 100 mg/day MLF for 4 weeks; (4) 100 mg/day MLF for 1 week+150 mg/day MLF for 3 weeks	(1) 93% (78–99); (2) 93% (78–99); (3) 97% (83–100); (4) 97% (83–100)	frequent GI toxicity in all arms
Sundar et al. ¹⁷⁷	India	Phase II, randomized, open label	45 adults ^b : (1) 17; (2) 18; (3) 10	28 days of: (1) 100 mg/day MLF; (2) 150 mg/day MLF; (3) 200 mg/day MLF	(1) 94% (71–100); (2) 100% (85–100); (3) 100% (74–100)	overall: 5 CTC-3 GI toxicities; 1 CTC-3 hepatotoxicity; 1 CTC-3 nephrotoxicity
Sundar et al. ¹⁷⁸	India	Phase II, randomized, dose finding	54 adults ^b : 18 in each arm	100 mg/day MLF for: (1) 14 days; (2) 21 days; (3) 28 days	(1) 89% (65–99); (2) 100% (85–100); (3) 100% (85–100)	moderate GI toxicity (weeks 1–2): (1) 67%; (2) 78%; (3) 61%
Sundar et al. ¹⁷⁹	India	Phase III, randomized, open label, comparative	398 adults ^b : (1) 299; (2) 99	(1) 28 days of 50 mg/day (≤ 25 kg) or 100 mg/day (> 25 kg) MLF; (2) 15 \times infusions of 1 mg/kg iv AMB qod	(1) 94% (91–97); (2) 97% (91–99)	(1) mild GI toxicity: vomiting 38% and diarrhoea 20%; \uparrow AST 17%; (2) mild GI toxicity: vomiting 20% and diarrhoea 6%; \uparrow ALT 18%; \uparrow Creat 35%
Sundar et al. ¹⁸⁰	India	Phase I/II, randomized, dose finding	39 children ^b : (1) 21; (2) 18	28 days of: (1) 1.5 mg/kg/day MLF; (2) 2.5 mg/kg/day MLF	(1) 90% (73 NR); (2) 83% (62 NR)	mild/moderate GI toxicity: (1) vomiting 33% and diarrhoea 5%, (2) vomiting 39% and diarrhoea 17%
Bhattacharya et al. ⁷⁵	India	Phase III, open label	80 children ^b	28 days of 2.5 mg/kg/day	94% (87 NR)	mild/moderate GI toxicity: vomiting 26% and diarrhoea 25%; \uparrow AST 55%
Singh et al. ¹⁸¹	India	randomized, open label	125 children (≤ 14 years): (1) 44; (2) 20; (3) 38; (4) 23	(1) 28 days of 2.5 mg/kg/day MLF, not pre-treated; (2) 28 days of 2.5 mg/kg/day MLF for 28 days, pre-treated; (3) 15 \times infusions of 1 mg/kg iv AMB qod, pre-treated; (4) 15 \times infusions of 1 mg/kg iv AMB qod (not pre-treated)	(1) 93%; (2) 95%; (3) 92%; (4) 91%	for (1) and (2): mild/moderate GI toxicity (vomiting 36% and diarrhoea 41%); \uparrow AST 48%; \uparrow ALT 61%; \uparrow BUN 13%; for (3) and (4): \uparrow AST 56%; \uparrow ALT 53%; \uparrow BUN 70%

Bhattacharya <i>et al.</i> ¹⁵³	India	Phase IV, open label	(1) 704 adults ^b (477 males); (2) 428 children ^b (247 males)	28 days of: (1) 50 mg/day (≤ 25 kg) or 100 mg/day (> 25 kg) MLF; (2) 2.5 mg/kg/day MLF	high % LTFU \rightarrow PP: (1) 96.6%; (2) 93.6% (intention-to-treat: overall 81.9%)	overall: GI toxicity 8% (week 1) and 3% (week 4); \uparrow ALT CTC-1 31%; \uparrow Creat CTC-1 14%
Rahman <i>et al.</i> ¹⁵⁸	Bangladesh	Phase IV, open label	977 adults ^b	28 days of 2.5 mg/kg/day MLF	high % LTFU \rightarrow PP: 85% (intention-to-treat: 72%)	vomiting 25% and diarrhoea 8% (mainly CTC-1/CTC-2); no liver/renal functions determined
Sundar <i>et al.</i> ¹⁵¹	India	open label, non-comparative	567 adults and children (≥ 6 years) ^b	28 days of 2.5 mg/kg/day (< 12 years), 50 mg/day (≤ 25 kg) or 100 mg/day (> 25 kg) MLF	90.3% (88–94)	vomiting 65% and diarrhoea 7% (mainly CTC-1/CTC-2), 1 patient died after GI intolerance; \uparrow ALT CTC-1 23%; \uparrow AST CTC-1 57%; \uparrow Creat CTC-1 29%
<i>Miltefosine in combination therapy for VL</i>						
Sundar <i>et al.</i> ¹⁸²	India	Phase II, randomized, non-comparative, group sequential	226 adults ^b : 45 in each arm, except (2) which had 46	(1) 1 \times infusion of 5 mg/kg iv L-AMB; (2) 1 \times infusion of 5 mg/ kg iv L-AMB+10 days of 100 mg/day MLF; (3) 1 \times infusion of 5 mg/kg iv L-AMB+14 days of 100 mg/day MLF; (4) 1 \times infusion of 3.75 mg/kg iv L-AMB+14 days of 100 mg/day MLF; (5) 1 \times infusion of 3.75 mg/ kg iv L-AMB+7 days of 100 mg/ day MLF	(1) 91% (78–97); (2) 98% (87–100); (3) 96% (84– 99); (4) 96% (84–99); (5) 98% (87–100)	mild/moderate GI toxicity in week 1: (1) 18%; (2) 37%; (3) 27%; (4) 24%; (5) 7%. \uparrow ALT CTC-1 in week 1: ~30% in all arms
Sundar <i>et al.</i> ²⁰	India	randomized, open label, parallel group, non-inferiority	634 children and adults ^b : (1) 157; (2) 160; (3) 158; (4) 159, of which 36%–45% were ≤ 18 years	(1) 15 \times infusions of 1 mg/kg iv AMB qod; (2) 1 \times infusion of 5 mg/kg iv L-AMB+7 days of: 50 mg/day MLF (≤ 25 kg) or 100 mg/day MLF (> 25 kg) for adults or 2.5 mg/kg/day MLF for children; (3) 1 \times infusion of 5 mg/kg iv L-AMB+10 \times injections of 11 mg/kg im PM base qd; (4) 10 days of MLF [for dosage see (2)]+10 \times injections of 11 mg/kg im PM base qd	(1) 93% (88–96); (2) 98% (93–99); (3) 98% (93– 99); (4) 99% (95–100)	most patients with AEs in (1): 91% versus 45%–52% in other arms; vomiting: (1) 19%, (2) 16%, (3) 3%, (4) 10%; diarrhoea: (1) 2%, (2) 2%, (3) 0%, (4) 3%; chills: (1) 72%, (2) 13%, (3) 13%, (4) 0%; \uparrow Creat: (1) 10%, (2) 0%, (3) 4%, (4) 4%

Continued

Table 2. Continued

Reference	Country	Study design	Patients enrolled	Treatments studied	Definite cure (95% CI) ^a	Tolerance
Miltefosine monotherapy for HIV/VL coinfection Ritmeijer et al. ¹⁵⁷	Ethiopia	randomized, open label, comparative	580 adults: 290 in each arm (all >25 kg), of which HIV+: (1) 33%, (2) 24%; HIV unknown: (1) 33%, (2) 38%	(1) 28 days of 100 mg/day MLF; (2) 30 injections of 20 mg/kg im SSG qd	at EOT: (1) 88% (84–92), (2) 88% (83–91); at 6 month FU: overall: (1) 60% (54–66), (2) 65% (59–71); in HIV+: (1) 46% (NR), (2) 57% (NR); relapse rate at 6 month FU: overall: (1) 10%, (2) 2%; in HIV+: (1) 25%, (2) 11%	vomiting: (1) 55%, (2) 32%; diarrhoea: (1) 51%, (2) 53%; bleeding: (1) 22%, (2) 22%; overall mortality: (1) 6%, (2) 12%

AE, adverse event; AMB, amphotericin B deoxycholate; BUN, blood urea nitrogen; Creat, creatinine; CTC-1/2/3, common toxicity criteria—grade 1/2/3; EOT, end of treatment; FU, follow-up; GI, gastrointestinal; im, intramuscular; iv, intravenous; L-AMB, liposomal amphotericin B; LTFU, lost to follow-up; MLF, miltefosine; NR, not reported; PM, paromomycin; PP, per protocol; qd, daily; qod, every other day; SSG, sodium stibogluconate.

^aDetermined at end of follow-up (ranging between 6 and 9 months) based on an intention-to-treat analysis, unless otherwise indicated. 95% CI is only given in the case that it was reported in the original study.

^bIn these studies, 'adult' was defined as ≥ 12 years of age and 'child' as < 12 years of age.

remains unreported. This is important, because besides specific species-related variation in the therapeutic response of VL, geographical variation has also been described.⁵ Various clinical trials of miltefosine are currently ongoing in Kenya and Sudan,⁶⁰ and also in Bangladesh,¹⁶⁰ to evaluate the efficacy and safety of miltefosine in VL in these geographical areas.

Combination miltefosine therapy

In a pre-clinical study, the *in vivo* activity of miltefosine was enhanced when combined with amphotericin B.¹⁶¹ Although the clinical relevance of this pre-clinical synergy remains unknown, there is nevertheless a broad consensus about the urgency of using combination regimens for VL.¹⁶² The rationale for this consensus is elaborately described by van Griensven et al.,¹⁶² and includes: reducing treatment duration, thereby reducing both the burden and costs of treatment; improving treatment efficacy for complicated cases; and delaying the emergence of drug-resistant parasites, thereby increasing the therapeutic lifespan of current drugs. This latter aspect may be refuted, if it is assumed that the selection of resistant parasites takes place mainly at the start of treatment with inadequate (initial) drug exposure. In that context, initial parasite clearance and the achievement of adequate drug concentrations immediately at the start of treatment would be more important, from a pharmacological perspective, to avoid the selection of resistant parasites, than the avoidance of long exposure to relatively low drug concentrations. To date, two studies, both in India, have been completed for combination miltefosine therapies.^{20,182} These showed that when miltefosine was combined with a single infusion of liposomal amphotericin B (5 mg/kg), the duration of miltefosine treatment could be reduced from 28 to 7 days, without affecting the efficacy of the drug (98% on an intention-to-treat basis) (summarized in Table 2). A Phase III study (Table 2) has also shown high efficacy with a 10 day combination of miltefosine and intramuscular paromomycin (98% on an intention-to-treat basis). For the African VL context, other combination therapies might be more appropriate, and are currently being explored in Kenya and Sudan.⁶⁰

HIV/VL coinfection

Only a single clinical trial has been reported that included HIV-positive patients in an area with high HIV prevalence in northern Ethiopia.¹⁵⁷ In the confirmed HIV-positive patients in this study, miltefosine was less effective than sodium stibogluconate, with more relapses at the end of treatment (18% versus 2%, respectively) and at 6 month follow-up (25% versus 11%, respectively) (also see Table 2). However, overall, miltefosine resulted in a lower mortality than sodium stibogluconate (6% versus 12%, respectively), which probably can be attributed to a better safety profile of miltefosine in HIV-positive patients. The high rates of relapsing and retreated patients, and the high percentage of patients with unknown HIV status complicate the interpretation of these results. The compassionate use of miltefosine in 39 HIV/VL-coinfected patients, outside a clinical trial and mainly from Europe, has been reported.¹⁶³ Initial cure was achieved in 64%, although almost all cured patients relapsed, and long-term tolerability of miltefosine treatment (up to 2 years) was demonstrated.¹⁶³

Table 3. Efficacy and tolerance of oral miltefosine in trials with CL patients and mucocutaneous leishmaniasis patients

Reference	Country	Study design	Patients enrolled	Causative species	Treatments studied	Definite cure (95% CI) ^a	Tolerance
<i>Miltefosine monotherapy for New World CL</i>							
Soto et al. ¹⁸³	Colombia	Phase I/II, open label, increasing dose, historic control	72 adults: (1) 16; (2) 19; (3) 17; (4) 20	<i>L. amazonensis</i> and <i>L. panamensis</i>	(1) 20 days of 50 mg/day MLF; (2) 7 days of 50 mg/day MLF + 13 days of 100 mg/day MLF; (3) 7 days of 100 mg/day MLF + 13 days of 150 mg/day MLF; (4) 28 days of 150 mg/day MLF	(1) 56%; (2) 63%; (3) 82%; (4) 80%	overall: 'motion sickness' 40%, increasing with MLF dose; vomiting or diarrhoea 21%; ↑AST/ALT: (1) 38%, (2) 42%, (3) 35%, (4) 20%
Soto et al. ¹⁸⁴	Colombia and Guatemala	randomized, placebo controlled, double blind	Colombia: 73 adults: (1) 49; (2) 24 Guatemala: 60 adults: (1) 40; (2) 20	<i>L. panamensis</i> (presumably) <i>L. braziliensis</i> and <i>L. mexicana</i> (presumably)	28 days of: (1) 100 mg/day (<45 kg) or 150 mg/day (≥45 kg) MLF; (2) oral placebo capsules 28 days of: (1) 100 mg/day (<45 kg) or 150 mg/day (≥45 kg) MLF; (2) oral placebo capsules	(1) 82%; (2) 38% (1) 50%; (2) 20%	Colombia and Guatemala together: ↑Creat CTC-1: (1) 31%, (2) 9%; nausea: (1) 36%, (2) 9%; vomiting: (1) 31%, (2) 5%; diarrhoea: (1) 6%, (2) 2%; ↑AST: (1) 8%, (2) 18%
Soto et al. ¹⁶⁴	Bolivia	randomized, open label	62 adults: (1) 44; (2) 18	<i>L. braziliensis</i>	(1) 28 days of ~2.5 mg/kg/day MLF; (2) 20 injections of 20 mg Sb ^V /kg im MA qd	(1) 80%; (2) 83%	GI toxicity: (1) 61%, (2) NR; arthralgia/local pain: (1) NR, (2) 72%
Vélez et al. ¹⁸⁵	Colombia	Phase III, randomized, open label	288 adults: (1) 145; (2) 143	<i>L. braziliensis</i> and <i>L. panamensis</i>	(1) 28 days of 150 mg/day MLF; (2) 20 injections of 20 mg Sb ^V /kg im MA qd	(1) 59% (50–67); (2) 72% (64–80)	vomiting: (1) 34%, (2) 12%; nausea: (1) 46%, (2) 21%; diarrhoea: (1) 5%, (2) 2%; ↑AST: (1) 5%, (2) 9%; ↑ALT: (1) 10%, (2) 18%
Machado et al. ¹⁸⁶	Brazil	randomized, open label	90 adults and children: (1) 60 (22 children); (2) 30 (10 children)	<i>L. braziliensis</i>	(1) 28 days of: 50 mg/day (≤29 kg), 100 mg/day (≤45 kg) or 150 mg/day (>45 kg) MLF; (2) 20 injections of 20 mg Sb ^V /kg im MA qd	(1) 75%; (2) 53%	vomiting: (1) 42%, (2) 3%; nausea: (1) 40%, (2) 10%; diarrhoea: (1) 10%, (2) 3%; arthralgia: (1) 0%, (2) 21%; myalgia: (1) 0%, (2) 21%
Chrusciak-Talhari et al. ¹⁸⁷	Brazil	Phase II/III, randomized, open label	90 adults and children: (1) 60 (20 children); (2) 30 (10 children)	<i>L. guyanensis</i>	(1) 28 days of: 30 mg/day (≤14 kg), 50 mg/day (≤29 kg), 100 mg/day (≤45 kg) or 150 mg/day (>45 kg) MLF; (2) 20 injections of 20 mg Sb ^V /kg im MA qd	(1) 67% (55–80); (2) 53% (34–72)	vomiting: (1) 48%, (2) NR; diarrhoea: (1) 7%, (2) NR; nausea: (1) 9%, (2) NR; arthralgia: (1) NR, (2) 33%
Rubiano et al. ¹⁸⁸	Colombia	randomized, open label, non-inferiority	116 children ^b : (1) 58; (2) 58	mixed (<i>L. panamensis</i> , <i>L. guyanensis</i> , <i>L. braziliensis</i>)	(1) 28 days of 1.5–2.5 mg/kg/day MLF; (2) 20 injections of 20 mg Sb ^V /kg im MA qd	(1) 83% (72–94); (2) 69% (55–83)	vomiting: (1) 26%, (2) 4%; diarrhoea: (1) 7%, (2) 5%; nausea: (1) 16%, (2) 4%; ↑AST: (1) 11%, (2) 32%; ↑ALT: (1) 5%, (2) 19%; ↑Creat: (1) 11%, (2) 23%
<i>Miltefosine monotherapy for Old World CL</i>							
Mohebbi et al. ¹⁸⁹	Iran	randomized, open label, comparative	63 adults: (1) 32; (2) 31	<i>L. major</i>	(1) 28 days of ~2.5 mg/kg/day MLF; (2) 14 injections of 20 mg Sb ^V /kg im MA qd	(1) 81%; (2) 81% ^c	week 1: nausea: (1) 41%, (2) 0%; vomiting: 28%, (2) 0%; diarrhoea: (1) 4%, (2) 3%; local pain: (1) 0%, (2) 10%
van Thiel et al. ⁷⁴	Afghanistan	observational, open label	34 adults, of which 31 were pre-treated with il Sb ^V	<i>L. major</i>	28 days of 150 mg/day MLF	88%	reduced ejaculate volume: 64%; complete absence of ejaculate: 6%; nausea: 76%; vomiting: 56%

Continued

Table 3. Continued

Reference	Country	Study design	Patients enrolled	Causative species	Treatments studied	Definite cure (95% CI) ^a	Tolerance
Miltefosine monotherapy for New World mucocutaneous leishmaniasis							
Soto et al. ¹⁹⁰	Bolivia	randomized, equivalency	97 adults: (1) 78; (2) 19	<i>L. braziliensis</i>	(1) 28 days of 2.5–3.3 mg/kg/day MLF; (2) 45–60 injections of 1 mg/kg iv AMB qd	(1) mild disease: 83%; extensive disease: 58%; (2) mild disease: 44%, extensive disease: 60% ^d	(1): vomiting: 10%; diarrhoea: 10%; ↑AST: 4%; ↑ALT: 1%; ↑BUN: 8%
Soto et al. ¹⁹¹	Bolivia	observational, open label	42 adults: (1) 21 (failed 4 weeks of MLF); (2) 21 (not pre-treated)	NR, presumably <i>L. braziliensis</i>	(1) secondary Rx, 6 weeks of ~2.5 mg/kg MLF; (2) primary Rx, 6 weeks of ~2.5 mg/kg MLF	(1) 65%; (2) 75% ^e	overall: mild GI toxicity: CTC-1 74% and CTC-2 5%; no liver or kidney toxicity

AMB, amphotericin B deoxycholate; BUN, blood urea nitrogen; Creat, creatinine; CTC-1/2, common toxicity criteria-grade 1/2; GI, gastrointestinal; il, intralesional; im, intramuscular; iv, intravenous; MA, meglumine antimoniate; MLF, miltefosine; NR, not reported; qd, daily; Rx, treatment.

^aDetermined at end of follow-up (ranging between 6 and 9 months) based on an intention-to-treat analysis. 95% CI is only given in the case that it was reported in the original study.

^bIn this study, ‘child’ was defined as ≤12 years of age.

^cDetermined at 3 month follow-up, rates were similar at 6 month follow-up.

^dDetermined at 12 month follow-up.

^eBased on a per-protocol analysis, determined at 12 month follow-up.

CL

Miltefosine has been administered both orally and topically for the treatment of CL, although the latter application has never been formally reported. The interpretation of efficacy rates of drugs in the treatment of CL is intricate and complex for various reasons. First, the geographical variation in efficacy and the additional variation in the susceptibility of the *Leishmania* (sub)species is even higher for CL than for VL. *Leishmania* species typing was not always performed for each individual in each clinical trial. Moreover, even within the same genetic (sub)-species, large variation in efficacy has been demonstrated.¹⁶⁴ Second, CL is in nature a self-healing disease and treatment might only incite an acceleration of the healing process. As such, the efficacy of miltefosine is preferably compared with an established control arm. In general, the overall quality of the reported clinical trials for CL is weak and potentially biased. Guidelines have been prepared to improve the quality of design and reporting of clinical trials for CL, which are urgently needed for miltefosine, certainly in Old World CL.¹⁶⁵ For post-kala-azar dermal leishmaniasis (PKDL), a non-ulcerating cutaneous complication of VL that can develop after initial successful treatment, miltefosine use has been generally limited to case reports that suggest reasonable efficacy when administered for an extended period of time.^{166–170} For Indian PKDL, treatment periods of 2 months (150 mg/day) or 3 months (100 mg/day) have been suggested.¹⁷⁰

Oral miltefosine for cutaneous and mucocutaneous leishmaniasis

The safety and efficacy data from clinical trials, plus two observational studies, of oral miltefosine in CL and mucocutaneous leishmaniasis are summarized in Table 3. Most trials were performed on New World CL, involving typical South American *Leishmania* species. More controlled clinical trials with Old World CL in Europe, the Mediterranean Basin, the Arabian Peninsula and Ethiopia are urgently needed.

The efficacy results for New World CL are mixed, showing large variation in clinical response between countries and (typed) species (see Table 3). Nevertheless, in most clinical trials, 28 days of miltefosine was more efficacious than the standard therapy (20 days of meglumine antimoniate) in both children and adults, and was also more efficacious than placebo. Also, against mucocutaneous leishmaniasis, 28 days of miltefosine performed better than 45–60 amphotericin B injections (1 mg/kg) and miltefosine might be the treatment of choice for this difficult-to-treat destructive cutaneous disease.¹⁹⁰ Extending miltefosine treatment from 4 to 6 weeks for mucocutaneous leishmaniasis does not seem to result in an added benefit to final cure rates determined at 12 months (Table 3).¹⁹¹ In Iran, miltefosine was demonstrated to be a good alternative to meglumine antimoniate for the treatment of *L. major* infections,¹⁸⁹ which was confirmed in an observational study with *L. major* infections originating from Afghanistan (Table 3).⁷⁴

Topical miltefosine for CL

No clinical trials or observational studies have been published on the use of topical miltefosine (available as Miltex[®]) for the

treatment of CL. Pre-clinical animal study results indicated a potential benefit of MilteX[®] in the treatment of experimental *L. mexicana* and *L. major* infections in BALB/c, CBA/J and C57BL/6 mice, leading to the reduction of lesion size.¹⁷¹ Previously published personal communications indicate, however, that this could not be confirmed in (unreported) clinical trials.¹⁷² Apparently, trials in Syria (16 patients) and in Colombia (19 patients) failed to demonstrate efficacy of the MilteX[®] formulation in the treatment of CL.¹⁷² It remains unknown whether these contradictory clinical observations are due to a lack of drug penetration in the lesion, insufficient dosage or treatment duration, or non-optimal formulation of this topical product, which all deserve further evaluation and proper reporting.

Perspective: role of miltefosine in the treatment of leishmaniasis

At present, miltefosine is mainly being utilized in the South Asian foci of VL, where it is a central part of a regional elimination strategy undertaken by the governments of India, Bangladesh and Nepal. The Regional Technical Advisory Group for Kala-azar Elimination has recommended miltefosine monotherapy as first-line therapy for the treatment of VL in these countries, depending on the local availability of the drug.^{173,174} The efficacy and effectiveness of this strategy in different clinical settings (primary health centres, zonal hospitals and tertiary treatment centres) is currently under study in the Kaladrug-R project, the results of which are to be published shortly.^{151,175} However, a recent WHO Expert Committee report on the control of leishmaniasis has not recommended the use of miltefosine as a first-line agent for VL in any geographical area, preferring instead monotherapies of either liposomal amphotericin B or combinations involving other drugs.¹⁷⁶ Since a terminated Phase II trial in Brazil,¹⁵⁹ there has been no further large-scale use or planned trial of the drug for VL in Latin America. Combination treatments for VL involving miltefosine are still being assessed in South Asia and East Africa, and have yet to be rolled out. In the latter region, a clinical trial and pharmacokinetic study on conventional miltefosine monotherapy and a combination involving liposomal amphotericin B is expected to be completed by the end of 2012.⁶⁰ Following this, registration of miltefosine in the region is expected. However, it remains to be seen whether the drug will be widely used in East Africa for not only primary VL, but also HIV/VL-coinfected cases and CL caused by *L. aethiopica*. Indeed, for CL, miltefosine is currently only recommended for use in *L. mexicana*, *L. guyanensis* and *L. panamensis* by the WHO Expert Committee.¹⁷⁶ For PKDL, miltefosine use has been relatively limited to a few specialist centres and it is currently not recommended for first-line use in Sudan and Bangladesh, where reported cases have been concentrated. Again, a lack of evidence and limitations of the drug itself have prevented usage of what should, in principle, be an ideal drug for cutaneous disease.

For whatever indication it will be used, pharmacovigilance for important safety events, especially birth defects, and treatment failure will remain a priority. This is especially important, since in the field context it is not clear how successfully contraceptive cover can be implemented for women of child-bearing potential. As mentioned earlier, another issue relating to the field context is

non-adherence to therapy, which could drastically limit the life-span of this essential oral drug. Particularly in India, strong concerns were raised about non-compliance, linked to the availability of (expensive) miltefosine in the private sector, the long treatment course needed and the rapid apparent clinical recovery from VL once treatment is initiated.^{46,47} Directly observed treatment, a ban on miltefosine from the private sector and a strictly regulated free-of-charge public distribution of miltefosine are urgent measures to overcome this specific issue.

Coupled with its long treatment course, possible teratogenicity and relatively high preferential price (which is only available per 200 000 capsule batch order), the uptake of miltefosine for human use remains relatively limited considering the global epidemiology of the leishmaniasis. Further research and development are therefore required to further optimize the use of the drug as well as identify better oral treatments that can be of much shorter course (e.g. 7 days), have a better safety profile, relatively high efficacy in all the main geographical foci and be more affordable (less than US\$10 per treatment).

Concluding remarks

In 2002, miltefosine was licensed in India as the first oral treatment for VL, which was a major breakthrough for the management of this neglected disease. Nevertheless, it took a further 8 years (2010) before the drug was included in the WHO Model List of Essential Medicines, which has arguably slowed down its use and adoption in other geographical areas where VL is endemic. Taking all therapies for VL into consideration, miltefosine is not the cheapest option available, but used in combination with paromomycin or liposomal amphotericin B it might well be the most cost-effective. The relatively easy production of *in vitro* resistant *Leishmania* clones, combined with the occurrence of relapses in immunocompetent patients, the presence of HIV/VL coinfections and high levels of anthroponotic transmission in both Africa and India, only increase the probability for the emergence of drug resistance in the field. The ultimate future of miltefosine in VL is therefore probably confined to its use in combination with other agents. Currently, several clinical trials with these combination regimens are ongoing in East Africa and the Indian subcontinent, most of them initiated by DNDi. For CL, more and better quality clinical trials are needed, certainly for Old World CL, to specifically define the role of miltefosine for the various *Leishmania* species. On the other hand, as the only oral drug available, miltefosine is sometimes logistically the only viable option for the treatment of patients. Over the years, awareness has increased for complicated cases, such as HIV-coinfected patients. As a well-tolerated and oral drug, miltefosine might play a fundamental role in the management of these patients, although clarification of the exact conditions for its use and possible complications, such as drug-drug interactions, needs to be prioritized.

Funding

This review was carried out as part of our routine work. T. P. C. D. was supported by a personal scholarship from the Academic Medical Center—Graduate School for Medical Sciences.

Transparency declarations

None to declare.

References

- Murray HW, Berman JD, Davies CR et al. Advances in leishmaniasis. *Lancet* 2005; **366**: 1561–77.
- Chappuis F, Sundar S, Hailu A et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat Rev Micro* 2007; **5**: 873–82.
- Sengupta PC. History of kala-azar in India. *Indian Med Gaz* 1947; **82**: 281–6.
- Croft SL. Monitoring drug resistance in leishmaniasis. *Trop Med Int Health* 2001; **6**: 899–905.
- Hailu A, Musa A, Wasunna M et al. Geographical variation in the response of visceral leishmaniasis to paromomycin in East Africa: a multicentre, open-label, randomized trial. *PLoS Negl Trop Dis* 2010; **4**: e709.
- Croft SL. Kinetoplastida: new therapeutic strategies. *Parasite* 2008; **15**: 522–7.
- 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**: 1104–7.
- Thakur CP, Narayan S, Ranjan A. Epidemiological, clinical and pharmacological study of antimony-resistant visceral leishmaniasis in Bihar, India. *Indian J Med Res* 2004; **120**: 166–72.
- 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.
- Edwards T, Omollo R, Khalil EAG et al. Single-dose liposomal amphotericin B (AmBisome®) for the treatment of visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. *Trials* 2011; **12**: 66.
- Sundar S, Chakravarty J, Agarwal D et al. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N Engl J Med* 2010; **362**: 504–12.
- Soto J, Fuya P, Herrera R et al. Topical paromomycin/methylbenzethonium chloride plus parenteral meglumine antimonate as treatment for American cutaneous leishmaniasis: controlled study. *Clin Infect Dis* 1998; **26**: 56–8.
- Jha TK, Olliaro P, Thakur CPN et al. Randomised controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *BMJ* 1998; **316**: 1200–5.
- Musa AM, Younis B, Fadlalla A et al. Paromomycin for the treatment of visceral leishmaniasis in Sudan: a randomized, open-label, dose-finding study. *PLoS Negl Trop Dis* 2010; **4**: e855.
- el-On J, Livshin R, Paz ZE et al. Topical treatment of cutaneous leishmaniasis. *Br Med J (Clin Res Ed)* 1985; **291**: 1280–1.
- Chunge CN, Owate J, Pamba HO et al. Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Trans R Soc Trop Med Hyg* 1990; **84**: 221–5.
- Thakur CP, Olliaro P, Gothoskar S et al. Treatment of visceral leishmaniasis (kala-azar) with aminosidine (= paromomycin)-antimonial combinations, a pilot study in Bihar, India. *Trans R Soc Trop Med Hyg* 1992; **86**: 615–6.
- Teklemariam S, Hiwot AG, Frommel D et al. 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**: 334–9.
- Seaman J, Pryce D, Sondorp HE et al. Epidemic visceral leishmaniasis in Sudan: a randomized trial of aminosidine plus sodium stibogluconate versus sodium stibogluconate alone. *J Infect Dis* 1993; **168**: 715–20.
- Sundar S, Sinha PK, Rai M et al. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet* 2011; **377**: 477–86.
- Dorlo TPC, Kager PA. Pentamidine dosage: a base/salt confusion. *PLoS Negl Trop Dis* 2008; **2**: e225.
- ClinicalTrials.gov. *Prophylaxis of Visceral Leishmaniasis Relapses in HIV Co-infected Patients with Pentamidine: A Cohort Study (NCT01360762)*. 2011. <http://clinicaltrials.gov/ct2/show/NCT01360762> (16 December 2011, date last accessed).
- Patel TA, Lockwood DN. Pentamidine as secondary prophylaxis for visceral leishmaniasis in the immunocompromised host: report of four cases. *Trop Med Int Health* 2009; **14**: 1064–70.
- 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.
- Smorenburg CH, Seynaeve C, Bontenbal M et al. Phase II study of miltefosine 6% solution as topical treatment of skin metastases in breast cancer patients. *Anticancer Drugs* 2000; **11**: 825–8.
- Verweij J, Planting A, van der Burg M et al. A dose-finding study of miltefosine (hexadecylphosphocholine) in patients with metastatic solid tumours. *J Cancer Res Clin Oncol* 1992; **118**: 606–8.
- Verweij J, Gandia D, Planting AS et al. Phase II study of oral miltefosine in patients with squamous cell head and neck cancer. *Eur J Cancer* 1993; **29A**: 778–9.
- Planting AS, Stoter G, Verweij J. Phase II study of daily oral miltefosine (hexadecylphosphocholine) in advanced colorectal cancer. *Eur J Cancer* 1993; **29A**: 518–9.
- Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. *Trans R Soc Trop Med Hyg* 2006; **100**: S17–20.
- Kuhlencord A, Maniera T, Eibl H et al. Hexadecylphosphocholine: oral treatment of visceral leishmaniasis in mice. *Antimicrob Agents Chemother* 1992; **36**: 1630–4.
- Sundar S, Rosenkaimer F, Makharia MK et al. Trial of oral miltefosine for visceral leishmaniasis. *Lancet* 1998; **352**: 1821–3.
- Engel J. Miltefosine, the story of a successful partnership: disease endemic country–TDR–pharmaceutical industry (Zentaris). *TDR News* 2002; **68**: 5.
- Paladin Labs Inc. *Application for Inclusion of Miltefosine on WHO Model List of Essential Medicines*. 2010. http://www.who.int/selection_medicines/committees/expert/18/applications/Miltefosine_application.pdf (13 December 2011, date last accessed).
- Ruiter GA, Zerp SF, Bartelink H et al. Anti-cancer alkyl-lysophospholipids inhibit the phosphatidylinositol 3-kinase-Akt/PKB survival pathway. *Anticancer Drugs* 2003; **14**: 167–73.
- Croft SL, Seifert K, Duchène M. Antiprotozoal activities of phospholipid analogues. *Mol Biochem Parasitol* 2003; **126**: 165–72.
- Brachwitz H, Vollgraf C. Analogs of alkyllysophospholipids: chemistry, effects on the molecular level and their consequences for normal and malignant cells. *Pharmacol Ther* 1995; **66**: 39–82.
- Achterberg V, Gercken G. Cytotoxicity of ester and ether lysophospholipids on *Leishmania donovani* promastigotes. *Mol Biochem Parasitol* 1987; **23**: 117–22.
- Leder GH, Fiebig HH, Wallbrecher E et al. In vitro and in vivo cytotoxicity of alkyl lysophospholipid ET-18-OCH₃ and thioether lipid BM 41.440. *Lipids* 1987; **22**: 958–61.

- 39 Hilgard P, Klenner T, Stekar J et al. D-21266, a new heterocyclic alkylphospholipid with antitumour activity. *Eur J Cancer* 1997; **33**: 442–6.
- 40 German Drug Registration Authorities. *Impavido 10/50 mg Kapseln—Fachinformation*. 2008. <http://www.pharmnet-bund.de/dynamic/de/index.html> (15 February 2010, date last accessed).
- 41 Central Drugs Standard Control Organization, Directorate General of Health Services—Government of India. *List of Finished Formulation Registered from 2003 to 2009*. 2009. http://cdsco.nic.in/html/finished_formulation_drug_registered.htm (14 December 2011, date last accessed).
- 42 Dorlo TPC, Eggelte TA, de Vries PJ et al. Characterization and identification of suspected counterfeit miltefosine capsules. *Analyst* 2012; **137**: 1265–74.
- 43 Dorlo TPC, Eggelte TA, Schoone GJ et al. A poor-quality generic drug for the treatment of visceral leishmaniasis: a case report and appeal. *PLoS Negl Trop Dis* 2012; **6**: e1544.
- 44 WHO. *WHO Model List of Essential Medicines—17th List*. 2011. http://whqlibdoc.who.int/hq/2011/a95053_eng.pdf (13 December 2011, date last accessed).
- 45 Murray HW. Leishmaniasis in the United States: treatment in 2012. *Am J Trop Med Hyg* 2012; **86**: 434–40.
- 46 Sundar S, Murray HW. Availability of miltefosine for the treatment of kala-azar in India. *Bull World Health Organ* 2005; **83**: 394–5.
- 47 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.
- 48 Dorlo TPC, Huitema ADR, Beijnen JH et al. Optimal dosing of miltefosine in children and adults with visceral leishmaniasis. *Antimicrob Agents Chemother* 2012; **56**: 3864–72.
- 49 Harhay MO, Olliaro PL, Vaillant M et al. Who is a typical patient with visceral leishmaniasis? Characterizing the demographic and nutritional profile of patients in Brazil, East Africa, and South Asia. *Am J Trop Med Hyg* 2011; **84**: 543–50.
- 50 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.
- 51 Olliaro P, Darley S, Laxminarayan R et al. Cost-effectiveness projections of single and combination therapies for visceral leishmaniasis in Bihar, India. *Trop Med Int Health* 2009; **14**: 918–25.
- 52 Meheus F, Balasegaram M, Olliaro P et al. Cost-effectiveness analysis of combination therapies for visceral leishmaniasis in the Indian subcontinent. *PLoS Negl Trop Dis* 2010; **4**: e818.
- 53 Dorlo TPC, Hillebrand MJX, Rosing H et al. Development and validation of a quantitative assay for the measurement of miltefosine in human plasma by liquid chromatography–tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **865**: 55–62.
- 54 Dorlo TPC, van Thiel PPAM, Huitema ADR et al. Pharmacokinetics of miltefosine in Old World cutaneous leishmaniasis patients. *Antimicrob Agents Chemother* 2008; **52**: 2855–60.
- 55 Woo EW, Messmann R, Sausville EA et al. Quantitative determination of perifosine, a novel alkylphosphocholine anticancer agent, in human plasma by reversed-phase liquid chromatography–electrospray mass spectrometry. *J Chromatogr B Biomed Appl* 2001; **759**: 247–57.
- 56 Knebel NG, Grieb S, Winkler M et al. Quantification of perifosine, an alkylphosphocholine anti-tumour agent, in plasma by pneumatically assisted electrospray tandem mass spectrometry coupled with high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 1999; **721**: 257–69.
- 57 Blanco-Prieto MJ, Campanero MA, Mollinedo F. Quantitative determination of the antitumor alkyl ether phospholipid edelfosine by reversed-phase liquid chromatography–electrospray mass spectrometry: application to cell uptake studies and characterization of drug delivery systems. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; **810**: 85–92.
- 58 Lindner LH, Eibl H, Hossann M et al. Quantification of erufosine, the first intravenously applicable alkylphosphocholine, in human plasma by isotope dilution liquid chromatography–tandem mass spectrometry using a deuterated internal standard. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **869**: 16–9.
- 59 Jiang H, Cannon MJ, Banach M et al. Quantification of CLR1401, a novel alkylphosphocholine anticancer agent, in rat plasma by hydrophilic interaction liquid chromatography–tandem mass spectrometric detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; **878**: 1513–8.
- 60 Omollo R, Alexander N, Edwards T et al. Safety and efficacy of miltefosine alone and in combination with sodium stibogluconate and liposomal amphotericin B for the treatment of primary visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. *Trials* 2011; **12**: 166.
- 61 ClinicalTrials.gov. *Pharmacokinetics of Miltefosine in Children and Adults (NCT01462500)*. 2011. <http://clinicaltrials.gov/ct2/show/record/NCT01462500> (20 January 2012, date last accessed).
- 62 Kötting J, Marschner NW, Neumüller W et al. Hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine: a comparison of hemolytic activity, serum binding and tissue distribution. *Prog Exp Tumor Res* 1992; **34**: 131–42.
- 63 Ménez C, Buyse M, Dugave C et al. Intestinal absorption of miltefosine: contribution of passive paracellular transport. *Pharm Res* 2007; **24**: 546–54.
- 64 Ménez C, Buyse M, Farinotti R et al. Inward translocation of the phospholipid analogue miltefosine across Caco-2 cell membranes exhibits characteristics of a carrier-mediated process. *Lipids* 2007; **42**: 229–40.
- 65 Ménez C, Buyse M, Chacun H et al. Modulation of intestinal barrier properties by miltefosine. *Biochem Pharmacol* 2006; **71**: 486–96.
- 66 Breiser A, Kim D, Fleer E et al. Distribution and metabolism of hexadecylphosphocholine in mice. *Lipids* 1987; **22**: 925–6.
- 67 Marschner N, Kötting J, Eibl H et al. Distribution of hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine in rat tissues during steady-state treatment. *Cancer Chemother Pharmacol* 1992; **31**: 18–22.
- 68 Müller G, Petry S. *Lipases and Phospholipases in Drug Development: From Biochemistry to Molecular Pharmacology*. Weinheim: Wiley-VCH, 2004.
- 69 Bianciardi P, Brovida C, Valente M et al. Administration of miltefosine and meglumine antimoniate in healthy dogs: clinicopathological evaluation of the impact on the kidneys. *Toxicol Pathol* 2009; **37**: 770–5.
- 70 Dorlo TPC, van Thiel PPAM, Schoone GJ et al. Dynamics of parasite clearance in cutaneous leishmaniasis patients treated with miltefosine. *PLoS Negl Trop Dis* 2011; **5**: e1436.
- 71 de Vallière S, Mary C, Joneberg JE et al. AA-amyloidosis caused by visceral leishmaniasis in a human immunodeficiency virus-infected patient. *Am J Trop Med Hyg* 2009; **81**: 209–12.
- 72 de Vries PJ, van der Meide WF, Godfried MH et al. Quantification of the response to miltefosine treatment for visceral leishmaniasis by QT-NASBA. *Trans R Soc Trop Med Hyg* 2006; **100**: 1183–6.
- 73 Dorlo T. Erratum: AA-amyloidosis caused by visceral leishmaniasis in a human immunodeficiency virus-infected patient. *Am J Trop Med Hyg* 2009; **81**: 732.
- 74 van Thiel PPAM, Leenstra T, Kager PA et al. Miltefosine treatment of *Leishmania major* infection: an observational study involving Dutch

- military personnel returning from northern Afghanistan. *Clin Infect Dis* 2010; **50**: 80–3.
- 75** Bhattacharya SK, Jha TK, Sundar S *et al.* Efficacy and tolerability of miltefosine for childhood visceral leishmaniasis in India. *Clin Infect Dis* 2004; **38**: 217–21.
- 76** Jha TK, Sundar S, Thakur CP *et al.* Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. *N Engl J Med* 1999; **341**: 1795–800.
- 77** Dorlo TPC, Balasegaram M, Lima MA *et al.* Translational pharmacokinetic modelling and simulation for the assessment of duration of contraceptive use after treatment with miltefosine. *J Antimicrob Chemother* 2012; **67**: 1996–2004.
- 78** Croft SL, Neal RA, Pendergast W *et al.* The activity of alkyl phosphorylcholines and related derivatives against *Leishmania donovani*. *Biochem Pharmacol* 1987; **36**: 2633–6.
- 79** Vermeersch M, da Luz RI, Toté K *et al.* In vitro susceptibilities of *Leishmania donovani* promastigote and amastigote stages to antileishmanial reference drugs: practical relevance of stage-specific differences. *Antimicrob Agents Chemother* 2009; **53**: 3855–9.
- 80** da Luz RI, Vermeersch M, Dujardin J-C *et al.* In vitro sensitivity testing of *Leishmania* clinical field isolates: preconditioning of promastigotes enhances infectivity for macrophage host cells. *Antimicrob Agents Chemother* 2009; **53**: 5197–203.
- 81** Seifert K, Escobar P, Croft SL. In vitro activity of antileishmanial drugs against *Leishmania donovani* is host cell dependent. *J Antimicrob Chemother* 2010; **65**: 508–11.
- 82** Yardley V, Croft SL, De Doncker S *et al.* The sensitivity of clinical isolates of *Leishmania* from Peru and Nepal to miltefosine. *Am J Trop Med Hyg* 2005; **73**: 272–5.
- 83** Escobar P, Matu S, Marques C *et al.* Sensitivities of *Leishmania* species to hexadecylphosphocholine (miltefosine), ET-18-OCH₃ (edelfosine) and amphotericin B. *Acta Trop* 2002; **81**: 151–7.
- 84** Lux H, Hart DT, Parker PJ *et al.* Ether lipid metabolism, GPI anchor biosynthesis, and signal transduction are putative targets for anti-leishmanial alkyl phospholipid analogues. *Adv Exp Med Biol* 1996; **416**: 201–11.
- 85** Zufferey R, Allen S, Barron T *et al.* Ether phospholipids and glycosylinositolphospholipids are not required for amastigote virulence or for inhibition of macrophage activation by *Leishmania major*. *J Biol Chem* 2003; **278**: 44708–18.
- 86** Lux H, Heise N, Klenner T *et al.* Ether-lipid (alkyl-phospholipid) metabolism and the mechanism of action of ether-lipid analogues in *Leishmania*. *Mol Biochem Parasitol* 2000; **111**: 1–14.
- 87** Rakotomanga M, Saint-Pierre-Chazalet M, Loiseau PM. Alteration of fatty acid and sterol metabolism in miltefosine-resistant *Leishmania donovani* promastigotes and consequences for drug-membrane interactions. *Antimicrob Agents Chemother* 2005; **49**: 2677–86.
- 88** Rakotomanga M, Blanc S, Gaudin K *et al.* Miltefosine affects lipid metabolism in *Leishmania donovani* promastigotes. *Antimicrob Agents Chemother* 2007; **51**: 1425–30.
- 89** Zufferey R, Mamoun CB. Choline transport in *Leishmania major* promastigotes and its inhibition by choline and phosphocholine analogs. *Mol Biochem Parasitol* 2002; **125**: 127–34.
- 90** Das M, Mukherjee SB, Shaha C. Hydrogen peroxide induces apoptosis-like death in *Leishmania donovani* promastigotes. *J Cell Sci* 2001; **114**: 2461–9.
- 91** Paris C, Loiseau PM, Bories C *et al.* Miltefosine induces apoptosis-like death in *Leishmania donovani* promastigotes. *Antimicrob Agents Chemother* 2004; **48**: 852–9.
- 92** Verma NK, Dey CS. Possible mechanism of miltefosine-mediated death of *Leishmania donovani*. *Antimicrob Agents Chemother* 2004; **48**: 3010–5.
- 93** Marinho F de A, Gonçalves KC da S, de Oliveira SS *et al.* Miltefosine induces programmed cell death in *Leishmania amazonensis* promastigotes. *Mem Inst Oswaldo Cruz* 2011; **106**: 507–9.
- 94** Khademvatan S, Gharavi MJ, Saki J. Miltefosine induces metacaspase and PARP genes expression in *Leishmania infantum*. *Braz J Infect Dis* 2011; **15**: 442–8.
- 95** Moreira W, Leprohon P, Ouellette M. Tolerance to drug-induced cell death favours the acquisition of multidrug resistance in *Leishmania*. *Cell Death Dis* 2011; **2**: e201.
- 96** Santa-Rita RM, Henriques-Pons A, Barbosa HS *et al.* Effect of the lysophospholipid analogues edelfosine, ilmofosine and miltefosine against *Leishmania amazonensis*. *J Antimicrob Chemother* 2004; **54**: 704–10.
- 97** Luque-Ortega JR, Rivas L. Miltefosine (hexadecylphosphocholine) inhibits cytochrome c oxidase in *Leishmania donovani* promastigotes. *Antimicrob Agents Chemother* 2007; **51**: 1327–32.
- 98** Zuo X, Djordjevic JT, Bijosono Oei J *et al.* Miltefosine induces apoptosis-like cell death in yeast via Cox9p in cytochrome c oxidase. *Mol Pharmacol* 2011; **80**: 476–85.
- 99** Zeisig R, Rudolf M, Eue I *et al.* Influence of hexadecylphosphocholine on the release of tumor necrosis factor and nitroxide from peritoneal macrophages in vitro. *J Cancer Res Clin Oncol* 1995; **121**: 69–75.
- 100** Wadhwa P, Maiti M, Agarwal R *et al.* Miltefosine promotes IFN- γ -dominated anti-leishmanial immune response. *J Immunol* 2009; **182**: 7146–54.
- 101** Escobar P, Yardley V, Croft SL. Activities of hexadecylphosphocholine (miltefosine), AmBisome, and sodium stibogluconate (Pentostam) against *Leishmania donovani* in immunodeficient scid mice. *Antimicrob Agents Chemother* 2001; **45**: 1872–5.
- 102** Hochhuth CH, Vehmeyer K, Eibl H *et al.* Hexadecylphosphocholine induces interferon-gamma secretion and expression of GM-CSF mRNA in human mononuclear cells. *Cell Immunol* 1992; **141**: 161–8.
- 103** Eue I. Hexadecylphosphocholine selectively upregulates expression of intracellular adhesion molecule-1 and class I major histocompatibility complex antigen in human monocytes. *J Exp Ther Oncol* 2002; **2**: 333–6.
- 104** Griewank K, Gazeau C, Eichhorn A *et al.* Miltefosine efficiently eliminates *Leishmania major* amastigotes from infected murine dendritic cells without altering their immune functions. *Antimicrob Agents Chemother* 2010; **54**: 652–9.
- 105** Weller K, Artuc M, Jennings G *et al.* Miltefosine inhibits human mast cell activation and mediator release both in vitro and in vivo. *J Invest Dermatol* 2008; **129**: 496–8.
- 106** Hartmann K, Siebenhaar F, Belloni B *et al.* Effects of topical treatment with the raft modulator miltefosine and clobetasol in cutaneous mastocytosis: a randomized, double-blind, placebo-controlled trial. *Br J Dermatol* 2010; **162**: 185–90.
- 107** Cummings HE, Barbi J, Reville P *et al.* Critical role for phosphoinositide 3-kinase gamma in parasite invasion and disease progression of cutaneous leishmaniasis. *Proc Natl Acad Sci USA* 2012; **109**: 1251–6.
- 108** Liu D, Zhang T, Marshall AJ *et al.* The p110 δ isoform of phosphatidylinositol 3-kinase controls susceptibility to *Leishmania major* by regulating expansion and tissue homing of regulatory T cells. *J Immunol* 2009; **183**: 1921–33.

- 109** van Blitterswijk WJ, Verheij M. Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects. *Curr Pharm Des* 2008; **14**: 2061–74.
- 110** Berkovic D, Sievers S, Haase D et al. Effects of hexadecylphosphocholine on phosphatidylcholine and phosphatidylserine metabolism in human lymphoma cells. *J Exp Ther Oncol* 2002; **2**: 85–92.
- 111** van der Luit AH, Budde M, Ruurs P et al. Alkyl-lysophospholipid accumulates in lipid rafts and induces apoptosis via raft-dependent endocytosis and inhibition of phosphatidylcholine synthesis. *J Biol Chem* 2002; **277**: 39541–7.
- 112** Carrasco MP, Jiménez-López JM, Segovia JL et al. Hexadecylphosphocholine interferes with the intracellular transport of cholesterol in HepG2 cells. *FEBS J* 2008; **275**: 1675–86.
- 113** Chakrabandhu K, Huault S, Hueber A-O. Distinctive molecular signaling in triple-negative breast cancer cell death triggered by hexadecylphosphocholine (miltefosine). *FEBS Lett* 2008; **582**: 4176–84.
- 114** Croft SL, Snowdon D, Yardley V. The activities of four anticancer alkyllysophospholipids against *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*. *J Antimicrob Chemother* 1996; **38**: 1041–7.
- 115** Konstantinov SM, Kaminsky R, Brun R et al. Efficacy of anticancer alkylphosphocholines in *Trypanosoma brucei* subspecies. *Acta Trop* 1997; **64**: 145–54.
- 116** Saraiva VB, Gibaldi D, Previato JO et al. Proinflammatory and cytotoxic effects of hexadecylphosphocholine (miltefosine) against drug-resistant strains of *Trypanosoma cruzi*. *Antimicrob Agents Chemother* 2002; **46**: 3472–7.
- 117** Lira R. Mechanism of action of anti-proliferative lysophospholipid analogues against the protozoan parasite *Trypanosoma cruzi*: potentiation of *in vitro* activity by the sterol biosynthesis inhibitor ketoconazole. *J Antimicrob Chemother* 2001; **47**: 537–46.
- 118** Santa-Rita RM, Santos Barbosa H, Meirelles MN et al. Effect of the alkyl-lysophospholipids on the proliferation and differentiation of *Trypanosoma cruzi*. *Acta Trop* 2000; **75**: 219–28.
- 119** Malaquias AT, Oliveira MM. Phospholipid signalling pathways in *Trypanosoma cruzi* growth control. *Acta Tropica* 1999; **73**: 93–108.
- 120** Seifert K, Duchêne M, Wernsdorfer WH et al. Effects of miltefosine and other alkylphosphocholines on human intestinal parasite *Entamoeba histolytica*. *Antimicrob Agents Chemother* 2001; **45**: 1505–10.
- 121** Walochnik J, Duchêne M, Seifert K et al. Cytotoxic activities of alkylphosphocholines against clinical isolates of *Acanthamoeba* spp. *Antimicrob Agents Chemother* 2002; **46**: 695–701.
- 122** Polat ZA, Obwaller A, Vural A et al. Efficacy of miltefosine for topical treatment of *Acanthamoeba* keratitis in Syrian hamsters. *Parasitol Res* 2012; **110**: 515–20.
- 123** Blaha C, Duchêne M, Aspöck H et al. In vitro activity of hexadecylphosphocholine (miltefosine) against metronidazole-resistant and -susceptible strains of *Trichomonas vaginalis*. *J Antimicrob Chemother* 2006; **57**: 273–8.
- 124** Shahiduzzaman M, Dyachenko V, Obwaller A et al. Combination of cell culture and quantitative PCR for screening of drugs against *Cryptosporidium parvum*. *Vet Parasitol* 2009; **162**: 271–7.
- 125** Sinkala E, Katubulushi M, Sianongo S et al. In a trial of the use of miltefosine to treat HIV-related cryptosporidiosis in Zambian adults, extreme metabolic disturbances contribute to high mortality. *Ann Trop Med Parasitol* 2011; **105**: 129–34.
- 126** Eissa MM, El-Azzouni MZ, Amer EI et al. Miltefosine, a promising novel agent for schistosomiasis mansoni. *Int J Parasitol* 2011; **41**: 235–42.
- 127** Bertão HG, da Silva RAR, Padilha RJR et al. Ultrastructural analysis of miltefosine-induced surface membrane damage in adult *Schistosoma mansoni* BH strain worms. *Parasitol Res* 2012; **110**: 2465–73.
- 128** Llull D, Rivas L, García E. In vitro bactericidal activity of the antiprotozoal drug miltefosine against *Streptococcus pneumoniae* and other pathogenic streptococci. *Antimicrob Agents Chemother* 2007; **51**: 1844–8.
- 129** Obando D, Widmer F, Wright LC et al. Synthesis, antifungal and antimicrobial activity of alkylphospholipids. *Bioorg Med Chem* 2007; **15**: 5158–65.
- 130** Huelves L, Del Prado G, Gracia M et al. In vitro and in vivo activity of miltefosine against penicillin-sensitive and -resistant *Streptococcus pneumoniae* strains. *J Chemother* 2008; **20**: 441–4.
- 131** Walochnik J, Obwaller A, Gruber F et al. Anti-*Acanthamoeba* efficacy and toxicity of miltefosine in an organotypic skin equivalent. *J Antimicrob Chemother* 2009; **64**: 539–45.
- 132** Widmer F, Wright LC, Obando D et al. Hexadecylphosphocholine (miltefosine) has broad-spectrum fungicidal activity and is efficacious in a mouse model of cryptococcosis. *Antimicrob Agents Chemother* 2006; **50**: 414–21.
- 133** Tong Z, Widmer F, Sorrell TC et al. In vitro activities of miltefosine and two novel antifungal biscationic salts against a panel of 77 dermatophytes. *Antimicrob Agents Chemother* 2007; **51**: 2219–22.
- 134** Chugh P, Bradel-Tretheway B, Monteiro-Filho CMR et al. Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. *Retrovirology* 2008; **5**: 11.
- 135** Lucas A, Kim Y, Rivera-Pabon O et al. Targeting the PI3K/Akt cell survival pathway to induce cell death of HIV-1 infected macrophages with alkylphospholipid compounds. *PLoS ONE* 2010; **5**: e13121.
- 136** Seifert K, Matu S, Javier Pérez-Victoria F et al. Characterisation of *Leishmania donovani* promastigotes resistant to hexadecylphosphocholine (miltefosine). *Int J Antimicrob Agents* 2003; **22**: 380–7.
- 137** Pérez-Victoria FJ, Castanys S, Gamarro F. *Leishmania donovani* resistance to miltefosine involves a defective inward translocation of the drug. *Antimicrob Agents Chemother* 2003; **47**: 2397–403.
- 138** Pérez-Victoria FJ, Gamarro F, Ouellette M et al. Functional cloning of the miltefosine transporter. A novel P-type phospholipid translocase from *Leishmania* involved in drug resistance. *J Biol Chem* 2003; **278**: 49965–71.
- 139** Pérez-Victoria FJ, Sánchez-Cañete MP, Castanys S et al. Phospholipid translocation and miltefosine potency require both *L. donovani* miltefosine transporter and the new protein LdRos3 in *Leishmania* parasites. *J Biol Chem* 2006; **281**: 23766–75.
- 140** Sánchez-Cañete MP, Carvalho L, Pérez-Victoria FJ et al. Low plasma membrane expression of the miltefosine transport complex renders *Leishmania braziliensis* refractory to the drug. *Antimicrob Agents Chemother* 2009; **53**: 1305–13.
- 141** Weingärtner A, Drobot B, Herrmann A et al. Disruption of the lipid-transporting LdMT-LdRos3 complex in *Leishmania donovani* affects membrane lipid asymmetry but not host cell invasion. *PLoS ONE* 2010; **5**: e12443.
- 142** Seifert K, Pérez-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.
- 143** Pérez-Victoria JM, Pérez-Victoria FJ, Parodi-Talice A et al. Alkyl-lysophospholipid resistance in multidrug-resistant *Leishmania tropica* and chemosensitization by a novel P-glycoprotein-like transporter modulator. *Antimicrob Agents Chemother* 2001; **45**: 2468–74.

- 144** Pérez-Victoria JM, Cortés-Selva F, Parodi-Talice A et al. Combination of suboptimal doses of inhibitors targeting different domains of LtrMDR1 efficiently overcomes resistance of *Leishmania* spp. to miltefosine by inhibiting drug efflux. *Antimicrob Agents Chemother* 2006; **50**: 3102–10.
- 145** Castanys-Muñoz E, Alder-Baerens N, Pomorski T et al. A novel ATP-binding cassette transporter from *Leishmania* is involved in transport of phosphatidylcholine analogues and resistance to alkyl-phospholipids. *Mol Microbiol* 2007; **64**: 1141–53.
- 146** Castanys-Muñoz E, Pérez-Victoria JM, Gamarro F et al. Characterization of an ABCG-like transporter from the protozoan parasite *Leishmania* with a role in drug resistance and transbilayer lipid movement. *Antimicrob Agents Chemother* 2008; **52**: 3573–9.
- 147** Coelho AC, Boisvert S, Mukherjee A et al. Multiple mutations in heterogeneous miltefosine-resistant *Leishmania major* population as determined by whole genome sequencing. *PLoS Negl Trop Dis* 2012; **6**: e1512.
- 148** Zerpa O, Ulrich M, Blanco B et al. Diffuse cutaneous leishmaniasis responds to miltefosine but then relapses. *Br J Dermatol* 2007; **156**: 1328–35.
- 149** Pandey BD, Pandey K, Kaneko O et al. Relapse of visceral leishmaniasis after miltefosine treatment in a Nepalese patient. *Am J Trop Med Hyg* 2009; **80**: 580–2.
- 150** Calvopina M, Gomez EA, Sindermann H et al. Relapse of New World diffuse cutaneous leishmaniasis caused by *Leishmania (Leishmania) mexicana* after miltefosine treatment. *Am J Trop Med Hyg* 2006; **75**: 1074–7.
- 151** Sundar S, Singh A, Rai M et al. Efficacy of miltefosine in the treatment of visceral leishmaniasis after a decade of use in India. *Clin Infect Dis* 2012; doi:10.1093/cid/cis474.
- 152** Cojean S, Houzé S, Haouchine D et al. *Leishmania* resistance to miltefosine associated with genetic marker. *Emerging Infect Dis* 2012; **18**: 704–6.
- 153** 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**: 591–8.
- 154** Soto J, Soto P. Miltefosine: oral treatment of leishmaniasis. *Expert Rev Anti Infect Ther* 2006; **4**: 177–85.
- 155** Rybczynska M, Liu R, Lu P et al. MDR1 causes resistance to the antitumour drug miltefosine. *Br J Cancer* 2001; **84**: 1405–11.
- 156** Márián T, Balkay L, Trón L et al. Effects of miltefosine on membrane permeability and accumulation of [99mTc]-hexakis-2-methoxyisobutyl isonitrile, 2-[18F]fluoro-2-deoxy-D-glucose, daunorubicin and rhodamine123 in multidrug-resistant and sensitive cells. *Eur J Pharm Sci* 2005; **24**: 495–501.
- 157** 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.
- 158** Rahman M, Ahmed B-N, Faiz MA et al. Phase IV trial of miltefosine in adults and children for treatment of visceral leishmaniasis (kala-azar) in Bangladesh. *Am J Trop Med Hyg* 2011; **85**: 66–9.
- 159** ClinicalTrials.gov. Miltefosine for Brazilian Visceral Leishmaniasis (NCT00378495). 2011. <http://clinicaltrials.gov/ct2/show/NCT00378495> (14 February 2012, date last accessed).
- 160** ClinicalTrials.gov. Phase III, Study of Three Short Course Combination Regimens (AmBisome®, Miltefosine, Paromomycin) Compared with AmBisome® Alone for the Treatment of Visceral Leishmaniasis in Bangladesh (NCT01122771). 2011. <http://clinicaltrials.gov/ct2/show/NCT01122771> (19 January 2012, date last accessed).
- 161** Seifert K, Croft SL. In vitro and in vivo interactions between miltefosine and other antileishmanial drugs. *Antimicrob Agents Chemother* 2006; **50**: 73–9.
- 162** van Griensven J, Balasegaram M, Meheus F et al. Combination therapy for visceral leishmaniasis. *Lancet Infect Dis* 2010; **10**: 184–94.
- 163** Sindermann H, Engel KR, Fischer C et al. Oral miltefosine for leishmaniasis in immunocompromised patients: compassionate use in 39 patients with HIV infection. *Clin Infect Dis* 2004; **39**: 1520–3.
- 164** Soto J, Rea J, Balderrama M et al. Efficacy of miltefosine for Bolivian cutaneous leishmaniasis. *Am J Trop Med Hyg* 2008; **78**: 210–1.
- 165** González U, Pinart M, Reveiz L et al. Designing and reporting clinical trials on treatments for cutaneous leishmaniasis. *Clin Infect Dis* 2010; **51**: 409–19.
- 166** Khandpur S, Chaturvedi P, Kumar U et al. Oral miltefosine in post-kala-azar dermal leishmaniasis—experience in three cases. *Int J Dermatol* 2010; **49**: 565–9.
- 167** Sundar S, Kumar K, Chakravarty J et al. Cure of antimony-unresponsive Indian post-kala-azar dermal leishmaniasis with oral miltefosine. *Trans R Soc Trop Med Hyg* 2006; **100**: 698–700.
- 168** Belay AD, Asafa Y, Mesure J et al. Successful miltefosine treatment of post-kala-azar dermal leishmaniasis occurring during antiretroviral therapy. *Ann Trop Med Parasitol* 2006; **100**: 223–7.
- 169** Ramesh V, Ansari NA, Jain RK et al. Oral miltefosine in the treatment of post-kala-azar dermal leishmaniasis. *Clin Exp Dermatol* 2008; **33**: 103–5.
- 170** Ramesh V, Katara GK, Verma S et al. Miltefosine as an effective choice in the treatment of post-kala-azar dermal leishmaniasis. *Br J Dermatol* 2011; **165**: 411–4.
- 171** Schmidt-Ott R, Klenner T, Overath P et al. Topical treatment with hexadecylphosphocholine (Miltex) efficiently reduces parasite burden in experimental cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 1999; **93**: 85–90.
- 172** Garnier T, Croft SL. Topical treatment for cutaneous leishmaniasis. *Curr Opin Investig Drugs* 2002; **3**: 538–44.
- 173** Sundar S, Mondal D, Rijal S et al. Implementation research to support the initiative on the elimination of kala azar from Bangladesh, India and Nepal—the challenges for diagnosis and treatment. *Trop Med Int Health* 2008; **13**: 2–5.
- 174** Government of India—National Vector Borne Disease Control Programme. Guideline on Use of Miltefosine. <http://nvbdcp.gov.in/Doc/Guidelines%20on%20miltefosine.pdf> (12 December 2011, date last accessed).
- 175** Kaladrag-R. Project: New Tools for Monitoring Drug Resistance and Treatment Response in Visceral Leishmaniasis in the Indian Subcontinent. http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/projects/002_en.html (9 March 2012, date last accessed).
- 176** WHO. WHO Technical Report Series 949: Control of the Leishmaniases. Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf (14 February 2012, date last accessed).
- 177** Sundar S, Gupta LB, Makharia MK et al. Oral treatment of visceral leishmaniasis with miltefosine. *Ann Trop Med Parasitol* 1999; **93**: 589–97.
- 178** Sundar S, Makharia A, More DK et al. Short-course of oral miltefosine for treatment of visceral leishmaniasis. *Clin Infect Dis* 2000; **31**: 1110–3.
- 179** Sundar S, Jha TK, Thakur CP et al. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 2002; **347**: 1739–46.
- 180** Sundar S, Jha TK, Sindermann H et al. Oral miltefosine treatment in children with mild to moderate Indian visceral leishmaniasis. *Pediatr Infect Dis J* 2003; **22**: 434–8.

- 181** Singh UK, Prasad R, Mishra OP *et al.* Miltefosine in children with visceral leishmaniasis: a prospective, multicentric, cross-sectional study. *Indian J Pediatr* 2006; **73**: 1077–80.
- 182** 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**: 1000–6.
- 183** Soto J, Toledo J, Gutierrez P *et al.* Treatment of American cutaneous leishmaniasis with miltefosine, an oral agent. *Clin Infect Dis* 2001; **33**: E57–61.
- 184** Soto J, Arana BA, Toledo J *et al.* Miltefosine for New World cutaneous leishmaniasis. *Clin Infect Dis* 2004; **38**: 1266–72.
- 185** Vélez I, López L, Sánchez X *et al.* Efficacy of miltefosine for the treatment of American cutaneous leishmaniasis. *Am J Trop Med Hyg* 2010; **83**: 351–6.
- 186** Machado PR, Ampuero J, Guimarães LH *et al.* Miltefosine in the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis* in Brazil: a randomized and controlled trial. *PLoS Negl Trop Dis* 2010; **4**: e912.
- 187** Chrusciak-Talhari A, Dietze R, Chrusciak Talhari C *et al.* Randomized controlled clinical trial to access efficacy and safety of miltefosine in the treatment of cutaneous leishmaniasis caused by *Leishmania (Viannia) guyanensis* in Manaus, Brazil. *Am J Trop Med Hyg* 2011; **84**: 255–60.
- 188** Rubiano LC, Miranda MC, Muvdi Arenas S *et al.* Noninferiority of miltefosine versus meglumine antimoniate for cutaneous leishmaniasis in children. *J Infect Dis* 2012; **205**: 684–92.
- 189** Mohebbali M, Fotouhi A, Hooshmand B *et al.* Comparison of miltefosine and meglumine antimoniate for the treatment of zoonotic cutaneous leishmaniasis (ZCL) by a randomized clinical trial in Iran. *Acta Trop* 2007; **103**: 33–40.
- 190** Soto J, Toledo J, Valda L *et al.* Treatment of Bolivian mucosal leishmaniasis with miltefosine. *Clin Infect Dis* 2007; **44**: 350–6.
- 191** Soto J, Rea J, Valderrama M *et al.* Efficacy of extended (six weeks) treatment with miltefosine for mucosal leishmaniasis in Bolivia. *Am J Trop Med Hyg* 2009; **81**: 387–9.