Clinical pharmacology in leishmaniasis: treatment optimization of a neglected disease
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Miltefosine: a review of its pharmacology and therapeutic efficacy

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Abstract

Miltefosine is an alkylphosphocholine drug with demonstrated activity against various parasites species and cancer cells as well as some pathogenic bacteria and fungi. Since 10 years it is 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 performed clinical trials of miltefosine, either alone or in combination, in the treatment of VL and CL.
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 *L. major* and *L. tropica* in the Old World (Europe, Africa, Central Asia and the Middle East) and by the *L. braziliensis*, *L. guyanensis* and *L. 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 (SbV) 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 formulation produced by 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, US) 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, ILL, US) [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 United Kingdom 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 (TDR) 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 license 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 (MSF) 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 term: (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. This alkylphosphocholine compounds are structurally related to the group of alkyllysophospholipids, which are synthetic analogs 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 C_{21}H_{46}NO_{4}P, yielding a molecular weight of 407.57 g/mol. Other alkylphosphocholine compounds that were tested for their antileishmanial or

![Figure 1. Structural formula of miltefosine](image)
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 alkylglycerophosphocholines, also known as ether lipids, which have only small structural modifications compared with miltefosine [36]. For example, edelfosine contains an ether-linked 2-methoxy-glycerol backbone and has an octadecyl alkyl chain, while ilmofosine contains a 2-mercapto-methoxy-methyl-glycerol backbone [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 dimethyl sulfoxide 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 visceral leishmaniasis 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 (EMA) 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 United States from the manufacturer through an Investigational New Drug (IND) application to the FDA in conjunction with local 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 & 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. 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 [48].

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 €45.28-54.92 for 56 capsules containing 50 mg miltefosine and between €34.36–39.30 for 56 capsules containing 10 mg 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$200 [46] 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 employs reversed-phase liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to detect miltefosine in human EDTA plasma with pretreatment by solid-phase extraction and has a lower limit of quantitation of 4 ng/mL using a 250 µL aliquot of plasma, which is sensitive enough to determine miltefosine levels 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], erucylphosphocholine [58] 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 & 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 will be started in 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 maximal concentration (Tmax) between 4 and 48 h [29]. In human, 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 2-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. using in vitro permeability testing with Caco-2 cells. Below 50 µM (20.4 µg/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 [¹⁴C]-radiolabeled miltefosine and in mice following single oral and intravenous administration [³H]-radiolabeled miltefosine (25 µg total dose) indicated a wide general distribution of miltefosine [29,66]. These studies demonstrated that the uptake of the 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 unlabeled miltefosine dose was administered, which demonstrated highest drug concentrations in the adrenal glands, kidneys and spleen [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 cerebrospinal fluid of patients treated for Balamuthia and Naegleria infections, although it was unclear how intact their blood-brain barrier was (T.P.C. Dorlo & G.S. Visvesvara, unpublished data). Placental distribution and transfer through the umbilical cord has not been investigated but should be assumed given the results from reproductive animal studies [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 CYP3A4 isoenzymes by miltefosine could be demonstrated in both male and female rats [29]. Metabolic drug-drug interactions at the cytochrome P450 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 hydrolyze 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 could be confirmed in mice in which the metabolic fate of [*H*-radiolabeled 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 radiolabeled 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 radiolabeled 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 less than 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% (± 4.86%) of the total miltefosine clearance [69]. Excretion into 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 miltefosine for 28 days, showed a median maximal concentration of 70 µg/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 concentrations between day 23-28 of treatment was 24 µg/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 CL [70] and with VL [71,72], and one larger and more detailed population pharmacokinetic study in CL patients [54] were published, which allowed a more extensive evaluation of its clinical pharmacokinetics. Absorption of miltefosine is slow with an absorption rate of 0.36 day\(^{-1}\) 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, amounted 14.6-15.6 µg/mL (100 mg/day, \(n = 1\)) [71,73], 11.9 µg/mL (150 mg/day, \(n = 1\)) [72], 29-38 µg/mL (150 mg/day, \(n = 2\)) [70] and a median 30.8 µg/mL (interquartile range 25.2–33.4 µg/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.

Population pharmacokinetic modelling of the pooled original pharmacokinetic data collected during the paediatric Indian phase II/III trial [75], the adult Indian phase II trial [76] 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 ¾ 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.

**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].

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

\(^{a}\) Estimates 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 \(V_3/F\).

\(^{b}\) Between-subject variabilities in CL/F and \(V_2/F\) correlated with a correlation coefficient of 0.92.

\(^{c}\) Range
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 reverse transcriptase PCR targeting the *Leishmania* 18S rRNA genome estimated a parasite clearance rate for miltefosine of around ~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 (*L. 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 both *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-dependant cell signalling pathways.

Antileishmanial activity & 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 dependant 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*, *L. aethiopica*, *L. tropica*, *L. panamensis*, *L. mexicana* and *L. major* laboratory strains, *L. donovani* was most susceptible with EC50 values of 3.3-4.6 µM (corresponding with 1.3-1.9 µg/mL), while *L. major* was least susceptible with EC50 values of 31.6–37.2 µM (corresponding with 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 variability is found between clinical isolates. All *L. donovani* isolates from Nepal were very susceptible to miltefosine with an EC\(_{50}\) of 0.04-8.7 µg/mL, while the isolates from Peru showed remarkable variability, with EC\(_{50}\) 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 *L. lainsoni* (n = 4) [82]. For comparison, the 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 emerged over the recent years, which 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.

**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 the ether-phospholipid metabolism was specified to the inhibition of the glycosomal alkyl-specific acyl-CoA acyltransferase [86], but also this pathway is probably not the primary target due to the high IC\(_{50}\) 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 the phospholipid metabolism and the parasite’s membrane composition: a decrease in phosphatidylcholine by inhibiting its synthesis through the cytidine 5’-diphosphocholine (CDP-choline) pathway, but an increase in phosphatidylethanolamine (PE) by stimulation of cytidine triphosphate:PE (CTP:PE) cytidylyltransferase 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 promastigotes [91] and intra-/extra-cellular 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-dependant matter 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 (scid) 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 upregulate 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 tumor 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 PI3K kinases (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 signaling 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 ED$_{50}$ values of miltefosine of 35.5 µM (14.5 µg/mL) and 47.0 µM (19.2 µg/mL), respectively [114], which was corroborated by limited life extension *in vivo* in mice [115]. Against all phenotypes of *Trypanosoma cruzi*, the South American *Trypanosoma* species and the etiologic agent of Chagas disease, the different reported ED$_{50}$, IC$_{50}$/72 h and IC$_{50}$/120 h values of miltefosine were 0.5 µM (0.2 µg/mL), 0.7 µM (0.3 µg/mL) and 1 µM (0.4 µg/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/kg [114] 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 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 µM (range 28-99 µM) [corresponding with 22 µg/mL (11-40 µg/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 µM (16 µg/mL) [121]. Both amoeba species displayed miltefosine-induced alterations of the membrane architecture [120,121]. The antiacanthamoebic activity of miltefosine was confirmed in a Syrian hamster model infected with *Acanthamoeba keratitis* in which topically applied miltefosine (160 µM [65 µg/mL], 28 days) resulted in complete cure of the infection in 85% of 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 µM (3.3 and 16.3 µg/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. 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* [126].

**Antibacterial activity**

Miltefosine has demonstrated significant bacteriocidal activity *in vitro* against pneumococcal bacteria. The determined minimal inhibitory concentration (MIC) ranged between 5 – 6.25 µM (2 – 2.5 µg/mL) for *Streptococcus pneumoniae* strains, and from 10 to 20 µM (4 to 8 µg/mL) for other pathogenic streptococci [128]. Against methicillin-resistant *Staphylococcus aureus* (MRSA) a higher MIC was demonstrated compared with susceptible *S. aureus*: 22 µM (9 µg/mL) versus 44 µM (18 µg/mL), respectively [129]. Miltefosine also had moderate activity against vancomycin-resistant *Enterococcus* (VRE) [MIC: 44 µM (18 µg/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*, and *Scedosporium apiospermum*, *Candida tropicalis*, *Aspergillus terreus* and *Candida parapsilosis* were 2 to 4 µg/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 µg/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 µg/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 µg/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 PI3K/Akt pathway is exploited by the HIV-1 virus to establish the maintenance of a cytoprotective effect of HIV-1 infection and thereby a prolonged 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 µg/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 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 noncatalytic 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 amastigotes 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 ATP-binding cassette (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) not only 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 were 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 neighboring 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-responding 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 gastro-intestinal tract and its potential teratogenicity as shown in pre-clinical reproductive animal studies. 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 Table 2 and 3), and were also the primary observed and most severe side effects in the large phase 4 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)
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 (Table 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- and aspartate-aminotransferase (ALT and 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**

Pre-clinical reproductive toxicity studies in animals showed both embryo- and foetotoxicity (in rabbits and rats) and teratogenic effects (in rats) of miltefosine at a lowest observed adverse effect level (LOAEL) 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**

Pre-clinical 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 cytochrome P450 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 (HAART) in VL patients co-infected 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

Visceral leishmaniasis

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 might be noted that almost all clinical trials of miltefosine for VL are performed in a single area of VL endemcity, 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. in Northern Ethiopia, a study by Rahman et al. in Bangladesh, and a phase II trial in adults and children in Brazil sponsored by the AB foundation, which was terminated early [157–159]. This is important, because besides specific species-related variation in the therapeutic response of VL, also geographical variation has been described [5]. Various clinical trials of miltefosine are currently ongoing in Kenya and Sudan [60], and also in Bangladesh and Nepal [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 a great consensus about the urgency
of using combination regimens for visceral leishmaniasis [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 the 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 levels 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 levels. To date, two studies, both in India, have been completed for combination miltefosine therapies. 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-visceral leishmaniasis 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 failures at end of treatment (18% vs. 2%, respectively) and at 6 months follow-up (25% vs. 11%, respectively) (also see Table 2). However, overall, miltefosine resulted in a lower mortality than sodium stibogluconate (6% vs. 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 co-infected 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 2. Efficacy and tolerance of oral miltefosine in trials with visceral leishmaniasis patients and HIV-visceral leishmaniasis co-infected patients.

<table>
<thead>
<tr>
<th>Author &amp; reference</th>
<th>Country</th>
<th>Study design</th>
<th>Patients enrolled</th>
<th>Treatments studied</th>
<th>Definite cure (95% CI)</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Miltefosine monotherapy for visceral leishmaniasis</strong></td>
<td></td>
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<tr>
<td>Sundar et al. [31]</td>
<td>India</td>
<td>Phase I, randomised, dose-finding</td>
<td>30 adults&lt;sup&gt;b&lt;/sup&gt;; 5 in each arm</td>
<td>28 days of: (1) 50 mg MLF q.o.d.; (2) 100 mg MLF q.o.d.; (3) 100 mg/day MLF; (4) 150 mg/day MLF; (5) 200 mg/day MLF; (6) 250 mg/day MLF</td>
<td>(1) 40%; (2) 20%; (3) 100%; (4) 80%; (5) 100%; (6) 80%</td>
<td>Dose limiting GI toxicity in arms (5) and (6)</td>
</tr>
<tr>
<td>Jha et al. [76]</td>
<td>India</td>
<td>Phase II, randomised, open-label</td>
<td>120 adults&lt;sup&gt;b&lt;/sup&gt;; 30 in each arm</td>
<td>(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</td>
<td>(1) 93% (78-99); (2) 93% (78-99); (3) 97% (83-100); (4) 97% (85-100)</td>
<td>Frequent GI toxicity in all arms</td>
</tr>
<tr>
<td>Sundar et al. [177]</td>
<td>India</td>
<td>Phase II, randomised, open-label</td>
<td>45 adults&lt;sup&gt;b&lt;/sup&gt;; (1) 17; (2) 18; (3) 10</td>
<td>28 days of: (1) 100 mg/day MLF; (2) 150 mg/day MLF; (3) 200 mg/day MLF</td>
<td>(1) 94% (71-100); (2) 100% (85-100); (3) 100% (74-100)</td>
<td>Overall: 5 CTC-3 GI toxicities; 1 CTC-3 hepatotoxicity; 1 CTC-3 nephrotoxicity</td>
</tr>
<tr>
<td>Sundar et al. [178]</td>
<td>India</td>
<td>Phase II, randomised, open-label</td>
<td>54 adults&lt;sup&gt;b&lt;/sup&gt;; 18 in each arm</td>
<td>100 mg/day MLF for: (1) 14 days; (2) 21 days; (3) 28 days</td>
<td>(1) 89% (65-99); (2) 100% (85-100); (3) 100% (85-100)</td>
<td>Moderate GI toxicity (week 1-2): (1) 67%; (2) 78%; (3) 61%</td>
</tr>
<tr>
<td>Sundar et al. [179]</td>
<td>India</td>
<td>Phase III, randomised, open-label, comparative</td>
<td>398 adults&lt;sup&gt;b&lt;/sup&gt;; (1) 299; (2) 99</td>
<td>(1) 28 days of 50 mg/day (&lt;25kg) or 100 mg/day (&gt;25kg) MLF; (2) 15x infusions of 1 mg/kg i.v. AMB q.o.d</td>
<td>(1) 94% (91-97); (2) 97% (91-99)</td>
<td>(1) Mild GI toxicity: vomiting 36% &amp; diarrhoea 20%; AST ↑ 17%; (2) Mild GI toxicity: vomiting 20% &amp; diarrhoea 6%; ALT ↑ 18%; Creat ↑ 35%</td>
</tr>
<tr>
<td>Sundar et al. [180]</td>
<td>India</td>
<td>Phase III, randomised, open-label</td>
<td>39 children&lt;sup&gt;b&lt;/sup&gt;; (1) 21; (2) 18</td>
<td>28 days of: (1) 1.5 mg/kg/day MLF; (2) 2.5 mg/kg/day MLF</td>
<td>(1) 90% (73-NR); (2) 83% (62-NR)</td>
<td>Mild-moderate GI toxicity for (1)/(2): vomiting 33%/39% &amp; diarrhoea 5%/17%</td>
</tr>
<tr>
<td>Bhattacharya et al. [75]</td>
<td>India</td>
<td>Phase III, open-label</td>
<td>80 children&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 days of 2.5 mg/kg/day</td>
<td>94% (87-NR)</td>
<td>Mild-moderate GI toxicity: vomiting 26% &amp; diarrhoea 25%; AST ↑ 35%</td>
</tr>
<tr>
<td>Singh et al. [181]</td>
<td>India</td>
<td>Randomised, open-label</td>
<td>125 children ≤14 yrs: (1) 44; (2) 20; (3) 38; (4) 23</td>
<td>(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) 15x infusions 1 mg/kg i.v. AMB q.o.d, pre-treated; (4) 15x infusions of 1 mg/kg i.v. AMB q.o.d, not pre-treated</td>
<td>(1) 93%; (2) 95%; (3) 92%; (4) 91%</td>
<td>For (1) &amp; (2): mild-moderate GI toxicity (vomiting 36% &amp; diarrhoea 41%); AST ↑ 48%; ALT ↑ 11%; BUN ↑ 13%. For (3) &amp; (4): AST ↑ 36%; ALT ↑ 53%; BUN ↑ 70%</td>
</tr>
<tr>
<td>Bhattacharya et al. [153]</td>
<td>India</td>
<td>Phase IV, open-label</td>
<td>(1) 704 adults&lt;sup&gt;b&lt;/sup&gt;; (477 males) and (2) 428 children&lt;sup&gt;c&lt;/sup&gt;; (247 males)</td>
<td>28 days of: (1) 50 mg/day (&lt;25kg) or 100 mg/day (&gt;25kg) MLF; (2) 2.5 mg/kg/day MLF</td>
<td>High % LTFU → PP: (1) 96.6%; (2) 93.6%. (ITT: overall 81.9%)</td>
<td>Overall: GI toxicity 8% (week 1) &amp; 3% (week 4); ALT CTC-1-3 31%; creat CTC-1-14%</td>
</tr>
<tr>
<td>Rahman et al. [158]</td>
<td>Bangladesh</td>
<td>Phase IV, open-label</td>
<td>977 adults&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 days of 2.5 mg/kg/day MLF</td>
<td>High % LTFU → PP: 85% (ITT: 72%)</td>
<td>Vomiting 25% &amp; diarrhoea 8% (mainly CTC-1/CTC-2); no liver/renal functions determined</td>
</tr>
<tr>
<td>Sundar et al. [151]</td>
<td>India</td>
<td>Open-label, noncomparative</td>
<td>567 adults &amp; children ≤12 yrs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 days of 2.5 mg/kg/day (&lt;12 yrs), 50 mg/day (&gt;25kg) MLF</td>
<td>90.3% (88-94)</td>
<td>Vomiting 65% &amp; diarrhoea 7% (mainly CTC-1/CTC-2); 1 patient died after GI intolerance: ALT CTC-1 23%; AST CTC-1 57.3%; Creat CTC-1 29.2%</td>
</tr>
</tbody>
</table>
### Table 2. Efficacy and tolerance of oral miltefosine in trials with visceral leishmaniasis patients and HIV-visceral leishmaniasis co-infected patients.

<table>
<thead>
<tr>
<th>Author &amp; reference</th>
<th>Country</th>
<th>Study design</th>
<th>Patients enrolled</th>
<th>Treatments studied</th>
<th>Definite cure (95% CI)</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Miltefosine in combination therapy for visceral leishmaniasis</strong></td>
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<tr>
<td>Sundar <em>et al</em>. [182]</td>
<td>India</td>
<td>Phase II, randomised, noncomparative, group-sequential</td>
<td>226 adults&lt;sup&gt;a&lt;/sup&gt;: 45 in each arm, except (2) which had 46</td>
<td>(1) 1x infusion of 5 mg/kg i.v. L-AMB; (2) 1x infusion of 5 mg/kg i.v. L-AMB + 10 days of 100 mg/day MLF; (3) 1x infusion of 5 mg/kg i.v. L-AMB + 14 days of 100 mg/day MLF; (4) 1x infusion of 3.75 mg/kg i.v. L-AMB + 14 days of 100 mg/day MLF; (5) 1x infusion of 3.75 mg/kg i.v. L-AMB + 7 days of 100 mg/day MLF</td>
<td>(1) 91% (78-97); (2) 98% (87-100); (3) 96% (84-99); (4) 96% (84-99); (5) 98% (87-100)</td>
<td>Mild-moderate GI toxicity in week 1: (1) 18%; (2) 37%; (3) 27%; (4) 24%; (5) 7%. ↑ALT (grade 1) in week 1: ~30% in all arms.</td>
</tr>
<tr>
<td>Sundar <em>et al</em>. [20]</td>
<td>India</td>
<td>Randomised, open-label, parallel-group, non-inferiority</td>
<td>634 children &amp; adults&lt;sup&gt;b&lt;/sup&gt;: (1) 157, (2) 160, (3) 158, (4) 159; of which between 36%-45% was ≤18 yrs</td>
<td>(1) 15x infusions of 1 mg/kg i.v. AMB q.o.d; (2) 1x infusion of 5 mg/kg i.v. L-AMB + 7 days of: 50 mg/day MLF (≤25kg) or 100 mg/day MLF (&gt;25kg) for adults or 2.5 mg/kg/day MLF for children; (3) 1x infusion of 5 mg/kg i.v. L-AMB + 10x injections of 11 mg/kg i.m. PM base q.d.; (4) 10 days of MLF (for dosage see (2)) + 10x injections of 11 mg/kg i.m. PM base q.d.</td>
<td>(1) 93% (88-96); (2) 98% (93-99); (3) 98% (93-99); (4) 99% (95-100)</td>
<td>Most patients with AEs in (1): 91% vs. 45%-52% in other arms; vomiting: (1) 19%, (2) 16%, (3) 3%, (4) 10%; diarrhoea: (1) 2%, (2) 2%, (3) - , (4) 3%; chills: (1) 72%, (2) 13%, (3) 13%, (4) - ; ↑Creat: (1) 10%, (2) - , (3) 4%, (4) 4%</td>
</tr>
</tbody>
</table>

| **Miltefosine monotherapy for HIV-visceral leishmaniasis co-infection** |
| Ritmeijer *et al*. [157] | Ethiopia | Randomised, open-label, comparative | 580 adults: 290 in each arm (all >25 kg), of which HIV+: (1) 33%, (2) 24%; HIV unknown: (1) 33%, (2) 36% | (1) 28 days of 100 mg/day MLF; (2) 30 injections of 20 mg/kg i.m. SSG q.d. | At EOT: overall: (1) 88% (84-92), (2) 88% (83-91). At 6 months FU: overall: (1) 60% (54-66), (2) 65% (59-71); in HIV+: (1) 46% (NR), (2) 57% (NR). Relapse rate at 6 months FU: overall: (1) 10%, (2) 2%; in HIV+: (1) 25%, (2) 11% | Vomiting: (1) 55%, (2) 32% diarrhea: (1) 51%, (2) 53%; bleeding: (1) 22%, (2) 22% overall mortality: (1) 6%, (2) 12%. |

<sup>a</sup> Determined 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.

<sup>b</sup> In these studies, ‘adult’ was defined as ≥12 years of age and ‘child’ as <12 years of age.

Abbreviations: ALT = alanine aminotransferase; AMB = amphotericin B deoxycholate; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CI = confidence interval; CTC-1/2 = Common Toxicity Criteria – grade 1/2; EOT = end of treatment; FU = follow-up; i.m. = intramuscular; i.v. = intravenous; L-AMB = liposomal amphotericin B; LTFU: lost to follow-up; MLF = miltefosine; NR = not reported; PM = paromomycin; PP = per-protocol; q.d. = daily; q.o.d. = every other day; SSG = sodium stibogluconate
Cutaneous leishmaniasis

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 cutaneous leishmaniasis is intricate and complex for various reasons. First, the geographical variation in the efficacy and the additional variation in the susceptibility of the \textit{Leishmania} (sub)species is even higher for CL than for VL. \textit{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 one observational study, 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 \textit{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 to 60 amphotericin B infusions and miltefosine might be the treatment of choice for this difficult-to-treat destructive cutaneous disease. 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). In Iran, miltefosine was demonstrated to be a good alternative to meglumine antimoniate for the treatment of \textit{L. major} infections, which was confirmed in an observational study with \textit{L. major} infections originating from Afghanistan (Table 3).
Table 3. Efficacy and tolerance of oral miltefosine in trials with cutaneous and mucocutaneous leishmaniasis patients

<table>
<thead>
<tr>
<th>Author &amp; reference</th>
<th>Country</th>
<th>Study design</th>
<th>Patients enrolled</th>
<th>Causing species</th>
<th>Treatments studied</th>
<th>Definite cure (99% CI)*</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Miltefosine monotherapy for New World cutaneous leishmaniasis</strong></td>
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<tr>
<td>Soto et al. [183]</td>
<td>Colombia</td>
<td>Phase II/III, open-label, increasing dose, historic control</td>
<td>72 adults (1) 16; (2) 19; (3) 17; (4) 20</td>
<td>L. amazonensis and L. panamensis</td>
<td>(1) 20 days of 50 mg/day MLF; (2) 7 days of 30 mg/day MLF; (3) 13 days of 100 mg/day MLF; (4) 7 days of 100 mg/day MLF + 15 days of 150 mg/day MLF; (5) 28 days of 150 mg/day MLF</td>
<td>(1) 50% (2) 63% (3) 82% (4) 80%</td>
<td>Overall: &quot;motional sickness&quot; 40%, increasing with MLF dose; vomiting or diarrhoea 21%; ↑AST/ALT: (1) 38%, (2) 42%, (3) 30%, (4) 20%</td>
</tr>
<tr>
<td>Soto et al. [184]</td>
<td>Colombia &amp; Guatemala</td>
<td>Randomised, placebo-controlled, double-blind</td>
<td>Colombia: 73 adults: (1) 49; (2) 24</td>
<td>L. panamensis (presumably)</td>
<td>28 days of: (1) 100 mg/day (&lt;45 kg) or 150 mg/day (&gt;45 kg); (2) oral placebo capsules</td>
<td>(1) 82% (2) 38%</td>
<td>Colombia &amp; Guatemala together: ↑Creat CTCA (1) 33%, (2) 9%; nausea (1) 36%, (2) 9%; vomiting: (1) 31%, (2) 3%; diarrhoea: (1) 16%, (2) 2%; ↑AST (1) 8%, (2) 38%</td>
</tr>
<tr>
<td>Soto et al. [164]</td>
<td>Bolivia</td>
<td>Randomised, open-label</td>
<td>62 adults: (1) 46; (2) 18</td>
<td>L. braziliensis</td>
<td>(1) 28 days of -2.5 mg/kg/day MLF; (2) 20 injections of 20 mg Sb/kg i.m. MA q.d.</td>
<td>(1) 80% (2) 83%</td>
<td>GI toxicity: (1) 61%; (2) NR; arthralgias/ local pain: (1) NR; (2) 72%</td>
</tr>
<tr>
<td>Machado et al. [186]</td>
<td>Brazil</td>
<td>Randomised, open-label</td>
<td>90 adults and children: (1) 60 (2 children); (2) 30 (10 children)</td>
<td>L. braziliensis</td>
<td>(1) 28 days of 50 mg/day (&lt;29 kg) or 100 mg/day (≥29 kg) or 150 mg/day (≥45 kg); (2) 20 injections of 20 mg Sb/kg i.m. MA q.d.</td>
<td>(1) 75% (2) 53%</td>
<td>Vomiting: (1) 42%, (2) 3%; nausea: (1) 40%, (2) 26%; diarrhoea: (1) 10%, (2) 13%; arthralgias: (1) 0%, (2) 21%; malaises: (1) 0%, (2) 21%</td>
</tr>
<tr>
<td>Cherukolo-Talbert et al. [187]</td>
<td>Brazil</td>
<td>Phase II/III, randomised, open-label</td>
<td>90 adults and children: (1) 60 (2 children); (2) 30 (10 children)</td>
<td>L. guyanensis</td>
<td>(1) 28 days of 30 mg/day (&lt;45 kg) or 100 mg/day (≥45 kg); (2) 20 injections of 20 mg Sb/kg i.m. MA q.d.</td>
<td>(1) 67% (2) 53% (54-72)</td>
<td>Vomiting: (1) 48%, (2) NR; diarrhoea: (1) 21%, (2) NR; nausea: (1) 9%, (2) NR; arthralgias: (1) NR, (2) 33%</td>
</tr>
<tr>
<td>Rubiano et al. [188]</td>
<td>Colombia</td>
<td>Randomised, open-label, non-intervention</td>
<td>116 children: (1) 98; (2) 18</td>
<td>Mixed (L. panamensis, L. guyanensis, L. braziliensis)</td>
<td>(1) 28 days of 12.5-2.5 mg/kg/day MLF; (2) 20 injections of 20 mg Sb/kg i.m. MA q.d.</td>
<td>(1) 83% (2) 69% (55-83)</td>
<td>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%; crCTCA (1) 11%, (2) 23%</td>
</tr>
</tbody>
</table>

**Miltefosine monotherapy for Old World cutaneous leishmaniasis**

<table>
<thead>
<tr>
<th>Author &amp; reference</th>
<th>Country</th>
<th>Study design</th>
<th>Patients enrolled</th>
<th>Causing species</th>
<th>Treatments studied</th>
<th>Definite cure (99% CI)*</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohebali et al. [189]</td>
<td>Iran</td>
<td>Randomised, open-label, comparative</td>
<td>63 adults (1) 32; (2) 31</td>
<td>L. major</td>
<td>(1) 28 days of -2.5 mg/kg/day MLF; (2) 14 injections of 20 mg Sb/kg i.m. MA q.d.</td>
<td>(1) 81% (2) 81%*</td>
<td>Week 1: Nausea: (1) 4%; (2) -; vomiting: 28%; (2) 0%; diarrhoea: (1) 4%, (2) 3%; local pain: (1) 0%, (2) 10%</td>
</tr>
<tr>
<td>Van Thiel et al. [74]</td>
<td>Afghanistan</td>
<td>Observational, open-label</td>
<td>34 adults, of which 31 were pre-treated with l. Sb</td>
<td>L. major</td>
<td>28 days of 150 mg/day MLF</td>
<td>88%</td>
<td>Reduced ejaculate volume: 64%; complete absence of ejaculate: 6%; nausea: 76%; vomiting: 56%</td>
</tr>
</tbody>
</table>

**Miltefosine monotherapy for New World mucocutaneous leishmaniasis**

<table>
<thead>
<tr>
<th>Author &amp; reference</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Soto et al. [190]</td>
<td>Bolivia</td>
<td>Randomised, equivalence</td>
<td>97 adults (1) 78; (2) 19</td>
<td>L. braziliensis</td>
<td>(1) 28 days of 25-3.3 mg/kg/day MLF; (2) 48-60 injections of 1 mg/kg i.v. AMB q.d.</td>
<td>(1) Mild disease: 83%, extensive disease: 38%; (2) Mild disease: 44%, extensive disease: 60%*</td>
<td>For arm (2) toxicity was NR. For arm (1): vomiting: 10%; diarrhoea: 3%; ↑AST: 4%; ↑ALT: 1%; ↑SUG: 8%</td>
</tr>
<tr>
<td>Soto et al. [191]</td>
<td>Bolivia</td>
<td>Observational, open-label</td>
<td>42 adults (1) 21 (failed 4 weeks of MLF); (2) 21 (not pre-treated)</td>
<td>NR, presumably L. braziliensis</td>
<td>(1) Secondary Rx, 6 weeks of -2.5 mg/kg MLF; (2) Primary Rx, 6 weeks of -2.5 mg/kg MLF</td>
<td>(1) 68% (2) 75%*</td>
<td>Overall: Mild GI toxicity: CTCA 174% and CTCA 5%; no liver or kidney toxicity</td>
</tr>
</tbody>
</table>

* Determined 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.
* In these studies, "adult" was defined as ≥12 years of age and "child" as <12 years of age.
* Determined at 3 months FU, rates were similar at 6 months FU.
* Determined at 12 months FU.
* Based on a per-protocol analysis, determined at 12 months FU.
Abbreviations: ALT = alanine aminotransferase; AMB = amphotericin B deoxycholate; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CTC = confidence interval; Creat = creatinine; CTCA = Common Toxicity Criteria – grade 1/2; FU = follow-up; i.l. = intralesional; i.m. = intramuscular; i.v. = intravenous; MA = meglumine antimoniate; MLF = miltefosine; NR = not reported; q.d. = daily; Rx = treatment; SSG = sodium stibogluconate.
**Topical miltefosine for cutaneous leishmaniasis**

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 clinical trials (further unreported). Both a trial in Syria (16 patients) and in Colombia (19 patients) apparently did not demonstrate efficacy of the Miltex® formulation against CL [172]. It remains unknown whether these contradictory clinical observations are due to a lack of drug penetration in the lesion, an 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 utilised 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 Kalaazar 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 co-infected 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 lifespan 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 leishmaniases. Further research and development is therefore required to further optimise 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).

Conclusive 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 refutably 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 co-infections 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 co-infected patients. As a well-tolerable and oral drug, miltefosine might play a fundamental role in the management of these patients, although clarification on the exact conditions for its use and possible complications, such as drug-drug interactions, needs to be prioritized.
References


Chapter 1.1

Miltosine: a review of its pharmacology and therapeutic efficacy

24:546–554.


211.


125. Sinkala E, Katabulushi M, Sianongo S, Obwaller A, Kelly P. In a trial of the use of miltefosine to treat HIV-related cryptosporidiosis in Zambian adults, extreme metabolic disturbances contribute to high


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...
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González U, Pinart M, Reveiz L, et al.


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