CHAPTER 3

From Bench to Bedside: Development and Optimization of Clinical Therapies for Visceral Leishmaniasis

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3.1 Introduction

Despite a considerable effort and investment in leishmaniasis drug discovery in the past several years, treatments for visceral leishmaniasis (VL) still rely on a few drugs that have limitations such as parenteral administration, poor tolerability and toxicity, long treatment duration and high cost. Given the scarcity of new compounds in the clinical pipeline in the last decade, efforts have been focussed on optimizing current available treatments according to the context of VL in different regions. In Southeast Asia, where pentavalent antimonials can no longer be used due to drug resistance, a single-dose regimen of liposomal amphotericin B and a combination regimen such as miltefosine–paromomycin for 10 days have proven to be

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highly efficacious and safe and are currently used on a routine basis. In Eastern Africa, the combination of sodium stibogluconate (SSG) with paromomycin for 17 days is recommended as first-line treatment; whilst in Brazil, where nearly 90% of VL cases from the Americas are reported, meglumine antimoniate given for 20 to 30 days is still used as first-line therapy, followed by treatment with liposomal amphotericin B over 7 days.

Despite improvements in current regimens through development of safer formulations or combinations, new oral treatments that are efficacious, safe, of short duration and easily implementable in remote areas where VL occurs are still badly needed. Unfortunately, the path that a new compound has to follow from early discovery to registration is costly and takes several years, and attrition rates due to safety issues or poor efficacy are high. In the era of new classes of anti-leishmanial compounds, there is a need to consider more systematic use of pharmacokinetic–pharmacodynamic (PK–PD) modelling and simulation for translation from preclinical discovery to early clinical development, and subsequently to further fine-tune and optimize the dosing regimen in later clinical development.

Hereafter, a summary of the *in vitro* and *in vivo* strategies usually used for VL in the early preclinical development stage to provide an indication of the susceptibility, target exposure and therapeutic index to support the transition from preclinical to clinical development and first-in-human testing is presented; followed by the example of miltefosine, the only oral drug currently available: its historical path through clinical development and post-registration, and the efforts to optimize its use for the treatment of VL using novel translational tools.

3.2 The Role of Preclinical Models in Preclinical to Clinical Translation

3.2.1 Strategies for *in vitro* Screening

Most current strategies for preclinical development assess ''cidal'' activity against *Leishmania. In vitro* assays can be categorized, depending on the stage (promastigotes, axenic amastigotes or intracellular amastigotes) of the parasite. However, whole-cell-based assays are generally considered the gold standard, as the intramacrophagic amastigote is the clinically relevant form of the parasite. A range of protocols has been developed by several groups, using slightly different conditions to measure parasite viability remaining after a defined exposure period to the drugs. Classical methods to detect parasites, such as Giemsa staining and microscopic counting or the use of the tetrazolium dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)[1] to assess viability are still in use, but development of phenotypic screening assays based on image analysis—known as high-content screening—has been a breakthrough in the recent years.[2,3] Laboratory strains of *Leishmania donovani* and *Leishmania infantum* are typically used here and, while promastigotes or axenic amastigotes are grown in culture medium,

activity against intracellular amastigotes is determined in macrophages, the natural host cells targeted by *Leishmania.* Macrophages are either derived from mouse peritoneum[4,5] or bone-marrow, or are differentiated by chemical agents such as phorbol 12-myristate 13-acetate from mouse (*e.g.* J-774[6]) or human (THP-1) cell lines. New compounds are selected based on the 50% and 90% inhibitory concentrations (IC_{50} and IC_{90}), *i.e.* the concentrations of drugs that decrease the cell growth of a fixed inoculum of parasites by 50% and 90%, respectively, as compared with the negative control.

In vitro promastigote or axenic amastigote assays allow a fast and straightforward screening of large compound collections, as they are compatible with a relatively large-scale format and require minimal amounts of compounds. However, several laboratories have shown that they result in a relatively high rate of false positives and may not identify intracellular-stage-specific compounds.[7–9] This could tentatively be explained by the physiological differences between the two types of assays, including pH, media composition and incubation times, but also by differences in protein expression in relation to[7] parasite growth and duplication. Promastigotes are fast growing while intramacrophageal amastigotes are only slowly replicating. Also, in intracellular assays, drugs have to penetrate the parasitophorous vacuole, a macrophage sub-cellular compartment in which parasites survive and that is acidic and rich in microbicidal peptides and hydrolytic enzymes.[10] Lastly, in a panel of macrophages from different origins, the anti-leishmanial activity of various drugs, including amphotericin B deoxycholate, paromomycin and miltefosine was demonstrated to be host-cell-dependent.[11]

3.2.2 Preclinical Animal Models for Visceral Leishmaniasis

Animal models of VL have largely contributed to a better understanding of the pathophysiological processes underlying infection and host response to the disease. They also have played a paramount role in the selection of preclinical candidates, based on reduction of macrophageal parasite burden in the liver and/or spleen.

3.2.2.1 Murine Models

Experimental challenge murine models have been extensively used for the study of immunopathology[12,13] and genetic regulation[14] and for assessing the potential efficacy of new chemical entities (NCEs) against VL.[6,15] These models are produced by inoculation with either *L. donovani* or *L. infantum.* Murine models are highly susceptible to a variety of factors, including mouse strain, age and immunity status, virulence, infectivity and parasite stage, number of inoculated parasites and route of inoculation.[16] An important issue with murine models is that they do not directly translate to the human situation, because of the capacity of the murine immune system to reduce parasitic load.

3.2.2.2 *Syrian Hamster Model*

The hamster model is also used to assess activity of compounds against leishmaniasis; these animals exhibit severe clinical symptoms that are similar to those observed in naturally infected dogs and humans.[17] In this species, clinical symptoms of VL can range from mild to progressive fatal visceral disease.[18] Hamsters are usually infected by intracardiac or intraperitoneal inoculation of *L. donovani* or *L. infantum*, which progressively induces hepatosplenomegaly, anaemia, cachexia and immunodepression.[19] The hamster model is most frequently favoured, not only because disease evolution is close to the clinical situation in humans, but also because assessing drug effects on the liver, spleen and bone marrow may provide precious information for predicting efficacy in the clinic. However, the benefits of the model need to be balanced by the unusual pharmacokinetics of many compounds in hamsters.

3.2.3 Issues in Interpretation of Preclinical Models

Determination of *in vitro* cidal effects is essential for selection of the most promising new agents. However, parasite expression, host response and clinical manifestations vary considerably by endemic region and species, ranging from asymptomatic to life-threatening VL. While the use of laboratory *Leishmania* strains has the merit of helping standardize the assays and, as a result, ranking cidal activities of new agents, one should bear in mind that these strains may not adequately represent the diversity of parasite, virulence, infectivity and pathogenicity observed in clinical conditions. Therefore, the cidal activity may not translate to efficacy in clinical trials. Implementing a panel of strains in the process of drug screening may be a useful approach to better characterizing variability in drug activity.

Characterization of efficacy of the most interesting candidates in murine or hamster models is also a key step for the translation of potency in *in vitro* assays to potential efficacy in the host. In this simple, linear progression strategy, the BALB/c mouse and hamster models are considered to be acute and chronic models of VL respectively.[20] However, no preclinical model fully captures the complexity of disease states in human, and the models described above all have drawbacks. In addition, it is also sometimes challenging to make cross-study comparisons because of differences in experimental conditions. It is therefore important that models are well characterized, established and harmonized, resulting in a stable tissue infection and confirm that positive benchmark controls show consistent and reproducible efficacy.

Data should in any case be interpreted carefully, and absorption, disposition, metabolism and elimination (ADME) properties of the compounds, which may vary considerably between species, should be taken into account. Although not systematically performed, blood or plasma PK assessments should be included or performed in parallel under the same conditions as

for *in vivo* efficacy, bearing in mind that inflammation and a variety of clinical manifestations may affect drug tissue penetration and distribution locally, as evidenced in brain tissue and cerebrospinal fluid in the presence of meningeal inflammation.[21]

3.2.4 How can Pre-clinical Data be Translated to Facilitate Clinical Development?

There is increasing attention being paid to pre-clinical to clinical translational science within the drug discovery and development paradigm for infectious diseases, and investments are being made to improve the understanding of PK–PD relationships in animal models to facilitate clinical development and to better understand the gaps between animal models and clinical application in humans.

For antibiotics, potency is generally defined by the minimum inhibitory concentration (MIC), which is the lowest concentration that completely inhibits the *in vitro* growth of a microorganism. Despite the fact that MIC is a good descriptor of a drug's antibiotic potential, it provides no information about the time course of anti-microbial activity. Integrating potency with PK characteristics, it is possible to define PK–PD parameters that describe antimicrobial effects in a dynamic way. Based on PK–PD profile, antibiotics can be roughly grouped into three categories. For drugs such as aminoglycosides the maximum plasma concentration (C_{max}) in relation to the MIC appears to be important for its effect (concentration-dependence, defined by the peak concentration to MIC ratio). The higher the drug concentration, the greater the bactericidal effect. Others, like penicillins or cephalosporins, require plasma levels to be above the MIC for a certain period of time during the dosing interval (time-dependence). While yet others, like tetracyclines or vancomycin, exhibit persistent effects for efficacy both related to time and concentration, which means total drug exposure is important. Defining the ideal dosing regimen for appropriate efficacy therefore tends to maximize drug concentrations in plasma in the first case, the duration of exposure in the second and the amount of drug in the latter case. PK–PD tools and strategies have been developed[22–24] and are routinely used in antibiotic development to assess preclinical and clinical PK targets, to predict clinical efficacy based on preclinical data, and to optimize dosing regimens in clinical trials.

In the case of drug development for VL, there is now a lot of interest and investment in research to develop PK–PD tools to facilitate the translation of new compounds to clinical development. Nevertheless, most drugs currently in use were developed several decades ago, when these tools were clearly not available. The two latest drugs registered for VL, miltefosine and paromomycin, in 2002 and 2006 respectively, had been initially developed for other indications and pragmatically adapted for leishmaniasis. The use of PK–PD tools in clinical trials using the currently available drugs provides an opportunity to better characterize exposure–response relationships in

different patient populations, and allows the use of state-of-the-art modelling and simulation to further optimize therapies. In order to illustrate this concept, we have chosen the case of the development of miltefosine for VL.

3.3 Challenges and Opportunities to Optimize Therapies for Leishmaniasis: The Case of Miltefosine

3.3.1 Discovery and Development History of Miltefosine for Leishmaniasis

The discovery of the anti-parasitic activity of alkylphosphocholine compounds, including miltefosine, was to a large extent serendipitous. A range of platelet-aggregating-factor analogues was initially synthesized in an effort to screen for anti-inflammatory properties, while at the same time their anti-tumour activity was screened in Germany as inhibitors of the membrane signalling pathway PI3K–Akt. In the 1980s it was discovered that miltefosine, one of these novel phospholipid compounds, was highly active against trypanosomatid parasites, and in 1987 Croft and colleagues reported its activity against *L. donovani* in intracellular amastigotes and *in vivo* after subcutaneous injection in a mouse infection model.[25]

In the pharmaceutical and clinical development of miltefosine, priority was given to its application in the treatment of cancer, particularly solid tumours. The various phase I and II studies that followed for oral miltefosine in severely ill cancer patients revealed good oral bioavailability of the compound in humans with a simple oral capsulated formulation. However, the high dose (150 $mg\,day^{-1}$ and higher) and prolonged treatment courses needed to reach the systemic exposure expected to result in clinical efficacy in oncological patients were associated with dose-limiting gastrointestinal toxicity, such as nausea, vomiting and loss of appetite.[26–29] This led in the mid-1990s to the abandonment and discontinuation of development of oral miltefosine's anti-cancer application.[30]

The activity against VL was confirmed more or less concurrently in an oral miltefosine study in a BALB/c mouse model by Kuhlencord *et al.*, which showed excellent activity of 20 $mg\,kg^{-1}\,day^{-1}$ oral miltefosine for 5 days and superiority over the standard drug sodium stibogluconate.[31] Based on the high oral bioavailability that was demonstrated in the phase I and II studies in cancer patients and the urgent need to have the first oral treatment for VL, a clinical development programme was pursued at Asta Medica. Considering the high number of VL patients reported in the Indian subcontinent (India, Bangladesh and Nepal), an agreement was reached in 1995 between Asta Medica (later the spin-off company Zentaris), the Special Programme for Research and Training in Tropical Diseases of the World Health Organization (TDR–WHO), the Indian government and various Indian academic collaborators. Given the available safety database from the Phase I and II

studies in cancer patients, which contained tolerability data for over 200 oncological patients, healthy volunteer studies were therefore not performed within the clinical VL development programme. Clinical phase II studies started in adult VL patients,[32,33] including a dose-finding study, with initial dose regimens largely based on the 'flat' 150 mg day^{-1} dosages that were deemed maximally tolerable in terms of gastrointestinal toxicity directly taken from the initial oncological studies.

3.3.2 Changing Patterns in Clinical Efficacy

3.3.2.1 Clinical Development (Phase II–III Studies)

The clinical development of miltefosine started in India in the late 1990s and already in 2002 a large amount of data had been generated in five clinical trials in adult patients (over 12 years of age).[32–36] Gastrointestinal toxicity appeared to be much less severe in Indian VL patients than initially described in cancer patients, which, for instance, made lead-in dosing unnecessary.[30,33] Among the phase II trials in adults, different treatment regimens were tested (ranging from 50 to 200 mg day^{-1} for 14–42 days), and, in general, the daily dose of 100 mg day^{-1} for 21–28 days gave satisfactory efficacy (above 90%) and was well tolerated. In the randomized open-label phase III clinical trial the efficacy and safety of miltefosine (100 mg day^{-1} orally for 28 days) was compared with amphotericin B deoxycholate (1 mg kg^{-1} day^{-1} for 14 days) in adult VL patients.[35] The efficacy of miltefosine in this phase III trial was highly satisfactory: 98% cure rate at the end of therapy (2% of patients did not have parasitological assessment done, but were clinically well) and 94% cure rate (282 out of 299, 95% confidence interval (CI) 91–97%) at the end of the six-months follow-up by intention-to-treat (ITT) analysis. The actual relapse rate at six-months was 3% (9 out of 299 patients), whereas another 3% of the patients were lost to follow-up and defined as failures in the ITT analysis.

Miltefosine was registered in India in 2002 for VL indication for patients aged two years or older on the basis of published data for adults and further studies in children which were published later.[35] The dose was defined as a linear regimen of 2.5 mg kg^{-1} day^{-1} for 28 days. In practice, considering the capsule strength of 50 mg, adult patients with weights of over 25 kg received a 100 mg daily dose, and adults with weights of 25 kg or less received a 50 mg daily dose for 28 days, whereas children were dosed on the basis of 2.5 mg kg^{-1} day^{-1} that could be adjusted using the 10 mg capsule, when available. Due to this practical limitation, the actual dose administered ranged from 2 to 4 mg kg^{-1} day^{-1}.

Having an oral drug available for the treatment of anthroponotic VL, together with the ability to identify VL patients following an easy and reliable diagnostic algorithm based on clinical findings (fever for more than two weeks and presence of splenomegaly), and a user-friendly, field-adapted rapid diagnostic test (rK39), prompted the launch in 2005 of the Kala-azar

Elimination Initiative by India, Bangladesh and Nepal. Apart from case management, other pillars of the elimination programme were vector control, surveillance, social mobilization and operational research, aiming to reduce VL incidence to 1 case per 10 000 population at district or sub-district level by 2015 (subsequently changed to 2020).[37]

3.3.2.2 Post-registration Studies

Further studies were performed in India, Bangladesh and Nepal over the following years. A phase IV study performed in India (2007) aimed to investigate the feasibility of miltefosine treatment in real-life settings.[38] A total of 1132 VL patients (38% under 12 years of age, and 62% aged 12 years or over) were enrolled in the study, which was implemented as out-patient care in 13 centres in Bihar. Treatment was monitored through weekly visits to the clinic and compliance was assessed. Patients were counselled and clearly instructed about the nature of expected side effects, especially gastrointestinal reactions. The efficacy observed at six-months follow-up was 81.9% by ITT analysis (927 out of 1132), with 14.2% (161 patients) of failures which were lost to follow-up at the six-months visit. Among the patients who completed the study, the final cure rate was 95.5% (927 out of 971), with a significantly higher relapse rate observed in children (23 out of 358, 6.4%) as compared to adults (21 out of 613, 3.4%; $p = 0.03$).[38]

In 2012, a new study reported the evolution of miltefosine efficacy in India after a decade of use.[39] A total of 567 VL patients [135 (24%) under 12 years of age, and 432 (76%) aged 12 years and over] were treated in a hospital setting, with an efficacy at the end of six-months follow-up of 90.3% (512 out of 567). The reasons for failure in the ITT analysis were nine patients who had the treatment discontinued due to adverse events, four who withdrew consent, 39 cases of relapse (6.8%) and five deaths (including one patient with treatment discontinued due to adverse events and one case of relapse). This was a significant decline in efficacy from 94% in 2002 to 90% in 2012 ($p = 0.04$), with a twofold increase in relapse rate from 3% to 6.8% ($p = 0.02$). The reasons for failure were unlikely to be related to lack of compliance, as treatment was directly observed in this trial. However, in the field, as miltefosine was available in the market at an unaffordable price for the local impoverished population (US$ 145 for a 28-days treatment), practices of incomplete treatment, availability over the counter without proper prescription and non-compliance to the full treatment duration together with the long half-life of miltefosine raised important concerns about the risk of resistance development.[40] Resistance development had already compromised the use of antimonials in the region, now miltefosine, after less than a decade of use, was at risk if no strict measures to control access through a public distribution system and proper use were implemented.[40]

In Nepal, miltefosine treatment has been introduced as part of the regional elimination initiative, replacing antimonials, to which resistance had been clearly documented. From 2009 to 2011, a prospective cohort of 120 VL

patients treated with miltefosine (as per recommendations in the guidelines) was followed up for 12 months after the end of treatment. Efficacy was assessed at the end of treatment, after 6- and 12-months follow-up, with cure rates of 95.8% (115 out of 120), 82.5% (99 out of 120) and 73.3% (88 out of 120), respectively. Failure attributed to relapses accounted for 10.8% at the six-months follow-up and 20% at the 12-months follow-up; these were confirmed to be due to the same parasite through fingerprinting (no re-infection), and no significant change in parasite susceptibility was identified pre *vs*. post treatment.[41] Relapse was most common among children less than 12 years old [incidence risk ratio (IRR) = 2.43, 95% CI 1.09–5.42].

Subsequently, data from the cohort of patients from Nepal and India (a total of 1016 VL patients) were analysed to identify clinical and epidemiological risk factors for failure. Paediatric patients were confirmed to be at higher risk of failure, with a 2.5-fold higher risk for the age group 10–14 years (95% CI 1.37–4.5) and 3.2-fold higher risk for the age group 2–9 years old (95% CI 1.77–5.77); male patients had a twofold higher risk of failure (95% CI 1.27–3.61). Possible factors considered to be associated with this difference in outcome in children were differences in immune response and drug pharmacokinetics.[42]

One of the approaches to maintaining a longer life for this drug was combining it with other anti-leishmanial treatments. The rationale for combining drugs was to reduce treatment duration, improve the safety profile and increase compliance, while maintaining high efficacy and avoiding the risk of resistance development. Different regimens were tested, including combining oral miltefosine (100 mg for adults of 25 kg or heavier, 50 mg for adults of less than 25 kg, and 2.5 $mg\,kg^{-1}\,day^{-1}$ for children less than 12 years of age) with paromomycin IM injections (11 $mg\,kg^{-1}\,day^{-1}$ base) over 10 days, or combining liposomal amphotericin B (one single injection IV, 5 $mg\,kg^{-1}$) with miltefosine treatment (as previously described) for 7 days. Both miltefosine combination regimens have proven to be highly efficacious, with ITT cure rates at six-months follow-up of 97.5% (156 out of 160, 95% CI 93.3–99.2) for liposomal amphotericin B and miltefosine; and 98.7% (157 out of 159, 95% CI 95.1–99.8%) for miltefosine and paromomycin, which were non-inferior to the amphotericin B deoxycholate comparator arm with a cure rate of 93% (146 out of 157, 95% CI 87.5–96.3%).

As described in Chapter 1, the epidemiology of VL is different in the three continents where the disease is endemic. Therefore, data from South Asia cannot be extrapolated to Eastern Africa or Latin America.

In Eastern Africa, miltefosine treatment was first described in Ethiopia by Médecins Sans Frontières (MSF), in the context of routine practice in a population with a high prevalence of HIV co-infection (29%). Adult male VL patients were randomized to receive miltefosine (100 mg for 28 days) or the standard-of-care treatment of sodium stibogluconate (SSG, 20 $mg\,kg^{-1}\,day^{-1}$ for 30 days). The cure rate at six-months follow-up among non-HIV-co-infected patients was 75.6% for miltefosine (95% CI 67.3–82.7%) and 77.4% for the SSG treatment (95% CI 69.4–84.1%).

More recently, in a phase II randomized multicentre clinical trial conducted in Kenya and Sudan, two study arms contained miltefosine, either as monotherapy (2.5 mg kg^{-1} day^{-1}) for 28 days, or in a combination of liposomal amphotericin B (10 mg kg^{-1} single injection) with miltefosine (2.5 mg kg^{-1} day^{-1}) for 10 days. Efficacy at six-months follow-up was 72% (95% CI 60–85%) for miltefosine alone and 77% (95% CI 64–90%) for the liposomal amphotericin B and miltefosine combination. Although the study was not powered for sub-group analysis, there was significantly lower efficacy in children (less than 12 years of age) compared with adults in the monotherapy arm (59% *vs.* 86%, $p = 0.05$), and the same trend was observed in the liposomal amphotericin B and miltefosine combination arm (74% *vs.* 90%, $p = 0.159$).[43]

The differences in cure rate between Asia and Eastern Africa could be related to differences in parasite, host and/or drug exposure. The *L. donovani* population in Eastern Africa is genetically different from that in India.[44] While resistance mechanisms have been described *in vitro* for miltefosine,[45] there is nevertheless, to date, no evidence of widespread resistant strains that can be isolated from relapsed patients in the Indian subcontinent or Eastern Africa.[41,46] In previous studies, there was clearly a higher risk of relapse in children under 12 years of age in the two regions. Considering the differences in metabolism and drug clearance between age groups, it was imperative to examine the pharmacokinetics of miltefosine in order to provide insights into how miltefosine therapy might be optimized, especially for the paediatric population.

3.3.3 Dose Optimization of Miltefosine for Future Combination Strategies

During the initial phase of the clinical development of oral miltefosine for VL in India, only limited attention was given to the clinical pharmacokinetics of this drug: the various phase II dose-finding studies that were performed lacked extensive pharmacokinetic assessments, and only very sparse descriptive data were reported in the registration documents that were initially filed in India (2002) and Germany (2004). Overall, miltefosine pharmacokinetics are mainly characterized by a very slow initial, and even slower terminal, elimination; the conventional 2.5 mg kg^{-1} day^{-1} for 28 days dosing regimen thus leads to a continued accumulation of the drug during the treatment period (Figure 3.1).[47] This means that individual end-of-treatment concentrations generally correlate to the overall exposure during treatment, but at the same time plasma concentrations in the first part of the treatment remain low and possibly suboptimal.

Miltefosine concentrations were analysed for the first time in VL patients enrolled in an early phase II dose-finding clinical trial in adults[33] and in a paediatric clinical trial in India,[48] however these data were not published at the time. Median miltefosine concentration around the end of treatment for

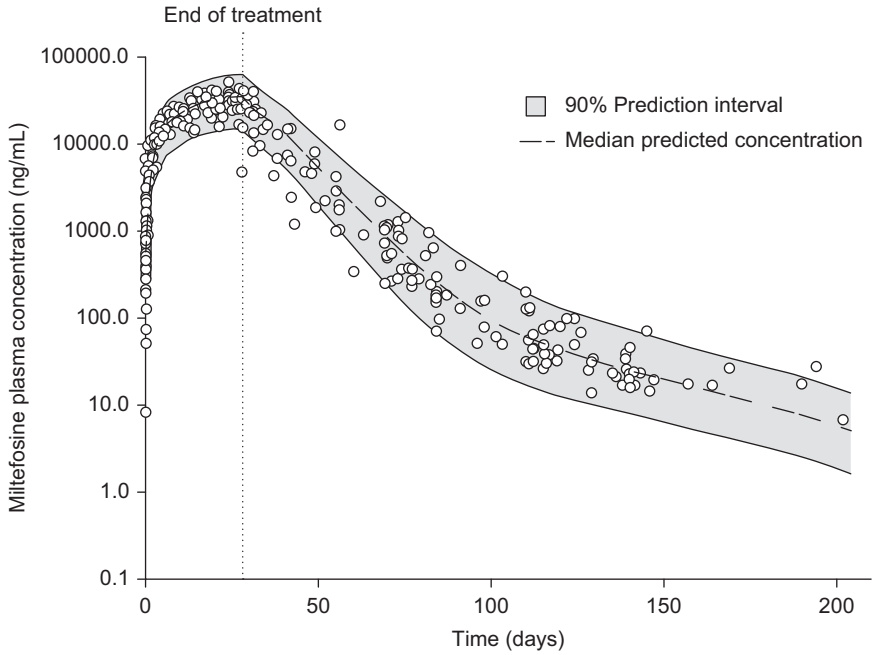

Figure 3.1 Visual predictive check of population pharmacokinetic model for miltefosine. Open circles represent observed data ($n = 382$) from 31 cutaneous leishmaniasis (*Leishmania major*) patients. All patients were treated with 150 mg day^{-1} miltefosine for 28 days. The grey area shows the 90% interval of the model predictions; the broken line indicates the median predicted concentrations.
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adult patients treated with 100 mg day^{-1} (median 2.9 mg kg^{-1} day^{-1}) for 28 days was 70 μg ml^{-1} (day 23), whereas for a group of children treated with a median daily dose of 2.6 mg kg^{-1} day^{-1} for 28 days this was 24 μg ml^{-1} (between day 26 and 28). After 14 days of treatment, the adult group of patients had already accumulated miltefosine plasma concentrations of between 24 and 69 μg ml^{-1}. This is consistent with clinical findings from another phase II trial, where treatments for 14, 21 and 28 days with 100 mg kg^{-1} day^{-1} resulted in an efficacy at six-months follow-up of 89% (95% CI 71–100%), 100%, and 100% (95% CI 85–100), indicating substantial efficacy with 14 days of treatment.[49] No pharmacokinetic evaluation has, to our knowledge, been performed to date in healthy individuals to investigate, in a controlled manner, the effect of body weight on the distribution and clearance of the drug, or absorption-related issues, such as the effect of food on the relative oral bioavailability of the compound.

Other studies confirmed the apparent differences in drug exposure between adults and children using the conventional 2.5 mg kg^{-1} day^{-1} dose,

with children generally defined as 12 years of age or less. A subset of patients of the Nepalese cohort study described above was included in a sparse pharmacokinetic assessment, which again demonstrated a pronounced difference in end-of-treatment concentrations between children and adults of approximately 30%.[50] Similar findings were observed in Eastern Africa, where patients with a lower body weight (less than 30 kg, mainly children between 7 and 12 years of age) were exposed to significantly lower levels of miltefosine than patients with a body weight of 30 kg or higher, both after 28 days of miltefosine monotherapy and after 10 days of miltefosine combination therapy, with average differences in end-of-treatment concentrations of 36% and 32%, respectively.[51]

Achieving adequate miltefosine drug exposure has nevertheless been shown to be important for VL, and various attempts have been made in the past five years to establish a PK–PD relationship, given the increased failure rates for this drug over time (see above). Treatment failure of miltefosine, in terms of relapse of disease within a 12-month follow-up period, was shown to be associated with lower drug exposure. The Nepalese cohort study mentioned earlier revealed that the probability of treatment failure was affected by the period of time that miltefosine concentrations where above ten times the *in vitro* IC_{50} susceptibility value of 17.9 $\mu g\,ml^{-1}$; the odds ratio for treatment failure decreased with increasing time-related drug exposure. As mentioned earlier, in previous studies Indian children barely reached this threshold value at day 28 of treatment, while Indian adults generally reached this value after just 14 days with 100 $mg\,kg^{-1}\,day^{-1}$.[52] Another exposure–response relationship was established for miltefosine in Eastern African VL patients, where miltefosine exposure time at levels greater than the IC_{90} inversely affected the relapse hazard and thus the time to relapse of infection.[53]

Many observations from various controlled and observational studies from both the Indian subcontinent and Eastern Africa indicate that children accumulate the drug to a lesser degree than adults under conventional 2.5 $mg\,kg^{-1}\,day^{-1}$ dosing, which is consistent with the clinical findings that children are more at risk of failing miltefosine treatment.[38,50,54] This, in combination with the established exposure–response relationship for miltefosine in VL, underlines the need to revise the miltefosine dosing guidelines for children.

From a mechanistic point of view, the lower miltefosine exposure in children can be explained by a standard physiological mechanism. It has long been recognized, over a wide range of animal species, that metabolic processes in the body are not linearly related to body mass or body size, but are related through a non-linear, so-called, allometric relationship between size and function.[55] From a PK perspective, this implies that clearance of drugs is not related to body weight linearly, but exponentially, with an allometric power-exponent of 3/4. This was also demonstrated for miltefosine when PK data from Indian and European patients with a wide distribution of body weights was combined; here, miltefosine clearance from

the central compartment of distribution was most accurately estimated when scaled allometrically, based on fat-free mass.[52] Miltefosine clearance is thus higher per kg of body weight in children compared with adults, which means that giving the same mg kg^{-1} day^{-1} dose will lead to lower accumulation of the drug in children during the treatment period. Given the importance of adequate miltefosine exposure in VL patients, as illustrated by the aforementioned exposure–response relationship for treatment failure, allometric dosing was therefore proposed to overcome the disparity in miltefosine drug exposure between paediatric and adult patients.[52] Although the reasons for treatment failure in VL are probably multifactorial, it is expected that allometric dosing, and thus increased exposure in children, will result in fewer paediatric VL relapses. The safety and clinical pharmacokinetics of the proposed allometric dosing regimen are currently being evaluated in paediatric VL patients in Kenya and Uganda (Clinicaltrials.gov NCT02431143) and in post-kala-azar dermal patients in Bangladesh (Clinicaltrials.gov NCT02193022).

The differences in PK profiles between adult VL patients in Asia and Africa appear to be significant. While not much has been published on the pharmacokinetics of miltefosine in Indian VL patients, the reported median concentration on day 23 of miltefosine treatment at 100 mg day^{-1} (median 2.9 mg kg^{-1} day^{-1}) over 28 days was 70 μg ml^{-1} in India. In Africa, adults treated with 2.5 mg kg^{-1} day^{-1} had a mean end of treatment concentration (day 28) of 31 μg ml^{-1}.[43] Initial results from a population PK analysis of the Eastern African data indicated that bioavailability is significantly reduced in the first week of treatment, which may lead to the much lower levels of exposure to miltefosine in African patients compared with Indian patients.[53] The mechanism behind the reduced bioavailability is not yet well-understood, but this unexpected observation of non-linearity highlights the difficulty of externally validating a finding when extrapolating clinical and PK observations between patient populations in distinct geographical regions.

3.3.4 Translational Approach to Manage Miltefosine's Main Safety Concern: Teratogenicity

Miltefosine treatment is generally well tolerated. The most common adverse events are related to gastrointestinal effects, with vomiting occurring in at least 20–30% of patients treated. The events are in general mild, occur mainly in the first week(s) of treatment, and less than 1% of the patients are expected to discontinue treatment due to intolerance.[38] Other common adverse events include mild increases in liver enzymes and creatinine.

The major safety concern for miltefosine is its teratogenic potential, which hampers its application in the clinic, particularly in rural areas. Women of reproductive age have been shown to make up 6.2–26.3% of the total VL population, depending on geographic area.[56] While there is no human data available that confirms teratogenicity, animal models have indicated embryo–foetal toxicity, including death and teratogenicity. Preclinical

reproductive toxicity studies in animals showed both embryotoxicity and fetotoxicity in rabbits and rats, while teratogenic effects were only demonstrated in rats at a lowest observed adverse effect level, which was lower than the recommended human dose.[57] Miltefosine is therefore contraindicated in pregnant women and contraceptive protection is strictly required in female patients of reproductive age. However, due to the extremely long elimination half-life of miltefosine (see above), making it detectable in the blood plasma of patients for as long as six months post-treatment, it remained unclear what would be an appropriate duration of contraception to avoid the risk of teratogenicity. To assess this, a translational animal-to-human PK modelling and simulation framework was designed to study and characterize the teratogenic risk, by translating the animal doses corresponding to the lowest observed adverse effect level in animals to human-equivalent doses, for which exposure was simulated in human female VL patients and compared with exposure after regular treatment.[58] This framework constituted a more rational teratogenic risk-management strategy and recommended increasing the current contraceptive cover periods to four months after the end of treatment for the standard 28-day miltefosine regimen, while for all shorter regimens (five, seven or ten days) two months may be considered adequate.

3.4 Final Remarks

This chapter has provided an overview of the different stages of progressing a new compound from early screening, through *in vivo* testing and potential translation, to clinical development, and the value of new PK–PD tools for supporting this process.

The case of miltefosine shows how PK–PD modelling and simulation can provide new insights into how to optimize currently available drugs, such as the use of allometric dosing of miltefosine to overcome the lower exposure in children and the practical example of the clinically recommended minimal duration of contraception for female patients of child-bearing age. The development of a gastro-resistant formulation may be explored for future optimization as a way to overcome the gastrointestinal effects of miltefosine. The new capsule may not only improve the tolerability of the treatment, but also potentially allow for improved regimens with loading dosing and shorter treatment duration.

These continuous improvements are necessary in the context of this single oral treatment that is available for VL, given the prospect that miltefosine may be the only option that can be considered in the near future for combination treatments with oral new chemical entities.

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References

1. P. Mukherjee, S. B. Majee, S. Ghosh and B. Hazra, *Int. J. Antimicrob. Agents*, 2009, **34**, 596.
2. J. L. Siqueira-Neto, S. Moon, J. Jang, G. Yang, C. Lee, H. K. Moon, E. Chatelain, A. Genovesio, J. Cechetto and L. H. Freitas-Junior, *PLoS Neglected Trop. Dis.*, 2012, **6**, e1671.
3. M. De Rycker, I. Hallyburton, J. Thomas, L. Campbell, S. Wyllie, D. Joshi, S. Cameron, I. H. Gilbert, P. G. Wyatt, J. A. Frearson, A. H. Fairlamb and D. W. Gray, *Antimicrob. Agents Chemother.*, 2013, **57**, 2913.
4. V. Yardley, F. Gamarro and S. L. Croft, *Antimicrob. Agents Chemother.*, 2010, **54**, 5356.
5. S. Shafi, F. Afrin, M. Islamuddin, G. Chouhan, I. Ali, F. Naaz, K. Sharma and M. S. Zaman, *Front. Microbiol.*, 2016, **7**, 1379.
6. S. Gupta, V. Yardley, P. Vishwakarma, R. Shivahare, B. Sharma, D. Launay, D. Martin and S. K. Puri, *J. Antimicrob. Chemother.*, 2015, **70**, 518.
7. G. De Muylder, K. K. H. Ang, S. Chen, M. R. Arkin, J. C. Engel and J. H. McKerrow, *PLoS Neglected Trop. Dis.*, 2011, **5**, e1253.
8. A. S. Nagle, S. Khare, A. B. Kumar, F. Supek, A. Buchynskyy, C. J. N. Mathison, N. K. Chennamaneni, N. Pendem, F. S. Buckner, M. H. Gelb and V. Molteni, *Chem. Rev.*, 2014, **114**, 11305.
9. A. Nühs, M. De Rycker, S. Manthri, E. Comer, C. A. Scherer, S. L. Schreiber, J. R. Ioset and D. W. Gray, *PLoS Neglected Trop. Dis.*, 2015, **9**, e0004094.
10. J. C. Antoine, E. Prina, T. Lang and N. Courret, *Trends Microbiol.*, 1998, **6**, 392.
11. K. Seifert, P. Escobar and S. L. Croft, *J. Antimicrob. Chemother.*, 2010, **65**, 508.
12. P. M. Kaye, M. Svensson, M. Ato, A. Maroof, R. Polley, S. Stager, S. Zubairi and C. R. Engwerda, *Immunol. Rev.*, 2004, **201**, 239.
13. C. R. Engwerda, M. Ato and P. M. Kaye, *Trends Parasitol.*, 2004, **20**, 524.
14. J. M. Blackwell, M. Fakiola, M. E. Ibrahim, S. E. Jamieson, S. B. Jeronimo, E. N. Miller, A. Mishra, H. S. Mohamed, C. S. Peacock, M. Raju, S. Sundar and M. E. Wilson, *Parasite Immunol.*, 2009, **31**, 254.
15. S. Patterson, S. Wyllie, L. Stojanovski, M. R. Perry, F. R. C. Simeons, S. Norval, M. Osuna-Cabello, M. De Rycker, K. D. Read and A. H. Fairlamb, *Antimicrob. Agents Chemother.*, 2013, **57**, 4699.
16. C. Loeuillet, A.-L. Bañuls and M. Hide, *Parasites Vectors*, 2016, **9**, 144.
17. M. Hommel, C. L. Jaffe, B. Travi and G. Milon, *Ann. Trop. Med. Parasitol.*, 1995, **89**(Suppl. 1), 55.
18. P. C. Melby, B. Chandrasekar, W. Zhao and J. E. Coe, *J. Immunol.*, 2001, **166**, 1912.
19. A. Nieto, G. Domínguez-Bernal, J. A. Orden, R. De La Fuente, N. Madrid-Elena and J. Carrión, *Vet. Res.*, 2011, **42**, 39.
20. S. Croft and V. Yadley, in *Handbook of Animal Models of Infection*, ed. O. Zak and M. A. Sande, Academic Press, 1999, pp. 783–787.

21. R. Nau, F. Sörgel and H. Eiffert, *Clin. Microbiol. Rev.*, 2010, **23**, 858.
22. T. Velkov, P. J. Bergen, J. Lora-Tamayo, C. B. Landersdorfer and J. Li, *Curr. Opin. Microbiol.*, 2013, **16**, 573.
23. G. L. Drusano, *Clin. Infect. Dis.*, 2007, **45**(Suppl. 1), S89.
24. E. Asin-Prieto, A. Rodriguez-Gascona and A. Isla, *J. Infect. Chemother.*, 2015, **21**, 319.
25. S. L. Croft, R. A. Neal, W. Pendergast and J. H. Chan, *Biochem. Pharmacol.*, 1987, **36**, 2633.
26. J. Verweij, A. Planting, M. van der Burg and G. Stoter, *J. Cancer Res. Clin. Oncol.*, 1992, **118**, 606.
27. J. Verweij, D. Gandia, A. S. Planting, G. Stoter and J. P. Armand, *Eur. J. Cancer*, 1993, **29A**, 778.
28. J. Verweij, K. Krzemieniecki, T. Kok, A. Poveda, C. van Pottelsberghe, M. van Glabbeke and H. Mouridsen, *Eur. J. Cancer*, 1993, **29A**, 208.
29. A. S. Planting, G. Stoter and J. Verweij, *Eur. J. Cancer*, 1993, **29A**, 518.
30. S. L. Croft and J. Engel, *Trans. R. Soc. Trop. Med. Hyg.*, 2006, **100**(Suppl. 1), S4.
31. A. Kuhlencord, T. Maniera, H. Eibl and C. Unger, *Antimicrob. Agents Chemother.*, 1992, **36**, 1630.
32. S. Sundar, F. Rosenkaimer, M. K. Makharia, A. K. Goyal, A. K. Mandal, A. Voss, P. Hilgard and H. W. Murray, *Lancet*, 1998, **352**, 1821.
33. T. K. Jha, S. Sundar, C. P. Thakur, P. Bachmann, J. Karbwang, C. Fischer, A. Voss and J. Berman, *N. Engl. J. Med.*, 1999, **341**, 1795.
34. S. Sundar, L. B. Gupta, M. K. Makharia, M. K. Singh, A. Voss, F. Rosenkaimer, J. Engel and H. W. Murray, *Ann. Trop. Med. Parasitol.*, 1999, **93**, 589.
35. S. Sundar, T. K. Jha, C. P. Thakur, J. Engel, H. Sindermann, C. Fischer, K. Junge, A. Bryceson and J. Berman, *N. Engl. J. Med.*, 2002, **347**, 1739.
36. S. Sundar, A. Makharia, D. K. More, G. Agrawal, A. Voss, C. Fischer, P. Bachmann and H. W. Murray, *Clin. Infect. Dis.*, 2000, **31**, 1110.
37. WHO, Regional Strategic Framework for Elimination of Kala-Azar From the South-East Asia Region (2005–2015). Regional Office for South-East Asia SEA-VBC-85 (Rev-1), New Delhi. World Health Organ, 2005.
38. S. K. Bhattacharya, P. K. Sinha, S. Sundar, C. P. Thakur, T. K. Jha, K. Pandey, V. R. Das, N. Kumar, C. Lal, N. Verma, V. P. Singh, A. Ranjan, R. B. Verma, G. Anders, H. Sindermann and N. K. Ganguly, *J. Infect. Dis.*, 2007, **196**, 591.
39. S. Sundar, A. Singh, M. Rai, V. K. Prajapati, A. K. Singh, B. Ostyn, M. Boelaert, J. C. Dujardin and J. Chakravarty, *Clin. Infect. Dis.*, 2012, **55**, 543.
40. S. Sundar and H. W. Murray, *Bull. W. H. O.*, 2005, **83**, 394.

41. S. Rijal, B. Ostyn, S. Uranw, K. Rai, N. R. Bhattarai, T. P. C. Dorlo, J. H. Beijnen, M. Vanaerschot, S. Decuypere, S. S. Dhakal, M. L. Das, P. Karki, R. Singh, M. Boelaert and J. C. Dujardin, *Clin. Infect. Dis.*, 2013, **56**, 1530.
42. B. Ostyn, E. Hasker, T. P. C. Dorlo, S. Rijal, S. Sundar, J. C. Dujardin and M. Boelaert, *PLoS One*, 2014, **9**, e100220.
43. M. Wasunna, S. Njenga, M. Balasegaram, N. Alexander, R. Omollo, T. Edwards, T. P. C. Dorlo, B. Musa, M. Hassan Sharaf Ali, M. Yasein Elamin, G. Kirigi, R. Juma, A. E. Kip, G. J. Schoone, A. Hailu, J. Olobo, S. Ellis, R. Kimutai, S. Wells, E. Awad Gasim Khalil, N. Strub Wourgaft, F. Alves and A. Musa, *PLoS Neglected Trop. Dis.*, 2016, **10**, e04880.
44. T. Gelanew, K. Kuhls, Z. Hurissa, T. Weldegebreal, W. Hailu, A. Kassahun, T. Abebe, A. Hailu and G. Schonian, *PLoS Neglected Trop. Dis.*, 2010, **4**, e889.
45. A. Mondelaers, M. P. Sanchez-ca, S. Hendrickx, E. Eberhardt, R. Garcia-Hernandez, L. Lachaud, J. Cotton, M. Sanders, B. Cuypers, H. Imamura, J. Dujardin, P. Delputte, P. Cos, G. Caljon, F. Gamarro, S. Castanys and L. Maes, *PLoS One*, 2016, **11**, e0154101.
46. V. K. Prajapati, S. Sharma, M. Rai, B. Ostyn, P. Salotra, M. Vanaerschot, J. C. Dujardin and S. Sundar, *Am. J. Trop. Med. Hyg.*, 2013, **89**, 750.
47. T. P. C. Dorlo, P. P. A. M. Van Thiel, A. D. R. Huitema, R. J. Keizer, H. J. C. De Vries, J. H. Beijnen and P. J. De Vries, *Antimicrob. Agents Chemother.*, 2008, **52**, 2855.
48. S. Sundar, T. K. Jha, H. Sindermann, K. Junge, P. Bachmann and J. Berman, *Pediatr. Infect. Dis. J.*, 2003, **22**, 434.
49. S. Sundar, A. Makharia, D. K. More, G. Agrawal, A. Voss, C. Fischer, P. Bachmann and H. W. Murray, *Clin. Infect. Dis.*, 2000, **31**, 1110.
50. T. P. C. Dorlo, S. Rijal, B. Ostyn, P. J. de Vries, R. Singh, N. Bhattarai, S. Uranw, J.-C. Dujardin, M. Boelaert, J. H. Beijnen and A. D. R. Huitema, *J. Infect. Dis.*, 2014, **210**, 146.
51. M. Wasunna, S. Njenga, M. Balasegaram, N. Alexander, R. Omollo, T. Edwards, T. P. C. Dorlo, B. Musa, M. H. S. Ali, M. Y. Elamin, G. Kirigi, R. Juma, A. E. Kip, G. J. Schoone, A. Hailu, J. Olobo, S. Ellis, R. Kimutai, S. Wells, E. A. G. Khalil, N. Strub Wourgaft, F. Alves and A. Musa, *PLoS Neglected Trop. Dis.*, 2016, **10**, e0004880.
52. T. P. C. Dorlo, A. D. R. Huitema, J. H. Beijnen and P. J. De Vries, *Antimicrob. Agents Chemother.*, 2012, **56**, 3864.
53. T. P. C. Dorlo, A. E. Kip, F. Alves, J. Alvar, A. Musa, E. Khalil, M. Wasunna and M. O. Karlsson, 2015, p. 24, Abstr 3567 [www.page-meeting.org/?abstract = 3567].
54. B. Ostyn, E. Hasker, T. P. C. Dorlo, S. Rijal, S. Sundar, J.-C. Dujardin and M. Boelaert, *PLoS One*, 2014, **9**, e100220.
55. B. J. Anderson and N. H. G. Holford, *Drug Metab. Pharmacokinet.*, 2009, **24**, 25.

56. M. O. Harhay, P. L. Olliaro, M. Vaillant, F. Chappuis, M. A. Lima, K. Ritmeijer, C. H. Costa, D. L. Costa, S. Rijal, S. Sundar and M. Balasegaram, *Am. J. Trop. Med. Hyg.*, 2011, **84**, 543.
57. H. Sindermann and J. Engel, *Trans. R. Soc. Trop. Med. Hyg.*, 2006, **100**(Suppl. 1), S17.
58. T. P. C. Dorlo, M. Balasegaram, M. A. Lima, P. J. de Vries, J. H. Beijnen and A. D. R. Huitema, *J. Antimicrob. Chemother.*, 2012, **67**, 1996.