Parasitology Research (2019) 118:2743–2752
https://doi.org/10.1007/s00436-019-06443-2

IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - REVIEW

# Need for sustainable approaches in antileishmanial drug discovery

Sarah Hendrickx[1] · G. Caljon[1] · L. Maes[1]

Received: 15 January 2019 / Accepted: 23 August 2019 / Published online: 31 August 2019


**Abstract**
Leishmaniasis is a neglected parasitic disease for which the current antileishmania therapeutics are hampered by drug toxicity, high cost, need for parenteral administration, increasing treatment failure rates, and emergence of drug resistance. The R&D pipeline had run fairly dry for several years, but fortunately some new drug candidates are now under (pre)clinical development. Identification of novel drugs will nevertheless remain essential to adequately sustain and improve effective disease control in the future. In this review, a package of standard and accessible R&D approaches is discussed with expansion to some alternative strategies focusing on parasite–host and vector–host interactions.

**Keywords** Leishmania · Drug evaluation · Vector and host interaction

## Leishmaniasis as a major health problem

Leishmaniasis is a neglected tropical disease (NTD) caused by the protozoan parasite *Leishmania* and spread by female sand flies of the genus *Phlebotomus* or *Lutzomyia*. With up to one million new cases reported annually, 12 million people being infected with about 25,000 deaths each year, and with over 350 million people worldwide considered at risk, the World Health Organization has classified leishmaniasis as a Category I (emerging or uncontrolled) disease (Charlton et al. 2018; WHO 2018). Its economic impact is estimated around 2.4 million disability adjusted life years (DALY's), which in the field of parasitic diseases is only topped by malaria, schistosomiasis, and lymphatic filariasis (Bern et al. 2008; Davies et al. 2003). Depending on the species, different clinical manifestations may develop: (1) visceral leishmaniasis (VL) in which spleen, liver, and bone marrow become severely parasitized and is fatal if left untreated; (2) cutaneous leishmaniasis (CL) which generally results in self-limiting but disfiguring skin lesions; (3) mucocutaneous leishmaniasis (MCL) which is associated with destruction of the nasal septum, lips, and palate; and (4) post-Kala-azar dermal leishmaniasis (PKDL) which presents as a cutaneous sequela after resolution of VL (Burza et al. 2018). Although leishmaniasis is mainly affecting poor populations in developing countries, over the last years, it has become a more widely emerging problem (Ready 2014). One of the biggest challenges for VL control is the increasing prevalence of HIV/VL co-infections which do not only impact on the spread and severity of the infection but also on the increased incidence of side effects and relapses (Alvar et al. 2012; van Griensven et al. 2014).

## Current antileishmanial therapeutics

Trivalent antimonials ($Sb^{III}$) were already used in Brazil in 1912 as the first effective treatment (Vianna 1912), and it was the Indian Nobel Prize laureate Brahmchari who first synthesized ureum stibamine in 1922 (Brahmachari 1922). Pentavalent antimonials ($Sb^{V}$) were introduced to reduce serious side effects. In 1945, sodium stibogluconate became the treatment option of choice for VL and remained so for over 6 decades (Frezard et al. 2009). However, the widespread use of $Sb^{V}$ is hampered by toxicity and by the high incidence of drug resistance particularly in the Indian subcontinent (Chakravarty and Sundar 2010; Sundar and Chakravarty 2010). Since the first manifestations of Sb treatment failure in the 1950s, pentamidine, amphotericin B (AmB), and paromomycin (PMM) have been explored and used to combat VL, but all proved unsatisfactory in terms of either efficacy, cost, ease of

Section Editor: Marta Teixeira

✉ Sarah Hendrickx
sarah.hendrickx@uantwerpen.be

[1] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Antwerp, Belgium

administration, and/or safety (McGwire and Satoskar 2014; Murray 2010) (Table 1). Many aspects relating to chemical structure, putative drug targets, mode-of-action, and side effects of the current and various new classes (synthetic and natural) of antileishmanial agents have indeed been extensively investigated (reviewed by Kumar et al. 2018; Sangshetti et al. 2015). Even though miltefosine (MIL) conquered a steady place in regions of widespread Sb resistance, its application is now threatened by increasing treatment failure rates and the slow but inevitable emergence of drug resistance (Cojean et al. 2012; Hendrickx et al. 2014; Rijal et al. 2013). Large-scale implementation of liposomal AmB (L-AmB) was initially unaffordable, but negotiations between WHO and Gilead Sciences led in 2007 to a significant price reduction, allowing AmBisome® to become the formulation of first choice in Asia when a cold-chain can be ensured (Alves et al. 2018). There are some indications of clinical AmB resistance (Purkait et al. 2012), hence emphasizing the need for continued R&D investments for novel antileishmanials.

## Antileishmanial drug R&D—lessons from the past

Notwithstanding the worrying rate at which drug resistance has been emerging, antileishmanial R&D pipelines have run dry for a fairly long time and the development of novel drugs has been moving forward at a disturbingly slow pace because of the different clinical manifestations, interregional differences in drug efficacy, slow adjustments in national control/public-health policies, lack of adequate financing and of inter-laboratory harmonization, and technology transfer (Alves et al. 2018; Freitas-Junior et al. 2012; Hendrickx et al. 2016b; Kumar et al. 2018). As full understanding of the functional genetics of the parasite is still lagging, drug resistance mechanisms are largely unidentified as well. However, in-depth understanding of the mode-of-action and the underlying reasons for treatment failure and drug resistance is not only essential in assuring optimal application of current drugs but will definitely also steer innovative drug discovery (Durieu et al. 2016b).

### De novo drug discovery

Drug discovery programs are slow, expensive, and prone to considerable risks, particularly for NTDs because the chances for return-of-investment are extremely slim considering that the daily income of an average VL-patient is less than 2 USD (WHO 2002). Given the inherent risk of failure, the standard development cost of a new chemical entity (NCE) starting from de novo discovery is estimated at about 100–150 million € and takes 15 years on average (Avorn 2015; DNDi 2019). For NTDs, this is not economically viable without significant

**Table 1** Antileishmanial drugs and their drawbacks

| Therapy | | Administration | Dosing | Toxicity | Other drawbacks | Cost (USD) |
|---|---|---|---|---|---|---|
| Antimonials (Sb) | Sodium stibogluconate<br>Meglumine-antimoniate | IV or IM | 20 mg/kg/day 30 consecutive days | Abdominal pain, vomiting, diarrhea, nausea, headache, fatigue, fever, cough, rash, pancreatitis, pneumonia, liver failure, nephrotoxicity and cardiotoxicity | Hospitalization required<br>Primary resistance in some endemic regions | 50–70 |
| Amphotericin B (AmB) | | IV | 1 mg/kg (15 mg/kg total dose) 30 days | Nephrotoxicity | | ~ 100 |
| Pentamidine | | IV or IM | 2–4 mg/kg/day alternate days 3–5 weeks | Cardiotoxicity, hypotension and gastrointestinal side effects,<br>induction of insulin-dependent diabetes mellitus | Poor efficacy | ~ 46 |
| Paromomycin (PMM) | | IM or TOP | 15 mg/kg/day 21 consecutive days | Nephrotoxicity, ototoxicity, hepatotoxicity | | ~ 10 |
| Miltefosine (MIL) | | PO | 1.5–2.5 mg/day 28 consecutive days | Gastrointestinal toxicity, nephrotoxicity, hepatotoxicity, teratogenicity | Increasing incidence of treatment failure | ~ 70 |

cross-subsidy (Charlton et al. 2018) and engagements in interdisciplinary initiatives between the public and private sector have been taken to accommodate this particular need (Kumar et al. 2018), also involving attempts to repurpose approved drugs developed for other indications.

## Drug repurposing

Almost all antileishmania drugs that came to the market after the introduction of Sb's have been developed through drug repositioning or repurposing, an option that was relatively straightforward given the lack of knowledge on validated targets and the pressing medical need for new treatment options to replace Sb in areas were drug resistance was emerging. For example, MIL was originally developed as an anticancer drug, but also proved to have excellent oral efficacy against *Leishmania* (Croft and Engel 2006). Against a rapidly proliferating intracellular parasite, it seems logical that some enzymes targeted by *anticancer* therapies could be exploited (Klinkert and Heussler 2006; Uliana and Barcinski 2009). More recently, kinases are considered a major target in oncology which encouraged the recent repurposing strategy of a range of kinase inhibitors for leishmaniasis (Charlton et al. 2018; Durieu et al. 2016b).

Various *antimicrobial* drugs have been repurposed as well. The polyene antifungal amphotericin B (AmB) has high binding affinity to ergosterol, causing pore formation in the cellular membrane and subsequent cellular leakage (de Souza and Rodrigues 2009). Other antifungals such as fluconazole and terbinafine can be administered orally and were shown to be safe and effective for the treatment of CL caused by *L. major* (Alrajhi et al. 2002). The aminoglycoside antibiotic paromomycin (PMM), used to treat bacterial infections, cryptosporidiosis, and amoebiasis, demonstrated adequate activity against VL in monotherapy; however, it was never implemented in Asia as first-line intervention because of the earlier introduction of MIL and L-AmB (Alves et al. 2018).

Other antileishmanial candidates have been identified by focused screening of other *antiparasitics*. Out of a collection of 400 compounds from the Medicines for Malaria Venture, substituted 14-amino-benzimidazoles were shown to have in vitro antileishmanial potential with $IC_{50}$ values ranging from 61 to 134 nM (Khraiwesh et al. 2016) and for which in vivo evaluation is currently ongoing. While the pharmaceutical industry avoids nitro-aromatics for the risk of mutagenicity and carcinogenicity, some nitro-aromatics demonstrated promising potential in the treatment of NTDs (Patterson and Wyllie 2014). Fexinidazole, which is currently in clinical development for the treatment of African sleeping sickness and Chagas disease, proved inadequately effective against VL (ClinicalTrials.gov 2015) but was briefly considered in the Drugs for Neglected Diseases *initiative* (DND*i*) portfolio as a potential combination with MIL in Eastern Africa (DNDi 2016). Its potency against VL in combination with the antitubercular drug delamanid is under investigation (Patterson et al. 2013, 2016). Next to this list, antileishmanial potential has also been demonstrated for various *other drugs*, including immunomodulators, antihistamines, and central nervous system and cardiovascular drugs (extensively reviewed by Charlton et al. 2018; Kumar et al. 2018; Nagle et al. 2014; Sangshetti et al. 2015).

Drug repurposing gained particular interest to more rapidly accommodate the unmet medical need for new treatment options. With most of the toxicological and preclinical data already available, the risks and costs to reach regulatory approval upon initial proof-of-concept studies demonstrating antileishmanial clinical efficacy are significantly reduced (Nwaka and Hudson 2006). On the other hand, repositioning old drugs may trigger reduced interest into identifying actual molecular targets of existing drugs and of new drug targets in support of the discovery of NCEs (Charlton et al. 2018).

## Initiatives to boost R&D commitments for NTDs

In 2012, WHO and representatives of the pharmaceutical industry, politicians, and financial donors launched an ambitious roadmap to control or eliminate 10 NTDs by 2020 by taking a series of commitments to provide more drugs, research, and funds, better known as the London Declaration for Neglected Diseases (Johnston et al. 2014). In the meantime, billions have been donated by the pharmaceutical industry to treat NTDs worldwide. Regardless of these increased drug supplies and donation campaigns, only one (Guinea worm) of the ten diseases outlined in the Declaration came close to eradication (Galán-Puchades 2017), again strongly emphasizing the need for a continued R&D commitment for novel drugs (Cohen et al. 2016).

Over the last decades, DND*i* has greatly invested in new treatments for NTDs by first focusing on improving VL therapy using the combination of existing drugs to shorten treatment duration and/or improve efficacy and safety. The combination sodium-stibogluconate–PMM is currently in use in Africa, while the PMM–MIL combination therapy has conquered a place as second option of choice on the Asian market (Alves et al. 2018). DND*i* aims to proactively tackle the unmet needs in VL treatment by clearly defining the desired target product profile (TPP) that should be considered as a planning/outcome tool for any promising NCE lead compound. This TPP takes critical factors into account, such as clinical efficacy, mode of delivery, safety and tolerability, dosage form, treatment duration, stability, contraindications, and cost (Breder et al. 2017; Freitas-Junior et al. 2012; Wyatt et al. 2011). Upon the London declaration, millions of compounds have been screened by DND*i,* the Genomics Institute of the Novartis Research Foundation (GNF Novartis), GlaxoSmithKline (GSK), and Dundee University (Cohen

et al. 2016), from which six drug candidates from five different chemical classes were identified, all showing over 95% reduction in parasite burdens in animal models after a 10-day monotherapy. Among these, four are now at the preclinical stage, with two about to finish phase-I clinical evaluation, e.g., the nitroimidazole DND*i*-0690, oxaborole DND*i*-6148, aminopyrazole DND*i*-5561, proteasome inhibitor LXE408, GSK3494245/DDD1305143, and the CRK-12 kinase inhibitor GSK3186899/DDD853651. With four more candidates in the pipeline aiming to reach preclinical development in late 2018 and 2019, DND*i* and its partners have developed a rich portfolio of oral drugs that now can also be evaluated in combinations to avoid or at least delay emergence of drug resistance. A detailed overview of the discovery, current status, application potential, and clinical trials performed in South Asia, Africa, and Latin America has recently been published (Alves et al. 2018).

## Different approaches in drug discovery—bridging past and future

It is well-accepted that different approaches can be followed to identify starting points for drug discovery. Typically only those based on experimental screening approaches are mentioned, i.e., the molecular target approach (usually referred to as target-based) and the phenotypic approach (also called target-free), both having advantages and disadvantages (Freitas-Junior et al. 2012; Zulfiqar et al. 2017b). The more recently developed in silico approaches that exploit existing data to identify potential new drug-disease associations deserve to be mentioned as well (Cha et al. 2018).

### Phenotypic screening

In phenotypic screening, drugs are evaluated for their direct efficacy towards the pathogen generally without any prior knowledge on the molecular targets or pathways involved. Given the difficulties in identifying and validating druggable targets for *Leishmania,* phenotypic screening is still largely the preferred method. The standard in vitro assay for phenotypic evaluation was defined already in 1986 and included (1) amastigotes as target, (2) a dividing (intracellular) parasite population, (3) quantifiable and reproducible endpoints of activity, and (4) activity at concentrations achievable in serum/tissues (Croft 1986). Quite recently, the Global Health Innovative Technology (GHIT) Fund defined some additional "hit" selection standards: a 50% effective concentration ($EC_{50}$ value) cut-off value of $\leq$ 10 μM for a potential in vitro hit and > 70% reduction in liver parasite burdens in mouse or hamster models infected with *L. infantum* or *L. donovani* upon five 50 mg/kg oral doses once or twice daily (Alcantara et al. 2018). Despite repeated calls towards assay standardization (Croft 2001; Croft and Olliaro 2011; Croft et al. 2006), a wide variety of assay formats are still in use today (Hendrickx et al. 2016b; Hendrickx et al. 2018; Zulfiqar et al. 2017b) whereby it is important to emphasize that the lack in harmonization can severely impact on the obtained hit rates. While a correlation between promastigote and amastigote susceptibility has been indicated for some drugs (Kulshrestha et al. 2013), there is general consensus on the requirement of using intracellular amastigotes for in vitro drug screening/evaluation. For reasons of being much easier, cheaper, and quicker, several groups nevertheless still prefer the (extracellular) promastigote stage, although these are never exposed to drugs as they reside in the sand fly vector. Hence, it is not surprising that only 4% of the "hits" originating from promastigote screening do translate into anti-amastigote hits, whereas about 50% of the hits from the intracellular amastigote screen can be confirmed in promastigotes (Freitas-Junior et al. 2012; Siqueira-Neto et al. 2012). Likewise, a high false-positive hit rate was also observed using the axenic amastigote assay (De Rycker et al. 2013). Although the amastigote-infected macrophage assay is undoubtedly the standard, its hit rate may be affected by slow amastigote replication (dependent on the strain used), hence complicating the detection of static versus cidal activity potential (Tegazzini et al. 2016).

Considerable attention is currently put on high content combined with high-throughput screening (HCS/HTS) assays on intracellular amastigotes using automated image analysis. This approach combines the efficiency of HTS with multiparameter readout allowing phenotypic information on the whole cell (Freitas-Junior et al. 2012; Siqueira-Neto et al. 2012). Although millions of compounds have passed in HTS (Pena et al. 2015; Zulfiqar et al. 2017a), very few progressed towards further development. One example of success is the selective inhibitor of the kinetoplastid proteasome (GNF6702) discovered by Novartis demonstrating excellent activity potential against leishmaniasis, Chagas disease, and sleeping sickness (Khare et al. 2016). It can further be argued that the hit-to-lead process should be supported by systematic studies on a panel of recent clinical isolates, "time-to-kill" assays and studies providing initial information about the pharmacokinetic/pharmacodynamics (PK/PD) properties (Alcantara et al. 2018; Hendrickx et al. 2018). Moreover, HCS/HTS data analysis is complex because not only the direct impact on the parasite is assessed but also the off-target host cell toxicity together with insight into compound cell permeability and stability within the unique host-parasite microenvironment (Zulfiqar et al. 2017b).

### Target-based screening

In target-based drug discovery, compounds are screened against a defined target considered to be essential for parasite survival, generally a protein of functional relevance belonging

to a specific pathway. Unfortunately, target-based screening for *Leishmania* has been applied much less given the lack of fully validated targets. After confirmation of activity, compounds are optimized for cellular activity, enzyme/pathway activity, and selectivity. However, as *Leishmania* is an intracellular organism, potential hits will require indispensable physicochemical properties facilitating transport across host cell membranes, stability within the acidic parasitophorous vacuole of the macrophage host cell, and protection from xenobiotic host cell metabolism to prevent fast compound degradation (Zulfiqar et al. 2017b).

Several putative drug targets have been proposed and include proteins and enzymes involved in the sterol pathway, thiol pathway, hypusine pathway, glycolytic pathway, purine salvage pathway, polyamine pathway, or are protein kinases, dihydrofolate reductases, and topoisomerases that are different from the mammalian equivalent (Sangshetti et al. 2015; Zulfiqar et al. 2017b). Although inhibition of these targets on promastigote level may have a profound effect because of their rapid expansion and high metabolism, their effect on the slower dividing intracellular amastigote may be much less pronounced. In the last couple of decades, different genetic deletion techniques such as CRISPR/Cas9 have become available to more easily validate molecular targets (Jones et al. 2018a) and will hopefully facilitate and increase the number of suitable targets for entry into HTS campaigns.

### Virtual screening

In recent years, a huge variety of innovative computational methods has emerged, enabling systematic screens through in silico approaches (Cha et al. 2018). If the molecular drug target is known, in silico docking studies can be applied to accurately predict target-drug interactions via bioinformatics. This approach is particularly valuable as it may accelerate drug discovery by more rapidly predicting the most likely hits to enter HTS while saving experimental resources (Mesa et al. 2015). Moreover, computer-assisted drug design has emerged as a promising alternative in medicinal chemistry to facilitate the experimental design, discovery of new drugs, and evaluation of the structure–activity relationship (QSAR). By using topological and 3D descriptors, it is now possible to correlate biological activities with the chemical structure, enabling development of QSAR models that can immediately assess the interaction of secondary metabolites. Such in silico predictions provide valuable knowledge on the drug's mechanism-of-action and can help identifying pharmacophore groups to increase the biological activity of molecules (Herrera Acevedo et al. 2017). These recent "chemoinformatics" have contributed significantly to trypanosomiasis and leishmaniasis drug discovery (Ferreira and Andricopulo 2018).

## Alternative approaches in drug discovery—a glance at the future

Although *Leishmania* relies on specific virulence factors to initiate and sustain infection, it is also known for its great genome plasticity. By frequent genome modifications and amplifying chromosomes in response to environmental factors, the parasite becomes a real escape artist that is able to adapt to many situations, including drug exposure. The parasite can often easily withstand drug pressure by acquiring mutations in target genes and/or uptake systems, or by amplification of efflux pumps hereby sometimes also altering parasite fitness (Hendrickx et al. 2016a; Ouakad et al. 2011; Turner et al. 2015; Vanaerschot et al. 2011). This wide genome plasticity also implies that strategies directly targeting the parasite are likely more prone to select for resistance (Lamotte et al. 2017) and is a strong argument why some alternative drug discovery approaches should gain interest as well.

### Targeting parasite–host interactions

Instead of focusing on the direct antiparasitic effect of a compound, *Leishmania* viability may also be targeted indirectly via mechanisms of host-parasite interaction, an approach that has already been adopted for malaria (Langhorne and Duffy 2016). This approach may be very versatile, and various strategies of host-directed therapy have already been explored for leishmaniasis. Macrophages, the primary host cell type for the parasite, present a high degree of plasticity and undergo phenotypic changes in response to various environmental stimuli. For example, *Leishmania* infection can be affected by manipulating the host cell cholesterol content (Kumar et al. 2016; Roy et al. 2014). This way, simvastatin has been shown to enhance host protection against *L. major* in both BALB/c and C57BL/6 mice (Parihar et al. 2016). Impairment of the macrophage actin cytoskeleton is another way of reducing intracellular parasite loads (Roy et al. 2014). The host macrophage response can also be altered by combination of antileishmanial drugs with immunomodulators or the use of immunomodulators alone (Dalton and Kaye 2010). Several studies have focused on imiquimod, a toll-like receptor 7 agonist with beneficial effects in combination with antileishmanial reference drugs (Arevalo et al. 2001; El-On et al. 2007; Khalili et al. 2011). Some anti-inflammatory drugs, such as the TNF-$\alpha$ inhibitor pentoxyphylline, have also been investigated but failed to demonstrate any additional effects over Sb treatment alone (Brito et al. 2017). More recently, promising results were achieved in *L. donovani*-infected mice with leptin, an adipocyte-derived hormone capable of regulating the immune response (Maurya et al. 2016). Also in canine leishmaniasis, the use of immunomodulatory compounds already proved to be successful with a clear improvement of clinical signs and cellular immunity upon treatment

with the protein aggregate magnesium–ammonium phospholinoleate–palmitoleate anhydride P-MAPA (Santiago et al. 2013).

Another indirect antiparasitic strategy is by targeting parasite modulation of host cell signaling and transcription, thereby disrupting the conditions favorable for intracellular survival. As this tactic falls beyond the direct genetic control of the parasite, inhibitors of these pathways may indeed be more refractory to drug resistance (Lamotte et al. 2017). Increasing knowledge on the protein kinases released by the parasite in the modulation of host cell signaling underlines their potential as drug target. Next to targeting the typical parasite kinases, such as CRK3 (cyclin-dependent kinase) and casein kinase (LmCK1.2) (Jones et al. 2018b), more than 400 putative parasite ectokinases have been identified of which the majority is involved in various biochemical processes (glycolytic pathways, nucleotide synthesis), implying an important role in regulating host cell metabolism (Silverman et al. 2008; Silverman et al. 2010). One of these is the serine/threonine protein kinase CK1.2 with a likely impact on host cell signaling and metabolism to establish favorable conditions for intracellular survival (Durieu et al. 2016a; Knockaert et al. 2000; Rachidi et al. 2014). Targeting these parasite-released signaling factors could be an interesting approach for the development of novel antileishmania drugs, as their inhibition may affect downstream host cell immune evasion and be detrimental for parasite survival. As several kinase inhibitors already hold promise in the treatment of various human pathologies, including diabetes, cancer, and inflammation, well-characterized focused libraries are now available and will facilitate drug discovery efforts directed against *Leishmania*-released kinases. As some pathogens can alter gene expression levels via modulation of host cell DNA methylation hereby altering expression of genes involved in their clearance or in promoting their growth (Pacis et al. 2015), epigenetic signaling upon infection may be a second potential drug target. Little is yet known about the epigenetic impact of *Leishmania* infection on the host cell, with only one study showing epigenetic variations in macrophage DNA methylation upon *L. donovani* infection with interference of genes implicated in host cell antimicrobial defense (Marr et al. 2014). A third target under investigation is the extracellular matrix (ECM). During tissue invasion, the parasite interacts with the host epithelium through high affinity binding of a promastigote surface protein to the ECM (Pina-Vazquez et al. 2012). It is likely that the binding of ECM proteins to the cell surface simultaneously may cause degradation of the ECM and signal transduction within parasites, resulting in changes in gene expression facilitating parasite uptake and/or transformation (Kulkarni et al. 2008). More recent research is now assessing the impact ECM degradation on parasite virulence (Pina-Vazquez et al. 2012).

## Targeting vector–host interactions

Complementary to the more traditional approaches of vector control, interest in vector stages as potential targets for novel transmission-blocking strategies to control and eliminate NTDs has markedly increased (Goncalves and Hunziker 2016). Thus far, three main strategies have been applied: the use of gametocidal drugs to reduce transmission of *Plasmodium* to mosquitoes, the development of transmission-blocking vaccines, and vector control via paratransgenesis (Wilke and Marrelli 2015).

### Transmission blocking vaccines

Various sand fly salivary proteins have been characterized for their impact on the biology of the vector–host–parasite interaction, which encouraged investigating their potential application in transmission blocking approaches (Lestinova et al. 2017; Martin-Martin et al. 2018). Although the biology of sand fly blood-feeding is very complex, unraveling this process might enable its implementation in vaccination strategies. During feeding, *Leishmania* parasites are embedded in promastigote secretory gel (PSG) that is inoculated in the mammalian host together with sand fly saliva containing many pharmacologically active components, called sialogenins. These molecules have anti-hemostatic, anti-inflammatory, and immunomodulatory properties, helping to initiate infection and to successfully finish the blood meal (Abdeladhim et al. 2014). Also, the PSG plug is composed of a diverse group of molecules with pharmacological and immunomodulatory properties, known to be beneficial for the recruitment of macrophages and neutrophils, and enhancing lesion size and parasite burdens (Lestinova et al. 2017). These salivary components are also able to induce specific cellular and humoral immunity which confers protection against subsequent exposure (Andrade and Teixeira 2012; Gomes and Oliveira 2012; McDowell 2015). As cross reactivity is observed between phylogenetically related vector species having more conserved salivary proteins (Lestinova et al. 2017), the development of a saliva-based vaccine that is applicable in endemic foci could be a very attractive option.

### Paratransgenesis

Although currently still more theoretical, alternative strategies to block transmission include genetic control methods that either allow replacement of a vector population by a disease-refractory population or the release of vectors carrying a lethal gene to suppress target populations, another approach gaining momentum in the control of malaria, Chagas disease, dengue, or lymphatic filariasis is paratransgenesis in which genetically modified symbiotic bacteria are introduced to colonize the vector species that either cause pathogenic effects in the host,

interfere with the host's reproduction, reduce the vector competence, or interfere with parasite multiplication (Hurwitz et al. 2011a; Wilke and Marrelli 2015).

Exploration of the importance of the sand fly gut microbiome on the survival of *Leishmania* revealed that not only the gut microbiota influences parasite survival and behavior inside the vector but also triggers local immune responses in the host stimulating neutrophil infiltration and enhancing parasite visceralization. As such, antibiotic-mediated perturbation of the midgut microbiome significantly disturbed parasite growth and metacyclogenesis and reduced host infection (Dey et al. 2018; Kelly et al. 2017). Although the presence of gut microbes influences sand fly vector competence, the relationships between parasite and gut bacteria are considered competitive (Telleria et al. 2018). For example, prefeeding of colony-raised *L. longipalpis* with bacteria (*Asaia sp.*, *Ochrobactrum sp.*) and a yeast-like fungus (*Pseudozyma sp.*) that had been isolated from wild and laboratory-reared female *L. longipalpis* prohibited parasite establishment in the midgut (Sant'Anna et al. 2014). Moreover, antibiotic treatment of colonized *P. duboscqi* sand flies resulted in extremely transmissible infections, which was attributed to the bacterial establishment in the midgut creating favorable conditions for parasite survival and metacyclogenesis (Louradour et al. 2017). While *Bacillus megaterium* and *Brevibacterium linens* have been suggested as candidates to prevent transmission of *Leishmania* via paratransgenesis (Hillesland et al. 2008; Hurwitz et al. 2011b), the more recent detection of *Bacillus subtilis* in *P. perniciosus* and in at least two other sand fly species in the Mediterranean area supports the explorative paratransgenic or biological approaches to control of sand fly populations and reduce transmission (Fraihi et al. 2017). Given the complexity of the insect vector, the protozoan pathogen, and the microbiota relationship, the relevance of sand fly microbiota could possibly be promising for future exploitation although its practical implementation and actual impact on transmission in the field may remain fairly questionable.

## Conclusion

So far, the number of antileishmanial drugs reaching the market has remained rather limited and was mostly dependent on the repurposing of drugs approved for other indications. The increasing treatment failure rates and the emergence of drug resistance against most of current first-line therapies urge for the approval of novel drugs to treat and/or prevent leishmaniasis. Although phenotypic screening has long remained the method of choice to identify new compounds, the application of high-quality genetic tools and technologies now facilitates the identification of druggable targets that will enable target-based HTS and virtual screening of large compound libraries. Alternative approaches focusing on parasite–host or vector–host interactions are gaining momentum and may yield promise in the future. By indirectly targeting the parasite, these approaches could be applied for transmission blocking rather than only aiming at disease resolution. Anyhow, the road towards VL elimination in the near future may still be long and difficult.

**Funding information** This work was funded by the Research Fund Flanders (FWO: project G051812N, G013118N, and 12I0317N), and research funds of the University of Antwerp (TT-ZAPBOF 33049 and TOP-BOF 35017). LMPH is a partner of the Antwerp Drug Discovery Network (ADDN, www.addn.be) and the Excellence Centre "Infla-Med" (www.uantwerpen.be/infla-med).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Abdeladhim M, Kamhawi S, Valenzuela JG (2014) What's behind a sand fly bite? The profound effect of sand fly saliva on host hemostasis, inflammation and immunity. Infect Genet Evol 28:691–703. https://doi.org/10.1016/j.meegid.2014.07.028

Alcantara LM, Ferreira TCS, Gadelha FR, Miguel DC (2018) Challenges in drug discovery targeting TriTryp diseases with an emphasis on leishmaniasis. Int J Parasitol Drugs Drug Resist 8(3):430–439. https://doi.org/10.1016/j.ijpddr.2018.09.006

Alrajhi AA, Ibrahim EA, De Vol EB, Khairat M, Faris RM, Maguire JH (2002) Fluconazole for the treatment of cutaneous leishmaniasis caused by Leishmania major. N Engl J Med 346(12):891–895. https://doi.org/10.1056/NEJMoa011882

Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, Boer M, the WHO Leishmaniasis Control Team (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoSOne 7(5): e35671. https://doi.org/10.1371/journal.pone.0035671

Alves F, Bilbe G, Blesson S, Goyal V, Monnerat S, Mowbray C, Muthoni Ouattara G, Pécoul B, Rijal S, Rode J, Solomos A, Strub-Wourgaft N, Wasunna M, Wells S, Zijlstra EE, Arana B, Alvar J (2018) Recent development of visceral Leishmaniasis treatments: successes, pitfalls, and perspectives. Clin Microbiol Rev 31(4). https://doi.org/10.1128/cmr.00048-18

Andrade BB, Teixeira CR (2012) Biomarkers for exposure to sand flies bites as tools to aid control of leishmaniasis. Front Immunol 3:121. https://doi.org/10.3389/fimmu.2012.00121

Arevalo I, Ward B, Miller R, Meng TC, Najar E, Alvarez E, Matlashewski G, Llanos-Cuentas A (2001) Successful treatment of drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator. Clin Infect Dis 33(11):1847–1851. https://doi.org/10.1086/324161

Avorn J (2015) The $2.6 billion pill–methodologic and policy considerations. N Engl J Med 372(20):1877–1879. https://doi.org/10.1056/NEJMp1500848

Bern C, Maguire JH, Alvar J (2008) Complexities of assessing the disease burden attributable to leishmaniasis. PLoSNeglTropDis 2(10):e313. https://doi.org/10.1371/journal.pntd.0000313

Brahmachari U (1922) Chemotherapy of antimonial compounds in kala-azar infection. Part I. Indian J Med Res 10(2):492–522

Breder CD, Du W, Tyndall A (2017) What's the regulatory value of a target product profile? Trends Biotechnol 35(7):576–579. https://doi.org/10.1016/j.tibtech.2017.02.011

Brito G et al (2017) Oral Pentoxifylline associated with pentavalent antimony: a randomized trial for cutaneous Leishmaniasis. Am J Trop Med Hyg 96(5):1155–1159. https://doi.org/10.4269/ajtmh.16-0435

Burza S, Croft SL, Boelaert M (2018) Leishmaniasis. Lancet 392(10151): 951–970. https://doi.org/10.1016/s0140-6736(18)31204-2

Cha Y, Erez T, Reynolds IJ, Kumar D, Ross J, Koytiger G, Kusko R, Zeskind B, Risso S, Kagan E, Papapetropoulos S, Grossman I, Laifenfeld D (2018) Drug repurposing from the perspective of pharmaceutical companies. Br J Pharmacol 175(2):168–180. https://doi.org/10.1111/bph.13798

Chakravarty J, Sundar S (2010) Drug resistance in leishmaniasis. J Global Infect Dis 2(2):167–176. https://doi.org/10.4103/0974-777X.62887

Charlton RL, Rossi-Bergmann B, Denny PW, Steel PG (2018) Repurposing as a strategy for the discovery of new anti-leishmanials: the-state-of-the-art. Parasitology 145(2):219–236. https://doi.org/10.1017/s0031182017000993

ClinicalTrials.gov (2015) US National Library of medicine, 1997, NCT01980199. Trial to Determine Efficacy of Fexinidazole in Visceral Leihmaniasis Patients in Sudan. Registered Nov 2013, https://clinicaltrials.gov/ct2/show/NCT01980199. Accessed 6/6/2019

Cohen JP, Silva L, Cohen A, Awatin J, Sturgeon R (2016) Progress report on neglected tropical disease drug donation programs. Clin Ther 38(5):1193–1204. https://doi.org/10.1016/j.clinthera.2016.02.031

Cojean S et al (2012) Leishmania resistance to miltefosine associated with genetic marker. Emerg Infect Dis 18(4):704–706. https://doi.org/10.3201/eid1804.110841

Croft SL (1986) In vitro screens in the experimental chemotherapy of leishmaniasis and trypanosomiasis. Parasitol Today (Personal ed) 2(3):64–69

Croft SL (2001) Monitoring drug resistance in leishmaniasis. Tropical Med Int Health 6(11):899–905

Croft SL, Engel J (2006) Miltefosine–discovery of the antileishmanial activity of phospholipid derivatives. Trans R Soc Trop Med Hyg 100 Suppl 1:S4–S8. https://doi.org/10.1016/j.trstmh.2006.03.009

Croft SL, Olliaro P (2011) Leishmaniasis chemotherapy—challenges and opportunities. Clin Microbiol Infect 17(10):1478–1483. https://doi.org/10.1111/j.1469-0691.2011.03630.x

Croft SL, Sundar S, Fairlamb AH (2006) Drug resistance in leishmaniasis. Clin Microbiol Rev 19(1):111–126. https://doi.org/10.1128/CMR.19.1.111-126.2006

Dalton JE, Kaye PM (2010) Immunomodulators: use in combined therapy against leishmaniasis. Expert Rev Anti-Infect Ther 8(7):739–742. https://doi.org/10.1586/eri.10.64

Davies CR, Kaye P, Croft SL, Sundar S (2003) Leishmaniasis: new approaches to disease control. Bmj 326(7385):377–382

De Rycker M et al (2013) Comparison of a high-throughput high-content intracellular Leishmania donovani assay with an axenic amastigote assay. Antimicrob Agents Chemother 57(7):2913–2922. https://doi.org/10.1128/aac.02398-12

de Souza W, Rodrigues JCF (2009) Sterol biosynthesis pathway as target for anti-trypanosomatid drugs. Interdiscip Perspect Infect Dis 2009: 1–19. https://doi.org/10.1155/2009/642502

Dey R et al (2018) Gut microbes egested during bites of infected sand flies augment severity of Leishmaniasis via Inflammasome-derived IL-1beta. Cell Host Microbe 23(1):134–143.e6. https://doi.org/10.1016/j.chom.2017.12.002

DND*i* (2016) Fexinidazole/miltefosine combination (VL). http://www.dndi.org/diseases-projects/portfolio/fexinidazole-vl/. Accessed 6/6/2019

DNDi (2019) https://www.dndi.org/about-dndi/business-model/lessons-learned/. Accessed 6/6/2019

Durieu E et al (2016a) From drug screening to target deconvolution: a target-based drug discovery pipeline using Leishmania casein kinase 1 isoform 2 to identify compounds with Antileishmanial activity. Antimicrob Agents Chemother 60(5):2822–2833. https://doi.org/10.1128/aac.00021-16

Durieu E, Prina E, Leclercq O, Oumata N, Gaboriaud-Kolar N, Vougogiannopoulou K, Aulner N, Defontaine A, No JH, Ruchaud S, Skaltsounis AL, Galons H, Späth GF, Meijer L, Rachidi N (2016b) From drug screening to target deconvolution: a target-based drug discovery pipeline using Leishmania casein kinase 1 isoform 2 to identify compounds with Antileishmanial activity. Antimicrob Agents Chemother 60(5):2822–2833. https://doi.org/10.1128/aac.00021-16

El-On J, Bazarsky E, Sneir R (2007) Leishmania major: in vitro and in vivo anti-leishmanial activity of paromomycin ointment (Leshcutan) combined with the immunomodulator Imiquimod. Exp Parasitol 116(2):156–162. https://doi.org/10.1016/j.exppara.2006.12.004

Ferreira LLG, Andricopulo AD (2018) Chemoinformatics strategies for Leishmaniasis drug discovery. Front Pharmacol 9:1278–1278. https://doi.org/10.3389/fphar.2018.01278

Fraihi W et al (2017) An integrated overview of the midgut bacterial flora composition of Phlebotomus perniciosus, a vector of zoonotic visceral leishmaniasis in the Western Mediterranean Basin. PLoS Negl Trop Dis 11(3):e0005484. https://doi.org/10.1371/journal.pntd.0005484

Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL (2012) Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int J Parasitol Drugs Drug Resist 2:11–19. https://doi.org/10.1016/j.ijpddr.2012.01.003

Frezard F, Demicheli C, Ribeiro RR (2009) Pentavalent antimonials: new perspectives for old drugs. Molecules 14(7):2317–2336. https://doi.org/10.3390/molecules14072317

Galán-Puchades MT (2017) WHO delays Guinea-worm disease eradication to 2020: are dogs the sole culprits? Lancet Infect Dis 17(11): 1124–1125. https://doi.org/10.1016/S1473-3099(17)30565-0

Gomes R, Oliveira F (2012) The immune response to sand fly salivary proteins and its influence on leishmania immunity. Front Immunol 3:110. https://doi.org/10.3389/fimmu.2012.00110

Goncalves D, Hunziker P (2016) Transmission-blocking strategies: the roadmap from laboratory bench to the community. Malar J 15:95. https://doi.org/10.1186/s12936-016-1163-3

Hendrickx S et al (2014) Experimental selection of paromomycin and miltefosine resistance in intracellular amastigotes of Leishmania donovani and L. infantum. Parasitol Res 113(5):1875–1881. https://doi.org/10.1007/s00436-014-3835-7

Hendrickx S, Beyers J, Mondelaers A, Eberhardt E, Lachaud L, Delputte P, Cos P, Maes L (2016a) Evidence of a drug-specific impact of experimentally selected paromomycin and miltefosine resistance on parasite fitness in Leishmania infantum. J Antimicrob Chemother 71:1914–1921. https://doi.org/10.1093/jac/dkw096

Hendrickx S, Guerin PJ, Caljon G, Croft SL, Maes L (2016b) Evaluating drug resistance in visceral leishmaniasis: the challenges. Parasitology 1-11 doi:https://doi.org/10.1017/s0031182016002031

Hendrickx S, Maes L, Croft SL, Caljon G (2018) The challenges of effective leishmaniasis treatment. In: Sucre AP (ed) Drug Resistance in Leishmania Parasites. Springer: 193–206

Herrera Acevedo C, Scotti L, Feitosa Alves M, Formiga Melo Diniz MF, Scotti MT (2017) Computer-Aided Drug Design Using Sesquiterpene Lactones as Sources of New Structures with Potential Activity against Infectious Neglected Diseases. Molecules (Basel, Switzerland) 22(1) doi:https://doi.org/10.3390/molecules22010079

Hillesland H et al (2008) Identification of aerobic gut bacteria from the kala azar vector, Phlebotomus argentipes: a platform for potential

paratransgenic manipulation of sand flies. Am J Trop Med Hyg 79(6):881–886

Hurwitz I, Fieck A, Read A, Hillesland H, Klein N, Kang A, Durvasula R (2011a) Paratransgenic control of vector borne diseases. Int J Biol Sci 7(9):1334–1344

Hurwitz I, Hillesland H, Fieck A, Das P, Durvasula R (2011b) The paratransgenic sand fly: a platform for control of Leishmania transmission. Parasit Vectors 4:82. https://doi.org/10.1186/1756-3305-4-82

Johnston KL, Ford L, Taylor MJ (2014) Overcoming the challenges of drug discovery for neglected tropical diseases: the A.WOL experience. J Biomol Screen 19(3):335–343. https://doi.org/10.1177/1087057113511270

Jones NG, Catta-Preta CMC, Lima A, Mottram JC (2018a) Genetically validated drug targets in Leishmania: current knowledge and future prospects. ACS Infect Dis 4(4):467–477. https://doi.org/10.1021/acsinfecdis.7b00244

Jones NG, Catta-Preta CMC, Lima APCA, Mottram JC (2018b) Genetically validated drug targets in Leishmania: current knowledge and future prospects. ACS Infect Dis 4(4):467–477. https://doi.org/10.1021/acsinfecdis.7b00244

Kelly PH, et al. (2017) The gut microbiome of the vector Lutzomyia longipalpis is essential for survival of Leishmania infantum. mBio 8(1) doi:https://doi.org/10.1128/mBio.01121-16

Khalili G, Dobakhti F, Mahmoudzadeh-Niknam H, Khaze V, Partovi F (2011) Immunotherapy with Imiquimod increases the efficacy of Glucantime therapy of Leishmania major infection. Iran J Immunol 8(1):45–51

Khare S, Nagle AS, Biggart A, Lai YH, Liang F, Davis LC, Barnes SW, Mathison CJN, Myburgh E, Gao MY, Gillespie JR, Liu X, Tan JL, Stinson M, Rivera IC, Ballard J, Yeh V, Groessl T, Federe G, Koh HXY, Venable JD, Bursulaya B, Shapiro M, Mishra PK, Spraggon G, Brock A, Mottram JC, Buckner FS, Rao SPS, Wen BG, Walker JR, Tuntland T, Molteni V, Glynne RJ, Supek F (2016) Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. Nature 537(7619):229–233. https://doi.org/10.1038/nature19339

Khraiwesh M, Leed S, Roncal N, Johnson J, Sciotti R, Smith P, Read L, Paris R, Hudson T, Hickman M, Grogl M (2016) Antileishmanial activity of compounds derived from the Medicines for Malaria Venture open access box against intracellular Leishmania major amastigotes. Am J Trop Med Hyg 94(2):340–347. https://doi.org/10.4269/ajtmh.15-0448

Klinkert MQ, Heussler V (2006) The use of anticancer drugs in antiparasitic chemotherapy. Mini-Rev Med Chem 6(2):131–143

Knockaert M, Gray N, Damiens E, Chang YT, Grellier P, Grant K, Fergusson D, Mottram J, Soete M, Dubremetz JF, le Roch K, Doerig C, Schultz PG, Meijer L (2000) Intracellular targets of cyclin-dependent kinase inhibitors: identification by affinity chromatography using immobilised inhibitors. Chem Biol 7(6):411–422

Kulkarni MM, Jones EA, McMaster WR, McGwire BS (2008) Fibronectin binding and proteolytic degradation by Leishmania and effects on macrophage activation. Infect Immun 76(4):1738–1747. https://doi.org/10.1128/iai.01274-07

Kulshrestha A et al (2013) Validation of a simple resazurin-based promastigote assay for the routine monitoring of miltefosine susceptibility in clinical isolates of Leishmania donovani. Parasitol Res 112(2):825–828. https://doi.org/10.1007/s00436-012-3212-3

Kumar GA, Roy S, Jafurulla M, Mandal C, Chattopadhyay A (2016) Statin-induced chronic cholesterol depletion inhibits Leishmania donovani infection: relevance of optimum host membrane cholesterol. Biochim Biophys Acta 1858(9):2088–2096. https://doi.org/10.1016/j.bbamem.2016.06.010

Kumar A, Pandey SC, Samant M (2018) Slow pace of antileishmanial drug development. Parasitol Open 4:e4. https://doi.org/10.1017/pao.2018.1

Lamotte S, Spath GF, Rachidi N, Prina E (2017) The enemy within: targeting host-parasite interaction for antileishmanial drug discovery. PLoS Negl Trop Dis 11(6):e0005480. https://doi.org/10.1371/journal.pntd.0005480

Langhorne J, Duffy PE (2016) Expanding the antimalarial toolkit: targeting host-parasite interactions. J Exp Med 213(2):143–153. https://doi.org/10.1084/jem.20151677

Lestinova T, Rohousova I, Sima M, de Oliveira CI, Volf P (2017) Insights into the sand fly saliva: blood-feeding and immune interactions between sand flies, hosts, and Leishmania. PLoS Negl Trop Dis 11(7):e0005600. https://doi.org/10.1371/journal.pntd.0005600

Louradour I, Monteiro CC, Inbar E, Ghosh K, Merkhofer R, Lawyer P, Paun A, Smelkinson M, Secundino N, Lewis M, Erram D, Zurek L, Sacks D (2017) The midgut microbiota plays an essential role in sand fly vector competence for Leishmania major. Cell Microbiol 19(10):e12755. https://doi.org/10.1111/cmi.12755

Marr AK, MacIsaac JL, Jiang R, Airo AM, Kobor MS, McMaster WR (2014) Leishmania donovani infection causes distinct epigenetic DNA methylation changes in host macrophages. PLoS Pathog 10(10):e1004419. https://doi.org/10.1371/journal.ppat.1004419

Martin-Martin I, Chagas AC, Guimaraes-Costa AB, Amo L, Oliveira F, Moore IN, DeSouza-Vieira TS, Sanchez EE, Suntravat M, Valenzuela JG, Ribeiro JMC, Calvo E (2018) Immunity to LuloHya and Lundep, the salivary spreading factors from Lutzomyia longipalpis, protects against Leishmania major infection. PLoS Pathog 14(5):e1007006. https://doi.org/10.1371/journal.ppat.1007006

Maurya R, Bhattacharya P, Ismail N, Dagur PK, Joshi AB, Razdan K, McCoy JP, Ascher J, Dey R, Nakhasi HL (2016) Differential role of leptin as an Immunomodulator in controlling visceral Leishmaniasis in Normal and leptin-deficient mice. Am J Trop Med Hyg 95(1):109–119. https://doi.org/10.4269/ajtmh.15-0804

McDowell MA (2015) Vector-transmitted disease vaccines: targeting salivary proteins in transmission (SPIT). Trends Parasitol 31(8):363–372. https://doi.org/10.1016/j.pt.2015.04.011

McGwire BS, Satoskar AR (2014) Leishmaniasis: clinical syndromes and treatment. QJM 107(1):7–14. https://doi.org/10.1093/qjmed/hct116

Mesa CVB, Gustavo A, Muñoz DL, Muskus CE, Flórez AF, Ochoa R, Vélez ID, Robledo SM (2015) In silico screening of potential drug with Antileishmanial Activty and validation of their activity by in vitro and in vivo studies. J Chem Chem Eng 9:375–402

Murray HW (2010) Treatment of visceral leishmaniasis in 2010: direction from Bihar state, India. Future Microbiol 5(9):1301–1303. https://doi.org/10.2217/fmb.10.92

Nagle AS, Khare S, Kumar AB, Supek F, Buchynskyy A, Mathison CJN, Chennamaneni NK, Pendem N, Buckner FS, Gelb MH, Molteni V (2014) Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis. Chem Rev 114(22):11305–11347. https://doi.org/10.1021/cr500365f

Nwaka S, Hudson A (2006) Innovative lead discovery strategies for tropical diseases. Nat Rev Drug Discov 5(11):941–955. https://doi.org/10.1038/nrd2144

Ouakad M et al (2011) Increased metacyclogenesis of antimony-resistant Leishmania donovani clinical lines. Parasitology 138(11):1392–1399. https://doi.org/10.1017/S0031182011001120

Pacis A et al (2015) Bacterial infection remodels the DNA methylation landscape of human dendritic cells. Genome Res 25(12):1801–1811. https://doi.org/10.1101/gr.192005.115

Parihar SP, Hartley MA, Hurdayal R, Guler R, Brombacher F (2016) Topical simvastatin as host-directed therapy against severity of cutaneous Leishmaniasis in mice. Sci Rep 6:33458. https://doi.org/10.1038/srep33458

Patterson S, Wyllie S (2014) Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects. Trends Parasitol 30(6):289–298. https://doi.org/10.1016/j.pt.2014.04.003

Patterson S, Wyllie S, Stojanovski L, Perry MR, Simeons FRC, Norval S, Osuna-Cabello M, de Rycker M, Read KD, Fairlamb AH (2013) The R enantiomer of the Antitubercular drug PA-824 as a potential Oral treatment for visceral Leishmaniasis. Antimicrob Agents Chemother 57(10):4699–4706. https://doi.org/10.1128/aac.00722-13

Patterson S, et al. (2016) The anti-tubercular drug delamanid as a potential oral treatment for visceral leishmaniasis. eLife 5. doi:https://doi.org/10.7554/eLife.09744

Pena I et al (2015) New compound sets identified from high throughput phenotypic screening against three kinetoplastid parasites: an open resource. Sci Rep 5:8771. https://doi.org/10.1038/srep08771

Pina-Vazquez C, Reyes-Lopez M, Ortiz-Estrada G, de la Garza M, Serrano-Luna J (2012) Host-parasite interaction: parasite-derived and -induced proteases that degrade human extracellular matrix. J Parasitol Res 2012:748206–748224. https://doi.org/10.1155/2012/748206

Purkait B et al (2012) Mechanism of amphotericin B resistance in clinical isolates of Leishmania donovani. Antimicrob Agents Chemother 56(2):1031–1041. https://doi.org/10.1128/AAC.00030-11

Rachidi N, Taly JF, Durieu E, Leclercq O, Aulner N, Prina E, Pescher P, Notredame C, Meijer L, Späth GF (2014) Pharmacological assessment defines Leishmania donovani casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection. Antimicrob Agents Chemother 58(3):1501–1515. https://doi.org/10.1128/aac.02022-13

Ready PD (2014) Epidemiology of visceral leishmaniasis. ClinEpidemiol 6:147–154. https://doi.org/10.2147/CLEP.S44267

Rijal S et al (2013) Increasing failure of miltefosine in the treatment of kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or noncompliance. Clin Infect Dis 56(11):1530–1538. https://doi.org/10.1093/cid/cit102

Roy S, Kumar GA, Jafurulla M, Mandal C, Chattopadhyay A (2014) Integrity of the actin cytoskeleton of host macrophages is essential for Leishmania donovani infection. Biochim Biophys Acta 1838(8):2011–2018. https://doi.org/10.1016/j.bbamem.2014.04.017

Sangshetti JN, Kalam Khan FA, Kulkarni AA, Arote R, Patil RH (2015) Antileishmanial drug discovery: comprehensive review of the last 10 years. RSC Adv 5(41):32376–32415. https://doi.org/10.1039/C5RA02669E

Sant'Anna MRV, Diaz-Albiter H, Aguiar-Martins K, al Salem WS, Cavalcante RR, Dillon VM, Bates PA, Genta FA, Dillon RJ (2014) Colonisation resistance in the sand fly gut: Leishmania protects Lutzomyia longipalpis from bacterial infection. Parasit Vectors 7(1):329. https://doi.org/10.1186/1756-3305-7-329

Santiago MEB, Neto LS, Alexandre EC, Munari DP, Andrade MMC, Somenzari MA, Ciarlini PC, V.M.F. L (2013) Improvement in clinical signs and cellular immunity of dogs with visceral leishmaniasis using the immunomodulator P-MAPA. Acta Trop 127(3):174–180. https://doi.org/10.1016/j.actatropica.2013.04.005

Silverman JM, Chan SK, Robinson DP, Dwyer DM, Nandan D, Foster LJ, Reiner NE (2008) Proteomic analysis of the secretome of Leishmania donovani. Genome Biol 9(2):R35. https://doi.org/10.1186/gb-2008-9-2-r35

Silverman JM, Clos J, de'Oliveira CC, Shirvani O, Fang Y, Wang C, Foster LJ, Reiner NE (2010) An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages. J Cell Sci 123(Pt 6):842–852. https://doi.org/10.1242/jcs.056465

Siqueira-Neto JL, Moon S, Jang J, Yang G, Lee C, Moon HK, Chatelain E, Genovesio A, Cechetto J, Freitas-Junior LH (2012) An image-based high-content screening assay for compounds targeting intracellular Leishmania donovani amastigotes in human macrophages. PLoS Negl Trop Dis 6(6):e1671. https://doi.org/10.1371/journal.pntd.0001671

Sundar S, Chakravarty J (2010) Antimony toxicity. Int J Environ Res Public Health 7(12):4267–4277. https://doi.org/10.3390/ijerph7124267

Tegazzini D, Díaz R, Aguilar F, Peña I, Presa JL, Yardley V, Martin JJ, Coteron JM, Croft SL, Cantizani J (2016) A replicative in vitro assay for drug discovery against Leishmania donovani. Antimicrob Agents Chemother 60(6):3524–3532. https://doi.org/10.1128/aac.01781-15

Telleria EL, Martins-da-Silva A, Tempone AJ, Traub-Csekö YM (2018) Leishmania, microbiota and sand fly immunity. Parasitology 145(10):1336–1353. https://doi.org/10.1017/S0031182018001014

Turner KG, Vacchina P, Robles-Murguia M, Wadsworth M, McDowell MA, Morales MA (2015) Fitness and phenotypic characterization of Miltefosine-resistant Leishmania major. PLoS Negl Trop Dis 9(7):e0003948. https://doi.org/10.1371/journal.pntd.0003948

Uliana SR, Barcinski MA (2009) Repurposing for neglected diseases. Science (New York, NY) 326(5955):935; **author reply 935**. https://doi.org/10.1126/science.326.5955.935-a

van Griensven J, Zijlstra EE, Hailu A (2014) Visceral Leishmaniasis and HIV coinfection: time for concerted action. PLoS Negl Trop Dis 8(8):e3023. https://doi.org/10.1371/journal.pntd.0003023

Vanaerschot M, De DS, Rijal S, Maes L, Dujardin JC, Decuypere S (2011) Antimonial resistance in Leishmania donovani is associated with increased in vivo parasite burden. PLoSOne 6(8):e23120. https://doi.org/10.1371/journal.pone.0023120

Vianna G (1912) Tratamento da leishmaniose tegumentar por injeções intravenosas de tártaro emético. An 7º Congr Bras Med Cirurg 4:426–428

WHO (2002) New treatment for leishmaniasis is 95% effective. Bull World Health Organ 80:688

WHO (2018) Leishmaniasis fact sheet. http://www.whoint/en/news-room/fact-sheets/detail/leishmaniasis. Accessed 6/5/2019

Wilke ABB, Marrelli MT (2015) Paratransgenesis: a promising new strategy for mosquito vector control. Parasit Vectors 8:342. https://doi.org/10.1186/s13071-015-0959-2

Wyatt PG, Gilbert IH, Read KD, Fairlamb AH (2011) Target validation: linking target and chemical properties to desired product profile. Curr Top Med Chem 11(10):1275–1283

Zulfiqar B, Jones AJ, Sykes ML, Shelper TB, Davis RA, Avery VM (2017a) Screening a natural product-based library against Kinetoplastid parasites. Molecules (Basel) 22(10). doi:https://doi.org/10.3390/molecules22101715

Zulfiqar B, Shelper TB, Avery VM (2017b) Leishmaniasis drug discovery: recent progress and challenges in assay development. Drug Discov Today 22(10):1516–1531. https://doi.org/10.1016/j.drudis.2017.06.004