



University
of Glasgow

Barrett, M.P., and Croft, S.L. (2012) Management of trypanosomiasis and leishmaniasis. *British Medical Bulletin*, 104 (1). pp. 175-196. ISSN 0007-1420

Copyright © 2012 The Authors

<http://eprints.gla.ac.uk/75815/>

Deposited on: 22th February 2013

Enlighten – Research publications by members of the University of Glasgow
<http://eprints.gla.ac.uk>

Management of trypanosomiasis and leishmaniasis

Michael P. Barrett[†], and Simon L. Croft^{‡*}

[†]Wellcome Trust Centre of Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK, and [‡]Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK

Background: The current treatments for human African trypanosomiasis (HAT), Chagas disease and leishmaniasis (collectively referred to as the kinetoplastid diseases) are far from ideal but, for some, there has been significant recent progress. For HAT the only advances in treatment over the past two decades have been the introduction of an eflornithine/nifurtimox co-administration and a shorter regime of the old standard melarsoprol.

Sources of data: PubMed.

Areas of Agreement: There is a need for new safe, oral drugs for cost-effective treatment of patients and use in control programmes for all the trypanosomatid diseases.

Areas of controversy: Cutaneous leishmaniasis is not on the agenda and treatments are lagging behind.

Growing points: There are three compounds in development for the treatment of the CNS stage of HAT: fexinidazole, currently due to entry into phase II clinical studies, a benzoxaborole (SCYX-7158) in phase I trials and a diamidine derivative (CPD-0802), in advanced pre-clinical development. For Chagas disease, two anti-fungal triazoles are now in clinical trial. In addition, clinical studies with benznidazole, a drug previously recommended only for acute stage treatment, are close to completion to determine the effectiveness in the treatment of early chronic and indeterminate Chagas disease. For visceral leishmaniasis new formulations, therapeutic switching, in particular AmBisome, and the potential for combinations of established drugs have significantly improved the opportunities for the treatment in the Indian subcontinent, but not in East Africa.

Areas timely for developing research: Improved diagnostic tools are needed to support treatment, for test of cure in clinical trials and for monitoring/surveillance of populations in control programmes.

*Correspondence address.
Department of Infectious
and Tropical Diseases,
London School of
Hygiene & Tropical
Medicine, London WC1E
7HT, UK. E-mail: simon.
croft@lshtm.ac.uk

Keywords: human African trypanosomiasis/Chagas disease/leishmaniasis/antiprotozoal drug treatment/antiprotozoal drug development

Accepted: October 4, 2012

Introduction

Leishmaniasis, human African trypanosomiasis (HAT) and Chagas disease are caused by taxonomically related kinetoplastid protozoan parasites that have similar structural and biochemical features. These include a single mitochondrion with a discrete structured DNA body: the kinetoplast, specific organelles for glycolysis; the glycosomes, a sub-pellicular microtubular corset and a unique thiol metabolism. Recent analysis of the genome sequences has identified both similar and different metabolic pathways, a new understanding about the pathogenesis of disease, and has led to further description of biochemical/molecular drug targets.¹

These kinetoplastid diseases, also classified as 'neglected diseases', are diseases of poverty that have received limited funding for discovery, development and delivery of new tools. The drugs that are currently used for the treatment of the leishmaniasis, Chagas disease and HAT (Table 1) suffer the limitations of toxicity, variable efficacy, requirements for parenteral administration and/or length of treatment regimens. For HAT, Chagas and cutaneous leishmaniasis (CL), there are few drugs or treatments in clinical development, although a pipeline is emerging. In contrast, for visceral leishmaniasis (VL) there has been significant progress in the Indian sub-continent with liposomal amphotericin B (AmBisome), miltefosine and paromomycin all proving effective and registered over the past decade. Importantly, single-dose liposomal amphotericin B and the co-administration of combinations from the above three listed drugs show potential for both treatment and control of VL. Considerable advances in the identification, validation and characterization of drug targets has accompanied the completion of the genomes for *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*² and new tools such as RNAi (in *T. brucei* and *L. braziliensis*) and gene knockout³ have been developed to help validate potential targets. New and established pharmacophores, based upon synthetic and natural product chemistry, are being identified through improved screening technologies to identify hits from libraries provided by the pharmaceutical industry and elsewhere.⁴ This is only one early part of the long and complex process of drug development. Attention is still required to improve predictive models of infection and pharmacokinetic studies to evaluate leads and appropriate diagnostic

Table 1 Drugs in use and on clinical trial.

	Drug	Comments
VL		
First-line drugs	Pentavalent antimonials: sodium stibogluconate (pentostam, generic SSG)	Parenteral administration; reported toxicities; resistance in Bihar, India
	Meglumine antimoniate (Glucantime)	
	Amphotericin B (Fungizone)	Slow i.v. infusion; known toxicities, used in India where there is resistance to antimonials
	Liposomal amphotericin B (AmBisome)	Further clinical studies on single-dose infusion in India
	Miltefosine	First oral drug; concerns over teratogenicity, compliance
Clinical trials	Paromomycin	Intramuscular administration; registered in India; less effective in Sudan
	Amphotericin B lipid formulations	Other formulations shown effective in clinical studies
	Co-administrations: AmBisome + miltefosine, AmBisome + paromomycin, miltefosine + paromomycin	Highly effective, short course treatment in phase III trials in India; in further clinical studies
CL		
First-line drugs	Pentavalent antimonials: sodium stibogluconate (Pentostam)	Standard treatments; variable efficacy between types and species of <i>Leishmania</i>
	Meglumine antimoniate (Glucantime)	
	Amphotericin B (Fungizone)	For specific types in South America
	Pentamidine	For specific types in South America
Clinical trials	Paromomycin (topical formulation)	Marketed formulation
	Paromomycin (topical formulation, Phase III)	Well-tolerated formulation, also containing 0.5% gentamicin
	Miltefosine (oral, Phase III)	
	Imiquimod	Topical immunomodulator, adjunct therapy to antimonials in Phase II
HAT		
Haemolympathic stage		
First-line drugs	Pentamidine	Used for <i>T. b. gambiense</i> disease
	Suramin	Used for <i>T. b. rhodesiense</i> disease
Clinical trial	Fexinidazole SCYX-7158	Phase II trials to start in 2012 Phase I trials in 2012
CNS stage		
First-line drugs	Melarsoprol	Only drugs available for <i>T. b. rhodesiense</i> disease
	Eflornithine Nifurtimox/eflornithine co-administration	Used for <i>T. b. gambiense</i> disease Approved for use by WHO in 2009
Clinical trial	Fexinidazole SCYX-7158	Used for <i>T. b. gambiense</i> disease Phase II trials in 2012 Phase I trials in 2012
Chagas disease		

Continued

Table 1 Continued

	Drug	Comments
Acute stage		
First-line drugs	Nifurtimox	Oral, long course, known toxicities and <i>T. cruzi</i> strain sensitivity variation
	Benznidazole	Oral, long course, known toxicities and <i>T. cruzi</i> strain sensitivity variation; paediatric formulation in late development with DNDi
Indeterminate stage/ early chronic stage	No standard treatments	
Clinical trials	Benznidazole	BENEFIT trial to report in 2012
	Antifungal triazoles: posaconazole and ravuconazole pro-drug	Entered clinical trials in 2011 with Merck and Eisai/DNDi

methods to improve interpretation of clinical trials. The emergence of Product Development Partnerships including the Geneva-based Drugs for Neglected Diseases initiative (DNDi) and the US-based Consortium for Parasitic Drug Development and Institute for One World Health (IoWH) provided formats to enable progression of compounds through clinical trials thus filling a gap created by the abandonment of these areas by the Pharmaceutical industry.

Management of Leishmaniasis

Leishmaniasis is endemic in 98 countries in Asia, Africa, South and Central America and southern Europe. Leishmaniasis is often considered a disease complex with two major manifestations, VL and CL. Additionally, there is a number of rarer forms, including mucocutaneous (MCL), diffuse (DCL) forms of CL and post-kala-azar dermal leishmaniasis (PKDL).⁵ There is an estimated incidence of around 0.5 million cases of VL and 1.5 million of CL. The *Leishmania* parasite is transmitted by female phlebotomine sandflies, in which the flagellated promastigote form divides and develops into an infective metacyclic form. Following the bite of the sandfly, the promastigote invades various macrophage populations where it transforms to the amastigote form that survives and multiplies in the phagolysosomal compartment of this host cell. In VL, normally caused by *Leishmania donovani* and *Leishmania infantum* (equivalent to *Leishmania chagasi* in S. America), the infection progresses to a potentially fatal disease if untreated. Different pathologies are associated with these species, even *L. donovani* having different pathologies in India and Sudan. This is

further complicated by the observations that some strains of *L. infantum* can cause CL and that post-treatment some *L. donovani*-infected patients develop into the DCL form, PKDL. Since 1985, cases of HIV-VL co-infection have been reported with increasing numbers of cases in East Africa (23% of all VL cases in NE Ethiopia).⁶ CL also presents in patients in many different forms, though most patients have limited cutaneous lesions that self-cure within 6–18 months leaving scarred tissue. CL has social and economic impact and is a stigmatizing disease as most lesions are on exposed regions of the skin, for example, face, arms and legs. Over 15 species of *Leishmania* cause CL in humans, with species such as *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica* in the Old World and *Leishmania mexicana*, *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania panamensis* and *Leishmania guyanensis* in the New World. Generally, *L. donovani* and *L. tropica* are the sources of anthroponotic infections, whereas *L. infantum* and the other CL-causing species are zoonotic infections.⁷

Four characteristics of *Leishmania* biology are particularly germane to drug efficacy, namely (i) the intracellular location of the amastigote target form in the macrophage's low pH phagolysosomal compartment; (ii) the different pharmacokinetic requirements of drugs that distribute to the liver, spleen, bone marrow in VL or the skin in CL; (iii) the significant differences in drug sensitivities of the 17 species of *Leishmania* that cause leishmaniasis in humans and (iv) the influence of the immune suppression associated with leishmaniasis, which can render some drugs less effective.

Clinical signs and diagnosis

The leishmaniasis present in a variety of ways. CL manifests as an open sore at the site of the insect bite and will frequently self-heal, albeit leaving a scar. DCL is more problematic causing lepromatous type lesions disseminated across the skin and can be more difficult to heal. The MCL form, endemic in parts of Latin America, starts with skin sores but these spread to the mucosal membranes of the face and profound inflammatory damage can lead to the erosion of nostrils and mouth in particular. VL, after initial skin lesions, takes 2–8 months to develop gross inflammatory reactions within the viscera (liver and spleen in particular) and is usually fatal unless treated. Microscopic identification of parasites has been central to diagnosis. The detection of amastigotes in the samples from the lymph nodes, bone marrow, liver, spleen or skin lesions is a first step. For VL, some techniques, like spleen aspiration, can cause life-threatening complications and should

be performed only by trained personnel. Bone marrow aspiration has a lower sensitivity. PCR is more sensitive than microscopic examination and has become the first-line test in referral hospitals and research centres. Quantitative PCR allows an accurate diagnosis in venous blood samples, thereby avoiding bone marrow aspiration.

Serological tests are the most widely used indirect method. Indirect fluorescence antibody, enzyme-linked immunosorbent assay (ELISA) or western blot require equipment that has not been optimized for field settings. Both the direct agglutination (DAT) and immunochromatographic tests (ICT) using the rK39 antigen (in a dipstick format), have proved to have a high sensitivity and specificity and translate readily to peripheral health centres. Serological tests have limitations as specific antibodies remain detectable for several years after cure, hence there is a need for a field-adapted antigen test. A latex agglutination test detecting a heat-stable, carbohydrate antigen in the urine of VL patients showed good specificity but low-to-moderate sensitivity and further innovation is needed in this area.⁸

Current drugs: VL

Pentavalent antimonials

Pentavalent antimonials (Fig. 1), the standard drugs for 70 years, are now almost obsolete in the key endemic area in Bihar, India due to drug resistance. However, sodium stibogluconate (Pentostam),

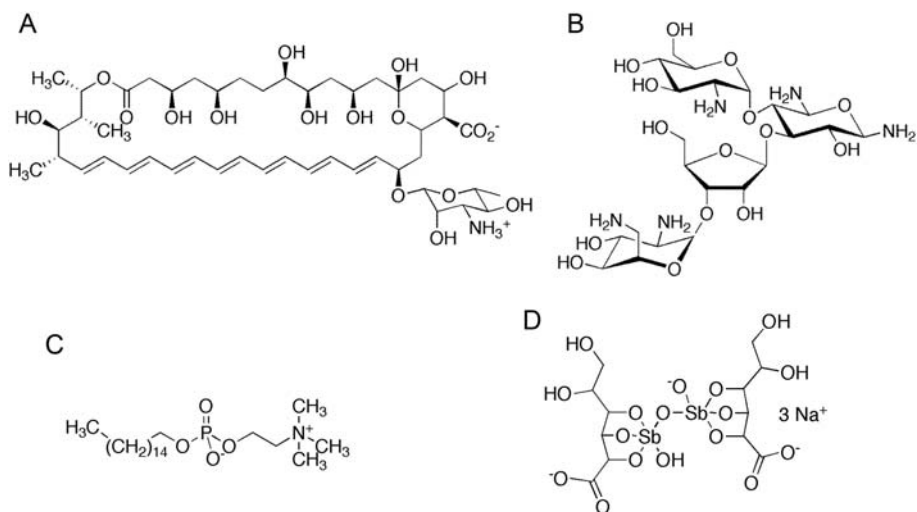


Fig. 1 Drugs used in leishmaniasis therapy: (A) amphotericin B; (B) paromomycin; (C) miltefosine; (D) sodium stibogluconate.

meglumine antimoniate (Glucantime) and a generic low-cost brand of sodium stibogluconate remain useful in treatments in the rest of the world. Here too, however, long courses (up to 30 days), parenteral administration and the known toxicity profile, including hepatotoxic, nephrotoxic and potentially fatal cardiotoxic effects, limit use.^{5,9} Their chemical structure remains poorly defined. Much current research is focused on the mechanisms of resistance to this class of drugs.¹⁰

Amphotericin B and lipid formulations

Amphotericin B (Fig. 1), normally considered a second-line drug, is now first-line treatment in Bihar, India following the loss of effectiveness of pentavalent antimonials. This drug is now at the forefront of VL treatment in a number of lipid formulations, developed during the 1980s for the treatment of systemic mycoses in immunocompromised patients. Of the several that have proved effective in the treatment of VL, only the liposomal formulation, AmBisome, has become a standard treatment, registered for the treatment of VL in a number of countries.¹¹ Recently, a single-course therapy of 10 mg/kg has been shown to cure 95% of patients in India.¹² A significant reduction in price negotiated by the World Health Organization (WHO) with the producers (Gilead), currently \$18 for a 50 mg ampoule) was followed in 2012 by a donation of 50 000 treatments. However, several ampoules are required for a single-course treatment, and adverse events and temperature stability (manufacturer guarantee 25°C) remain issues. A recurring issue around all the VL treatments, discussed in this section, are regional differences in response rates. Clinical studies have suggested that AmBisome was most effective in the treatment of VL patients in India, less so in East Africa and even less so against *L. infantum* (*L. chagasi*) in South America.¹³ The worldwide use of AmBisome for VL requires further study, especially in East Africa (www.dndi.org).

Paromomycin (aminosidine, monomycin)

Over 50 years ago, the aminoglycoside paromomycin (Fig. 1) was shown to possess anti-leishmanial activity. Following the studies that showed a parenteral formulation could cure VL in the 1980s, paromomycin moved slowly through clinical trials with WHO/TDR in the 1990s and the IoWH in the 2000s. Phase III clinical trials in India demonstrated 94% efficacy (15 mg/kg for 21 days, i.m.) leading to registration for treatment of VL in India in 2006.¹⁴ Despite the low

cost (estimated \$10.00 per course), the drug has not yet become part of the treatment options for VL patients in the subcontinent, although a phase IV trial in 2008–09 showed a cure rate of 94.2% at 6 months post-treatment (P. Desjeux, IoWH, pers comm.). A phase III VL clinical trial with the same dose in East Africa, conducted by DNDi, showed lower efficacy, particularly in Sudan where the 15 mg/kg regime gave a <50% cure rate, whilst a dose of 20 mg/kg for 21 days gave only an 85% cure rate, insufficient for consideration as a monotherapy.¹⁵ The reason for this difference is not understood.

Miltefosine

The anti-leishmanial activity of the phospholipid derivative, miltefosine (Fig. 1), identified in the 1980s led to the first oral treatment for VL. Clinical trials supported by WHO/TDR and Zentaris (the then manufacturing company), showed 94% efficacy in adults and children and miltefosine was registered for treatment of VL in India in 2002.¹⁶ It was the first anti-leishmanial to undergo phase IV studies and has been used in the VL Elimination Programme for the Indian sub-continent. Issues around the use of miltefosine include (i) potential teratogenicity, which requires women of child-bearing age to take contraception for up to 3 months post-treatment due to long half-life of the drug, (ii) the 28 days oral treatment which can lead to poor compliance and relapses and (iii) concerns about drug resistance (Fig. 1).

Other new treatments

Drug combinations shorten courses of therapy, reduce toxicities through lower dosage and diminish the selection of resistant mutations. Drug combinations are not yet available for VL, although co-administration (either concomitant or sequential) of three available anti-leishmanial drugs has been tested following experimental and pre-clinical toxicokinetic studies.¹⁷ Phase III trials on VL in India have shown that co-administrations of AmBisome + miltefosine (single dose iv + oral 7 days, sequential), AmBisome + paromomycin (single dose iv + 10 days i.m., sequential) and miltefosine + paromomycin (10 days + 10 days i.m., concomitant) gave a 98% cure rate. This reduces treatment time (30 days compared with a potential 8 days) and should have an impact on disease control. Antimonials were not included due to drug resistance in India. In Sudan, a phase III trial on a co-administration of sodium stibogluconate and paromomycin (17 days) has recently demonstrated equivalent efficacy to the longer courses of monotherapies.

Other forms of VL

Some VL-treated patients develop the cutaneous manifestation, PKDL, with parasite persistence within the context of an immunological response. Differences in Indian and Sudanese PKDL include frequency, age profile and rates of self-cure. Whether PKDL results from specific types of drug treatment is unlikely. Treatment based upon long courses of antimonial drugs, and small studies with miltefosine and AmBisome have reported improved cure rates. An immunotherapeutic approach, antimonial drug plus BCG, showed a higher cure rate than drug alone in Sudan.

HIV/Leishmaniasis co-infections

The standard drugs have been used in various regimens to treat VL in co-infection cases. Relapse rates are variable but up to 85% and frequently recorded.⁶ Co-infection increases the risk of VL developing in an infected human by 100–2300-fold, with accompanying issues of reduced response to therapy, and greatly increased probability of relapse. Currently, there is no successful defined treatment regimen for HIV-VL patients. Different policies have been adopted by countries with some recommending treatment with anti-retrovirals followed by treatment with anti-leishmanial drugs, whilst others have recommended maintenance therapy. In Southern Europe, this maintenance therapy is frequently based upon lipid amphotericin B formulations.

Current drugs: CL

In comparison to VL there are limited proven treatments for CL. Concerns over the reliability of data in the absence of randomized placebo controlled trials was reported in two Cochrane analyses of trials on New World CL and Old World CL, concluding that most clinical studies could not be analysed as they did not meet acceptable standards.^{18,19} A standardized protocol allowing clinical trials to present comparative data, defines start points (recruitment of patients with new or old lesions) and endpoints (resolution of lesion or complete re-epithelialization) has been proposed.²⁰

Pentavalent antimonials, amphotericin B and pentamidine

The three traditional standard drugs have been used widely in the treatment of CL but today are only recommended for the treatment of specific forms. Pentavalent antimonials have proved inconsistent in their effectiveness across the different *Leishmania* species whilst the use of pentamidine and amphotericin B are limited to specific types of CL in South America.

Paromomycin (aminosidine, monomycin)

Paromomycin has been used in various formulations for CL over several decades. Focus in the past three decades has been on topical formulations with a 15% paromomycin ointment (with the penetrating enhancement agent, 12% methylbenzethonium chloride), proving effective in clinical studies and currently available from TEVA (Israel). The search for more effective and less irritant topical formulations continues and a formulation of 12% paromomycin (with 0.5% gentamicin and 10 surfactants), which showed efficacy against *L. major* CL in a phase II trial in Tunisia, is now in phase III studies.²¹

Miltefosine

Oral miltefosine also has some variable, species-dependent effectiveness against CL. It is registered for the treatment of CL in Colombia and has been shown effective against various forms of CL. Again variation in species sensitivity has proved an issue in the New World and improved diagnostics are required.

Other treatments

For *L. major* and *L. tropica* infections there is evidence that the antifungal azoles, fluconazole and itraconazole have some effect. Recently, the triazole, posaconazole, which showed potential in experimental studies, was effective in a clinical study. One of the drugs identified in the Cochrane analyses as contributing towards cure was the anti-inflammatory drug pentoxifylline, used as adjunct therapy to antimonials.²² As CL treatment can also aim to accelerate self-cure through immune mediated means, adjunct therapy with immunomodulators also has potential. BCG has been used extensively in combination with antimonials in Venezuela, and the anti-viral TLR7 agonist, imiquimod, in Peru.²³ A trial on CL patients in Peru country showed 75% cure for imiquimod plus antimonials compared with 58% for antimonials alone.²³

Management of HAT

HAT, often referred to as sleeping sickness in its second stage when neurological manifestations associated with parasites in the brain become apparent, is endemic to sub-Saharan Africa. Its prevalence had grown to an estimated 300 000 cases by 1998 from a position of near control in the 1960s.^{24,25} Enhanced surveillance, distribution of free

drugs and implementation of several clinical trials led to a steady decline in incidence in the twenty-first century with reported case numbers <7000 and a hope of elimination by 2020.

Trypanosoma brucei gambiense is found in West and central Africa. *Trypanosoma brucei rhodesiense* is found in East and southern Africa. The parasites can be distinguished by genetic markers, e.g. the serum resistance associated (*sra*) gene defines *T. b. rhodesiense*²⁶ and protects against a human trypanosome lytic factor (TLF), or apolipoprotein L1 (ApoL1), that is responsible for immune-independent lysis of most trypanosome species. *Trypanosoma brucei gambiense* does not have the *sra* gene, avoiding TLF lysis by other means.²⁶ *Trypanosoma evansi*, generally infectious only to non-primate mammals has been found to infect humans with rare genetic defects in ApoL1.²⁶

The process of antigenic variation makes vaccination an unlikely option in HAT control. Trypanosomes are transmitted by tsetse flies, *Glossina* spp., and the distribution of the disease is restricted to areas where tsetse thrives. Tsetse clearance can be effective in control; the Island of Zanzibar, for example, was declared trypanosome free following intense efforts at tsetse control. However, translation to a continent-wide approach is difficult and chemotherapy remains at the forefront of HAT management.²⁷

Clinical signs and diagnosis

Patients exposed to infected tsetse fly bites will develop the disease. In the first, hemolymphatic stage parasites proliferate in the blood and lymphatic systems, and symptoms such as headache and general malaise ensue. At Stage 2 the parasites have invaded the central nervous system including the brain, and progressive neurological breakdown, including psychiatric disorders, depression and altered sleep-wake patterns, ensues. *T. b. rhodesiense* progresses to Stage 2 in weeks to months, whereas it takes on average 18 months for gambiense disease to reach Stage 2.

Diagnosis in active screening campaigns involves a primary serological screen using the Card Agglutination Test for Trypanosomiasis (CATT), which targets antibodies produced against the common LiTat1.3 variant surface antigen which is expressed early in most *T. b. gambiense* infections. However, variable sensitivity (and failure to detect rhodesiense parasites which lack the CATT antigen) means microscopical identification of parasites in the lymph or blood is required for confirmation,²⁸ whilst diagnosis at Stage 2 (necessary as therapy is stage dependent) requires the identification of either parasites, or else white cells in CSF. Novel sensitive molecular diagnostics

using PCR or an isothermal DNA amplification technique (the so-called LAMP approach) along with several other tests for new biomarkers and parasite-enrichment techniques might soon yield improved diagnostics.

Current drugs

Current drugs for HAT are unsatisfactory: with varying degrees of toxicity, a need for parenteral administration and efficacy <100% with resistance a growing problem. For stage 1 HAT, pentamidine is used for *T. b. gambiense* and suramin for *T. b. rhodesiense* infection. For Stage 2, the arsenical melarsoprol remains the only option for rhodesiense disease, although for the majority of cases (>95% of cases being *T. b. gambiense*) eflornithine has become first-line treatment, particularly in combination with nifurtimox, in the nifurtimox–eflornithine combination therapy (NECT). The gratis donation to WHO of eflornithine, melarsoprol and pentamidine by Sanofi-Aventis and suramin and nifurtimox by Bayer has been critical in the successful campaign against HAT in the twenty-first century.

Pentamidine

Pentamidine (Fig. 2) has been used to treat first-stage *T. b. gambiense* disease since the 1940s.²⁹ It is given at 4 mg kg⁻¹ once per day, usually over a 7-day period intramuscularly (intravenous injection induces a

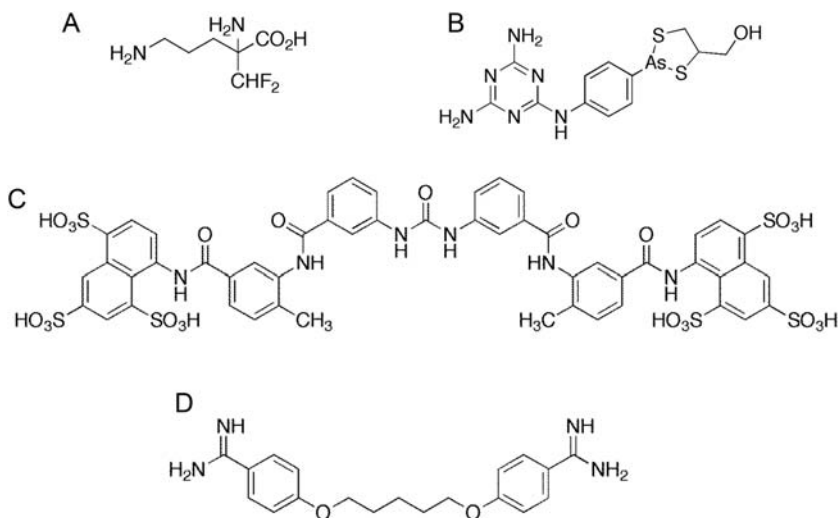


Fig. 2 Drugs used in human African trypanosomiasis therapy: (A) eflornithine; (B) melarsoprol; (C) suramin and (D) pentamidine.

potentially dangerous hypoglycaemic response). Injection-site pain, nephrotoxicity, leucopenia and liver enzyme abnormalities are common adverse events. The drug is potent, with *in vitro* trypanocidal IC₅₀ values in the order of 1–10 nM. Accumulation of drug to high concentrations by several transporter systems appears to contribute to selectivity, although loss of uptake can also contribute to resistance.²⁹ The pharmacology of pentamidine presents various difficulties including extensive tissue retention and binding to serum proteins giving a large volume of distribution and long-terminal half-life, while little drug can enter the brain. Trials of a shortened, 3 day, course of pentamidine are ongoing and will reduce dose and hospitalization time if successful.

An orally available analogue of pentamidine, pafuramidine maleate (DB289), is a methoxy prodrug that penetrates intestinal epithelia and converts systemically to the diamidine furamidine (DB75) (Fig. 2).²⁹ A phase III trial showed the oral formulation to be of comparable efficacy to injected pentamidine; however, an extended phase I safety trial using 14 day dosing (instead of the successful 10 day dosing in the phase III trial) demonstrated unacceptable nephrotoxicity and development was halted.²⁹

Suramin

Suramin was first used against HAT in 1922.³⁰ In a typical course it is given by slow intravenous injection once every 3–7 days, over a 4 week period. Suramin is >99% protein bound in serum with a terminal half-life of 41–78 days. Blood–brain barrier permeation is minimal meaning it is useful only in Stage 1. The uptake into trypanosomes is via ISG75 receptor-mediated endocytosis and interference with multiple points in the endocytic pathway leads to decreased suramin sensitivity,³¹ although how it actually kills cells once inside remains unknown. Adverse reactions include pyrexia, nephrotoxicity, nausea, urticaria, neuropathy and anaemia, particularly when used at high doses as a potential inhibitor of HIV infection.³⁰

Melarsoprol

Melarsoprol (Fig. 2) emerged in the 1940s as a safer drug than previous arsenic derivatives. However, an often fatal reactive encephalopathy afflicts 5–10% patients taking the drug. Other adverse effects include pyrexia, headache, pruritus, thrombocytopenia and heart failure. Melarsoprol is given by intravenous injection as a 3.6% solution in propylene glycol, usually now over a 10-day course³² which

replaced earlier regimens where it was given, with interruptions, over a month. Oral dosing of cyclodextrin–melarsoprol conjugates cured a stage 2 infection in mice, with reduced host toxicity.³³ Co-administration of corticosteroids (e.g. prednisolone) also provides limited protection against the reactive encephalopathy.

Selective uptake via specific transporters plays a key role in the selective toxicity of melarsoprol and loss of transport relates to resistance (including cross-resistance to pentamidine if the appropriate set of transporters is lost²⁷). Treatment failures reached alarming levels in many foci in the 1990s and 2000s. Trypanocidal metabolites of melarsoprol only reach levels in brain at 1–2% of maximum plasma levels which means that a relatively low drop in parasite sensitivity to drug could cause treatment failure and recrudescence from the brain.

Eflornithine and Nifurtimox eflornithine combination therapy (NECT)

Eflornithine (D,L- α -difluoromethyl ornithine) inhibits the polyamine biosynthetic enzyme ornithine decarboxylase, which turns over more rapidly in man than trypanosomes, partly explaining the selective effect of the drug. In monotherapy it is given at 100 mg kg⁻¹ body weight at 6 h intervals (i.e. 400 mg kg⁻¹ per day) by intravenous infusion for 14 days.³⁴ This high frequency of administration relates to a short plasma half-life (~3 h) and poor potency (*in vitro* IC₅₀ values of 81–693 μ M), although the action of the immune system also contributes to *in vivo* activity.³⁴ The CSF to plasma ratios are between 0.1 and 0.9 in humans. Adverse effects include fever, headache, hypertension, macular rash, peripheral neuropathy and tremor, gastrointestinal problems including diarrhoea. Infusion-associated septicaemia has been a major issue.

An amino-acid transporter, TbAAT6, mediates the uptake of eflornithine into trypanosomes and resistance relates to loss of the transporter.³⁵ The introduction of a combination of eflornithine with the unregistered trypanocide nifurtimox³⁶ aims to delay the emergence of resistance and improve administration regimens. The combination regimen uses intravenous eflornithine at 200 mg/kg every 12 h for 7 days (i.e. 14 rather than 56 infusions), with nifurtimox being given orally three times a day for 10 days. The advantages in cost and convenience have rapidly made NECT the treatment of choice for stage 2 disease (the cost issue currently being mitigated by the gratis provision of drugs to WHO).

New drug candidates for HAT

Orally available drugs, preferably of reduced administration duration for HAT, are highly desirable. A 'pipeline' of new compounds entering clinical trials has now emerged (Fig. 3).³⁷

Fexinidazole

Fexinidazole (Fig. 3) is a 2-substituted 5-nitroimidazole now being taken forward by the Drugs for Neglected Diseases initiative (DNDi) over the past 6 years. Although of modest potency (IC_{50} values around $1 \mu M$), the compound is active in mouse models of stage 1 and stage 2 diseases,³⁸ curing the stage 2 model at 100 mg/kg twice per day oral dosing for 5 days (Fig. 3).

It is metabolized to sulphoxide and sulphone derivatives (with similar potency to the parent compound) and has good CNS penetration. As most nitro-heterocycles, fexinidazole is positive in bacterial Ames genotoxicity tests but not in mammalian assays. Other safety tests also indicated no adverse events up to 200 mg/kg in rats and the drug entered phase I clinical trials in 2010, with phase II trials planned to start in 2012.

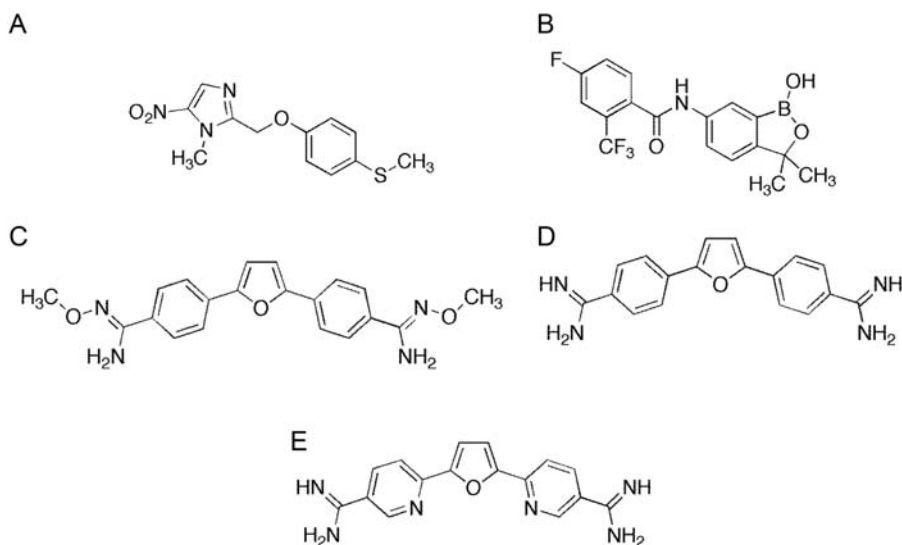


Fig. 3 Drugs in or approaching clinical trials for HAT therapy: (A) fexinidazole; (B) SCYX-7158; (C) DB289 (pafuramidine); (D) DB75 (furamidine) and (E) CPD0801.

SCYX-7158

The benzoxaborole class was developed against *Trypanosoma brucei* in a collaborative venture between Anacor Pharmaceuticals, SCYNEXIS and DNDi.³⁹ A 6-carboxamido-based series was chosen for optimization based on trypanocidal activity and pharmacokinetic properties, including brain permeation. Ultimately, SCYX-7158 (Fig. 3) was selected for further development. Its modest *in vitro* potency (IC₅₀ around 1 μM) was offset by good pharmacokinetic properties giving 100% cure in a mouse model of stage 2 disease following a 25 mg/kg once a day for 7 days oral dosing.³⁹ Toxic effects have been minimal in preclinical evaluation and the compound entered phase I trials early in 2012.

Stage 2 active dicationic molecules

Several aza analogues of pafuramidine's active diamidine product, furamidine (DB75) (Fig. 3), including CPD-0802 (or DB-829) (Fig. 3), DB-868 and 28DAP010 cured mouse and primate models of stage 2 trypanosomiasis.²⁹ CPD-0802 at 20 mg/kg once daily, intraperitoneally, for 10 days cured the GVR35 CNS mouse model while a daily 5 mg/kg intramuscular injection for 5 days cured a vervet monkey model of stage 2 (*T. b. rhodesiense*) disease. The methoxy prodrug of CPD0802 also cleared the GVR35 mouse model when given orally. Renal toxicity is an issue that requires consideration in the development of diamidines; CPD-0802 accumulates in the rat kidney at concentrations around 10 times lower than furamidine. This potentially improved safety profile and the higher risk–benefit margin required for stage 2 drugs places these compounds in the pre-clinical part of the pipeline for HAT drug development.

Management of American trypanosomiasis

American trypanosomiasis or Chagas' disease, caused by *Trypanosoma cruzi*, afflicts 8–10 million people, mainly in Central and South America. Prevalence in non-endemic countries is increasing as emigration from endemic countries brings hundreds of thousands of cases to the USA, Europe and elsewhere.⁴⁰ Natural transmission depends on reduviid bugs of the genus *Triatoma* that convey parasites in their faeces whilst taking blood meals from their mammalian hosts. Blood transfusion is also a key route of transmission. *Trypanosoma cruzi* infects several mammalian species in addition to humans. Molecular analysis has revealed that at least six distinct lineages can be classified,

with genetic distances between these classified types being as far as those that divide *Leishmania* into distinct species. A definitive link between different epidemiology and different clinical symptoms has yet to be made.

Following entry into the host, parasites invade host cells and differentiate into replicative amastigote forms (in the cytosol rather than phagolysosome as seen in *Leishmania*). The acute stage of infection involves inflammatory reactions to the parasites and general symptoms such as fever and malaise. After this patients enter an indeterminate phase: characterized by a lack of symptoms and failure to find parasites in biopsy, or even detect their presence by sensitive DNA based techniques. This phase can last 5–15 years (or more), many individuals dying of causes not related to the trypanosome infection. Others will go on to develop a symptomatic chronic disease where pathologies ranging from cardiomyopathy to megaesophagus or megacolon lead to death. Persistence of parasites eventually leads to the inflammatory responses that underlie these chronic Chagasic pathologies.⁴⁰

Coordinated programmes to destroy the triatomine vectors have been effective in reducing Chagas' incidence,⁴⁰ although the longevity of infection makes it probable that human or other wild animal reservoirs will maintain an infectious cycle beyond the dates when sustained insect control becomes non-viable. Although hope exists that immunization could be used in Chagas control, useful vaccines are not available yet.

Clinical signs and diagnosis

Infection follows the entry of parasites from insect faeces in lesions following the feed of the reduviid bugs. Facial swelling, especially around the eye (Romana's sign), is common and general malaise accompanies the acute phase following infection. A long, asymptomatic, indeterminate phase can follow where parasites are difficult to find. This can last in excess of 10 years. In some cases inflammatory responses to residual parasites induce progressive and sometimes fatal inflammatory damage to the heart, oesophagus, colon or other organs.⁴⁰

In addition to symptomatic diagnosis and microscopy in the acute stage when trypomastigotes can be found in the blood, serological diagnosis using *T. cruzi* antigen to detect antibody responses is routinely performed.⁴⁰ PCR techniques have also been evaluated and particularly in the indeterminate and chronic phases, and have been adopted for use in clinical trials and surveillance studies. Traditionally, xenodiagnosis, involving feeding laboratory reared triatomid nymphs on blood of patients, was used along with haemoculture as classical parasitological methods.

Current drugs

Only two drugs, both nitroheterocycles, are recommended for treatment of Chagas disease.⁴¹ Nifurtimox is a nitrofuran from Bayer, whilst benznidazole, produced for many years by Roche, is a 2-nitroimidazole. The latter's supply has been a cause of ongoing concern since Roche stopped the production with transfer of manufacture to the Brazilian government associated pharmaceutical company, LAFEPE laboratories.

Nifurtimox

Nifurtimox (Fig. 4) was introduced in the 1960s to treat acute Chagas disease.⁴¹ The drug is trypanocidal, against both circulating trypomastigotes and amastigotes. Its use for Chagas declined for several years but the enhanced manufacture to contribute to NECT use for HAT, and recent interruptions in availability of benznidazole, have led to increased use. Clearance of nifurtimox from blood is fast (plasma elimination half-life of around 3 h) and trypanocidal potency is weak and so the drug is given for prolonged periods (in tablet form, adults receiving 8–10 mg/day for 90 days while juveniles receive 15 mg/day). Nifurtimox appears to act primarily through the metabolism to a reactive trinitrile species and different *T. cruzi* lineages show significant differences in susceptibility possibly because of varying expression levels of nitroreductase enzymes required for activation. Toxic effects to the central and peripheral nervous systems and gastrointestinal problems (nausea, stomach cramps, vomiting and diarrhoea) are common.

Benznidazole

Benznidazole (Fig. 4), introduced in the 1970s, has variable activity across different *T. cruzi* strains, relating to variable reductive activation by metabolism. It is given orally (5–7 mg/day for 60 days in adults and 5–10 mg/day in juveniles).⁴¹ A new formulation to facilitate paediatric administration is currently under development through DNDi. Side

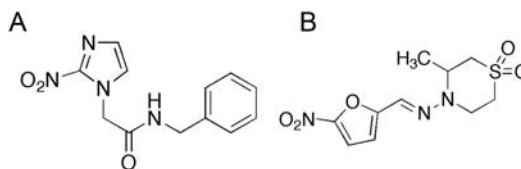


Fig. 4 Drugs used in Chagas disease therapy: (A) benznidazole and (B) nifurtimox.

effects are also common, principally hypersensitivity reactions manifesting themselves in dermal lesions, but intestinal and neurological side effects are reported too. Clear-cut evidence of enhanced risk of cancer is not reported for either drug, but in the absence of long-term follow-up studies this might be missed. An important trial (benznidazole evaluation for interrupting trypanosomiasis, BENEFIT trial) evaluating the use of the drug in chronic stage Chagas is under way with results due late in 2012.⁴²

Treatment of the symptomatic complications of Chagas disease is also useful, thus for Chagasic heart disease, salt restriction, diuretics, digitalis, angiotensin converting enzyme inhibitors, beta blockers, anti-coagulant treatment for atrial fibrillation, pacemakers or automatic cardiofibrillator implantation are all used when necessary. Surgery too can be used including heart transplantation for patients with advanced chagasic cardiomyopathy, or surgical removal of afflicted areas of oesophagus and colon in the other megasyndromes.

Drugs under development for Chagas disease

Development of new drugs for Chagas disease is fraught with difficulty, not least because patients entering the indeterminate stage are asymptomatic and the difficulty to detect parasites then and in early chronic infections complicates any definitive assessment of cure. Notwithstanding, several compounds are in clinical trials for Chagas disease. Two antifungal lanosterol-14 alpha-demethylase inhibitors, posaconazole (Fig. 5) (developed by Schering Plough, now with Merck).⁴³ and E1224 (a water-soluble prodrug of ravuconazole—developed by Eisai Pharmaceuticals with DNDi) are showing particular promise. Both are orally available and in early stages of trials given (400 mg twice per day for 60 days alone or with benznidazole for posaconazole). A cysteine protease (cruzipain) inhibitor, K777—a vinylsulfone (Fig. 5), has also shown efficacy in chronic rodent models and is in preclinical development.⁴⁴

Conclusion

The diseases caused by protozoa of the Order Kinetoplastida, collectively known as the trypanosomiasis and leishmaniasis, afflict millions of the world's poorest people. Current treatments still rely upon toxic arsenicals and antimonials and genotoxic nitroheterocycles. However, in the past decade we have witnessed a surge in interest and commitment to develop new drugs for these diseases and several new therapies

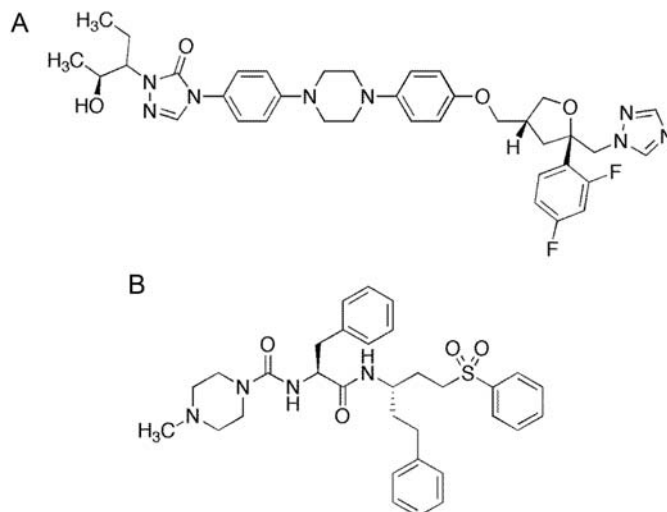


Fig. 5 Drugs in or approaching clinical trials for Chagas disease therapy: (A) Posoconazole and (B) K-777.

have become available while other compounds are in clinical trials.⁴⁵ Yet, given the known high attrition rates in drug development sustained efforts to bolster and diversify the pipelines are still required if the efforts to eliminate HAT in Africa and VL in India, as well as bringing Chagas and other forms of leishmaniasis under control by 2020 are to be successful.

Funding

The authors are grateful to the Bill and Melinda Foundation, The Medical Research Council, Biotechnology and Biological Sciences Research Council and the EU FP7 Programme for funding research into chemotherapy of trypanosomiasis and leishmaniasis. The Wellcome Trust Centre for Molecular Parasitology is supported by core funding from the Wellcome Trust [085349]. We are grateful to Alan Sewall for drawing structures of compounds. Funding to pay the Open Access publication charges for this article was provided by the Wellcome Trust.

References

- 1 Stuart K, Brun R, Croft S *et al*. Kinetoplastids: related protozoan pathogens, different diseases. *J Clin Invest* 2008;118:1301–10.
- 2 El-Sayed NM, Myler PJ, Blandin G *et al*. Comparative genomics of trypanosomatid parasitic protozoa. *Science* 2005;309:404–9.

- 3 Frearson JA, Wyatt PG, Gilbert IH *et al*. Target assessment for antiparasitic drug discovery. *Trends Parasitol* 2007;23:589–95.
- 4 Jacobs RT, Nare B, Phillips MA. State of the art in African trypanosome drug discovery. *Curr Top Med Chem* 2011;11:1255–74.
- 5 Alvar J, Croft S, Olliaro P. Chemotherapy in the treatment and control of leishmaniasis. *Adv Parasitol* 2006;61:223–74.
- 6 Alvar J, Aparicio P, Aseffa A *et al*. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev* 2008;21:334–59.
- 7 Reithinger R, Dujardin J-C, Louzir H *et al*. Cutaneous leishmaniasis. *Lancet Infect Dis* 2007;7:581–96.
- 8 Chappuis F, Sundar S, Hailu A *et al*. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature Rev Microbiol* 2007;5:S7–16.
- 9 Croft SL, Olliaro P. Leishmaniasis chemotherapy—challenges and opportunities. *Clin Microbiol Infect* 2011;17:1478–83.
- 10 Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin Microbiol Rev* 2006;19:111–26.
- 11 Bern C, Adler-Moore J, Berenguer J *et al*. Liposomal amphotericin b for the treatment of visceral leishmaniasis. *Clin Infect Dis* 2006;43:917–24.
- 12 Sundar S, Chakravarty J, Agarwal D *et al*. Single-dose liposomal Amphotericin B for visceral leishmaniasis in India. *N Engl J Med* 2010;362:504–12.
- 13 Berman JD, Badaro R, Thakur CP *et al*. Efficacy and safety of liposomal amphotericin B (Ambisome) for visceral leishmaniasis in endemic developing countries. *Bull World Health Organ* 1998;76:25–32.
- 14 Sundar S, Jha TK, Thakur CP *et al*. Injectable paromomycin for visceral leishmaniasis in India. *N Engl J Med* 2007;356:2571–81.
- 15 Hailu A, Musa A, Wasunna M *et al*. Geographical variation in the response of visceral leishmaniasis to paromomycin in east africa: a multicentre, open-label, randomized trial. *PLoS Negl Trop Dis* 2010;4:e709.
- 16 Sundar S, Jha TK, Thakur CP *et al*. Oral miltefosine for Indian visceral leishmaniasis. *N Engl J Med* 2002;347:1739–46.
- 17 Sundar S, Sinha PK, Rai M *et al*. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet* 2011;377:477–86.
- 18 Gonzalez U, Pinart M, Rengifo-Pardo M *et al*. Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst Rev* 2009;CD004834.
- 19 Gonzalez U, Pinart M, Reveiz L *et al*. Interventions for old world cutaneous leishmaniasis. *Cochrane Database Syst Rev* 2008;CD005067.
- 20 Gonzalez U, Pinart M, Reveiz L *et al*. Designing and reporting clinical trials on treatments for cutaneous leishmaniasis. *Clin Infect Dis* 2010;51:409–19.
- 21 Ben Salah A, Buffet PA, Morizot G *et al*. WR279,396, a third generation aminoglycoside ointment for the treatment of leishmania major cutaneous leishmaniasis: a phase 2, randomized, double blind, placebo controlled study. *PLoS Negl Trop Dis* 2009;3:e432.
- 22 Lessa HA, Machado P, Lima F *et al*. Successful treatment of refractory mucosal leishmaniasis with pentoxifylline plus antimony. *Am J Trop Med Hyg* 2001;65:87–9.
- 23 Miranda-Verastegui C, Tulliano G, Gyorkos TW *et al*. First-line therapy for human cutaneous leishmaniasis in Peru using the tlr7 agonist imiquimod in combination with pentavalent antimony. *PLoS Negl Trop Dis* 2009;3:e491.
- 24 Barrett MP, Burchmore RJ, Stich A *et al*. The trypanosomiasis. *Lancet* 2003;362:1469–80.
- 25 Brun R, Blum J, Chappuis F *et al*. Human African trypanosomiasis. *Lancet* 2010;375:148–59.
- 26 Vanhollenbeke B, Pays E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Mol Microbiol* 2010;76:806–14.
- 27 Barrett MP, Boykin DW, Brun R *et al*. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br J Pharmacol* 2007;152:1155–71.
- 28 Chappuis F, Loutan L, Simarro P *et al*. Options for field diagnosis of human African trypanosomiasis. *Clin Microbiol Rev* 2005;18:133–46.
- 29 Paine MF, Wang MZ, Generaux CN *et al*. Diamidines for human African trypanosomiasis. *Curr Opin Investig Drugs* 2010;11:876–83.

- 30 Voogd TE, Vansterkenburg EL, Wilting J *et al.* Recent research on the biological activity of suramin. *Pharmacol Rev* 1993;45:177–203.
- 31 Alsford S, Eckert S, Baker N *et al.* High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature* 2012;482:232–6.
- 32 Burri C, Nkunku S, Merolle A *et al.* Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. *Lancet* 2000;355:1419–25.
- 33 Rodgers J, Jones A, Gibaud S *et al.* Melarsoprol cyclodextrin inclusion complexes as promising oral candidates for the treatment of human African trypanosomiasis. *PLoS Negl Trop Dis*. 2011;5:e1308.
- 34 Burri C, Brun R. Eflornithine for the treatment of human African trypanosomiasis. *Parasitol Res* 2003;90(Suppl. 1):S49–52.
- 35 Vincent IM, Creek D, Watson DG *et al.* A molecular mechanism for eflornithine resistance in African trypanosomes. *PLoS Pathog* 2010;6:e1001204.
- 36 Priotto G, Kasparian S, Mutombo W *et al.* Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. *Lancet* 2009;374:56–64.
- 37 Brun R, Don R, Jacobs RT *et al.* Development of novel drugs for human African trypanosomiasis. *Future Microbiol* 2011;6:677–91.
- 38 Torrele E, Bourdin Trunz B *et al.* Fexinidazole—a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS Negl Trop Dis* 2010;4:e923.
- 39 Jacobs RT, Nare B, Wring SA *et al.* SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. *PLoS Negl Trop Dis* 2011;5:e1151.
- 40 Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. *Lancet* 2010;375:1388–402.
- 41 Le Loup G, Pialoux G, Lescure FX. Update in treatment of Chagas disease. *Curr Opin Infect Dis* 2011;24:428–34.
- 42 Coura JR, Borges-Pereira J. Chronic phase of Chagas disease: why should it be treated? A comprehensive review. *Mem Inst Oswaldo Cruz* 2011;106:641–5.
- 43 Pinazo MJ, Espinosa G, Gállego M *et al.* Successful treatment with posaconazole of a patient with chronic Chagas disease and systemic lupus erythematosus. *Am J Trop Med Hyg* 2010;82:583–7.
- 44 McKerrow JH, Doyle PS, Engel JC *et al.* Two approaches to discovering and developing new drugs for Chagas disease. *Mem Inst Oswaldo Cruz* 2009;104(Suppl. 1):263–9.
- 45 Chatelain E, Ioset JR. Drug discovery and development for neglected diseases: the DNDi model. *Drug Des Dev Ther* 2011;5:175–81.