Review

A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis

Peter Babokhov[1], Adekunle O. Sanyaolu[2], Wellington A. Oyibo[3], Adetayo F. Fagbenro-Beyioku[3], Nnaemeka C. Iriemenam[3]

[1]Department of Biology, University of Massachusetts, Boston, MA, USA, [2]Saint James School of Medicine, Anguilla, BWI, [3]Department of Medical Microbiology and Parasitology, College of Medicine of the University of Lagos, Idi-araba, Lagos, Nigeria

Despite the recent advances in drug research, finding a safe, effective, and easy to use chemotherapy for human African trypanosomiasis (HAT) remains a challenging task. The four current anti-trypanosomiasis drugs have major disadvantages that limit more widespread use of these drugs in the endemic regions of sub-Saharan Africa. Pentamidine and suramin are limited by their effectiveness against the only first stage of *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, respectively. In addition, melarsoprol and eflornithine (two second stage drugs) each have disadvantages of their own. The former is toxic and has increasing treatment failures while the latter is expensive, laborious to administer, and lacks efficacy against *T. b. rhodesiense*. Furthermore, melarsoprol's toxicity and decreasing efficacy are glaring problems and phasing out the drug as a frontline treatment against *T. b. gambiense* is now possible with the emergence of competent, safe combination chemotherapies such as nifurtimox–eflornithine combination treatment (NECT). The future of eflornithine, on the other hand, is more promising. The drug is useful in the context of combination chemotherapy and potential orally administered analogues. Due to the limits of monotherapies, greater emphasis should be placed on the research and development of combination chemotherapies, based on the successful clinical tests with NECT and its current use as a frontline anti-trypanosomiasis treatment. This review discussed the current and future chemotherapy strategies for the treatment of HAT.

Keywords: Human African trypanosomiasis, Sub-Saharan Africa, Chemotherapy, Resistance, Control

Background on African Trypanosomiasis

Human African trypanosomiasis (HAT), commonly known as sleeping sickness is endemic to some regions of sub-Saharan Africa, covering 36 countries with about 70 millions of people at risk. It remains one of the most serious constraints to economic development in sub-Saharan Africa, impacting the health of the people as well as their domestic livestock.[1–7] In addition, the risk of HAT has now extended to non-disease endemic countries through travel and migration. Between 2000 and 2010, 94 cases of HAT were reported in 19 non-disease endemic countries and 72% were the Rhodesiense form while 28% were the Gambiense.[7] However, after continued control efforts, the total numbers of reported cases between 2000 and 2009 have dropped to 175 572 cases in 19 countries, with the number of reported cases decreasing to less than 10 000 in 2009 alone. This downward trend continued in 2011, with 6743 reported cases, reducing the impact of HAT to the lowest in 50 years. Sleeping sickness is a tropical disease caused by two subspecies of the eukaryotic parasite *Trypanosoma brucei*. The first subspecies, *Trypanosoma brucei gambiense*, accounts for 98% of all reported cases of sleeping sickness and causes a chronic infection while *Trypanosoma brucei rhodesiense* accounts for 2% of all reported cases and causes acute infection. This obligate parasite uses the tsetse fly (*Glossina* spp.) as its vector, and it is injected into the mammalian bloodstream after an infected tsetse fly takes a blood meal. Other means of transmitting the disease include mother-to-child infections and accidental infections in the laboratory.

Even though the number of new cases has decreased below 10 000 during the latter half of the last decade, HAT continues to be a considerable challenge to scientists. The disease still affected an

Correspondence to: Peter Babokhov, Department of Biology, University of Massachusetts, 100 William T Morrissey Blvd, Boston, MA 02125, USA. Email: pbabokhov@gmail.com

DOI 10.1179/2047773213Y.0000000105

estimated 50 000–70 000 people annually in sub-Saharan Africa as late as 2005–2006, and killed an estimated 60 000 people a year. The largest numbers of recent HAT cases have been reported in endemic regions in Sudan, Chad, Angola, with the Central African Republic and the Democratic Republic of Congo being the two most affected countries. Human African trypanosomiasis is primarily endemic in the rural areas, where infrastructure is lacking and there is little access to health services. This means that the actual number of cases could be higher than the current reported cases.

There are two variants of HAT, West African trypanosomiasis and East African trypanosomiasis. West African trypanosomiasis, the primary variant of HAT, is caused by the subspecies *T. b. gambiense*. This disease is found in West and Central Africa and is responsible for 98% of all reported trypanosomiasis cases, with the number of cases decreasing from 26 117 in 2001 to 6631 in 2011. Nevertheless, 82.2% of the population in endemic regions remains at risk for the disease. West African trypanosomiasis causes a chronic infection, akin to an incubation period, in affected patients. The initial symptoms of infected individuals tend to be similar to non-fatal diseases. The major symptoms of West African trypanosomiasis appear months or years after initial infection. By that point, the infection has already reached an advanced stage and the parasite has crossed the blood–brain barrier and entered the central nervous system (CNS). According to Pepin and Khonde,[8] the length of the infection is the reason West African trypanosomiasis is able to create epidemics in areas that it thrives. Additionally, the length of the infection ensures that the disease is able to spread to previously unaffected areas due to mass movements of infected individuals.[9] East African trypanosomiasis is caused by the subspecies, *T. b. rhodesiense* and it occurs primarily in southern and eastern Africa. This variant of sleeping sickness is responsible for about 2% of all reported trypanosomiasis cases. As with West African trypanosomiasis, reported cases of East African trypanosomiasis reduced from 755 in 2001, to 112 in 2011. A majority of *T. b. rhodesiense* infections, i.e. 72% of all reported cases, occurred outside endemic regions. Additionally, most of these cases were a result of stage I infection.[7] Overall, 17.8% of the population in sub-Saharan Africa remains at risk of *T. b. rhodesiense*.[7] East African trypanosomiasis causes an acute infection in affected patients. Infected patients are symptomatic as early as a few weeks after initial infection and the parasite rapidly crosses the blood–brain barrier into the CNS.[6]

Pathogenesis

The clinical course of HAT has two stages, a first or early stage in which the parasite is found in the peripheral circulation but has not yet invaded the CNS and a second or late stage during which the parasite crosses the blood–brain barrier and infects the CNS. The first stage involves eliciting immune response to the pathogen. There are continuous waves of parasitaemia, with some parasites surviving and evading the subsequent immune response.[10] Trypanosomes are able to evade immune response through a process called antigenic variation. During this process, the trypanosome switches its variable surface glycoprotein (VSG) coat to a new VSG coat that is not recognized by the host immune system. This action is continuous and thus exhausts the host immune defenses. The process of evasion also includes endocytosis of VSG-antibody complexes, allowing them to escape detection by the antibodies responsible for complement-mediated killing.[11] Antigenic variation prolongs the time that trypanosomes spend within the host, thus allowing for further proliferation and transmission to other hosts via the tsetse vector. Lastly, this process allows trypanosomes to infect hosts that already recognize VSGs from prior infections.[12] The symptoms of the first stage include fever, headaches, pain in the joints and, in 19% of cases, irritation of the skin at the site of infection. Fever is the most significant symptom of this stage, lasting from a day to about a week, with intervals between episodes ranging from days to about a month. The fever episodes are related to the waves of parasitaemia and the continuous type I and type II immune responses.[13] The second stage of a trypanosomal infection, known as the meningoencephalitic stage, involves the parasite breaching the blood–brain barrier, entering the CNS and settling in the cerebrospinal fluid (CSF). The symptoms of the second stage infection include confusion and poor coordination, tremors, general motor weaknesses, irritability and aggressive behaviour. The most important symptom of the second stage infection is the disruption of body's natural circadian sleep/wake rhythm, resulting in irregular and fragmented sleeping patterns. This is what gives the disease the name 'sleeping sickness'. If left untreated or treated inadequately, HAT infections result in death unless treatment is applied at the beginning or early stages of the disease. The subspecies of *T. brucei* determines whether a trypanosomiasis infection is acute or chronic in nature.

Overview of the Current Anti-HAT Drugs

The biggest obstacle in the complete treatment of HAT is that there is still no effective vaccine against both human pathogenic subspecies. Creating any sort of vaccine against trypanosomes is difficult due to antigenic variation. The constant changes of the VSG coat allow the parasite to evade immune defences. Thus, the only viable anti-trypanosomiasis measures available are chemotherapies.[14–16] The available

drugs have limited effectiveness, are complicated to administer (especially during the second stage of the disease), and can cause severe adverse reactions. Until recently, there have been only four drugs utilized as HAT treatments: pentamidine, suramin, melarsoprol, and eflornithine (Table 1). The utilization of these drugs depends on the disease stage and causative pathogen.[13] Additionally, the oral drug nifurtimox, usually used against 'Chagas diseases', has been utilized as off-label compassionate treatment for those infected with Western African trypanosomiasis. Each of these drugs has a number of disadvantages with respect to such issues as toxicity, resistance and the aforementioned logistical problems. The combination therapy involving the oral drug nifurtimox and eflornithine has been the most recent breakthrough in anti-trypanosomiasis drug research (Table 1). This therapy, known as nifurtimox–eflornithine combination treatment (NECT), was added to World Health Organisation's list of essential medicines in 2009. Since 2010, NECT has been used to treat 59% of all reported *T. b. gambiense* cases. Treatment with NECT has been shown to be non-inferior against *T. b. gambiense* cases when compared to treatment with eflornithine alone. In addition, it is less costly and time-intensive than the latter monotherapy.

Pentamidine: Drug used against Early Stage *T. b. gambiense* Infections

Pentamidine isethionate, more commonly known as pentamidine, is a positively-charged aromatic diamidine compound that is effective against the first stage of *T. b. gambiense* infections. The origins of pentamidine stem from the diamidine synthalin, which had trypanocidal properties among animals. In 1937, the British chemist Arthur James Ewins, who worked for May and Baker, synthesised a number of related compounds, where the polar amidine groups were separated by aromatic groups. Two particular compounds, stilbamidine and pentamidine, had high trypanocidal activity. While stilbamidine was eventually abandoned due to neurological issues, pentamidine is still used today.[17] Pentamidine was first used in 1940 to treat cases of West African trypanosomiasis.

The standard treatment regimen with pentamidine involves intramuscular injections of 4 mg/kg every day for a total of 7–10 injections. Pentamidine's charged diamidine groups ensure a high affinity towards plasma proteins and has a long half-life, with the range of 22–47 hours. The drug is used to treat the initial stages of the infection caused by the *T. b. gambiense* parasite due to its selective toxicity for trypanosome parasites, accumulating in these organisms, while generally avoiding the host cells.[18] While pentamidine is relatively well tolerated by most recipients of the drug, there are reported side effects including hypotension, hypoglycaemia, and nephrotoxicity. There have also been rarer and more severe side effects reported such as highly abnormal pancreatic and hepatic functions, and cases of the potentially fatal Stevens–Johnson syndrome. The occurrence and severity of these side effects is largely dependent upon the dose of pentamidine given, usually any dose above the standard 4 mg/kg per day. Additionally, any allergic reaction against the drug also tends to increase the severity of side effects. One of the biggest advantages of pentamidine is that there has been no reported trypanosomal drug resistance in the field.[19]

Pentamidine has a number of disadvantages. One is that it is not as effective against *T. b. rhodesiense* as it is against *T. b. gambiense* and it is completely ineffective against stage II African trypanosomiasis. This is due

Table 1 Five current anti-human African trypanosomiasis (HAT) chemotherapies

Drugs	Mechanism	Advantages	Disadvantages
Pentamidine (pentamidine Isethionate)	Accumulates in trypanosomes; disrupts mitochondrial processes	Effective against stage I *Trypanosoma brucei gambiense*	Ineffective against stage II *T. b. gambiense* and both stages of *Trypanosoma brucei rhodesiense*
Suramin* (Bayer 205, Germanin)	Binds to enzymes in the glycosome; disrupts glycolysis	Effective against stage I *T. b. rhodesiense*	Ineffective against stage II *T. b. rhodesiense* and stage II *T. b. gambiense*
Melarsoprol (Mel B)	Disrupts trypanosomal redox metabolism and glycolysis	Effective against both subspecies at both stages	Toxic; around 5% of patients die as a result of post-treatment reactive encephalopathy (PTRE); trypanosomal resistance reported to be as high as 30%
Eflornithine (difluoromethylornithine)	Irreversibly inhibits ODC; disruption of proliferation and vulnerability to oxidative attack	Effective against stage II *T. b. gambiense*	Ineffective against both stages of *T. b. rhodesiense*; treatment is time-consuming
NECT (nifurtimox–eflornithine combination treatment)	Eflornithine inhibits ODC; nifurtimox induces oxidative attack upon weakened trypanosomes	High cure rate for both stages of *T. b. gambiense*; low rate of adverse effects; no death rates	Potential for resistance to the treatment in the field

*Suramin is also effective against stage I *T. b. gambiense*, but its use remains confined to infections causes by stage I *T. b. rhodesiense*.

to pentamidine's inability to cross the blood–brain barrier at an efficient rate and thus, making the concentrations of pentamidine not sufficient enough to affect the trypanosomes located in the CNS. One possible explanation is that the cationic diamidine groups on pentamidine bind to the membranes in the capillary endothelium, thus preventing sufficient amounts of the drug from crossing the barrier.[20] Theoretically, one potential way of getting pentamidine past the blood–brain barrier is by coupling it to either a P-glycoprotein inhibitor or to a multidrug resistance-associated protein (MRP) inhibitor, as long as such inhibitor is specific for a single transporter.[20] Unfortunately, this theory has not been tested experimentally.

Suramin and its Effectiveness against Early Stage *T. b. rhodesiense* Infections

Suramin, also known as Bayer 205, is a drug that is utilized against the first stage of *T. b. rhodesiense* infection. Suramin, a polysulphonated naphthylamine-based compound is derived from naphthylamine dyes such as trypan red and trypan blue. The origins of suramin began in 1906 when the Bayer pharmaceutical company of Germany donated a number of benzopurpurine dyes to the Pasteur Institute. Their aim was to see if any 31 of these dyes contained trypanocidal properties.[17] Paul Ehrlich, a major investigator within the synthetic dyestuff industry, described a compound called Trypan blue, which was shown to have trypanocidal properties, but was a coloured dye and thus unacceptable for use in humans. One of Ehrlich's colleagues, Wilhelm Roehl, decided to synthesize a naphthalene derivative that was completely colourless. In 1917, after the testing of a large number of compounds for both trypanocidal properties and lack of colour, a compound named Bayer 205 was shown to cure trypanosomiasis in both animal and human subjects. The new molecule was first renamed Germanin and subsequently given its present name, suramin. Suramin was first used to treat trypanosomiasis cases in the field in 1922.

The common treatment regimen with suramin involves five intravenous injections of 20 mg/kg of the drug, every 3–7 days over a total period of 4 weeks. Suramin stays in the body for weeks at a time due to its long half-life of around 44–54 days and 99.7% affinity for proteins in the serum. Currently, intravenous injection is the only effective way of getting suramin into the body, as well as being the most pleasant way for the patients. Orally administered suramin is poorly absorbed by the intestines and intramuscular administration of the drug leads to local irritation at the site of injection.[15] The main side effects of suramin include fatigue, neuropathy, renal problems, anaemia, nausea, and anaphylactic shock. Since these side effects primarily occur at higher concentrations, the rate of suramin introduced into the bloodstream should be kept at no more than 1 g per injection. Suramin is primarily effective against the bloodstream forms of *T. brucei* and is much less active in the presence of procyclic forms. The drug acts upon *T. b. rhodesiense* by inhibiting the glycosomal enzymes involved in glycolysis, the bloodstream form's only source of energy. This inhibition happens at a slow and gradual rate and it is even possible that suramin might affect newly-synthesized glycolytic enzymes within the cytosol before they are imported into the glycosome, due to the drug having direct access to those enzymes. Other trypanosomal enzymes inhibited by suramin are enzymes of the pentose phosphate pathway, such as 6-phosphogluconate dehydrogenase. Currently, it is not known whether suramin has an inhibitory action upon multiple enzymes or if it is limited to just one of the pathways mentioned. Suramin most likely enters trypanosomes by way of receptor-mediated endocytosis. One proposed example is that suramin binds with high affinity to serum proteins such as low-density lipoprotein (LDL) and utilizes this binding to enter *T. b. rhodesiense*'s bloodstream form. Unfortunately, this hypothesis has not yet been successfully tested and an alternative hypothesis has suggested that endocytosis could proceed without the need for any specific receptor.

The main advantages of suramin are that it is highly effective against *T. b. rhodesiense* and there have been no drug resistances reported in the field. The lack of resistance could be in part due to suramin's inhibition of multiple enzymes and metabolic pathways. Another advantage of suramin is that it shows a reasonable level of synergism for the second stage drugs such as eflornithine, nifurtimox and debatably, melarsoprol thus, making suramin very useful as a pre-treatment drug, clearing the body of haemolymphatic *T. b. gambiense* and *T. b. rhodesiense* forms, in preparation for the treatment with stage II drugs. Additionally, studies in murine models induced with second stage HAT, showed that suramin co-administered with CNS drugs resulted in higher cure rates in mice. One hypothesis states that these high cure rates were in part due to suramin's inhibition of the P-glycoprotein, thus preventing the expulsion of stage II drugs from the CNS.[21] The main disadvantage of suramin is that it is only effective against the first stage of trypanosomiasis. While suramin also has efficacy against *T. b. gambiense*, there were reports of treatment failures in some foci during the 1950s. Additionally, pentamidine is preferable for use against West African trypanosomiasis because of the ease of administration due to the relatively quick intramuscular injection.[15] Thus suramin remains confined to the treatment of trypanosomiasis caused by *T. b. rhodesiense*. The main reason for suramin's ineffectiveness against the second stage of

HAT is the drug's inability to breach the blood–brain barrier, which is likely due to the large size of the molecule, the presence of a number of tight junctions near the endothelial membrane and the lack of transport vesicles that could otherwise facilitate entry into the CNS and CSF. This could account for the low concentrations of suramin in the CSF ($\sim$1%) when compared to the serum. Another disadvantage of suramin is that combination chemotherapy involving the drug has not been successfully implemented. Suramin and pentamidine combination chemotherapy has not worked as the former drug inhibits the actions of the latter. Despite its ineffectiveness against stage II HAT, suramin continues to be an invaluable part of the chemotherapy repertoire available to healthcare workers in endemic regions due to its action against stage I *T. b. rhodesiense* infections and its synergism with a number of the second stage HAT drugs.

Melarsoprol: Drug used against Second Stage HAT Infections

The organic arsenical drug melarsoprol, introduced in 1949, was the most commonly used treatment against HAT until the introduction of the nifurtimox–eflornithine combination treatment in 2009. This drug was originally derived from the melamine arsenical melarsen. Melarsen was first synthesized in 1938 by Dr Ernst A. H. Friedheim, a pathologist, microbiologist, and chemist from Zurich, Switzerland. In 1939, following further studies of the compound's effects on trypanosomes, Friedheim synthesized an analogue to melarsen called melarsen oxide, a trivalent compound with an arsenical group devoid of both hydroxides. This synthesis was based on the premise that melarsen oxide was a more potent trypanocide than the pentavalent compound it was derived from.[17] The unfortunate side effect of additional potency was higher toxicity. In order to reduce the toxic effects of melarsen oxide, Friedheim added a disulphide chelating agent called dimercaprol or British 'anti-Lewisite' (BAL) in an attempt to negate some of the effects of the arsenic group. This finalized compound, dubbed melarsoprol, was less toxic than melarsen oxide and still potent trypanocide.

Treatment of HAT with melarsoprol consists of intravenous injection of a 3.6% solution dissolved in propylene glycol inside a 5 ml ampoule. There are a number of different regimens used for the treatment with melarsoprol. Two examples of such a regimen are published earlier.[22] One variant involves a 1.2 mg/kg injection on day 1, followed by a 2.4 mg/kg injection on day 2, and 3.6 mg/kg on days 3 and 4. Another version calls for 3 days of injection with 3.6 mg/kg of melarsoprol per day. Both of these involve a 7–10-day rest period between each day of injection. These regimens are utilized to treat infection caused by *T. b. rhodesiense*. Recently, the ten-day regimen has become the more widely used treatment protocol. This regimen consists of injections of approximately 2.2 mg/kg of melarsoprol per day over a period of 10 days. It was shown to be more effective against trypanosomes than the older regimens, resulting in a cure rate of 93.9% initially, and 86.2%, 2 years post-treatment. Additionally, this regimen reduced the cost, drug dosage, treatment time, and increased the chances of post-treatment follow-ups.[23] This allows for faster and more efficient treatment, especially in the regions of sub-Saharan Africa where HAT is the most endemic and where resources are scarce. More importantly, this treatment regimen has been shown to be very effective against *T. b. rhodesiense* infections, with cure rates as high as 96% 1 year after treatment.[24] For either regimen, if there is any treatment relapse (a recurrence in HAT between the time of treatment and the follow-up period) then that specific treatment is counted as a failure. The specific trypanocidal mechanisms of melarsoprol are currently unknown, but the effects of the drug upon trypanosomes have been observed. Parasites exposed to even a low dose of melarsoprol lyse rapidly. Scientists postulate that this lysis occurs due to interruption of glycolysis and the redox metabolism of trypanosomes.

There are two main advantages of using melarsoprol as an anti-trypanosomiasis chemotherapy. The first advantage is that the drug is capable of being used against the second stage of HAT, when trypanosomes have moved past the blood–brain barrier and infected the CNS. The second and perhaps the most important advantage of melarsoprol is that it can be used against both the *T. b. gambiense* and *T. b. rhodesiense* subspecies. In fact, it is the only agent that can be used against the second stage East African trypanosomiasis. Melarsoprol is highly toxic due to the presence of the arsenic group. Around 5–10% of patients treated experience post-treatment reactive encephalopathy (PTRE), with seizures, high fever, headaches, nausea, vomiting and dizziness. Additionally, up to 50% of those affected by PTRE end up dying within 48 hours of being treated. Death rates from PTRE are similar whether the standard treatment regimen or the ten-day treatment regimen is used.[22] The exact causes of PTRE are currently unknown, but one possible explanation is that the propylene glycol found in the treatment mixture is responsible for the complications.[25] Another possible culprit is the dimercaprol group that makes up melarsoprol's molecular structure.[26] Possible treatments for people suffering from PTRE have included corticosteroids (such as prednisolone), eflornithine, substance P agonists, and immunosuppressant azathioprine. These treatments either reduce the severity of encephalopathy, or they act to prevent it.[27–30] If patients make successful recovery from PTRE, then melarsoprol

treatment has to be started with a smaller initial dose than the previous session.[27] Another significant disadvantage of melarsoprol is the increasing resistance of trypanosomes towards the drug. In recent years, the rates of treatment failure due to trypanosomal relapse have ranged from about 10% to about 30% in some endemic regions within Angola, the DRC, Sudan, and Uganda. Owing to these disadvantages, the use of melarsoprol as a frontline treatment has decreased by 57% in 2009. In 2010, only 12% of all stage II *T. b. gambiense* cases were treated with melarsoprol, a historical low for the six-decade old drug. The exact mechanism of trypanosomal resistance against melarsoprol is currently unknown but could be due to loss or modification of the P2/TbAT1 nucleoside transporter. This transporter is the primary means of melarsoprol entry into the trypanosome. Additionally, the loss or modification of the transporter implicated in trypanosomal resistance to pentamidine, HAPT1, also confer cross resistance to melarsoprol. Previous findings indicated that when trypanosomes had both P2/TbAT1 transporter and the HAPT1 knocked out, they displayed high levels of resistance to melarsoprol. However, if only one of the two pathways is disabled, the resistance does not change significantly. The evidence presented lent credence to the theory that melarsoprol enters trypanosomes via more than one pathway.[15] The most recent research identified a loss-of-function mutation in aquaglyceroporin 2 (AQP2), a membrane channel protein involved in drug uptake and accumulation, as a possible cause of melarsoprol resistance detected in trypanosomes.[31]

Eflornithine and its Effects on HAT

The drug alpha-Difluoromethylornithine (DFMO), more commonly known as eflornithine, is the newest of the four anti-trypanosomiasis monotherapies. Initially, it was used to treat patients with *T. brucei* strains that were refractory for melarsoprol as they were detected in the blood during the post-treatment follow-up. Eflornithine is primarily effective against the first and second stages of trypanosomal infections caused by *T. b. gambiense*. Conversely, it is not effective against infections caused by *T. b. rhodesiense*. Eflornithine underwent studies during the 1970s as a potential chemotherapeutic agent against cancer. The drug is involved in the inhibition of ornithine decarboxylase (ODC), an enzyme responsible for the synthesis of polyamines, a process necessary for facilitating cell division and proliferation. As recently as 2007, the drug was still not registered as an anti-cancer agent,[15] as a result of having little effect against cancerous cells due to the rapid turnover rate of the ODC found in human cells (t1/2 of human ODC=10–30 minutes).

The anti-trypanosomiasis properties of eflornithine were first observed by biologist Cyrus Bacchi in 1980.[17] It was thought that if this drug was potent against cancer cells, perhaps the inhibition of ODC activity could have a deleterious effect upon trypanosomes. Bacchi showed that eflornithine cured mice infected by *T. b. brucei*, and no apparent signs of toxicity or other deleterious side effects were present. After a decade of clinical trials, DFMO/eflornithine was approved for use against *T. b. gambiense* in 1990. The standard dose regimen for eflornithine involves 56 intravenous infusions at 100 mg/kg (150 mg/kg for children) every 6 hours a day for a total of 14 days. An alternative regimen that was studied called for 28 intravenous infusions at 100 mg/kg every 6 hours a day for a total of 7 days. In addition, there have been clinical trials with orally administered eflornithine. Ultimately, both the short course intravenous infusion regimen and oral administration were shown to have low efficacy against trypanosomiasis. While the short course eflornithine regimen had potential to be used as an alternative treatment in the event of melarsoprol relapse, the introduction of nifurtimox–eflornithine combination treatment (NECT) in 2009 rendered this regimen unnecessary.

Eflornithine has a trypanostatic effect upon *T. b. gambiense* parasites, irreversibly inhibiting trypanosome's ODC functions, by binding to the catalytic site on the enzyme. This results in a depletion of essential polyamines such as the putrescine and spermidine, and contributes to reduced trypanosomal proliferation by blocking the ability of the bloodstream forms to divide through binary fission. The damage to trypanosomes is exacerbated by their inability to replace any polyamines that they lose. Eventually, the trypanosomes are transformed into the short stumpy forms, which can be cleared by the body's immune defences. In addition to a shutdown of cell proliferation, levels of S-adenosyl methionine are increased within the trypanosome, disturbing proper methylation of proteins, nucleic acids and lipids. Eflornithine is especially effective against *T. b. gambiense* due to a slower ODC turnover rate when compared to the mammalian counterpart (18–19 hours compared to 10–30 minutes, respectively). Conversely, eflornithine is not very effective against *T. b. rhodesiense*. The most likely reason for this ineffectiveness is that this subspecies has a very rapid ODC turnover rate of about 4.3 hours, thus making eflornithine less able to inhibit ODC activity.

The biggest advantage of eflornithine is its greater effectiveness against *T. b. gambiense* when compared to melarsoprol. This increase in efficacy is coupled with a dramatically lower fatality rate of 1.4% versus up to 5% for melarsoprol.[32] A recent study on drug efficacy performed by Balasegaram *et al.*[22] in Uganda, Sudan, Angola, and the DRC showed that the highest average cure rates for eflornithine was 94% compared

to highest average melarsoprol cure rates of 84% for the standard course regimen and around 83.5% for the short course regimen. The combination of high initial cure rates, high cure rates after 1 year of follow-up and low fatality rates provide support for more frequent use of eflornithine in the field. The common side effects of eflornithine, such as diarrhoea, dizziness, headaches, and seizures are usually reversed by administering slightly lower doses of the drug. Additional side effects include anaemia, leukopaenia, and thrombocytopaenia, similar to other chemotherapies with anticancer origins.[26]

Eflornithine's main disadvantage is that the treatment is time-consuming and complex to administer. The mean half-life of eflornithine is between 1.5 and 5 hours and 80% of it is excreted in the urine in its unaltered form 24 hours post-injection. Additionally, there is little protein binding to eflornithine in the plasma indicating that prolonged intravenous infusion is needed to keep the drug actively working during the treatment process. Since both the short course treatment and oral application have little efficacy, the 14-day slow infusion is the only effective way to administer eflornithine. This decreases the overall efficiency of the treatment and increases the overall costs and labour; cost partially offset by the free donations of eflornithine by Sanofi.[33] Nevertheless, transportation and setting up of the treatment kits is costly, requiring specific training to administer the infusions, and each kit is cumbersome and expensive. A single eflornithine kit weighs 40 kg, has a volume of 190 dm^3, allows for two treatments and costs an estimated €554 for a single treatment.[33] This ultimately means that the use of eflornithine in regions that are resource poor or where medical infrastructure is non-existent, presents a logistical challenge. Since a successful eflornithine treatment session needs a functional immune system in order to clear out trypanosomes weakened by the drug, immunodeficiency diseases such as HIV/AIDS decrease the drug's cure rate. The second most significant disadvantage facing eflornithine is its ineffectiveness against *T. b. rhodesiense* due to the aforementioned rapid ODC turnover rate. Another additional disadvantage of eflornithine is that its penetration of the blood–brain barrier is more limited than previously thought. According to Sanderson *et al.*,[34] eflornithine was not able to cross the blood–brain barrier of a healthy mouse and that eflornithine concentrations were able to increase when suramin was co-administered. The relatively limited blood–brain barrier penetration could be another reason why eflornithine needs to be given as a prolonged 14-day intravenous infusion.[34] It could also help to explain its effectiveness as a secondary drug for melarsoprol-refractory patients,[1] as recurring trypanosome infections weaken the integrity of the blood–brain barrier.

Nifurtimox, an Oral Anti-Trypanosomiasis Drug

Nifurtimox, also known as Lampit, is an orally administered trypanocide derived from nitrofuran. The drug was first developed in 1960 by the Bayer Company and was first used in 1967 as a treatment for Chagas disease, an ailment caused by *T. cruzi*, a trypanosome species native to the Americas. The drug underwent clinical trials during the 1970s and 1980s, but because these tests were performed using differing drug regimens, the results were irregular and difficult to compare. Nifurtimox was prescribed off-label in compassionate use against melarsoprol-refractory *T. b. gambiense* infections. Nifurtimox has been shown to have efficacy for both the first stage and second stage infective variants of *T. b. gambiense*. Conversely, the levels of efficacy that nifurtimox has upon *T. b. rhodesiense* are considerably lower.[35] Other derivatives of nitrofuran have also been tested for efficacy against HAT. One such drug, nitrofurazone, entered trials during the 1960s and 1970s, but was found to be too toxic and future development was cancelled.

The mechanism of nifurtimox is unknown, but is thought to rely on generation of free radicals as a by-product of nitrofuran reduction. These free radicals then interact with the trypanosomal membrane and the associated DNA and proteins. This mode of action attacks processes such as the trypanosomal redox metabolism, creating reduced oxygen metabolites. Another mode of action for nifurtimox involves the type I nitroreductase (NTR) pathway, which reduces nifurtimox into open-chain nitrile products with cytotoxic properties.[36] The drug has limited efficacy against stage II HAT, with cure rates ranging from around 50–80%. This could be due to the lower concentration of the drug within the blood–brain barrier when compared to the plasma concentrations. Experiments from the 1990s and the experiments performed by Bisser *et al.*[37] showed that administration of nifurtimox as a monotherapy resulted in high treatment failure rates. Additionally, prolonged administration has the tendency to induce adverse neurological and gastrointestinal reactions in patients. Resistance to the drug by *T. brucei* is most likely due to point-mutation of trypanosomal NTR, which then decreases the processing of nifurtimox and other nitro-based drugs within cells.

NECT: Nifurtimox–Eflornithine Combination Treatment

The most recent chemotherapy against *T. b. gambiense* infection is NECT. Nifurtimox was already shown to have a level of synergism with melarsoprol and the combination therapy was also shown to be

more effective at treating trypanosomiasis, with little or no relapses when compared to nifurtimox and melarsoprol monotherapies. One major concern about the utilization of the nifurtimox–melarsoprol combination therapy has been its safety. There are few published data about this combination therapy, aside from few experiments[37–39] and a review.[40] The apparent lack of more published experimental data may be one of the reasons why the safety of the nifurtimox–melarsoprol combination therapy was brought into question. In the experiments performed by Priotto and colleague, a combination of nifurtimox and eflornithine as well as a nifurtimox–melarsoprol combination underwent a randomized clinical trial, along with an additional combination therapy, consisting of melarsoprol and eflornithine. The data from this trial showed that the nifurtimox–eflornithine combination therapy was superior to the nifurtimox–melarsoprol combination therapy in both effectiveness and safety. This report paved the way for future tests involving a case series in Uganda,[41] a phase III randomized clinical trial in the Republic of Congo[42] and most recently, a phase III non-inferiority trial, comparing NECT to a standard eflornithine monotherapy regimen. All three experimental trials were part of a wider neglected tropical diseases control programme initiated and funded by Medecins sans Frontieres.[43] The experiments were subsequently followed by a report that proposed adding NECT to the World Health Organization's list of essential medicines for treatment of neglected tropical diseases.[44]

Shortly after its inclusion, NECT was distributed amongst the various NSSCPs in the countries endemic for *T. b. gambiense*. The treatment regimen of NECT involves three daily oral doses of nifurtimox for a total of 10 days and 14 infusions of eflornithine for a total of 7 days. This was significantly lower than the 56 doses over 14 days required for the eflornithine treatment. Medical personnel previously trained by eflornithine implementation programmes could utilize the combination treatment much faster and more efficiently. Last, the reduced doses of each drug meant that more amount of the drug could be transported at a lower cost, when compared to eflornithine alone. A single NECT kit weighs 30 kg, has a volume of 100 dm^3, allows for four treatments and costs an estimated €288 for a single treatment, a major improvement over eflornithine (Table 2). Taken together, these advantages have made NECT the main frontline drug against stage II *T. b. gambiense*, accounting for 59% of all cases treated in 2010.[33] The main drawback of treatment with NECT is that it remains relatively labour-intensive and logistically complicated to implement. The aforementioned dosing regimen requires a minimum of four nurses to give the eflornithine infusions to the patient. Additionally, a doctor has to be there to prescribe the therapy in the first place and to monitor the patient for any adverse reactions.[9] The need for the medical personnel to have specific training in handling eflornithine is also a factor. There are side effects such as vomiting, nausea, headaches, abdominal pain, joint pains, seizures, and insomnia. Fortunately, the side effects are less severe than the previous drugs. The cost per single treatment rose from €288–336 in 2010.[33] These reasons could potentially make the use of NECT unsustainable in the long term (Table 2) coupled with the relatively easy selection of NECT resistance in the laboratory.[45] Thus newer therapies against stage II *T. b. gambiense* infection are required. These drugs must provide treatment that is safe, cheap, and simple to administer.

New Drug Candidates

One of the novel drug candidates is the nitroimidazole analogue fexinidazole. This compound was rediscovered by the drugs for neglected diseases *initiative* (DND*i*) during a screening campaign of nitroimidazoles. Both the 2-substituted 5-nitroimidazole, and its principal metabolites (fexinidazole sulphoxide and fexinidazole sulphone) have been characterized and shown to have potential for effective oral treatment against both stages of the *T. b. gambiense* and *T. b. rhodesiense* infections. In 2011, fexinidazole underwent phase I clinical trials and the dosage choice was selected. According to the trial, the drug is to be taken daily with food for a period of 10 days, with 1800 mg/day for 4 days and then 1200 mg/day for 6 days. Fexinidazole is currently undergoing preparations for phase II/III trials, following an ethics review conducted in Paris in February 2012 and clinical trials began in mid-2012. Another family of nitroimidazoles, known as 1-aryl-4-nitro-1H-imidazoles, is also seen as a promising drug candidate for the treatment of HAT.[46] The two specific compounds in question are (trimethoxy)-phenyl-based and chlorophenyl-based aryl nitroimidazoles. In murine models, these compounds

Table 2 Nifurtimox–eflornithine combination treatment (NECT) and eflornithine monotherapy comparison

	NECT	Eflornithine monotherapy
Mass of a single kit	38 kg	40 kg
Volume of a single kit	110 dm^3	190 dm^3
Number of patients that a single kit can treat	4	2
Estimated cost per single treatment (2010 prices)	€288	€554

Adapted from Ref. 33.

were able to cure both, the chronic infection at a dose 50–100 mg/kg for 5 days, and the acute infection at a dose of 25–50 mg/kg for 4 days.[46] In addition to their observed effectiveness against HAT, 1-aryl-4-nitro-1H-imidazoles lack the genotoxicity against mammalian cells that was observed in megazol[47] due to a lack of activity against strains lacking NTRs specific to bacteria. This makes 1-aryl-4-nitro-1H-imidazoles potential drug candidates for stage II HAT.[46]

Oxaborole SCYX-7158, an orally active benzoxaborole, is another new effective, safe, and orally active treatment for HAT. The compound was identified via a whole-cell assay and it was confirmed to have efficacy against stage II HAT, clearing the CNS of mice administered with 25 mg/kg of the drug per day for a total of 7 days.[48] Additionally, the biological and pharmacokinetic properties suggest that SCYX-7158 will be safe and efficacious to treat stage II HAT. Pre-clinical studies with SCYX-7158 were completed at the end of 2011[49] and the drug entered phase I clinical trials in March of 2012.[50]

Another promising series of new drugs have been the diamidine analogues of pentamidine, first developed by the consortium of parasitic drug development (CPDD).[51] The first series of drugs that underwent studies were DB75, also known as furamidine and its prodrug, DB289, also known as pafuramidine maleate. Furamidine was shown to have efficacy for HAT in 1977 by Das and Boykin, but was not shown to be superior to pentamidine. As DB75 was poorly absorbed by the gastrointestinal tract due to the presence of positively-charged diamidine groups, DB289, a drug that could be administered orally, was synthesized. This drug became the first orally administered treatment to undergo clinical trials, leading up to a phase III trial for potential use against second stage HAT infection. This clinical trial took place between 2005 and 2008, with 273 patients enrolled. After the success of this trial, an extended phase I safety assessment was undertaken in Africa. The results of the assessment showed that the drug caused severe liver toxicity and additionally, delayed renal insufficiency, similar to pentamidine. Owing to these problems, the development of DB289 as an anti-trypanosomiasis treatment was discontinued. Although DB289 has been withdrawn due to high toxicity, another set of diamidine analogues have recently been developed by CPDD. CPD0802 (DB829) and its prodrug, DB868 are more promising. In animal studies, DB868 and DB829 were shown to have a significant potency against *T. brucei*, but also without the liver toxicity and renal insufficiency that was displayed with DB289. This has made the DB829/DB868 diamidine series a potential candidate for clinical trials against stage II HAT.

There have also been a number of other compounds that have trypanocidal potential, but have not undergone full drug trials. One such therapy involves the use of DNA topoisomerase inhibitors, which are often utilized in anti-cancer therapies.[52–55] Another compound with trypanosomal potential is Genz-644131, an inhibitor of AdoMetDC in trypanosomes.[48] Experiments with this drug in mice provided evidence of significant brain penetration and complete cure of HAT. Lodamine is a known oral anti-cancer agent and has the ability to inhibit *T. brucei* Hexokinase 1 (TbHK1), an enzyme that catalyses the first step of the glycolysis process, and thus is essential for the trypanosome's survival.[48] Another pair of compounds that has the potential to be effective against the second stage of HAT is cordycepin and deoxycoformycin. A combination therapy involving this pair of compounds began to undergo pre-clinical trials in 2009. Since then, there has been no news about this therapy, leaving its potential effectiveness against HAT in the field in question. While there are a number of treatments against *T. b. gambiense* undergoing study, there are no treatments against *T. b. rhodesiense* infections. Nevertheless, a promising chemotherapy involves the orally administered combination of melarsoprol with two cyclodextrin inclusion complexes. The two combinations tested were melarsoprol hydroxypropyl-b-cyclodextrin, and melarsoprol randomly-methylated-b-cyclodextrin. According to Rodgers *et al.*,[56] both combination therapies were tested in mice over a course of 7 days, with the dose protocol of 0.005 mmo/kg of drug a day. The results showed that both combinations cured stage II *T. b. rhodesiense* infection, with no toxicity. Taken together, this set of experiments offer a promising orally administered treatment for East African trypanosomiasis, while avoiding the time-intensive and costly process of IV transfusion of melarsoprol.[56]

Combination Chemotherapy against HAT

After many years of neglect, the recent developments of chemotherapies for the treatment of HAT have been one of the main focuses of trypanosomiasis research. There are a number of advantages that make combination chemotherapy preferable to monotherapy. One advantage is that in an ideal situation, combination chemotherapy could be used against either (or both) *T. brucei* subspecies or alternatively, during both stage I and stage II HAT infections. It would reduce the need for staging, a procedure where a lumbar puncture is made and CSF samples are drawn from the patient. This process is necessary in order to detect the stage of the HAT infection. It is also a painful procedure that makes patients infected with HAT more hesitant to undergo treatment for second stage trypanosomiasis. The second advantage that combination chemotherapy has over monotherapy is

the ability of two drugs to work against *T. brucei* within a short time span of each other, or perhaps even work in tandem against the parasite, provided both drugs are synergistic for one another. The third advantage is that each sample of the drug within the combination chemotherapy contains a lower dose, avoiding toxicity, while retaining efficacy. The fourth advantage is that the lower dose of each drug means that the overall combination treatment will be less expensive, less labour-intensive, and ultimately, more accessible. The fifth advantage is that treatment with combination chemotherapy would lead to either little resistance by trypanosomes or at the very least, a delay in resistance.

With the introduction of NECT and its increasing usage, there is a greater hope for future combination therapies. There are a number of novel combination chemotherapies currently undergoing experimentation. A combination chemotherapy involving diaminotriazine or SIPI 1029 in conjunction with eflornithine is an example of such treatment. Another combination therapy involves the use anti-cancer agents, cordycepin and deoxycoformycin.[57] Even more examples of combination chemotherapies involve the use of inhibitors, receptor antagonists, and antibiotics. One of these alternative therapies involves a combination of existing drugs and DNA topoisomerase inhibitors, inhibitors of factors responsible for drug resistance (such as P-glycoprotein and MRP), and combinations with secondary treatments such as melarsoprol–aprepitant, or melarsoprol–cyclodextrin inclusion complex combination therapy.

In summary, concerted international efforts (with chemotherapy as the cornerstone) have reduced the notified cases of HAT to less than 10 000. The knowledge gained from melarsoprol treatment failures and eflornithine resistance (easily selected in the laboratory) should be put into action in tracking possible field resistance, in order to avoid disease resurgence. In addition, new drugs that act through mechanisms that will not facilitate cross resistance are urgently needed. Furthermore, these drugs must be safe, economical, effective, and easy to administer. Additional studies are however needed to validate the pre-clinical pharmacological and safety data on the newest drugs, which have the potential for treating advanced stage sleeping sickness with an easy treatment regimen.

Conflicts of Interest

None of the authors has a financial disclosure or conflict of interest.

Acknowledgement

The authors thank Drs Samuel Black and Dennis J. Grab of the University of Massachusetts, USA for their immense contributions and suggestions, and for proof-reading the manuscript.

References

1 Barrett MP, Burchmore RJ, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, *et al*. The trypanosomiases. Lancet. 2003;362:1469–80.
2 WHO. WHO/NTD Report update 2011. http://wwwwhoint/neglected_diseases/2010report/WHO_NTD_report_update_2011pdf, 1–25. 2011.
3 WHO. Neglected Tropical Diseases Booklet 2010. Neglected tropical diseases, hidden successes, emerging opportunities. 2010. http://whqlibdoc.who.int/publications/2009/9789241598705_eng.pdf, 1–71.
4 Simarro PP, Cecchi G, Paone M, Franco JR, Diarra A, Ruiz JA, *et al*. The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. Int J Health Geogr. 2010;9:57.
5 Simarro PP, Diarra A, Ruiz Postigo JA, Franco JR, Jannin JG. The human African trypanosomiasis control and surveillance programme of the World Health Organization 2000–2009: the way forward. PLoS Negl Trop Dis. 2011;5:e1007.
6 WHO. African trypanosomiasis (sleeping sickness). 2012. http://wwwwhoint/mediacentre/factsheets/fs259/en/ (accessed 2012 April 4).
7 Simarro PP, Franco JR, Cecchi G, Paone M, Diarra A, Ruiz Postigo JA, *et al*. Human African trypanosomiasis in non-endemic countries (2000–2010). J Travel Med. 2012;19:44–53.
8 Pepin J, Khonde N. Relapses following treatment of early-stage *Trypanosoma brucei gambiense* sleeping sickness with a combination of pentamidine and suramin. Trans R Soc Trop Med Hyg. 1996;90:183–6.
9 Tong J, Valverde O, Mahoudeau C, Yun O, Chappuis F. Challenges of controlling sleeping sickness in areas of violent conflict: experience in the Democratic Republic of Congo. Confl Health. 2011;5:7.
10 Stuart K, Brun R, Croft S, Fairlamb A, Gurtler RE, McKerrow J, *et al*. Kinetoplastids: related protozoan pathogens, different diseases. J Clin Invest. 2008;118:1301–10.
11 Baral TN. Immunobiology of African trypanosomes: need of alternative interventions. J Biomed Biotechnol. 2010;2010:389153.
12 Stockdale C, Swiderski MR, Barry JD, McCulloch R. Antigenic variation in *Trypanosoma brucei*: joining the DOTs. PLoS Biol. 2008;6:e185.
13 Brun R, Blum J, Chappuis F, Burri C. Human African trypanosomiasis. Lancet. 2010;375:148–59.
14 Matovu E, Seebeck T, Enyaru JC, Kaminsky R. Drug resistance in *Trypanosoma brucei* spp., the causative agents of sleeping sickness in man and nagana in cattle. Microbes Infect. 2001;3:763–70.
15 Barrett MP, Boykin DW, Brun R, Tidwell RR. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. Br J Pharmacol. 2007;152:1155–71.
16 Worthen C, Jensen BC, Parsons M. Diverse effects on mitochondrial and nuclear functions elicited by drugs and genetic knockdowns in bloodstream stage *Trypanosoma brucei*. PLoS Negl Trop Dis. 2010;4:e678.
17 Steverding D. The development of drugs for treatment of sleeping sickness: a historical review. Parasit Vectors. 2010;3:15.
18 De Koning HP. Uptake of pentamidine in *Trypanosoma brucei brucei* is mediated by three distinct transporters: implications for cross-resistance with arsenicals. Mol Pharmacol. 2001;59:586–92.
19 Wenzler T, Boykin DW, Ismail MA, Hall JE, Tidwell RR, Brun R. New treatment option for second-stage African sleeping sickness: *in vitro* and *in vivo* efficacy of aza analogs of DB289. Antimicrob Agents Chemother. 2009;53:4185–92.
20 Sanderson L, Dogruel M, Rodgers J, De Koning HP, Thomas SA. Pentamidine movement across the murine blood–brain and blood–cerebrospinal fluid barriers: effect of trypanosome infection, combination therapy, P-glycoprotein, and multidrug resistance-associated protein. J Pharmacol Exp Ther. 2009;329:967–77.
21 Sanderson L, Khan A, Thomas S. Distribution of suramin, an antitrypanosomal drug, across the blood–brain and blood–cerebrospinal fluid interfaces in wild-type and P-glycoprotein transporter-deficient mice. Antimicrob Agents Chemother. 2007;51:3136–46.
22 Balasegaram M, Young H, Chappuis F, Priotto G, Raguenaud ME, Checchi F. Effectiveness of melarsoprol and eflornithine as first-line regimens for gambiense sleeping sickness in nine

Medecins Sans Frontieres programmes. Trans R Soc Trop Med Hyg. 2009;103:280–90.
23 Schmid C, Richer M, Bilenge CM, Josenando T, Chappuis F, Manthelot CR, *et al*. Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis: confirmation from a multinational study (IMPAMEL II). J Infect Dis. 2005;191:1922–31.
24 Kuepfer I, Schmid C, Allan M, Edielu A, Haary EP, Kakembo A, *et al*. Safety and efficacy of the 10-day melarsoprol schedule for the treatment of second stage Rhodesiense sleeping sickness. PLoS Negl Trop Dis. 2012;6:e1695.
25 Rodgers J, Bradley B, Kennedy PG. Combination chemotherapy with a substance P receptor antagonist (aprepitant) and melarsoprol in a mouse model of human African trypanosomiasis. Parasitol Int. 2007;56:321–4.
26 Bouteille B, Oukem O, Bisser S, Dumas M. Treatment perspectives for human African trypanosomiasis. Fundam Clin Pharmacol. 2003;17:171–81.
27 Kennedy PG. Human African trypanosomiasis of the CNS: current issues and challenges. J Clin Invest. 2004;113:496–504.
28 Barrett MP, Croft SL. Management of trypanosomiasis and leishmaniasis. Br Med Bull. 2012;104:175–96.
29 Kennedy PG. The pathogenesis and modulation of the post-treatment reactive encephalopathy in a mouse model of Human African Trypanosomiasis. J Neuroimmunol. 1999;100:36–41.
30 Hunter CA, Jennings FW, Kennedy PG, Murray M. The use of azathioprine to ameliorate post-treatment encephalopathy associated with African trypanosomiasis. Neuropathol Appl Neurobiol. 1992;18:619–25.
31 Baker N, de Koning HP, Maser P, Horn D. Drug resistance in African trypanosomiasis: the melarsoprol and pentamidine story. Trends Parasitol. 2013;29:110–8.
32 Priotto G, Pinoges L, Fursa IB, Burke B, Nicolay N, Grillet G, *et al*. Safety and effectiveness of first line eflornithine for *Trypanosoma brucei gambiense* sleeping sickness in Sudan: cohort study. BMJ. 2008;336:705–8.
33 Simarro PP, Franco J, Diarra A, Postigo JA, Jannin J. Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis. Parasitology. 2012;139:842–6.
34 Sanderson L, Dogruel M, Rodgers J, Bradley B, Thomas SA. The blood–brain barrier significantly limits eflornithine entry into *Trypanosoma brucei brucei* infected mouse brain. J Neurochem. 2008;107:1136–46.
35 Kaiser M, Bray MA, Cal M, Bourdin Trunz B, Torreele E, Brun R. Antitrypanosomal activity of fexinidazole, a new oral nitroimidazole drug candidate for treatment of sleeping sickness. Antimicrob Agents Chemother. 2011;55:5602–8.
36 Hall BS, Bot C, Wilkinson SR. Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J Biol Chem. 2011;286:13088–95.
37 Bisser S, N'Siesi FX, Lejon V, Preux PM, Van Nieuwenhove S, Miaka Mia Bilenge C, *et al*. Equivalence trial of melarsoprol and nifurtimox monotherapy and combination therapy for the treatment of second-stage *Trypanosoma brucei gambiense* sleeping sickness. J Infect Dis. 2007;195:322–9.
38 Priotto G, Fogg C, Balasegaram M, Erphas O, Louga A, Checchi F, *et al*. Three drug combinations for late-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Uganda. PLoS Clin Trials. 2006;1:e39.
39 Chappuis F. Melarsoprol-free drug combinations for second-stage Gambian sleeping sickness: the way to go. Clin Infect Dis. 2007;45:1443–5.
40 Lutje V, Seixas J, Kennedy A. Chemotherapy for second-stage Human African trypanosomiasis. Cochrane Database Syst Rev. 2010;8:CD006201.
41 Checchi F, Piola P, Ayikoru H, Thomas F, Legros D, Priotto G. Nifurtimox plus Eflornithine for late-stage sleeping sickness in Uganda: a case series. PLoS Negl Trop Dis. 2007;1:e64.
42 Priotto G, Kasparian S, Ngouama D, Ghorashian S, Arnold U, Ghabri S, *et al*. Nifurtimox-eflornithine combination therapy for second-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Congo. Clin Infect Dis. 2007;45:1435–42.
43 Yun O, Priotto G, Tong J, Flevaud L, Chappuis F. NECT is next: implementing the new drug combination therapy for *Trypanosoma brucei gambiense* sleeping sickness. PLoS Negl Trop Dis. 2010;4:e720.
44 Torreele E, Bourdin Trunz B, Tweats D, Kaiser M, Brun R, Mazue G, *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.
45 Barrett MP, Vincent IM, Burchmore RJ, Kazibwe AJ, Matovu E. Drug resistance in human African trypanosomiasis. Future Microbiol. 2011;6:1037–47.
46 Trunz BB, Jedrysiak R, Tweats D, Brun R, Kaiser M, Suwinski J, *et al*. 1-Aryl-4-nitro-1*H*-imidazoles, a new promising series for the treatment of human African trypanosomiasis. Eur J Med Chem. 2011;46:1524–35.
47 Nesslany F, Brugier S, Mouries MA, Le Curieux F, Marzin D. *In vitro* and *in vivo* chromosomal aberrations induced by megazol. Mutat Res. 2004;560:147–58.
48 Jacobs RT, Plattner JJ, Nare B, Wring SA, Chen D, Freund Y, *et al*. Benzoxaboroles: a new class of potential drugs for human African trypanosomiasis. Future Med Chem. 2011;3:1259–78.
49 DND*i*. A needs-driven collaborative R&D model for neglected diseases. Geneva, Switzerland: R&D portfolio (Drugs for Neglected Diseases *Initiative*); 2011.
50 DND*i*. DND*i* launches phase I in-human clinical trial for promising oral drug for sleeping sickness. Press Releases, 2012. http://wwwdndiorg/press-releases/press-releases-2012html (accessed 2012 March 29).
51 Barrett MP. Potential new drugs for human African trypanosomiasis: some progress at last. Curr Opin Infect Dis. 2010;23:603–8.
52 Steverding D, Wang X. Evaluation of anti-sleeping-sickness drugs and topoisomerase inhibitors in combination on *Trypanosoma brucei*. J Antimicrob Chemother. 2009;63:1293–5.
53 Diaz-Gonzalez R, Perez-Pertejo Y, Prada CF, Fernandez-Rubio C, Balana-Fouce R, Reguera RM. Novel findings on trypanosomatid chemotherapy using DNA topoisomerase inhibitors. Mini Rev Med Chem. 2009;9:674–86.
54 Bakshi RP, Sang D, Morrell A, Cushman M, Shapiro TA. Activity of indenoisoquinolines against African trypanosomes. Antimicrob Agents Chemother. 2009;53:123–8.
55 Deterding A, Dungey FA, Thompson KA, Steverding D. Anti-trypanosomal activities of DNA topoisomerase inhibitors. Acta Trop. 2005;93:311–6.
56 Rodgers J, Jones A, Gibaud S, Bradley B, McCabe C, Barrett MP, *et al*. Melarsoprol cyclodextrin inclusion complexes as promising oral candidates for the treatment of human African trypanosomiasis. PLoS Negl Trop Dis. 2011;5:e1308.
57 Vodnala SK, Ferella M, Lunden-Miguel H, Betha E, van Reet N, Amin DN, *et al*. Preclinical assessment of the treatment of second-stage African trypanosomiasis with cordycepin and deoxycoformycin. PLoS Negl Trop Dis. 2009;3:e495.