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Antiprotozoals for human African trypanosomiasis: the heart of darkness at dawn

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"We should keep up the vision of making human African trypanosomiasis an 'ordinary' disease, with diagnosis and treatment integrated in the public health system, drugs that fulfill the criteria of 'access' and without the need for lumbar punctures..."

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In December 2012 the WHO convened an expert meeting on elimination of human African trypanosomiasis (HAT; sleeping sickness). The participants concluded that the decision to eliminate the disease should be maintained with a goal of elimination as a public health problem by 2020 and absence of transmission resulting in zero cases reported in all discrete geographic areas of endemicity, or 'foci', by 2030 [1]. The participants were optimistic about reaching those ambitious goals, which may put the end to a vicious threat to numerous people living in remote areas of sub-Saharan Africa, termed in literature 'the heart of darkness'. Elimination has already twice been attempted – and failed. So what makes this almost sorcerous disease so special, what are the developments spurring so much optimism and where are the major caveats in reaching the set goals?

HAT is one of 17 so-called 'neglected diseases' according to the WHO. It is caused by two subspecies of the protozoan parasite *Trypanosoma brucei – Trypanosoma brucei gambiense* in central and west Africa and *Trypanosoma brucei rhodesiense* in eastern and southern Africa. The disease is transmitted by different species of blood-sucking tsetse flies of the genus *Glossina* and is limited to those foci where tsetse flies exist [2,3]. However, disease epidemiology is extremely complex and for reasons that are so far unexplained, there are many regions where tsetse flies are found but sleeping sickness is not [4]. However, there are major differences between the subspecies that are of high relevance for control and elimination activities: trypanosomiasis in *T. b. rhodesiense* is a zoonosis that is usually transmitted from animals to man; there are two distinct settings where either cattle or game animals are the most important reservoir. Trypanosomiasis in *T. b. gambiense* is anthroponotic; that is, it mostly depends on human-to-human transmission [2,5].

The disease progresses in two stages; a first or hemolymphatic stage where trypanosomes proliferate at the bite site, travel to local lymph nodes and establish infection in the bloodstream; and a second or meningoencephalitic stage in which trypanosomes invade the CNS [6]. Clinically, the two disease forms are distinct: *T. b. gambiense* HAT is characterized by a chronic progressive course with an estimated duration of almost 3 years, evenly split between the first and the second stages. Chronic and intermittent fever, headache, pruritus, lymphadenopathy and to a lesser extent hepatosplenomegaly are the leading signs and symptoms of the first stage. In the second stage, a fragmentation and change of the sleeping pattern and neuropsychiatric disorders dominate the clinical presentation. The latter include tremor, fasciculations, general motor weakness, paralysis of an extremity, hemiparesis, akinesia and abnormal movements [7]. In contrast to the long-established paradigm that *T. b. gambiense* HAT was inevitably fatal without treatment, evidence

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has recently emerged showing that long-term survivors exist [8]. *T. b. rhodesiense* HAT is classically described as an acute disease progressing to the second stage within a few weeks and death within 6 months. Clinical presentation is highly variable in different foci, possibly due to different strains. In the second-stage pruritus, sleeping disorders, reduced consciousness or neurological signs and symptoms such as tremor, abnormal movements or walking disabilities may predominate. Compared with *T. b. gambiense* HAT, thyroid dysfunction, adrenal insufficiency, hypogonadism are more frequently found and myocarditis is more severe and may even be fatal [7].

In 2011, 6631 cases of *T. b. gambiense* and 112 cases of *T. b. rhodesiense* HAT have been reported and approximately 70 million people estimated to be at various levels of HAT risk in Africa, which corresponds to 10% of the total population and 7.4% of the total area of the endemic countries [3,9]. In addition, between 2000 and 2010, 94 travelers were infected, 72% of whom were infected by *T. b. rhodesiense*. The case number seems negligible but should not conceal the menace and impact of HAT. The clinical characteristics and focal distribution of the disease result in an enormous socioeconomic impact on affected villages. HAT reduces labor resources and indirectly impacts society as the disease is highly stigmatizing because it causes mental disturbances. Disease control involves exorbitantly expensive active case detection by specialized mobile teams followed by significant collateral treatment costs for those diagnosed (income loss, transport, food, hospitalization and ancillary drugs). In addition, in several geographic areas, patients treated for late-stage disease are still advised to observe a rest period of up to 6 months after treatment, which excludes, for example, working in the fields and sexual intercourse. The origin of this unsubstantiated practice is unclear and should clearly be abandoned. All those factors create major barriers to participate in screening programs and to seek care [10]. HAT was systematically under reported by over 50% for decades and only recent efforts, paired with modern reporting and mapping technology, decreased the estimated disagreement to below 10% [3,8]. Above all, the disease has a dangerous potential for re-emergence. Three major epidemics have ravaged the African continent, the first, which affected equatorial Africa, took place between 1896 and 1906 and killed an estimated 800,000 people. The second occurred between 1920 and the late 1940s when annual reported cases in western and central Africa reached a record 50,000 and prompted the colonial powers to invest in vector control and mobile teams to undertake active surveillance of the population. These control mechanisms, which are still the pillars of control, were effective and the disease was almost eliminated in the early 1960s. However, after the arrival of independence, the surveillance and control activities collapsed in most countries [6]. This led to a progressive re-emergence of the disease, which reached a peak in the late 1990s in the Democratic Republic of the Congo, Angola, Central African Republic, southern Sudan and Uganda when 45,000 cases of sleeping sickness were reported and 300,000–500,000 were estimated [11]. At this point the burden of disease caused by HAT reached 2.05 million disability-adjusted life-years lost, which placed it at the third place of parasitic diseases after malaria (45 million) and lymphatic filariasis (4.9 million) [12].

Since then, a global alliance led by the WHO set first control and then elimination as the goal of its strategy against HAT. With huge joint efforts between nongovernmental organizations (NGOs), endemic and donor governments, philanthropic foundations and academic research centers, the trend could be changed. In the beginning the situation looked very desperate; drugs were outdated and no new compounds on the horizon, even the production of the old drugs was in jeopardy because of the insufficient return on investment, and the political will was very limited at best. In 2001 I wrote in an editorial "… *although a huge amount of knowledge has been acquired in basic trypanosomiasis research, unfortunately there has been comparatively little progress in terms of improved methods for diagnosis and treatment, and we have to admit that the situation is not much better now than it was 100 years ago"* [101]. Nevertheless, some events around this time gave hope and I concluded "*The goal must now be to maintain the momentum*" [101]. A 1997 WHO resolution strongly advocated access to diagnosis; treatment; the reinforcement of surveillance and control activities; and concurrently setting up a network to strengthen coordination among those actively concerned by the problem [4]. Substantial advocacy work by NGOs and the WHO led to confirmation of new disease awareness at the 1999 International Scientific Committee of Trypanosomiasis Research and Control meeting and the Organisation of African Unity Heads of State meeting in Lomé (Togo) in 2000. New structures like the Program Against African Trypanosomiasis and the WHO Sleeping Sickness Treatment and Drug Resistance Network were created. The Bill & Melinda Gates Foundation was discussing a grant supporting the development of a new, orally applicable first-stage drug for sleeping sickness and the foundation meeting, for what today is the Drugs for Neglected Diseases Initiative, was in preparation. In the field, major NGOs, above all various sections of Médecins sans Frontières, had continuously increased emergency treatment efforts and the Belgian and French Cooperation had started

to substantially support the national sleeping sickness programs (NSSCPs) in the most affected countries. Finally, in 2006 the WHO and the Foundation for Innovative New Diagnostics signed a 5-year memorandum of understanding to promote the development of simple, more sensitive and specific diagnostic tests [5].

In 2001, the WHO's efforts resulted in signed contracts, which were prolonged several times but continue to remain valid todate, with the two manufacturers Aventis (now Sanofi) and Bayer – who committed themselves to ensure manufacturing and free donation to the WHO for distribution of the essential drugs to treat HAT [6]. The grants allowed provision of technical assistance, training and treatments to the developing countries affected. Between 2001 and 2011, 59,200 vials of melarsoprol, 5,763,765 vials of pentamidine, 477,542 vials of eflornithine and 13,597 vials of suramin were distributed to the national sleeping sickness programs [5]. Between 1986 and 2010, Médecins sans Frontières alone screened nearly 3 million people and treated more than 51,000 for the disease in several countries [13]. In 2011, the elimination of HAT as a public health problem was considered a feasible goal by the WHO Strategic and Technical Advisory Group on Neglected Tropical Diseases. The goal was included in the WHOs roadmap on neglected tropical diseases [9] and in 2012 major players including pharmaceutical companies, philanthropic donors, endemic countries and NGOs committed to support the roadmap through the London Declaration [9].

Finally in 2012, the WHO discussed the variability of the epidemiological settings and strategies to meet the different levels of endemicity proposed [1]. A combination of active-case finding, through mobile teams, directed by cases reported and surveillance, passive-case finding involving available health facilities and vector control, was proposed. The respective measures will be adapted depending on the respective epidemiological setting defined by the intensity of transmission, the accessibility and capabilities of existing health facilities, and vector knowledge including the sites where human–vector contact occurs. The efforts will be closely monitored trough baseline and follow-up surveys in sentinel and spot-check sites, evaluated based on specific criteria and verified [1].

A patient's perspective is principally guided by whether and at what conditions diagnosis can be provided and secured, whether and at what conditions treatment is available, as well as how successful treatment is. Access to treatment has five main dimensions: availability, accessibility, affordability, adequacy and acceptability. Treatment of a disease with a very complex epidemiology and biology, which is almost always fatal if untreated, causes incapacitating mental disturbances in those afflicted and occurs mainly in populations living in remote, rural areas with incomes of US$2 per day is likely to pose major challenges to access to treatment at all levels.

Research on HAT treatment received a lot of attention in the first half of the 20th century and very prominent researchers like Paul Ehrlich and Louise Pearce were involved in the early phase. Most of the numerous molecules invented (atoxyl [1905], suramin [1920] and pentamidine [1940] – the latter two still in use) were not able to cure second-stage sleeping sickness. Major progress was achieved with organoarsenic compounds, for example, tryparsamide was active against *T. b. gambiense* second-stage but was tainted with very lengthy treatment, dose-dependent optic nerve toxicity and drug resistance (1919). A long series of experimentation led to the association of dimercaptopropanol (British antilewisite) with melarsenoxide by Friedheim (1949), the first compound to be active against the second-stage of both disease forms. Melarsoprol became the backbone of second stage treatment for over 50 years. However, its use is associated with severe adverse drug reactions, the most important being an encephalopathic syndrome, which occurs at a very variable frequency in an average 4.7% of *T. b. gambiense* and 8.0% of *T. b. rhodesiense* patients, with a case fatality rate of 44 and 57%, respectively. After elucidation of the pharmacokinetics in the mid 1990s the inpatient treatment could be reduced from over 25 to 10 days [6]. The reduction of hospitalization time to less than half meant a major socioeconomic advantage, saving patients and their family costs for fees and food away from home. Nevertheless, the abridged melarsoprol regimen could not be regarded as a breakthrough since the frequency of encephalopathic syndromes did not decrease.

However, the Impamel program was crucial for further drug development since it comprised the first clinical trial on HAT executed according to GCP, as well as a large-scale multinational field study executed under real-life conditions. Those trials demonstrated the feasibility of modern clinical trials in extremely resource-limited conditions.

Meanwhile, the activity of two other drugs originally not developed for HAT was discovered and they together led to the first true breakthrough in sleeping sickness treatment history. In the 1980s, an antineoplastic drug, eflornithine (D,L,α-difluoromethyleflornithine), received attention for its activity against *T. b. gambiense* and the compound was eventually registered by the US FDA for this indication in 1990. Eflornithine is significantly better tolerated than melarsoprol but still produces several adverse drug reactions similar to those of other cytostatic drugs,

including bone marrow toxicity leading to anaemia, leukopenia, and thrombocytopenia (25–50%), gastrointestinal symptoms (10–39%) and convulsions (7%). Treatment lasts 2 weeks and because of the short half-life of the drug, four short infusions per day are necessary, requiring complex logistics and trained health staff. Hence, the use of drug remained limited to emergency interventions by NGOs [6]. Attempts were made to reduce the difficulty of drug administration by use of an oral formulation, but they were abandoned after a pharmacokinetic trial gave discouraging results [2]. In 2006, the WHO started to support NSSCPs with logistics and training to allow a switch from melarsoprol to eflornithine, and a prepackaged medical kit containing all the necessary drugs, infusion materials and accessories to administer eflornithine treatments was designed. The kit's weight for two patients was 40 kg, its volume 190 dm^3 and the direct and indirect drug costs per patient were €554, including transport to health facilities in the HAT 'foci'. Such support was only possible through the availability of free resources and those provided through the contract mentioned earlier with the respective pharmaceutical companies. Subsequently, the use of eflornithine increased from 12 to 64% coverage between 2006 and 2009. In 2007, most NSSCP had switched the first line drug from melarsoprol to eflornithine and the NSSCPs had passed the NGOs in terms of overall eflornithine utilization [14]. In parallel, the number of deaths related to treatment toxicity sharply decreased from an estimated 830 (5% of cases treated) in 2001 to 32 (0.9% of cases treated) in 2010 [14]. Despite those significant achievements, major parameters of sustainable access were still unfulfilled. A fair portion of patients, particularly in remote areas, still had no access to the modern treatment; treatment of HAT remained restricted to specialized health facilities. Eflornithine is only affordable due to massive subvention by the donation program and; saying this, the drug is far from being an adequate treatment, even if it appears to be much more acceptable to the population than melarsoprol (eflornithine was termed the 'resurrection drug') the very long hospitalization time excluded the poorest patients.

In the early 1990s, nifurtimox, an earlier development for Chagas disease, was used in experimental settings, mainly to treat melarsoprol-refractory HAT cases. The compound only had a very limited activity when used as a monotherapy and it also led to significant adverse drug reactions [6].

The availability of various compounds raised the interest of scientists and, subsequently, many combinations of registered and experimental compounds were tested on animals [6]. As a result, a series of trials assessing combinations of eflornithine, melarsoprol and nifurtimox were conducted in humans. In all trials, the efficacy was better in a combination therapy compared with monotherapy. However, combinations containing melarsoprol invariably resulted in very high frequencies of severe adverse drug reactions and were rapidly abandoned. In a multicenter trial, nifurtimox–eflornithine combination therapy (NECT) was initiated in the Republic of Congo and the Democratic Republic of the Congo, the results were compared with the standard eflornithine therapy [6]. The proportion of patients who had major drug-related adverse events (AE) was lower in the NECT group than in the eflornithine group (14.0 vs 28.7%, respectively), mainly because of a lower frequency of severe fever, infections, neutropenia, hypertension and diarrhea [15]. Based on these favorable trial results, NECT was included for treatment of second-stage *T. b. gambiense* HAT into the WHO's Essential Medicines List in May 2009 and based on further results from a field trial into WHO's Essential Medicines List for Pediatric Use in May 2013. A cohort study and targeted pharmacovigilance monitoring underlined the advantages of NECT over eflornithine and melarsoprol. In a cohort of 684 second-stage HAT patients (including 120 children) 85% of patients experienced at least one AE. Most AEs were mild (37.9%) or moderate (54.7%). Severe AEs included vomiting (n = 32), dizziness (n = 16), headache (n = 11) and convulsions (n = 11). The in-hospital case fatality rate was low at 0.15% and relapses were rare (n = 14) [16]. In a pharmacovigilance study, data were collected from 1735 patients treated with NECT. 60% of patients experienced at least one AE and serious AE were reported from 19 patients (1.1%) leading to death in nine (0.5%). Convulsions (n = 8), fever (n = 7) and coma (n = 6) were the most frequent serious AEs [17].

In comparison with the obsolete treatments, those figures are impressively positive. However, the complexity of NECT application and the frequency of adverse drug reactions still limit the use to second-stage disease and thus lumbar punctures are still required for disease staging and NECT use is restricted in specialized facilities. In addition, the use of eflornithine against *T. b. rhodesiense* is not advised because this organism is innately less susceptible to the drug than is *T. b. gambiense* and NECT is also unlikely to be effective, so melarsoprol is still left as the only drug option [6].

Diagnosis of *T. b. gambiense* HAT still follows a classic pathway, with serologic screening followed by microscopic confirmation of the parasite and staging [6]. The process is very demanding for various reasons and thus restricted to specialized health facilities. Since HAT has

no distinctive early signs and symptoms, the first step is a serological test by the card agglutination test for trypanosomiasis (CATT)/*T. b. gambiense* invented in 1978, which requires a cold chain [18]. Those who are serologically positive are considered 'suspect' and are subjected to parasitological confirmation of the parasite in blood, lymph node aspirate and cerebrospinal fluid; several techniques exist to concentrate the parasites in those fluids [6]. As a result of the relative toxicity and complex use of all drugs active against second-stage HAT, a lumbar puncture is performed for staging of the disease and the selection of the appropriate drug. Either the presence of the parasite or an increased number of white blood cells in the CSF is considered indicative for second-stage infection. Recently, very simple molecular tools were suggested (loop-mediated isothermal amplification technique) but they are not yet fully validated [18]. In addition, a new format of the CATT test was proposed, reducing the vial size from 50 to ten patients and eliminating the cold chain [18]. With this, the test becomes a possible tool for use in public health facilities. Last, and most importantly, the availability of a rapid diagnostic test was just announced [Ndung'u J, Pers. Comm.]; this must be considered a major breakthrough because it will finally make diagnosis possible in a general health facilities.

However, another major difficulty in HAT treatment needs a solution; drugs used for HAT are not 100% effective and relapses occur over a long time after treatment. Post-treatment followup, consisting of parasitological examination, are therefore recommended for 2 years' duration with visits at 3, 6, 12, 18, and 24 months after treatment. Since in relapses, trypanosomes are mainly found in CSF and rarely in the blood at each visit a lumbar puncture is performed [6]. This procedure is a major burden for patients and it is easy to understand that compliance with scheduled visits decreases over time, from 65–85% at 12 months to 25–70% at 24 months, whereas 40–90% of relapses occur within 12 months and 70–90% occur within 18 months. Over the past few years, significant efforts were made to either find easily accessible markers for relapse or to make use of calculation algorithms of the white blood cells counted in the CSF for prediction of cure [18]. In a recent publication the combination of criteria for failure and cure was suggested and a shortening of the follow up to a maximum of 12 months suggested [6]. In terms of access to diagnosis, only the very recent developments will make a change [18]. If sleeping sickness can eventually be easily diagnosed, lengthy delays causing transition to second stage and expensive referral can be avoided. Despite the significant improvement, follow up after treatment will remain an encumbrance.

In my view, several conditions need to be fulfilled in order to see the goal at the end of this bumpy road:

- The on-going development programs for two oral drugs (fexinidazole, currently in Phase II/III and SCYX-7158 currently in Phase I testing) need to be completed against all odds to allow replacement of NECT – a very demanding task that is becoming even more difficult due to the constant decrease of case numbers in areas with reasonable accessibility and logistics [19];
- The new diagnostic point of care tools (new format CATT and particularly the rapid tests) will need to be broadly implemented in the public health system;
- The approaches and tools will require abundant training and information – staff in public health are not familiar with HAT and the disease is noticeably stigmatized, which today negatively affects participation in screening programs and surveys [10];
- To put in place and sustain a surveillance system over a long period of time;
- Stability where HAT prevails is a *sine qua non* for success of elimination and;
- Donor fatigue is not permissible until the last focus has been declared free of trypanosomes.

Those conditions are requirements and not optional and it will require a lot of work at all levels, patience, stamina from the donors and politicians supporting this case and no repetition of the mistakes made in HAT and malaria in the past. We should keep up the vision of making HAT an 'ordinary' disease, with diagnosis and treatment integrated in the public health system, drugs that fulfill the criteria of 'access' and without the need for lumbar punctures – and eventually erradicate it entirely to finally bring light into the heart of darkness. For this 'we must now maintain the momentum'.

Financial & competing interest disclosure

C Burri is Head of Medicines Research Department, at the Swiss Tropical Institute (Swiss TPH) and Professor for clinical pharmacology and pharmacy at the Department of Pharmaceutical Sciences, University of Basel (Switzerland). The Pharmaceutical Medicine Unit of the Swiss TPH, which is in the sphere of influence of C Burri was significantly involved in the development of NECT and in the on-going clinical trials on fexinidazole on behalf of the Drugs for Neglected Diseases Initiative. The author has no other relevant affiliation or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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