New developments in human African trypanosomiasis
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Purpose of review
To review recent literature on human African trypanosomiasis, focussing on genome sequencing, diagnosis and drug discovery, and typing of trypanosomes.

Recent findings
The most important recent development has been the completion of the *Trypanosoma brucei* genome which will greatly facilitate the discovery of new drug targets and genetic markers. Correct staging of the disease is of key importance for treatment. The analysis of sleep patterns is a promising new method to this end and has advanced enough to begin thorough clinical trials. In terms of novel drug candidates, dicationic molecules show the most promise with one oral diamidine in phase 3 clinical trials. New targets and classes of molecules which show in vitro trypanocidal activity are also described. Two new methods – MGE-PCR and microsatellites – allow analyses without parasite cultivation, eliminating a major impediment to efficient sampling for population studies. The finding that several wild animal species harbour *T. b. gambiense,* and that parasite transmission is efficient even from very low parasitaemias, sheds a new light on the importance of animal reservoirs.

Summary
The use of *T. brucei* as model system for molecular and cell biology is regularly producing new technologies exploitable for diagnosis and new drugs. Drug discovery and development experience a revival through new public-private partnerships and initiatives. The challenge remains to translate this progress into improvements for affected people in disease endemic areas.

Keywords
animal reservoir, chemotherapy, population genetics, sleeping sickness, strain distinction, *Trypanosoma brucei*

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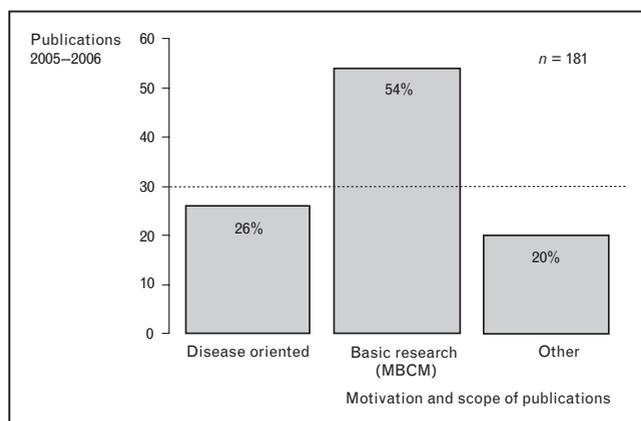Tel: +41 61 284 82 31; e-mail: reto.brun@unibas.ch

Abbreviations

HAT   human African trypanosomiasis
PCR   polymerase chain reaction

Introduction
Human African trypanosomiasis (HAT) or sleeping sickness is caused by flagellated protozoan parasites (*Trypanosoma brucei*) and transmitted by tsetse flies (*Glossina* spp.). The disease is fatal if not treated and is among the most neglected diseases. It affects individuals in over 30 African countries, and resources are lacking to control the disease properly and to improve diagnosis and chemotherapy. Uganda is the only country harbouring both forms of HAT, caused by *T. brucei gambiense* and *T. brucei rhodesiense*, respectively. The two forms of disease do not overlap yet, but there are indications that this may change in the near future because both areas are expanding [1]. *T. brucei rhodesiense* has recently moved from the south-eastern focus at Lake Victoria northward into Soroti and Kaberamaido [2]. As diagnosis is unreliable and active surveillance is lacking in many countries, it is likely that most cases go undetected. For example, Odiit *et al.* [3•] calculated that approximately 90% of HAT fatalities are not detected in epidemics in Uganda. Similar studies in *T. brucei gambiense* areas should be encouraged. In the period under review, the first human case of a pathogenic *Trypanosoma evansi* infection has also been reported from India [4•]. It is, however, not clear if this case involves a *T. evansi* strain that has acquired human infectivity (the strain could not be isolated) or if the patient was lacking the trypanolytic factor in his blood plasma.

Diagnosis and chemotherapy are both problematical areas: diagnosis is either insensitive or laborious, whereas chemotherapy depends on drugs that are old, inefficient, toxic and expensive. The pharmaceutical industry is currently not pursuing research and development for new drugs for HAT. Fortunately, this gap can be filled partly by new initiatives such as the Tropical Disease Research/World Health Organization committee on Genomics and Discovery Research, or the Drugs for Neglected Diseases initiative. In addition, as a result of several special features of its biology, *T. brucei* is an intensely studied model system for molecular, biochemical and cell biology. As a literature search reveals, only approximately a quarter of the published research articles on *T. brucei* are medically relevant, whereas the majority is fundamentally molecular, biochemical, cell or microbiological research with no disease-oriented applied objectives (Fig. 1). This produces abundant information, however, to suggest new targets for diagnosis and treatment and a rich array of molecular tools that greatly facilitate applied research.

**Figure 1 Proportion of journal articles published on _Trypano-
soma brucei_ or sleeping sickness in 2005–2006 that are disease
oriented, represent basic non-applied research in molecular,
biochemical, cell, or microbiology or fall into other categories**

Methods: All journal articles published in 2005 and the first half of 2006
containing the terms '_Trypanosoma brucei_' or 'sleeping sickness' in their
title were extracted from ISI Web of Science. The 181 identified articles
were placed into three groups based on their subject categories listed in
Web of Science. 'Disease oriented' articles have some relevance to the
disease problem, 'Basic research' articles focus on basic molecular,
biochemical, cell biological, and microbiological (MBCM) research with-
out an obvious connection to disease, and 'Other' articles did not fit any
of these categories. Articles were classified as 'Disease oriented' if they
contained the subject categories 'Tropical Medicine', 'General and
Internal Medicine', 'Medicinal Chemistry', 'Pharmacology and Pharmacy',
'Veterinary Sciences', or 'Pathology'. The remaining articles were
assigned to 'Basic research' if they contained the subject categories
'Biochemistry and Molecular Biology', 'Cell Biology', or 'Microbiology' or
to 'Other' otherwise. Articles with the (only) subject category 'Parasitol-
ogy' were assigned to the three groups based on their title. Note that
articles of both disease and basic interest were included as 'Disease
oriented'. No articles were assigned twice.

In this review we focus on progress in five areas we
consider of key importance for reducing the disease
burden in the intermediate term (diagnosis, staging,
and chemotherapy) and for devising sustainable control
strategies in the long term (population genetics and
animal reservoirs).

Diagnosis and staging

Field diagnosis of HAT has only seen slow progress over
past decades [5[•],6], while molecular diagnosis advances
steadily. Njiru _et al._ [7[•]] reported the development of an
improved molecular assay that targets the internal tran-
scribed spacer, capable of distinguishing different patho-
genic African trypanosomes with a single polymerase
chain reaction (PCR). Faster progress can be expected
in the future thanks to the completion of the genome
sequences of _T. brucei_ and related species [8[••]–11[••],
12–14] and the availability of the first genetic map
[15[•]]. Besides greatly facilitating the identification of
molecular markers and primer construction, the genome
sequence holds promise for in silico identification of
target proteins as serodiagnostic markers [16]. A new

approach to HAT diagnosis is proteomic signature
analysis [17,18[•]], which recognizes the typical fingerprint
of HAT infections in the protein content of patient
samples. The method is extremely sensitive and specific
(100 and 98.6%), but requires expensive equipment and
extensive expertise. It is therefore not directly applicable
in the field, but may help identify specific proteins
exploitable for serodiagnostics in the field.

There is an urgent need for better diagnostics for HAT
stage determination. Buguet _et al._ [19[••]] demonstrated
that entry into the second stage of the disease is associ-
ated with marked changes in the sleep pattern, observ-
able with relatively simple devices. This is a very
promising new avenue of research, especially as it appears
that rats exhibit the same two-staged disease course [20[•]],
so that an appropriate experimental system is at hand to
develop this system further. Finally, a new dot-enzyme-
linked immunosorbent assay for stage determination in
the field has been described by Courtioux _et al._ [21[•]].
After validation it may replace or complement other
available field assays [5[•]].

Chemotherapy

The search for new drugs is of paramount importance and
can follow several strategies: the improved use of existing
drugs, new drug combinations, therapeutic switching,
and the search for new chemical entities that should
be efficacious, safe and affordable for disease-endemic
countries. A 10-day melarsoprol treatment schedule was
validated in a multinational study in over 2000 patients
[22[••]]. The cure rate was 94% 24 h after treatment and
was still 86% after 2 years. The fatality rate was 5.9%, and
4% of treated patients died from an encephalitic syn-
drome. In a previous study [23], it was shown that the
efficacy and safety of the 10-day treatment schedule are
comparable to a standard 26-day treatment schedule in
three courses. The new treatment schedule reduces
treatment duration, the amount of drug and costs, and
thus increases the capacity of hospitals for HAT treat-
ments. On the basis of data from southern Sudan,
Chappuis _et al._ [24] showed that eflornithine is a safe
and effective drug for second-stage disease. Mortality
during treatment and adverse effects were much less
dramatic in patients treated with eflornithine than with
melarsoprol. The authors recommend the use of this
14-day treatment schedule with four daily intravenous
infusions not only in areas with high melarsoprol relapse
rates but wherever possible. The feasibility of this eflor-
nithine treatment schedule is, however, questionable in
rural areas considering that more than 50 intravenous
infusions are needed and the drug is enormously costly.

The most promising new compounds in the clinical or
preclinical phase are the aromatic diamidines, which
represent lead compounds against various protozoan

parasites and fungi [25[••]]. Several classes of dicationic molecules were recently synthesized and their in-vitro and in-vivo activity was demonstrated. Dicationic guanidine and reversed amidine derivatives were able to cure a *T. brucei rhodesiense* mouse model after multiple parenteral applications. Carbamate prodrugs could cure the mouse model even with an oral application [26[••]]. Biphenyl benzimidazole derivatives were synthesized and their DNA binding was measured [27]. All molecules showed strong DNA affinities and also a very high in-vitro activity against African trypanosomes and malaria parasites. Two compounds cured a *T. brucei rhodesiense* mouse model at 20 mg/kg with intraperitoneal application. Another interesting class of antitrypanosomal dications are the imidazopyridines [28]. Five parent compounds were able to cure the *T. brucei rhodesiense* mouse model at 20 mg/kg intraperitoneally. Prodrugs did not have the same efficacy, probably because of poor oral bioavailability. Athri *et al.* [29[•]] have generated three-dimensional quantitative structure–activity relationship maps based on a library of heterocyclic diamidines, and have built a model with additional descriptors for donor/acceptor and hydrophobic properties to help design new molecules with improved DNA binding characteristics and improved antiparasitic activity.

Progress has also been made on non-diamidine targets and lead compounds. Cordycepin (3'-deoxyadenosine) in combination with the adenosine deaminase inhibitor coformycin was found to cure a *T. brucei brucei* mouse model [30]. The authors noted that adenosine analogues are already on the market that are resistant to adenosine deaminase, which would allow one to omit the co-administration of coformycin. Baliani *et al.* [31[•]] reported the synthesis of new hybrid molecules containing the melamine moiety as an adenosine transporter recognition motive and a trypanocidal nitro heterocycle. The in-vitro activities of such hybrids are comparable to the standard drug melarsoprol, and two molecules were able to cure a *T. brucei brucei* mouse model. Biphosphonate inhibitors were shown to inhibit recombinant *T. brucei* soluble vacuolar pyrophosphatase in the low µmol range and with a selectivity against KB cells (human carcinoma cells) of greater than 100 [32[•]]. Another interesting report covered novel alkylpolyaminoguanidines as trypanothione reductase inhibitors with good in-vitro activities but no in-vivo data [33]. Vicik *et al.* [34] tested inhibitors of rhodesain, the major cysteine protease of *T. brucei rhodesiense*. Inhibition of the enzyme was excellent but in-vitro antitrypanosomal activity was completely missing (IC_{50} between 10 and 30 µmol) and has to be improved before in-vivo efficacy can be expected.

Strain distinction and population genetics

The ability to distinguish different genotypes (or strains) is key to understanding trypanosome population dynamics, in which individual organisms can not be followed through space and time. It allows us to assess the contribution of different genotypes to various observed phenomena, such as human infectivity, drug resistance, virulence, or adaptation to specific host genotypes. It also allows us to investigate the population structure and population dynamics of the parasite in detail to answer questions such as: to what degree are subpopulations (e.g. foci) separated? How much gene flow (parasite exchange) is there between foci? How did and do the parasites spread? Are new outbreaks caused by resident or newly introduced parasites? An understanding of these questions would help us devise more efficient and sustainable control strategies.

All genotyping methods traditionally applied have major limitations, arguably the worst being that most require a large amount of parasite material and thus depend on parasite cultivation. Cultivation, however, is a complex and time-consuming process that has limited sample sizes to levels below those required for many population studies. Furthermore, because parasite genotypes vary widely in if or how well they can be cultured, cultivation represents a selection process determining what genotypes are included in such studies [35], which introduces the possibility for a strong bias. Two new molecular markers promise to overcome these limitations and greatly improve our ability to study different *T. brucei* genotypes in the field. Simo *et al.* [36[••]] demonstrated the utility of mobile genetic element PCR (originally developed by Hide and Tilley [37]) to distinguish different strains of *T. brucei* across all three 'subspecies' and notably among *T. brucei gambiense*, which is at the same time the most medically relevant and the least genetically variable *T. brucei* subspecies. The beauty of this approach is that it requires only a single PCR, and is thus fast and relatively inexpensive. It apparently only amplifies Trypanozoon DNA and can thus be directly applied to field samples. The drawbacks of the method are that one does not know what exactly is amplified, and that given the marker's nature, the results can not be used for population genetic or phylogenetic inferences. To address this problem, Balmer *et al.* [38[••]] characterized a set of microsatellite markers. Microsatellites are extremely variable stretches of repeated DNA sequence that vary even among close relatives. Balmer *et al.* [38[••]] presented 14 microsatellite loci and demonstrated that they are highly variable and thus capable of distinguishing many different *T. brucei* genotypes. The great advantage microsatellites offer compared with all other available genotyping approaches is that they are selectively neutral and conform to a defined mutation model. They are thus ideal markers for population genetic analyses of historic and present population structure and the population dynamics of *T. brucei*. As the method is PCR based, it can be applied directly to field samples (host blood, tsetse flies) without relying on cultivation.

The main drawback of microsatellites is that their analysis is technically demanding and best performed with a DNA sequencer.

MacLeod *et al.* [39•] have used microsatellites in another way to give a statistically robust demonstration that alleles segregate in a Mendelian fashion in *T. brucei*. Although their data, contrary to their explicit claim in the title, do not prove the involvement of meiosis, the results are very comforting to population geneticists because they confirm the validity of a key assumption for population genetic analyses.

The need to distinguish *T. brucei* genotypes further arises in laboratory studies investigating interactions between different genotypes and effects of multiple genotype infections. The most powerful way to do this is to use markers that make different genotypes phenotypically distinguishable. Balmer and Tostado [40•] described genetic constructs that allow fluorescence expression in four different colours and notably in all parasite life stages. They demonstrate that these constructs are readily integrated into the genomes of various strains of *T. brucei brucei* and *T. brucei rhodesiense* (but not *T. brucei gambiense*, it seems) and that fluorescence expression is stable in all life stages. By transfecting two strains with two different colours they become distinguishable by eye. Relative population sizes can thus be followed under the fluorescence microscope or even more efficiently using a fluorescence-activated cell sorter, without the need to genotype or clone. The ability to use a fluorescence-activated cell sorter is a big step forward because it not only allows the very efficient daily tracking of large numbers of infections, but even allows the separation of strains again after experimental mixed infections.

Animal reservoir

The great significance of domestic and wild animal hosts for Rhodesian sleeping sickness is undisputed. As a result of the close proximity of livestock to humans and the relative ease with which they can be treated, Welburn *et al.* [41] propose that sleeping sickness would most effectively be controlled locally by fighting the parasite in livestock as part of farming practices. Ng'ayo *et al.* [42] show that domestic animals (here a sheep, a goat and a pig) harbour *T. brucei rhodesiense* even in areas with few recent cases of sleeping sickness. The role of animal reservoirs is much more controversial in Gambian sleeping sickness. An unbiased observer would be astonished how strongly workers in this field seem willing to dismiss the important role of animals in *T. brucei gambiense* epidemiology without much proper data from systematic surveys. Clearly, this mindset has not helped us gain a realistic view of sleeping sickness epidemiology in west Africa. Pigs have been shown repeatedly to harbour *T. brucei gambiense* [36••]. Even though pigs can clear infections in less than 6 months, as Penchenier *et al.* [43] demonstrate, they are still capable of maintaining parasite populations outside the human cycle. Njiokou *et al.* [44••] now show evidence that eight wild animal species (out of 36 species sampled) belonging to four orders (primates, artiodactyls, rodents, carnivores), host *T. brucei gambiense* group 1 parasites (Fig. 2). Even if these animal species play minor roles during epidemics, they probably hold the key to where the parasites are maintained between epidemics and where new epidemics start. Hopefully, the accumulated evidence on animals carrying *T. brucei gambiense* group 1 parasites will finally lead to the thorough rethinking of the role of animal hosts in Gambian sleeping sickness. Two next steps would be particularly desirable:

Figure 2 The greater white-nosed monkey, *Cercopithecus nictitans*, and its central and west African range

The recent discovery that this and several other wild animal species harbour *Trypanosoma brucei gambiense* group 1 parasites [44••] (see text) strongly suggests that animal reservoirs must play a more important role in west African sleeping sickness than generally acknowledged. A better understanding of their role will help control efforts in the long run. Foto© CERCOPAN – Witzens, map© Istituto di Ecologia Applicata IEA, Rome.
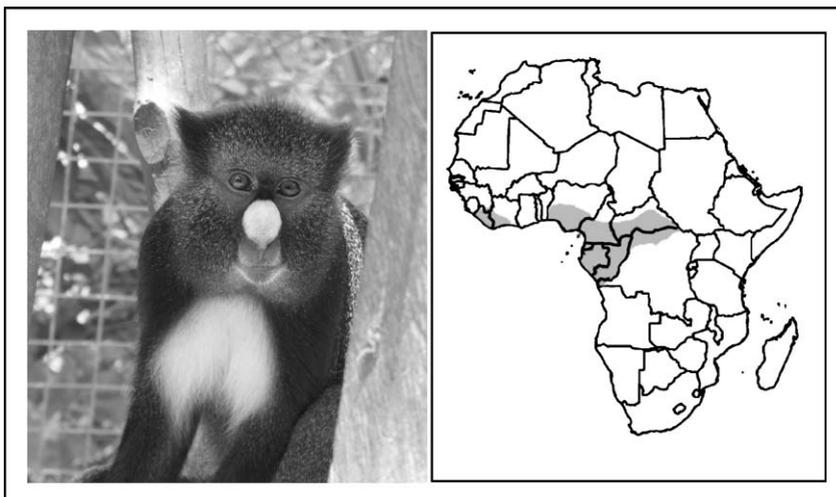
First, the identified *T. brucei gambiense* group 1 samples should be investigated with a wider array of genetic markers to eliminate any doubts about their identity, and to place them within the other isolates from the area to see if they are the same genotypes also found in humans. Second, a systematic geographical sampling independent of 'foci' would be desirable, also for *T. brucei rhodesiense* in east Africa, to assess how parasite presence and disease occurrence corelate.

Van den Bossche *et al.* [45••] further highlighted the potential impact of animal reservoirs by showing that the transmissibility of *T. brucei rhodesiense* to tsetse flies is independent of parasitemia and is efficient even at very low parasitemias. This means that each tsetse bite on an infected host has a similar probability of leading to a mature infection, and that even animals with very low parasite loads play an important epidemiological role, a result that is also of great importance for disease modelling.

Conclusion

Research in the field of HAT has progressed during the past 2 years in the areas of genomics, diagnosis and chemotherapy, but also in the development of new tools for the better characterization of trypanosomes. HAT largely benefits from basic research that uses *T. brucei* as a model organism to tackle genetic, molecular and biochemical questions. Research for diagnosis and drug discovery/development, abandoned by big pharmaceutical companies for the past 20 years, receives more attention through new public–private partnerships such as the Foundation for Innovative New Diagnostics, Geneva, the Tropical Disease Research/World Health Organization committee on Genomics and Discovery Research or the Drugs for Neglected Diseases initiative, Geneva. It can be expected that such non-profit organizations will deliver new tools for the diagnosis and treatment of HAT. For a long-term perspective it is also important that epidemiological work on the distribution of infected humans, vectors and animal reservoir hosts is intensified, especially in *T. brucei gambiense* areas.

Acknowledgement

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