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# Receptor-mediated endocytosis for drug delivery in African trypanosomes: fulfilling Paul Ehrlich's vision of chemotherapy

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**Bloodstream-form cells of *Trypanosoma brucei* exhibit massively increased endocytic activity relative to the insect midgut stage, enabling rapid recycling of variant surface glycoprotein and antibody clearance from the surface. In addition, recent advances have identified a role for receptor-mediated endocytosis in the uptake of the antitrypanosomal drug, suramin, via invariant surface glycoprotein 75, and in the uptake of trypanosome lytic factor 1 via haptoglobin–haemoglobin receptor. Here, we argue that receptor-mediated endocytosis represents both a validated drug target and a promising route for the delivery of novel therapeutics into trypanosomes.**

## African trypanosomes: toxins and receptors

The African trypanosomes have been a scourge of Africa for centuries, rendering large swathes of the continent unusable for livestock-based agriculture, and causing several epidemics of human African trypanosomiasis (HAT) in the 20th century [1]. There are currently five drugs used to treat HAT. Although these drugs can be effective, they are far from ideal due to their subspecies specificity, toxicity to patients, the complexity of their administration, and the emergence of resistance (Box 1) [2]. Although no new drugs have been approved for the treatment of HAT since the 1980s, when eflornithine was introduced, there have been some recent successes, including development of nifurtimox–eflornithine combination therapy [3], and the transition of new and promising orally administered compounds into early-stage clinical testing [2].

Suramin, the oldest anti-HAT drug currently in use, was developed in the early 20th century following on from the work of Paul Ehrlich, and remains in use for the treatment of early-stage East African HAT. A century ago, Ehrlich recognised that a full characterisation of the receptor complement of a pathogen could potentially lead to the development of effective combination therapies.

> When once we are acquainted with the majority of the chemioreceptors of a particular kind of parasite . . . we shall have far-reaching possibilities of simultaneous attack by various agencies. Ehrlich, 1913 [4]

Recent work has identified several of the key proteins responsible for the uptake and intracellular transit of suramin, highlighting the importance of receptor-mediated endocytosis to this process [5] and identifying it as an effective drug delivery strategy in *Trypanosoma brucei*. The characterisation of several cell surface receptors, including the invariant surface glycoprotein of 75 kDa (ISG75) [6], responsible for the uptake of suramin [5], and the haptoglobin–haemoglobin receptor (HpHbR), responsible for the uptake of human trypanolytic factor 1 (TLF1) [7], has brought us a step closer to realising Ehrlich's vision.

## Paul Ehrlich: 'chemiotherapy' and the development of suramin

In the early 1900s, Paul Ehrlich tested arsenicals and synthetic dyes as potential chemotherapies against trypanosomiasis [8]. Dyes were particularly useful at the time for this purpose, as efficient staining could reflect efficient compound uptake, and staining could be compared between parasite cells and mammalian host cells. The azo dyes, trypan red and trypan blue, stain and kill trypanosomes; trypan blue may be familiar to many as a widely used analytical reagent for assessing viability due to exclusion from most live cells and tissues. Strong dyes are not ideal chemotherapeutic agents, however, so it was important to develop colourless derivatives. Accordingly, the naphthalene urea, known as Bayer 205, emerged, which also goes by the names Germanin and suramin.

Studies on drug-resistant or 'drug-fast' strains yielded insights into how drugs are 'fixed' by trypanosomes. By selecting resistant strains, Ehrlich found that trypanosomes resistant to a specific drug were also resistant to other compounds in the same class, but not to those in other classes. He concluded that parasites express

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*Keywords:* *Trypanosoma brucei*; endocytosis; suramin; trypanolytic factor; drug delivery

1471-4922/$ – see front matter
 http://dx.doi.org/10.1016/j.pt.2013.03.004

**Box 1. Anti-HAT chemotherapy: the need for new therapeutic approaches**

HAT is generally regarded as fatal if left untreated, and due to parasite antigenic variation it is unlikely that a vaccine will be developed against *T. brucei*. Hence, effective chemotherapy is essential. Although there are five drugs available for treatment of HAT, efficacy is limited by subspecies and disease-stage specificity, as well as toxicity and complex administration requiring hospitalisation [2]. Pentamidine and suramin are used to treat stage one *T. b. gambiense* and *T. b. rhodesiense*, respectively. Eflornithine is used to treat late-stage *T. b. gambiense*, whereas melarsoprol is the only drug effective against both forms of the parasite during late-stage HAT. Nifurtimox, originally used to treat Chagas disease, is now used in combination with eflornithine to treat late-stage *T. b. gambiense* HAT; the reduced dosing required with nifurtimox–eflornithine combination therapy (NECT) has led to greater patient compliance [3]. Treatment failures have been documented for all available HAT therapies, although these do not necessarily indicate the presence of bona fide drug-resistant parasites, as mis-staging of disease and patient compliance will impact treatment efficacy. However, melarsoprol-resistant trypanosomes have been identified in the field [47], and parasites resistant to any of the available drugs can be easily generated in the laboratory. All of these factors highlight the pressing need to develop new, more effective therapies.

'different specific chemioreceptors' that bind these drugs, with reduction in drug affinity explaining the class-specific resistance [4].

Ehrlich recognised the importance of identifying the 'chemioreceptors' responsible, stating that a complete knowledge of all possible receptors was an essential component of any successful treatment – the more receptors identified, the greater the possibility of a successful chemotherapy. He concluded that the first necessary condition of a specific therapy must be an affinity for a parasite receptor, the second being toxicity. Thus, Ehrlich suggested a 'poisoned arrow' with haptophoric and toxophoric groups for receptor binding and poisoning, respectively. It was these, and related studies, that formed the foundations for the guiding principles of the receptor concept and its importance to chemotherapy [4,8]. This 100-year-old view still reflects the key principles of modern chemotherapy and, in our opinion, these principles can now be more effectively applied, given our current knowledge of several of these receptors and the downstream mechanisms for trafficking drugs and other toxins in *T. brucei*.

## Human trypanolytic factors and the endocytic system

Although two subspecies of *T. brucei*, *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and *Trypanosoma brucei gambiense* (*T. b. gambiense*), cause disease in humans, serum trypanolytic factors (TLFs) confer resistance against other African trypanosomes, including *Trypanosoma brucei brucei* (*T. b. brucei*) and *Trypanosoma congolense* [9]. TLFs come in two forms, TLF1, a component of high-density lipoprotein [10,11], and TLF2, an IgM/apolipoprotein-A1 complex [12,13]. Apolipoprotein-L1 (apoL1) is present in both complexes [14] and is responsible for trypanolysis due to the formation of pores in the lysosomal membrane of the parasite and subsequent osmotic swelling [14,15]. TLF1 also contains haptoglobin-related protein, and it is via the interaction between this protein and the *T. brucei* HpHbR in the flagellar pocket membrane that TLF1 is able to enter the parasite, delivering its lytic cargo into the endocytic system and ultimately the lysosome [7,16]. Therefore, according to Ehrlich's scheme, haptoglobin-related protein represents the haptophoric group and apoL1 represents the toxophoric group [4]. It is unknown how TLF2 enters the cell [17], but it has been postulated that entry may be via low-affinity binding to the variant surface glycoprotein (VSG) coat, whose abundance, rapid turnover, and recycling through the endocytic system may explain the efficiency of TLF2-mediated killing [9].

The African trypanosomes have used multiple strategies to circumvent the innate immunity conferred by TLF and apoL1, resulting in the modification of its endocytic system to either limit TLF1 uptake or to prevent apoL1 interacting with the lysosomal membrane. *T. b. rhodesiense* expresses serum resistance-associated antigen, or SRA, a truncated VSG [18], which is localised in the endocytic system where it is able to interact with apoL1 and prevent its lytic effect [19,20]. *T. b. gambiense* has evolved at least three mechanisms to avoid lysis by TLF1: group 1 parasites have (i) specific amino acid substitutions in HpHbR [21,22] and (ii) exhibit reduced HpHbR expression [23], leading to reduced TLF1 binding and uptake; and (iii) group 2 parasites, although they take up and traffic TLF1 to the lysosome, are resistant to its effects [24]. Exposure over millennia to this parasite has led to the evolution of apoL1 variants in West African populations, capable of lysing *T. b. rhodesiense*, although at the cost of a higher propensity to develop kidney disease [25]. Recognising the power of TLF1 as a parasite toxin, researchers have now joined forces with the innate immune system in the arms race against the African trypanosome. For example, truncated apoL1-conjugated nanobodies internalised via VSG endocytosis [26], and apoL1 variants that are no longer neutralised by SRA [27], represent potent antitrypanosomal treatments in experimental

**Box 2. RITseq and screening RNAi libraries for toxin efficacy determinants**

The *T. brucei* RNAi library, developed in the Englund laboratory and first used in procyclic insect-stage cells [48], has recently been imported into the bloodstream-stage parasite [38]. Using primers specific for common sequences flanking the integrated RNAi expression cassette, the RNAi targets remaining within a population following selection can be identified either by PCR and sequencing of individual products [49], or high-throughput sequencing [5]. This allows for rapid identification of proteins critical to the efficacy of the selective agent.

The power of this approach is 2-fold. Firstly, it provides an unbiased loss-of-function approach to assess the contribution of virtually the full *T. brucei* gene complement to the efficacy of any selective agent. Secondly, even proteins regarded as essential to parasite survival can be identified if the effect of their depletion on parasite growth is outweighed by the advantage gained under selection. For example, depletion of AP-1, p67, or cathepsin-L all generate significant growth defects in cultured bloodstream-form *T. brucei* [5,33,50]; however, all three were identified by selection of the RNAi library in suramin and were subsequently confirmed as contributing to suramin efficacy [5]. We were able to identify the role of such proteins in suramin uptake because, although the targeting fragments in the RNAi library have an average length of ~600 bp, their length actually ranges from ~100 bp to ~2 kbp; the shorter the fragment that overlaps the mRNA, the less the target mRNA is thought to be depleted, meaning that the contribution of otherwise essential proteins to a process can be observed.

infections. Several other approaches have also been suggested, including the attachment of surrogate ligands to TLF1 and the development of SRA-binding inhibitors, thus bypassing the need for TbHpHbR and enabling apoL1 to access the lysosomal membrane [28].

### Suramin: uptake and intracellular transit

Suramin is a highly charged molecule, preventing it from simply entering the cell by passive diffusion. The charged nature also gives suramin a high affinity for serum proteins, resulting in one of the longest *in vivo* half-lives of any drug [29]. Indeed, suramin binding to low-density lipoprotein (LDL) and receptor-mediated endocytosis was proposed for suramin uptake by *T. brucei* 20 years ago [30], although there is evidence for a non-LDL route of uptake in insect-stage cells [31]. More recently, high-throughput sequencing of a suramin-selected RNAi library (Box 2) identified a cohort of proteins contributing to drug efficacy [5]. Principal amongst these were the invariant surface glycoprotein, ISG75, proteins influencing its copy number, and several components of the endocytic apparatus, including AP-1 (adaptin complex-1), GLP-1 (Golgi/lysosomal protein-1), EMP70 (endosomal membrane protein 70), MFST (major facilitator superfamily transporter), p67 (major lysosomal glycoprotein), and cathepsin-L (Figure 1).

The identification of a substantial cohort of endosomal genes is consistent with a model whereby ISG75 acts as a major receptor for suramin (or the serum component to which it is bound) delivering the drug into the degradative arm of the endocytic pathway [5]. We speculate that suramin is delivered to the lysosome by either the serum protein carrier being cleaved by cathepsin-L upon reaching the lysosome or ISG75 being degraded at the late endosome. Once free, suramin may inhibit lysosomal enzymes and may also escape into the cytoplasm via the MFST channel, resulting in inhibition of other cellular processes.

### Exploiting receptor-mediated endocytosis for toxin delivery

Both TLF and suramin enter *T. brucei* by receptor-mediated endocytosis and, in common with suramin, TLF efficacy probably depends upon multiple factors, not limited to the presence of a surface receptor, but encompassing the many proteins regulating and constituting the endocytic system of the parasite. The efficacy of these two trypanocides may be influenced by overlapping cohorts of proteins. Indeed, the lysosomal protein, p67, has been shown to contribute to *T. b. brucei* human serum susceptibility [32,33] and suramin efficacy [5]. At present, other than HpHbR and the importance of the acidic environment in the lysosome [7,15], the parasite intrinsic determinants of TLF efficacy are largely unknown. Selecting the bloodstream-form *T. brucei* RNAi library in normal human serum will reveal the proteins that contribute to the trypanocidal action of TLF, including components of the endocytic system.

As outlined above, many determinants of suramin efficacy are now known. The invariant surface glycoproteins, including ISG75, probably constitute the major proteins being trafficked through the degradative arm of the endocytic pathway (Box 3) [34–36]. This may explain why suramin (as well as trypan blue, which is excluded from most other cell types) can enter *T. brucei*. Although there is probably >500-fold more VSG than ISG on the surface of the African trypanosome, and the VSG is endocytosed at a high rate [37], both VSGs and ISGs may represent a pathway into the cell that could be exploited for therapy.

It is curious that only ISG75, and not ISG65, was identified following suramin selection; this is unlikely to be a false negative as previous analysis suggests that, in culture at least, ISG65 is nonessential, and hence unlikely

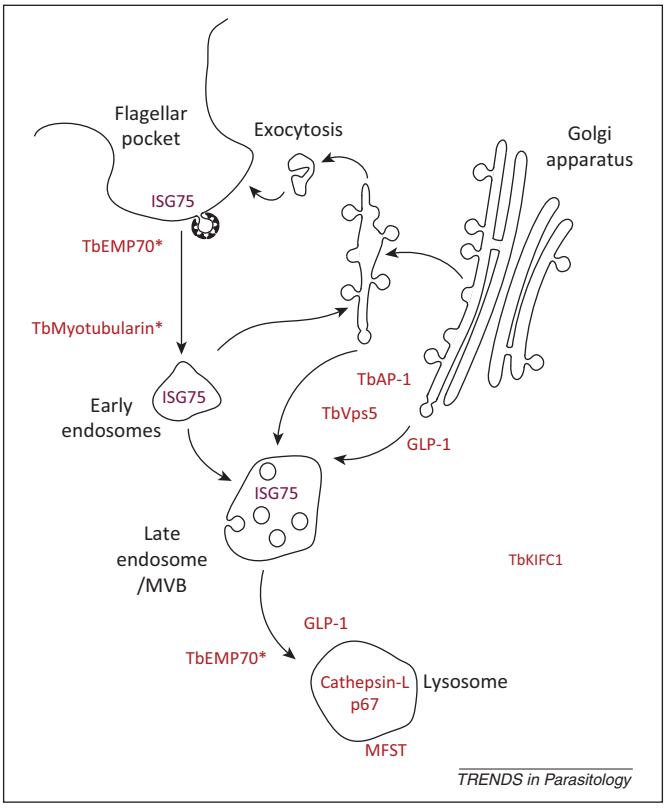


**Figure 1**. The *Trypanosoma brucei* endosomal apparatus and suramin uptake. The locations of suramin efficacy determinants are shown. ISG75 is shown at the flagellar pocket and early and late endosomes. The established marker for the trypanosome lysosome, p67, is well characterised, and cathepsin-L and the major facilitator superfamily transporter (MFST) have been localised to the same compartment [5]. At least two proteins are associated with the endocytic pathway to the lysosome, the trypanosome orthologues of EMP70 and myotubularin; the former may participate at both early and late points in the endosomal system [51], whereas myotubularin in higher eukaryotes is known to associate with phosphatidylinositol phosphate-5, placing it most probably at the early endosome in trypanosomes [52]. Three gene products that associate with the post-Golgi/endosome/lysosomal network were also identified: all four subunits of TbAP-1, TbVps5, and GLP-1. TbAP-1 mediates transport from the Golgi complex; although a role for lysosomal delivery has been reported, this does not seem to affect p67 and its precise function remains unclear [50,53]. GLP-1 is a protein of unknown function, but it has been localised to both Golgi-associated membranes and the lysosome, suggesting a role in trafficking between these compartments [54], whereas TbVps5 is a component of retromer, which mediates transport between the Golgi and late endosomal system. In trypanosomes, TbVps5 seems to preferentially affect ISG75 degradation, rather than ISG65, and is primarily located at late endosomal compartments, rather than the lysosome [39]. Not shown are ubiquitin hydrolases, which are likely to be cytoplasmic, and mediate their effects via modulation of ISG75 copy number. '*' designates locations based solely on analogy with orthologues in other organisms, and no data are available for trypanosomes at this time. TbKIFC1 is associated with acidocalcisomes, with no evidence for a function within the endosomal apparatus. However, these data are based on mainly chemical biology approaches, and therefore should be treated with caution [55].

**Box 3. Eyeing up the Big Eye: the endocytic apparatus of African trypanosomes**

The endocytic apparatus of all trypanosomatids is focused at the flagellar pocket, a small invagination towards the posterior pole of the cell, which provides a sheath around the flagellum as it enters the cell body. Many intracellular compartments, including the endosomes, are maintained in a close, coordinated manner at the same pole of the cell as the pocket [46]. All evidence indicates that the flagellar pocket membrane is the exclusive site for endocytic activity, and all endocytosis is clathrin-mediated in trypanosomes, with a number of specialisations that may serve to facilitate an extremely high flux in the bloodstream form [56]. These specialisations include the loss of the AP-2 sorting complex and dynamin from the endocytic apparatus in bloodstream stages. VSGs can be rapidly taken up by the endocytic system and recycled back to the cell surface, a process which we and others have suggested acts as an adjunct to antigenic variation [57]. Rapid endocytosis allows removal from the surface and subsequent degradation of surface-bound antibodies, but efficient recycling maintains an intact VSG coat [58].

The molecular atlas of the endocytic apparatus indicates that trypanosomes have a conventional, although simplified, endocytic system, consisting of early, sorting, and late endosomal compartments, together with a multivesicular body (MVB)/ESCRT system for the sorting of ubiquitylated proteins, and a terminal lysosome for degradation (Figure 1). Most recently, studies of 65 and 75 kDa *trans*-membrane domain ISG families revealed that these molecules are both efficiently endocytosed and likely to be recycled; moreover, they are present at fairly high copy number (~1 to 10 × $10^4$ per cell) [34]. Where these molecules differ from VSGs, however, is that their endocytosis is dependent on ubiquitylation [VSGs are glycosylphosphatidylinositol (GPI)-anchored proteins and hence cannot receive this modification] [36,59]. Both ISG65 and ISG75 are turned over rather rapidly, with half-lives of ~4 h, substantially shorter than VSGs, estimated as in excess of 30 h. The combination of high abundance and short half-life results in ISG65 and ISG75 being probably the major endocytic cargo trafficked through to the lysosome via the MVB, and potentially explains why one of them is involved in suramin sensitivity.

The extreme rate of bloodstream-form endocytosis also makes the pathway a potential Achilles' heel. Specifically, inhibition of endocytic activity by knockdown of various gene products results in rapid lethality due to enlargement of the flagellar pocket, a morphology referred to as Big Eye, due to the appearance of a phase light 'eye' in the cells [56]. Further, *N*-myristoyltransferase inhibitors also appear to target endocytosis, as they essentially phenocopy clathrin ablation [60], and accumulation of specific nanobodies at the flagellar pocket membrane also elicits a Big Eye phenotype and rapid cell death, potentially due to de-energisation [43]. Hence, the endocytic pathway is a vulnerability in itself and offers a viable route into the parasite for trypanocidal compounds.

to have been excluded by the screen [38]. Notably, both ISG65 and ISG75 are type I *trans*-membrane domain proteins with similar copy numbers, are comparatively similar in architecture, and rapidly turned over by related ubiquitylation-dependent pathways [36]. Recent data suggest differences between the trafficking of ISG65 and ISG75. Knockdown of TbVps5, part of the retromer complex involved in transport between late endosomes and the Golgi complex, has a stabilising effect on ISG75 but not ISG65 [39]. Conversely, knockdown of RME-8, which mediates late endosomal and recycling pathways, has a more profound effect on ISG65 than ISG75 turnover [34]. Additionally, ISG75 has a shorter half-life [36]. These data suggest that ISG65 and ISG75 trafficking is nonequivalent. Furthermore, the retention of these two gene families across the African trypanosomes is evidence that they are nonredundant. It is unknown what features of ISG75, compared with ISG65, determines its ability to interact with and mediate the toxicity of suramin. Also, it is unknown whether there is an additional suramin receptor beyond ISG75.

Regardless of the precise cellular mechanisms behind the role of ISG75 in suramin accumulation by *T. brucei*, these data indicate that ISG75 (and ISG65) represents a novel means to access the parasite and deliver trypanocides. This has appeal, as the ISGs are specific to the parasite, and also is conceptually related to oncology therapies exploiting similarly novel or overexpressed antigens for clinical gain [40]. Most obviously, the design of compounds related to suramin or screened for binding to ISG75 (or even ISG65) may represent a targeted route by which novel trypanocidal drug entities can be identified. Several pre-existing trypanocides have been noted to possess similar properties to suramin [41], and efforts are underway to determine if these compounds also enter the cell via ISG75, which would go part way towards justifying such a strategy.

A second, more complex route to utilise the ISGs is as a portal into the trypanosome for an immunotoxin. Again, this is a strategy that has its origins in oncology, where efforts to deliver toxins (either small molecule drugs or peptide based) coupled to an antibody or antibody fragment have long been seen as 'magic bullets' [42]. The presence of VSGs, and the issue of antigenic variation, has impacted consideration of this as a strategy for treating African trypanosomiasis, but at least one study has demonstrated trypanocidal action of nanobodies directed towards VSG epitopes [26]. The poor level of characterisation of the remainder of the bloodstream stage surface proteome and identification of alternative surface proteins for targeting has been an additional barrier. It is clear that nanobodies against VSGs elicit rather violent trypanocidal actions [26,43], indicating that these small molecules can efficiently access the flagellar pocket. The full exploitation of ISGs, which potentially avoids issues with antigenic variation, requires production of antibodies that recognise the protein while it is embedded in the VSG surface coat on living cells. This may be difficult to achieve, as the coat is conformationally distinct in live versus fixed parasites [44], and although ISG65 can be recognised on living cells [45], such an approach will require significant refinement before becoming a viable possibility. The use of nanobodies may provide superior access to invariant epitopes, offering an attractive strategy. Further, the ability of TLF1 and suramin to access the cell interior via TbHpHbR and ISG75, respectively, indicates that these receptors are accessible for the binding and intracellular delivery of toxins.

## Concluding remarks and future perspectives

A high rate of endocytosis is necessary for bloodstream-form *T. brucei* to maintain the VSG coat and remove bound antibodies [37], thus preventing complement-mediated destruction. We propose exploitation of this Achilles' heel for drug delivery. This strategy could minimise the development of resistance through reduced drug uptake, as endocytosis is essential for parasite survival [46]; clearly, the development of drugs that bind essential receptors

would be desirable in this regard. Alternatively, combination therapies may be used to minimise the development of resistance, and these should ideally bind different receptors and have different intracellular targets. A particular challenge is the development of selective drugs that are not toxic to the mammalian host. As each trypanosome receptor is expected to display an affinity for a particular host nutrient, it is likely that certain host receptors will also display affinity for these same factors. It will, therefore, be important to develop toxins with specific affinity for trypanosome receptors and/or with specific toxicity for trypanosomes. TLF clearly shows that selectivity is readily achievable, providing a precedent for the generation of further specific, drug-like ligands. There are some validated starting points in terms of known receptors, but an understanding of all exploitable trypanosome receptors and their ligands is also an important goal for future research.

## Update

There may be multiple additional candidate target receptors identified in this study: [Jackson, A.P. *et al.* (2013) A cell-surface phylome for African trypanosomes. *PLoS Negl. Trop. Dis.* 7, e2121].

## Acknowledgements

Work in the authors' laboratories is supported by the Wellcome Trust (SA, Institutional Strategic Support Fellowship; M.C.F., grant number 082813; D.H., grant number 093010/Z/10/Z) and the Medical Research Council (S.A., grant number MR/K011987/1; M.C.F., grant number MR/K008749/1; D.H., grant number MR/K000500/1).

## References

1 Steverding, D. (2008) The history of African trypanosomiasis. *Parasit. Vectors* 1, 3
2 Barrett, M.P. and Croft, S.L. (2012) Management of trypanosomiasis and leishmaniasis. *Br. Med. Bull.* 104, 175–196
3 Priotto, G. *et al.* (2009) Nifurtimox–eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, Phase III, non-inferiority trial. *Lancet* 374, 56–64
4 Ehrlich, P. (1913) Address in pathology, on chemiotherapy. Delivered before the Seventeenth International Congress of Medicine. *Br. Med. J.* 2, 353–359
5 Alsford, S. *et al.* (2012) High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature* 482, 232–236
6 Overath, P. *et al.* (1994) Invariant surface proteins in bloodstream forms of *Trypanosoma brucei*. *Parasitol. Today* 10, 53–58
7 Vanhollebeke, B. *et al.* (2008) A haptoglobin–hemoglobin receptor conveys innate immunity to *Trypanosoma brucei* in humans. *Science* 320, 677–681
8 Prull, C.R. (2003) Part of a scientific master plan? Paul Ehrlich and the origins of his receptor concept. *Med. Hist.* 47, 332–356
9 Vanhollebeke, B. and Pays, E. (2010) The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Mol. Microbiol.* 76, 806–814
10 Hajduk, S.L. *et al.* (1989) Lysis of *Trypanosoma brucei* by a toxic subspecies of human high density lipoprotein. *J. Biol. Chem.* 264, 5210–5217
11 Rifkin, M.R. (1978) Identification of the trypanocidal factor in normal human serum: high density lipoprotein. *Proc. Natl. Acad. Sci. U.S.A.* 75, 3450–3454
12 Raper, J. *et al.* (1999) Characterization of a novel trypanosome lytic factor from human serum. *Infect. Immun.* 67, 1910–1916
13 Tomlinson, S. *et al.* (1995) High-density-lipoprotein-independent killing of *Trypanosoma brucei* by human serum. *Mol. Biochem. Parasitol.* 70, 131–138
14 Molina-Portela, M.P. *et al.* (2005) Trypanosome lytic factor, a subclass of high-density lipoprotein, forms cation-selective pores in membranes. *Mol. Biochem. Parasitol.* 144, 218–226
15 Perez-Morga, D. *et al.* (2005) Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. *Science* 309, 469–472
16 Vanhollebeke, B. *et al.* (2007) Distinct roles of haptoglobin-related protein and apolipoprotein L-I in trypanolysis by human serum. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4118–4123
17 Bullard, W. *et al.* (2012) Haptoglobin–hemoglobin receptor independent killing of African trypanosomes by human serum and trypanosome lytic factors. *Virulence* 3, 72–76
18 Xong, H.V. *et al.* (1998) A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* 95, 839–846
19 Stephens, N.A. and Hajduk, S.L. (2011) Endosomal localization of the serum resistance-associated protein in African trypanosomes confers human infectivity. *Eukaryot. Cell* 10, 1023–1033
20 Vanhamme, L. *et al.* (2003) Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature* 422, 83–87
21 Higgins, M.K. *et al.* (2013) Structure of the trypanosome haptoglobin–hemoglobin receptor and implications for nutrient uptake and innate immunity. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1905–1910
22 Symula, R.E. *et al.* (2012) *Trypanosoma brucei gambiense* group 1 is distinguished by a unique amino acid substitution in the HpHb receptor implicated in human serum resistance. *PLoS Negl. Trop. Dis.* 6, e1728
23 Kieft, R. *et al.* (2010) Mechanism of *Trypanosoma brucei gambiense* (group 1) resistance to human trypanosome lytic factor. *Proc. Natl. Acad. Sci. U.S.A.* 107, 16137–16141
24 Capewell, P. *et al.* (2011) Differences between *Trypanosoma brucei gambiense* groups 1 and 2 in their resistance to killing by trypanolytic factor 1. *PLoS Negl. Trop. Dis.* 5, e1287
25 Genovese, G. *et al.* (2010) Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329, 841–845
26 Baral, T.N. *et al.* (2006) Experimental therapy of African trypanosomiasis with a nanobody-conjugated human trypanolytic factor. *Nat. Med.* 12, 580–584
27 Lecordier, L. *et al.* (2009) C-terminal mutants of apolipoprotein L-I efficiently kill both *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *PLoS Pathog.* 5, e1000685
28 Stephens, N.A. *et al.* (2012) Trypanosome resistance to human innate immunity: targeting Achilles' heel. *Trends Parasitol.* 28, 539–545
29 Burri, C. (2010) Chemotherapy against human African trypanosomiasis: is there a road to success? *Parasitology* 137, 1987–1994
30 Vansterkenburg, E.L. *et al.* (1993) The uptake of the trypanocidal drug suramin in combination with low-density lipoproteins by *Trypanosoma brucei* and its possible mode of action. *Acta Trop.* 54, 237–250
31 Pal, A. *et al.* (2002) Evidence for a non-LDL-mediated entry route for the trypanocidal drug suramin in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 122, 217–221
32 Lumb, J.H. *et al.* (2011) Rab28 function in trypanosomes: interactions with retromer and ESCRT pathways. *J. Cell Sci.* 124, 3771–3783
33 Peck, R.F. *et al.* (2008) The LAMP-like protein p67 plays an essential role in the lysosome of African trypanosomes. *Mol. Microbiol.* 68, 933–946
34 Koumandou, V.L. *et al.* (2013) Evidence for recycling of invariant surface transmembrane domain proteins in African trypanosomes. *Eukaryot. Cell* 12, 330–342
35 Leung, K.F. *et al.* (2008) Evolution of the multivesicular body ESCRT machinery; retention across the eukaryotic lineage. *Traffic* 9, 1698–1716
36 Leung, K.F. *et al.* (2011) Ubiquitylation and developmental regulation of invariant surface protein expression in trypanosomes. *Eukaryot. Cell* 10, 916–931
37 Engstler, M. *et al.* (2007) Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell* 131, 505–515
38 Alsford, S. *et al.* (2011) High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Res.* 21, 915–924
39 Koumandou, V.L. *et al.* (2011) Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei*. *J. Cell Sci.* 124, 1496–1509
40 Di Cosimo, S. and Baselga, J. (2008) Targeted therapies in breast cancer: where are we now? *Eur. J. Cancer* 44, 2781–2790
41 Morgan, H.P. *et al.* (2011) The trypanocidal drug suramin and other trypan blue mimetics are inhibitors of pyruvate kinases and bind to the adenosine site. *J. Biol. Chem.* 286, 31232–31240

42 Madhumathi, J. and Verma, R.S. (2012) Therapeutic targets and recent advances in protein immunotoxins. *Curr. Opin. Microbiol.* 15, 300–309
43 Stijlemans, B. *et al.* (2011) High affinity nanobodies against the *Trypanosoma brucei* VSG are potent trypanolytic agents that block endocytosis. *PLoS Pathog.* 7, e1002072
44 Schwede, A. *et al.* (2011) The VSG C-terminal domain is inaccessible to antibodies on live trypanosomes. *Mol. Biochem. Parasitol.* 175, 201–204
45 Chung, W.L. *et al.* (2004) Cytoplasmic targeting signals in transmembrane invariant surface glycoproteins of trypanosomes. *J. Biol. Chem.* 279, 54887–54895
46 Field, M.C. and Carrington, M. (2009) The trypanosome flagellar pocket. *Nat. Rev. Microbiol.* 7, 775–786
47 Matovu, E. *et al.* (2001) Genetic variants of the TbAT1 adenosine transporter from African trypanosomes in relapse infections following melarsoprol therapy. *Mol. Biochem. Parasitol.* 117, 73–81
48 Morris, J.C. *et al.* (2002) Glycolysis modulates trypanosome glycoprotein expression as revealed by an RNAi library. *EMBO J.* 21, 4429–4438
49 Baker, N. *et al.* (2011) Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. *Mol. Biochem. Parasitol.* 176, 55–57
50 Allen, C.L. *et al.* (2007) Dileucine signal-dependent and AP-1-independent targeting of a lysosomal glycoprotein in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* 156, 175–190
51 Aguilar, P.S. *et al.* (2010) A plasma-membrane E-MAP reveals links of the eisosome with sphingolipid metabolism and endosomal trafficking. *Nat. Struct. Mol. Biol.* 17, 901–908
52 Jean, S. *et al.* (2012) Sbf/MTMR13 coordinates PI(3)P and Rab21 regulation in endocytic control of cellular remodeling. *Mol. Biol. Cell* 23, 2723–2740
53 Tazeh, N.N. *et al.* (2009) Role of AP-1 in developmentally regulated lysosomal trafficking in *Trypanosoma brucei*. *Eukaryot. Cell* 8, 1352–1361
54 Lingnau, A. *et al.* (1999) Characterization of tGLP-1, a Golgi and lysosome-associated, transmembrane glycoprotein of African trypanosomes. *J. Cell Sci.* 112, 3061–3070
55 Dutoya, S. *et al.* (2001) A novel C-terminal kinesin is essential for maintaining functional acidocalcisomes in *Trypanosoma brucei*. *J. Biol. Chem.* 276, 49117–49124
56 Allen, C.L. *et al.* (2003) Clathrin-mediated endocytosis is essential in *Trypanosoma brucei*. *EMBO J.* 22, 4991–5002
57 Field, M.C. *et al.* (2009) Macromolecular trafficking and immune evasion in African trypanosomes. *Int. Rev. Cell Mol. Biol.* 278, 1–67
58 Pal, A. *et al.* (2003) Rab5 and Rab11 mediate transferrin and anti-variant surface glycoprotein antibody recycling in *Trypanosoma brucei*. *Biochem. J.* 374, 443–451
59 Chung, W.L. *et al.* (2008) Ubiquitylation is required for degradation of transmembrane surface proteins in trypanosomes. *Traffic* 9, 1681–1697
60 Frearson, J.A. *et al.* (2010) *N*-Myristoyltransferase inhibitors as new leads to treat sleeping sickness. *Nature* 464, 728–732