

University of Dundee

**Chimerization at the AQP2-AQP3 locus is the genetic basis of melarsoprol-pentamidine cross-resistance in clinical *Trypanosoma brucei gambiense* isolates**

Graf, Fabrice E.; Baker, Nicola; Munday, Jane C.; de Koning, Harry P.; Horn, David; Mäser, Pascal

*Published in:*  
International Journal for Parasitology: Drugs and Drug Resistance

*DOI:*  
[10.1016/j.ijpddr.2015.04.002](https://doi.org/10.1016/j.ijpddr.2015.04.002)

*Publication date:*  
2015

*Licence:*  
CC BY

*Document Version*  
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

*Citation for published version (APA):*

Graf, F. E., Baker, N., Munday, J. C., de Koning, H. P., Horn, D., & Mäser, P. (2015). Chimerization at the AQP2-AQP3 locus is the genetic basis of melarsoprol-pentamidine cross-resistance in clinical *Trypanosoma brucei gambiense* isolates. *International Journal for Parasitology: Drugs and Drug Resistance*, 5(2), 65-68. <https://doi.org/10.1016/j.ijpddr.2015.04.002>

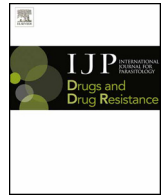
**General rights**

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

**Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



## Brief Report

Chimerization at the *AQP2–AQP3* locus is the genetic basis of melarsoprol–pentamidine cross-resistance in clinical *Trypanosoma brucei gambiense* isolatesFabrice E. Graf<sup>a,b</sup>, Nicola Baker<sup>c</sup>, Jane C. Munday<sup>d</sup>, Harry P. de Koning<sup>d</sup>, David Horn<sup>c</sup>, Pascal Mäser<sup>a,b,\*</sup><sup>a</sup> Swiss Tropical and Public Health Institute, CH-4051 Basel, Switzerland<sup>b</sup> University of Basel, CH-4000 Basel, Switzerland<sup>c</sup> Biological Chemistry & Drug Discovery, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK<sup>d</sup> Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, G12 8TA, UK

## ARTICLE INFO

## Article history:

Received 2 February 2015

Received in revised form 28 April 2015

Accepted 29 April 2015

Available online 7 May 2015

## Keywords:

Human African trypanosomiasis

Sleeping sickness

*Trypanosoma brucei gambiense*

Drug resistance

Melarsoprol

Pentamidine

Aquaporin

Reverse genetics

## ABSTRACT

Aquaglyceroporin-2 is a known determinant of melarsoprol–pentamidine cross-resistance in *Trypanosoma brucei brucei* laboratory strains. Recently, chimerization at the *AQP2–AQP3* tandem locus was described from melarsoprol–pentamidine cross-resistant *Trypanosoma brucei gambiense* isolates from sleeping sickness patients in the Democratic Republic of the Congo. Here, we demonstrate that reintroduction of wild-type *AQP2* into one of these isolates fully restores drug susceptibility while expression of the chimeric *AQP2/3* gene in *aqp2–aqp3* null *T. b. brucei* does not. This proves that *AQP2–AQP3* chimerization is the cause of melarsoprol–pentamidine cross-resistance in the *T. b. gambiense* isolates.

© 2015 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

*Trypanosoma brucei gambiense* is the causative agent of West-African sleeping sickness and responsible for 98% of today's cases of human African trypanosomiasis (HAT) (Brun et al., 2010). HAT is a fatal disease whose treatment exclusively relies on chemotherapy. Only five drugs are available: suramin and pentamidine for the first, haemolymphatic stage of the disease, melarsoprol and nifurtimox/eflornithine combination therapy for the second stage, when the parasites have infested the central nervous system. These drugs cause severe side effects and are difficult to administer (Brun et al., 2010). New drug candidates are in clinical development (Mäser et al., 2012), but until they are available for treatment, the current drugs must be used sustainably. Therefore understanding the molecular mechanism of drug resistance is a prerequisite. Drug resistance studies with *Trypanosoma brucei brucei* lab strains have identified loss of drug uptake as the major mechanism of drug re-

sistance in trypanosomes. This is due to mutations in the transporters responsible for drug uptake. The clinical drugs melarsoprol and pentamidine share two common transporter systems, the adenosine transporter 1 (TbAT1, also called P2; Carter and Fairlamb, 1993; Carter et al., 1995; Mäser et al., 1999) and aquaglyceroporin 2 (Baker et al., 2012). Genetic knock-out of either transporter gene, but particularly of *AQP2*, led to melarsoprol–pentamidine cross-resistance (MPXR) (Matovu et al., 2003; Baker et al., 2012, 2013).

Drug resistance of *T. b. gambiense* in the field has been controversial. The occurrence of mutant *TbAT1* alleles correlated to some extent with melarsoprol treatment failures (Matovu et al., 2001; Maina et al., 2007; Kazibwe et al., 2009), but no unambiguous genetic marker for resistance has been established so far. Recently, mutations at the *AQP2–AQP3* (Tb927.10.14170/Tb927.10.14160) tandem locus were found in *T. b. gambiense* isolates from the Democratic Republic of the Congo (Graf et al., 2013). In particular, a set of 41 isolates from Mbuji-Mayi, a HAT focus of exceptionally high melarsoprol treatment failure rates (Pyana et al., 2014), all carried a chimeric aquaglyceroporin, presumably formed by homologous recombination between *AQP2* and *AQP3*; a putative single-strand annealing mechanism accompanied by deletion of segments of *AQP2* and *AQP3* (Graf et al., 2013). The *AQP2/3*<sub>(814)</sub> chimera, the first 813

\* Corresponding author. Swiss Tropical and Public Health Institute, Socinstrasse 57, 4051 Basel, Switzerland. Tel.: +41 61 284 8338; fax: +41 61 284 8101.

E-mail address: [pascal.maeser@unibas.ch](mailto:pascal.maeser@unibas.ch) (P. Mäser).

b derived from *AQP2* and the last 126 b from *AQP3*, was in-frame, transcribed and homozygous. A second *AQP2/3* chimeric gene, *AQP2/3*<sub>(880)</sub> with just the last 60 bp derived from *AQP3*, had been described in the *T. b. gambiense* isolates from Mbuji-Mayi (Pyana et al., 2014). In the present study we did not detect the *AQP2/3*<sub>(880)</sub> gene either with direct sequencing of PCR products or after cloning of the PCR products into expression vectors. The isolates carrying the chimeric gene exhibited a markedly decreased melarsoprol sensitivity *in vivo* (Pyana et al., 2014). Those that were tested *in vitro* were cross-resistant to melarsoprol and pentamidine; to our knowledge, the first example of MPXR from clinical *T. b. gambiense* isolates (Graf et al., 2013).

Thus, the important question remained: Is the MPXR phenotype of the *T. b. gambiense* isolates from Mbuji-Mayi caused by the observed chimerization at the *AQP2–AQP3* locus? And if so, is it the presence of the *AQP2/3*<sub>(814)</sub> chimera or the absence of wild-type *AQP2* that causes drug resistance? Here, we answer these questions by (i) re-introducing wild-type *AQP2* into one of the mutant *T. b. gambiense* isolates and (ii) expressing the chimeric *AQP2/3*<sub>(814)</sub> gene from *T. b. gambiense* in *T. b. brucei*.

## 2. Materials and methods

### 2.1. Cell lines, cell culture and *in vitro* drug sensitivity assay

*T. b. brucei* 2T1 cells (Alsford et al., 2005) and 2T1 *aqp2–aqp3* double knock-out cells (Alsford et al., 2012) were maintained in HMI-11 medium. Puromycin (0.2 µg/ml) and phleomycin (0.5 µg/ml) were added for 2T1 cells. For 2T1 *aqp2–aqp3* double knock-out cells blasticidin (10 µg/ml) and G418 (2 µg/ml) were added in addition. Hygromycin (2.5 µg/ml), instead of puromycin, was added after transfection with chimeric *AQP2/3*<sub>(814)</sub>. *T. b. gambiense* 40AT (MHOM/CD/INRB/2006/07; Pyana et al., 2011) were cultured in HMI-9 medium with 15% FCS and 5% human serum, plus blasticidin (5 µg/ml) after transfection. *In vitro* drug sensitivities were determined as described (Graf et al., 2013). For the inducible cells, 1 µg/ml tetracycline (tet) was added 24 h prior to the assay.

### 2.2. Plasmids and transfection

The chimeric *AQP2/3*<sub>(814)</sub> gene (GenBank accession KF564935) was amplified by PCR with primers *AQP\_HindIII\_F* (ccgcaagcttatgca gagccaaccagac) and *AQP\_BamH1\_R* (ccgcgatccttagtggcacaataatt), or *AQP\_Xba1\_F* (ccgctctagaatgcagaccaaccagac) and *AQP\_BamH1\_R*, and cloned into the pRPa-series of tetracycline-inducible expression vectors (<http://www.lifesci.dundee.ac.uk/groups/david-horn/resources>). Vector inserts were checked for fidelity by Sanger sequencing (Microsynth). Bloodstream-form *T. b. brucei* were transfected as previously described (Baker et al., 2012). Clones were obtained by limiting dilution in standard HMI-11 medium plus antibiotics (see above). The *AQP2* gene was amplified from wild-type *T. brucei* 427 parasites and the *AQP2/3*<sub>(569–841)</sub> gene from the derived, pentamidine-resistant, strain B48, using proof-reading polymerase and oligonucleotides which added an Apal site to the 5′ end and a BamHI site to the 3′ end of the genes. The genes were ligated into pGEM-T Easy vector, and digested out using the added restriction sites. They were then ligated into similarly digested pHD1336 vector, to give plasmids pHDK21 (*AQP2*) and pHDK34 (*AQP2/3*<sub>(569–841)</sub>). Both plasmids were checked by Sanger Sequencing (Eurofins MWG Operon). Bloodstream-form *T. b. gambiense* were transfected with pHDK21 and pHDK34 as follows:  $4 \times 10^7$  cells were resuspended in 100 µl Tb-BSF nucleofection buffer (Schumann Burkard et al., 2011) (90 mM NaHPO<sub>3</sub>, 5 mM KCl, 0.15 mM CaCl<sub>2</sub>, 50 mM HEPES, pH 7.3) including 10 µg linearized plasmid DNA and placed in the nucleofection cuvette in the Amaxa Nucleofector (Lonza). Cells were electroporated using the program Z-001 and immediately trans-

ferred into 25 ml of pre-warmed HMI-9 medium containing 15% FCS, 5% human serum, and 20% sterile-filtered conditioned medium. Stable clones were obtained by limiting dilution and blasticidin selection (5 µg/ml). Correct integration was assessed by PCR on genomic DNA with primers *AQP2\_int\_F* (gtattggtgtggctgtcagc), *AQP3\_int\_R* (cccgttgagtaaccgatgtt), *pAQP\_F* (aacacaccgtaccgtcatt) and *pAQP\_R* (cttctctgtgctgtacg).

Western blots of GFP-*AQP2/3*<sub>(814)</sub> in 2T1 *aqp2–aqp3* null cells were performed as described (Baker et al., 2012). Western blots with GFP-*AQP2/3*<sub>(814)</sub> in 2T1 wild-type cells were performed as follows: cells were lysed in NUPAGE® LDS sample buffer (Life Technologies), samples separated on precast 4–12% Bis-Tris Gradient Gels (NuPAGE Novex®, Life Technologies) and transferred to nitrocellulose membranes using the iBlot dry-blotting system (Novex®, Life Technologies) according to the manufacturer's recommendations. Western blots were developed with the ECL Western Blotting Substrate (Pierce) using a ChemiDoc™ MP Gel Imaging System (Biorad). Primary Antibody: rabbit anti-GFP (Abcam, Ab290); secondary antibody: goat anti-rabbit (SouthernBiotech, 4050-05).

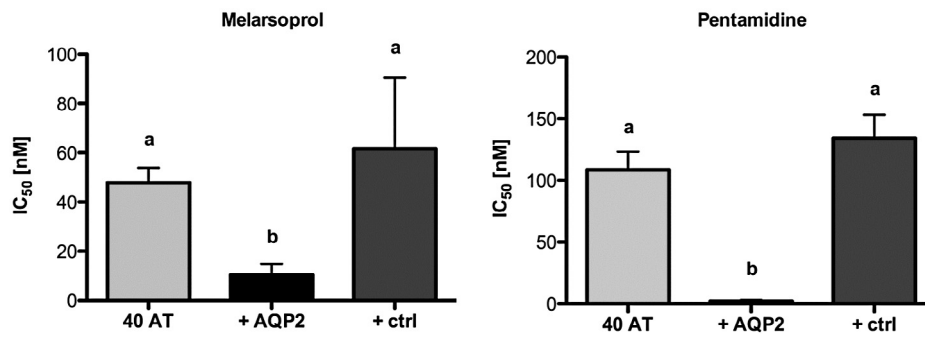
## 3. Results and discussion

### 3.1. Expression of wild-type *AQP2* re-sensitizes drug-resistant *T. b. gambiense*

To test whether the lack of *bona fide* *AQP2* activity contributes to drug resistance in the isolates from Mbuji-Mayi, we introduced a 'wild-type' copy of *AQP2* into *T. b. gambiense* 40AT, isolated from a melarsoprol-relapse patient after treatment (Pyana et al., 2011). The gene was integrated into the highly transcribed *rRNA*-spacer locus. This shifted the IC<sub>50</sub> of pentamidine from 108 nM to 2 nM and the IC<sub>50</sub> of melarsoprol from 47 nM to 10 nM (Fig. 1), a level similar to the fully susceptible *T. b. gambiense* reference isolate STIB930 (which had an IC<sub>50</sub> of 2 nM for pentamidine and 10 nM for melarsoprol; Graf et al., 2013). No shifts were observed with diminazene aceturate, a diamidine that is not an *AQP2* substrate (Munday et al., 2014), or with phenylarsine oxide (data not shown), an arsenical that diffuses through the plasma membrane. The same results were obtained with three additional clones. As a negative control, we transfected the 40AT cells with a non-functional *AQP2* mutant from the MPXR *T. b. brucei* clone B48 (Munday et al., 2014). As expected, this did not affect susceptibility to melarsoprol or pentamidine (Fig. 1). These results demonstrate that *AQP2* is key to drug susceptibility in the MPXR *T. b. gambiense* isolate.

### 3.2. Expression of the chimeric *AQP2/3*<sub>(814)</sub> in an *aqp2–aqp3* null background

To test whether the chimeric *AQP2/3*<sub>(814)</sub> can complement *AQP2* function with regard to drug uptake, we stably integrated the chimeric *AQP2/3*<sub>(814)</sub> gene from *T. b. gambiense* 40AT, either untagged or GFP-tagged, under the control of the tetracycline operator in a *T. b. brucei* host strain that expressed the tet repressor, and that carried a complete deletion of the *AQP2–AQP3* locus (Alsford et al., 2011). Tetracycline-inducible (1 µg/ml) expression of the chimeric *AQP2/3*<sub>(814)</sub> protein was confirmed by immuno-fluorescence microscopy (data not shown) and by Western blotting with an anti-GFP antibody (Fig. 2C). Drug sensitivities were determined *in vitro* for melarsoprol and pentamidine. None of the transfected cell lines showed a significant difference in IC<sub>50</sub> to pentamidine or melarsoprol when expression of *AQP2/3*<sub>(814)</sub> had been induced with tetracycline as compared to non-induced cells (Fig. 2A). This held true irrespective of the presence of the GFP tag. Thus no potential function in drug susceptibility could be attributed to the *AQP2/3* chimera. Expression of 'wild-type' *AQP2* using the same over-expression system (untagged and GFP-tagged) did not just reverse MPXR but



**Fig. 1.** Introduction of *AQP2* into mutant *T. b. gambiense*. *In vitro* drug sensitivity of bloodstream-form *T. b. gambiense* 40AT (grey) transfected with *AQP2* (black) or dysfunctional *AQP2* (ctrl, dark grey). Error bars are standard errors of the mean.  $n = 6$  independent experiments, each in duplicate. Small letters indicate significance groups as determined by one-way ANOVA and Tukey's post test using GraphPad Prism 5.0.

actually hypersensitized the *aqp2*–*aqp3* double null *T. b. brucei* to pentamidine and melarsoprol (Baker et al., 2012).

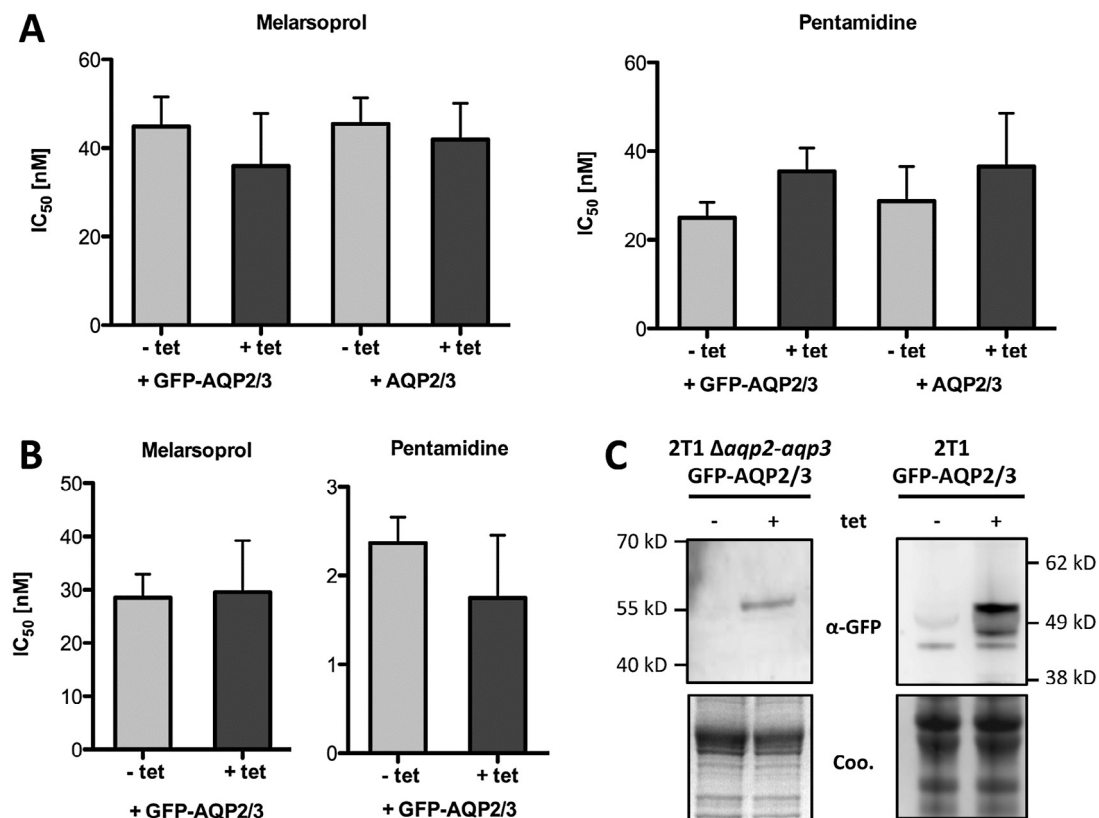
### 3.3. Expression of chimeric *AQP2/3*<sub>(814)</sub> in wild-type cells does not affect drug sensitivity

Aquaporins form homotetramers where each monomer constitutes a single pore. Work on human aquaporins involved in diabetes insipidus has revealed that the expression of a mutant aquaporin can give rise to dominant negative effects (Mulders et al., 1998). To test for negative interactions of *AQP2/3*<sub>(814)</sub> with 'wild-type' *AQP2*, the chimera was expressed in parental *T. b. brucei* 2T1 cells. The same

tetracycline-inducible over-expression system was used. Again, no significant difference was observed regarding sensitivity to pentamidine and melarsoprol in tetracycline-induced versus uninduced cells (Fig. 2B). Hence, the *AQP2/3*<sub>(814)</sub> chimera does not interfere with endogenous *AQP2* function in *T. b. brucei* bloodstream-form cells.

## 4. Conclusion

Previous work on the correlation of occurrence of the chimeric *AQP2/3*<sub>(814)</sub> gene in *T. b. gambiense* isolates from the DRC with *in vitro* drug sensitivity (Graf et al., 2013) suggested a functional link between the chimera and MPXR. However, proof of a causal relationship was



**Fig. 2.** Expression of the *AQP2/3*<sub>(814)</sub> chimera in *T. b. brucei*. *In vitro* drug sensitivity of bloodstream-form *T. b. brucei* 2T1 *aqp2*–*aqp3* double null mutants (A) and parental 2T1 cells (B) transfected with a tetracycline (tet) inducible *AQP2/3*<sub>(814)</sub> chimera. Dark bars, tet (1  $\mu\text{g}/\text{ml}$ ) was added 24 h prior to the drug assay. Error bars are standard error of the mean.  $n = 4$ –5 independent experiments, each in duplicate. (C) Western blot with anti-GFP antibody demonstrating inducible expression of GFP-tagged *AQP2/3*<sub>(814)</sub> (Coo, Coomassie stain). The GFP-*AQP2/3*<sub>(814)</sub> fusion proteins ran below their predicted molecular mass (approximately 60 kDa), which often applies for proteins with many transmembrane domains. The lower of the inducible bands in the blot on the right may represent unprocessed (e.g. unglycosylated) GFP-*AQP2/3*.

lacking. The AQP2/3<sub>(814)</sub> chimeric protein consists mostly of AQP2 sequence, including the atypical second filter sequence (Baker et al., 2012). Overall, AQP2/3<sub>(814)</sub> of the *T. b. gambiense* from Mbuji-Mayi has only 9 amino acid differences with AQP2. Moreover, the different *T. b. gambiense* isolates that harboured the chimeric gene were probably of clonal origin (Pyana et al., 2015) and may therefore not count as independent samples for the correlation of AQP2/3<sub>(814)</sub> genotype to MPXR phenotype. Thus reverse genetic engineering of bloodstream-form trypanosomes was required to establish a direct link between chimerization at the AQP2–AQP3 locus in *T. b. gambiense* isolates and MPXR. This was only feasible because some of the isolates from Mbuji-Mayi had been adapted to axenic growth *in vitro* (Pyana et al., 2011).

The MPXR *T. b. gambiense* isolate 40AT was completely re-sensitized to melarsoprol and pentamidine when transfected with a wild-type copy of AQP2. This proves that the observed chimerization at the AQP2–AQP3 locus is indeed the genetic basis of MPXR. The AQP2/3<sub>(814)</sub> chimeric protein did not exhibit any role in conferring drug sensitivity when over-expressed in *T. b. brucei*, neither in a *aqp2–aqp3* null background nor in the AQP2–AQP3 wild-type background. This further demonstrates that it is the absence of ‘wild-type’ AQP2, and not the presence of the AQP2/3<sub>(814)</sub> chimera, that causes the MPXR phenotype. Deletion-based gene-fusion at the AQP2–AQP3 locus by homologous recombination is likely facilitated by the high degree of sequence identity between AQP2 and AQP3. Taken together, our findings strongly indicate that chimerization at the AQP2–AQP3 locus causes melarsoprol–pentamidine cross-resistance in *T. b. gambiense*. This consequently increases the risk of treatment failures in problematic HAT foci such as Mbuji-Mayi of the Democratic Republic of the Congo. This is the first example where a genetic basis for drug-resistant sleeping sickness has been confirmed.

## Acknowledgements

We wish to thank Remo Schmidt and Christina Kunz-Renggli for their help in the lab. This work was supported by the Swiss National Science Foundation (grant 31003A\_135746 to PM), from the UK Medical Research Council (MRC; grant 84733 to HdK), from the MRC and Department for International Development, UK under the MRC/DFID Concordat agreement (grant MR/K000500/1 to DH), and from the Wellcome Trust (grant 100320/Z/12/Z; Senior Investigator Award to DH).

## Conflict of interest

The authors declared that there is no conflict of interest.

## References

- Alsford, S., Kawahara, T., Glover, L., Horn, D., 2005. Tagging a *T. brucei* RRNA locus improves stable transfection efficiency and circumvents inducible expression position effects. *Mol. Biochem. Parasitol.* 144, 142–148.
- Alsford, S., Turner, D.J., Obado, S.O., Sanchez-Flores, A., Glover, L., Berriman, M., et al., 2011. High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Res.* 21, 915–924.
- Alsford, S., Eckert, S., Baker, N., Glover, L., Sanchez-Flores, A., Leung, K.F., et al., 2012. High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature* 482, 232–236.
- Baker, N., Glover, L., Munday, J.C., Aguinaga Andres, D., Barrett, M.P., de Koning, H.P., et al., 2012. Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 10996–11001.
- Baker, N., de Koning, H.P., Mäser, P., Horn, D., 2013. Drug resistance in African trypanosomiasis: the melarsoprol and pentamidine story. *Trends Parasitol.* 29, 110–118.
- Brun, R., Blum, J., Chappuis, F., Burri, C., 2010. Human African trypanosomiasis. *Lancet* 375, 148–159.
- Carter, N.S., Fairlamb, A.H., 1993. Arsenical-resistant trypanosomes lack an unusual adenosine transporter. *Nature* 361, 173–175.
- Carter, N.S., Berger, B.J., Fairlamb, A.H., 1995. Uptake of diamidine drugs by the P2 nucleoside transporter in melarsen-sensitive and -resistant *Trypanosoma brucei*. *J. Biol. Chem.* 270, 28153–28157.
- Graf, F.E., Ludin, P., Wenzler, T., Kaiser, M., Brun, R., Pyana, P.P., et al., 2013. Aquaporin 2 mutations in *Trypanosoma brucei gambiense* field isolates correlate with decreased susceptibility to pentamidine and melarsoprol. *PLoS Negl. Trop. Dis.* 7, e2475.
- Kazibwe, A.J., Nerima, B., de Koning, H.P., Mäser, P., Barrett, M.P., Matovu, E., 2009. Genotypic status of the TbAT1/P2 adenosine transporter of *Trypanosoma brucei gambiense* isolates from Northwestern Uganda following melarsoprol withdrawal. *PLoS Negl. Trop. Dis.* 3, e523.
- Maina, N., Maina, K.J., Mäser, P., Brun, R., 2007. Genotypic and phenotypic characterization of *Trypanosoma brucei gambiense* isolates from Ibba, South Sudan, an area of high melarsoprol treatment failure rate. *Acta Trop.* 104, 84–90.
- Matovu, E., Geiser, F., Schneider, V., Mäser, P., Enyaru, J.C.K., Kaminsky, R., 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.
- Matovu, E., Stewart, M.L., Geiser, F., Brun, R., Mäser, P., Wallace, L.J., et al., 2003. Mechanisms of arsenical and diamidine uptake and resistance in *Trypanosoma brucei*. *Eukaryot. Cell* 2, 1003–1008.
- Mäser, P., Sütterlin, C., Kralli, A., Kaminsky, R., 1999. A nucleoside transporter from *Trypanosoma brucei* involved in drug resistance. *Science* 285, 242–244.
- Mäser, P., Wittlin, S., Rottmann, M., Wenzler, T., Kaiser, M., Brun, R., 2012. Antiparasitic agents: new drugs on the horizon. *Curr. Opin. Pharmacol.* 12, 562–566.
- Mulders, S.M., Bichet, D.G., Rijss, J.P., Kamsteeg, E.J., Arthus, M.F., Lonergan, M., et al., 1998. An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J. Clin. Invest.* 102, 57–66.
- Munday, J.C., Eze, A.A., Baker, N., Glover, L., Clucas, C., Aguinaga Andres, D., et al., 2014. *Trypanosoma brucei* aquaglyceroporin 2 is a high-affinity transporter for pentamidine and melaminophenyl arsenic drugs and the main genetic determinant of resistance to these drugs. *J. Antimicrob. Chemother.* 69, 651–663.
- Pyana, P., Van Reet, N., Mumba Ngoyi, D., Ngay Lukusa, I., Karhemere Bin Shamamba, S., Buscher, P., 2014. Melarsoprol sensitivity profile of *Trypanosoma brucei gambiense* isolates from cured and relapsed sleeping sickness patients from the Democratic Republic of the Congo. *PLoS Negl. Trop. Dis.* 8, e3212.
- Pyana, P.P., Ngay Lukusa, I., Mumba Ngoyi, D., Van Reet, N., Kaiser, M., Karhemere Bin Shamamba, S., et al., 2011. Isolation of *Trypanosoma brucei gambiense* from cured and relapsed sleeping sickness patients and adaptation to laboratory mice. *PLoS Negl. Trop. Dis.* 5, e1025.
- Pyana, P.P., Sere, M., Kabore, J., De Meeus, T., MacLeod, A., Bucheton, B., et al., 2015. Population genetics of *Trypanosoma brucei gambiense* in sleeping sickness patients with treatment failures in the focus of Mbuji-Mayi, Democratic Republic of the Congo. *Infect. Genet. Evol.* 30, 128–133.
- Schumann Burkard, G., Jutzi, P., Roditi, I., 2011. Genome-wide RNAi screens in bloodstream form trypanosomes identify drug transporters. *Mol. Biochem. Parasitol.* 175, 91–94.