Isolation and *in vivo* drug sensitivity profiling of *Trypanosoma brucei gambiense* from cured and relapsed sleeping sickness patients

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Introduction

• Until recently, neurological *gambiense* sleeping sickness stage treated with melarsoprol

• HAT foci with high relapse rates after melarsoprol treatment in Uganda, Sudan, Angola, D.R. Congo

• No definite explanation yet

• Working hypothesis: drug resistant *T.b. gambiense*
Working hypotheses

1. High relapse rates caused by drug resistance of parasites
   - Phenotypic and genotypic differences between strains isolated from cured and relapsed patients
   - Phenotypic characterisation
     - In vivo, in vitro drug sensitivity
   - Genotypic characterisation
     - Gene mutations
     - Gene expression

2. Parasites are not resistant to drugs
   - Phenotypic and genotypic differences between cured and relapsed patients
Study design

• Mbuji-Mayi, East Kasai, DRC: 360 patients with complete 2 years follow-up; 37% relapses

• Isolation of strains from relapsed and cured patients

• Primary isolation through *Grammomys surdaster* and *Mastomys natalensis*
Study design

• Adaptation of the isolates to *Mus musculus* and to axenic *in vitro* culture

• Drug sensitivity profiling of the strains
  – Assess minimum melarsoprol dose to cure mice infected with strains from cured patients
  – Test all strains and reference isolates (wild type and *TbAT1* knock out) with this minimal dose

• Testing for molecular drug resistance markers

• Testing for genetic diversity of strains
Methods

• Specimen collection at treatment center
  – Patient blood and CSF mixed with bull semen cryomedium (Triladyl®)
  – Cryopreservation in liquid nitrogen for 1 to > 24 months
  – Before treatment from all patients
  – After treatment from relapsing patients

• Isolation in susceptible rodents, adaptation to *Mus musculus*, and to *in vitro* medium
Methods

Isolation of *Trypanosoma brucei gambiense* from cured and relapsed sleeping sickness patients and adaptation to laboratory mice.


*Trypanosoma brucei gambiense*: HMI-9 medium containing methylcellulose and human serum supports the continuous axenic in vitro propagation of the bloodstream form.

Van Reet N, Pyana PP, Deborggraeve S, Büscher P, Claes F.
Methods

• *In-vivo* sensitivity testing
  – Acute infection model = brain not yet invaded
  – 6 mice per drug dose
  – Infection with 50,000 trypanosomes per mouse
  – Treatment at days 6,7,8,9
  – Follow-up for 90 days
  – Check for differences between strains isolated from cured and from relapsed patients
Methods

• Molecular characterisation for isolate diversity

  – *T.b. gambiense* specific PCR TgsGP

  – Mobile Genetic Element PCR
    “Trypanosoma brucei s.l.: Characterisation of stocks from Central Africa by PCR analysis of mobile genetic elements”.

  – Microsatellites
    Simo et al. Infect Genet Evol 2009, 10: 68-76
Methods

• Molecular characterisation for drug resistance

  – P2 adenosine transporter resistance specific PCRs
    “The P2 aminopurine transporter, encoded by TbAT1 ....in the Trypanosoma brucei group, carries melaminophenyl arsenical and diamidine drugs into these parasites.”

  – Aquaglyceroporin 2/3 (AQP2 and AQP3)
    Baker et al. PNAS 2012;10996-11001
    “Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes”.
Results

• 85 strains isolated from Kasaï and 6 from Masi

• 12 paired strains (same patient before and after TTT)

• 13 strains from cured patients

• 40 strains adapted to mice

• 5 strains adapted to *in vitro*
In vivo melarsoprol sensitivity test in acute infection model
All, except 3, *T. b. gambiense* strains are sensitive to 10 mg/kg melarsoprol

<table>
<thead>
<tr>
<th>strain</th>
<th>8mg/kg</th>
<th>10mg/kg</th>
<th>12mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. b. gambiense 348BT</em></td>
<td>relapsed</td>
<td>cure</td>
<td>cure</td>
</tr>
<tr>
<td><em>T. b. gambiense 15BT</em></td>
<td>not tested</td>
<td>relapsed*</td>
<td>cure</td>
</tr>
<tr>
<td><em>T. b. gambiense 163AT</em></td>
<td>not tested</td>
<td>relapsed*</td>
<td>relapsed</td>
</tr>
<tr>
<td><em>T. b. gambiense 346AT</em></td>
<td>not tested</td>
<td>relapsed*</td>
<td>relapsed</td>
</tr>
<tr>
<td><em>T. b. brucei</em> wild type</td>
<td>not tested</td>
<td>sensitive</td>
<td>sensitive</td>
</tr>
<tr>
<td><em>T. b. brucei</em> TbAT1 knock out</td>
<td>not tested</td>
<td>sensitive</td>
<td>sensitive</td>
</tr>
</tbody>
</table>

Wild type=sensitive strain; Knock out= resistant strain; BT=before treatment; AT=after treatment; *= tested two times at 10mg/kg
Results

Molecular characterisation

1. TgsGP: all strains confirmed *T.b.gambiense*
2. MGE-PCR: absence of intra-specific length variation of RIME elements
3. Microsatellites: analysis ongoing at Glasgow University/IRD
4. P2 Adenosine: no *TbAT1* gene mutations/deletion observed
5. Aqualyceroporin 2/3: ongoing
Conclusions

• Unique collection of *T.b. gambiense* strains from cured and relapsed patients with paired strains BT and AT

• Some evidence of drug resistance in few strains

• *In vivo* drug sensitivity and molecular characterisation continues
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