Review of recent preclinical information and pharmacokinetic-pharmacodynamic data in Chagas disease
Isabela Ribeiro
Chagas Disease Clinical Trials - 2008

- Two randomised clinical trial of BZN in adults
  - TRAENA (started in 03/1999 – 12/2012)
  - BENEFIT (11/2004 – ongoing)

- Decades with no new clinical trials for new treatment options in Chagas disease

- R&D and access stalled by existing knowledge gaps
  - Relevance of animal models
  - Limited data on:
    - the importance of different parasite strains to human disease
    - coexistence of infection
    - mechanisms of resistance
  - PK/PD in Chagas largely unknown
  - No consensus on reference treatment
  - Lack of early test of cure
  - Limited sensitivity of PCR test
Consensus 2008

• Azoles class of compounds represented the drug class with highest potential for treatment of Chagas disease
• Existing treatments for Chagas disease were more effective against *T. cruzi* infections in acute stage than in chronic stage
• Indications that children responded to Chagas disease treatment better than adults – better efficacy and safety profile
Results Adult Clinical Trials
NCT0116967 and NCT01489228

Parasitological Success with BZN

<table>
<thead>
<tr>
<th>Sustained Clearance At 12 Months</th>
<th>No n (%)</th>
<th>Yes n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=47) 43 (91.5)</td>
<td>4 (8.5)</td>
<td></td>
</tr>
<tr>
<td>(N=48) 44 (91.7)</td>
<td>4 (8.3)</td>
<td></td>
</tr>
<tr>
<td>(N=46) 41 (89.1)</td>
<td>5 (10.9)</td>
<td></td>
</tr>
<tr>
<td>(N=45) 32 (71.1)</td>
<td>13 (28.9)</td>
<td></td>
</tr>
<tr>
<td>(N=45) 8 (19.0)</td>
<td>37 (81.0)</td>
<td></td>
</tr>
<tr>
<td>(N=231) 168 (72.7)</td>
<td>63 (27.3)</td>
<td></td>
</tr>
</tbody>
</table>
Findings of Adult Clinical Trials

- Highlighted Major Translational Challenges
  - Need to translate research data to assays compatible with Drug Discovery & Development process
  - Better translation in vitro/in vivo models and the clinic
  - Address the right questions in our models
  - Better understanding of PK/PD relationships

- Importance of generating clinical trial data with standardised methodologies
Nitroheterocyclic compounds are more efficacious than CYP51 inhibitors against *Trypanosoma cruzi*: implications for Chagas disease drug discovery and development

Carolina B. Moraes¹,², Miriam A. Giardini³, Hwayoung Kim¹, Caiio H. Franco², Adilberto M. Araújo Junior⁴, Sergio Schenkman⁴, Eric Chatelain⁴ & Lucio H. Freitas Junior¹,³

Nitroderivatives showed Potent In Vitro Activity across all T. cruzi DTUs
- >90% maximum activity
- Panel of different strains Dm28c (DTU I, Y (DTU II), ARMA13 cl1 (DTU III), ERA cl2 (DTU IV), 92-80 cl2 (DTU V), CL Brener (DTU VI), and Tulahuen (DTU VI)

Azoles variable pattern
- <<50% maximum activity for some of the tested strains/lineages
- Considerable dispersion
Nitroheterocyclic compounds are more efficacious than CYP51 inhibitors against *Trypanosoma cruzi*: implications for Chagas disease drug discovery and development

*Time to kill experiments Y strain*

- BZN and Nifurtimox showed fast trypanocidal activity eliminating intracellular T.cruzi within 96 hours of continuous exposure *in vitro*

- Azole compounds exhibited slow trypanocidal activity, which with prolonged exposure reduces but did not eliminate intracellular infection
Assessing anti-*T. cruzi* candidates *in vitro* for sterile cidality

Monica Cal\(^a, b\), Jean-Robert Ioset\(^c\), Matthia A. Fügi\(^a, b\), Pascal Mäser\(^a, b\), Marcel Kaiser\(^a, b\), *

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### In vitro evaluation of cidal activity

- **Mouse peritoneal macrophage system** – long term testing and washout experiments
- **Giemsa readout**: >> sensitive and accurate
- **Nitros response versus Azoles**
  - **Azoles** –
    - flat dose response curve
    - Inability to kill all parasites in 96h
  - **Nitros**
    - 100% clearance in infected cells
    - BZN 40uM <1 parasite/100 macrophage

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**In vitro sensitivity after 96 hours of exposure** – B-galactosidase method

**In vitro sensitivity after 96 hours of exposure** – Giemsa

**Estimated number of amastigotes/100 macrophages after 96 hours of exposure**

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*University of Basel, CH-4003, Basel, Switzerland*

*Drs for Neglected Diseases Initiative, CH-1202, Geneva, Switzerland*
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In vitro evaluation of cidal activity
- Washout experiments
- Nitros response versus Azoles
  - Azoles —
    - Shift >200% in IC50
    - Viable amastigotes
  - Nitros
    - No significant change

**Drug sensitivity**

**Estimated number of amastigotes/100 macrophages after 96h washout and 168 hours recovery**

Drug sensitivity % infected cells after 96h washout and 168 hours recovery

Estimated number of amastigotes/100 macrophages after 96h washout and 168 hours recovery
Nitroheterocyclic drugs cure experimental *Trypanosoma cruzi* infections more effectively in the chronic stage than in the acute stage

Amanda Fortes Francisco, Shiromani Jayawardhana, Michael D. Lewis, Karen L. White, David M. Shackleford, Gong Chen, Jessica Saunders, Maria Osuna-Cabello, Kevin D. Read, Susan A. Charman, Eric Chatelain & John M. Kelly

Cl Brener model – evaluation of nifurtimox, benznidazole, fexinidazole and fexinidazole sulphone
Nitroheterocyclic drugs cure experimental Trypanosoma cruzi infections more effectively in the chronic stage than in the acute stage.

### Cure rates – Dose and duration dependent

<table>
<thead>
<tr>
<th>T. cruzi CL Brener strain</th>
<th>Chronic Infection Cure Rate</th>
<th>Acute Infection Cure Rate</th>
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</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Mouse strain</td>
<td>Dose (mg kg⁻¹)</td>
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<td></td>
</tr>
<tr>
<td>BZ</td>
<td>BALB/c</td>
<td>10 qd</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>30 qd</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>100 qd</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>50 bid</td>
</tr>
<tr>
<td>NF</td>
<td>BALB/c</td>
<td>30 qd</td>
</tr>
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<td></td>
<td>BALB/c</td>
<td>100 qd</td>
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<td></td>
<td>BALB/c</td>
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</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>50 bid</td>
</tr>
</tbody>
</table>

### Chronic stage infection

- NT 15 20 10 5
- BZ 13 14 20 10
- NF 13 14 23 35
- FX 14 13 138 35
- FXS 14 13 156 49

### Acute stage infection

- NT 20 10
- BZ 20 10
- NF 23 35
- FX 42 49
- FXS 42 49

### Figures

- **BZN 30 mg/kg**
- **5 days treatment**
Pharmacokinetic and cure data for BZN

A. Plasma concentration versus time after 100 mg/kg uninfected, acute and chronic
   • No difference in PK between acute and chronic

B. Plasma concentration with single dose 10, 30, 100 mg/kg

C. Cure rates after 10, 20 days in chronic stage mice

D. Cure rates after 10, 20 days in acute stage mice
   • Association between dose/exposure and effectiveness
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Implications for R&D

“The greatest enemy of knowledge is not ignorance, but the illusion of knowledge”

Stephen Hawking
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