Drug discovery and the epidemiology of Chagas disease
T. cruzi isolates differ in biological, pathological, immunological, molecular and ecoepidemiological criteria.

### Tissue tropism

- **Spleen**

### DNA content

- **Genome size variation**
  - 80 to 120 Mbp

### Drug susceptibility

- **Susceptible**
- **Resistant**

*Fig. 2. Individual sensitivity of 47 T. cruzi strains to nifurtimox and benznidazole determined in experimentally infected mice.*
At present, the taxon *T. cruzi* is divided into six lineages, named Discrete Typing Units (DTUs), TcI-TcVI.

### Protocol for genotyping the 6 DTUs

<table>
<thead>
<tr>
<th>24Sα rDNA PCR product</th>
<th>110bp only</th>
<th>125bp only</th>
<th>110bp only</th>
<th>120pb*</th>
<th>110bp or 110+125bp</th>
<th>125bp only</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP60- EcoRV PCR-RFLP</td>
<td>1 band</td>
<td>1 band</td>
<td>2 bands</td>
<td>1 band</td>
<td>3 bands</td>
<td>3 bands</td>
</tr>
<tr>
<td>GPI-HhaI PCR-RFLP</td>
<td>2 bands</td>
<td>3 bands</td>
<td>2 bands</td>
<td>3 bands**</td>
<td>4 bands</td>
<td>4 bands</td>
</tr>
</tbody>
</table>

- **TcI**
- **TcII**
- **TcIII**
- **TcIV**
- **TcV**
- **TcVI**

*Zingales et al., 2009
Zingales et al., 2012*
Association between DTUs and sylvatic reservoirs

TcI, TcII, TcIV, TcV and TcVI rares

opossums

anteaters

rodents

TcIII, TcI, TcIV, TcV and TcVI rares

armadillos

golden lion tamarins
TcI, TcII, TcV and TcVI are major agents of Chagas disease

Zingales et al., 2012
Differential drug susceptibility of *T. cruzi* strains

**Nifurtimox and Benznidazole**

Drug Resistance is not associated to DTU group

**Ergosterol biosynthesis inhibitors**
Comparison of Bz and Ravuconazole – Cure in a *murine model*

<table>
<thead>
<tr>
<th>Strain</th>
<th>BZ (100 mg/Kg/20 doses)</th>
<th>Ravuconazole (15 mg/Kg/40 doses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (TcVI)</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>(BZ-susceptible)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y (TcII)</td>
<td>9/12</td>
<td>7/12</td>
</tr>
<tr>
<td>(BZ-intermediate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colombiana (Tcl)</td>
<td>4/12</td>
<td>0/10</td>
</tr>
<tr>
<td>(BZ-resistant)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Colombiana (Tcl) – resistant to both drugs

Urbina et al., 2003

Fig. 2. Individual sensitivity of 47 *T. cruzi* strains to nifurtimox and benznidazole determined in experimentally infected mice.
Activity of compounds against intracellular amastigotes

Activity of compounds against intracellular amastigotes

Moraes et al., 2014, DOI: 10.1038/srep04703
Posaconazole Phase IIa Trial (Barcelona)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Benznidazole (N = 26)</th>
<th>Posaconazole HD (N = 26)</th>
<th>Posaconazole LD (N = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolivia (TcV)</td>
<td>24</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Brazil (TcII)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paraguay (TcV; TcVI)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Posa - Therapeutic failures**

**BZ still better**

Molina et al., 2014. The New England Journal of Medicine
Drug discovery for Chagas disease: selection of strains representing *Trypanosoma cruzi* diversity
Recommendations for high throughput screening

B. Zingales, M.A. Miles, C.B. Moraes, A. Luquetti, F. Guhl, A.G. Schijman, I. Ribeiro
Mem. Inst. Oswaldo Cruz, 2014

- Screening should be performed on intracellular amastigotes

- Initial screening against two DTUs prevalent in Humans (TcI and TcII, and/or TcV and/or TcVI).

- Secondary screening against two representatives of each human DTU
  - Different geographic origin
  - Resistant to BZ

Note: Strains available upon request. Shipment problems
Research lines of my laboratory
1. Therapeutic failures to benznidazole (natural and acquired BZ resistance)

- Participation of one ABC transporter – sub-family G (TcABCG1)

- Participation of a type I mitochondrial nitroreductase (TcNTR) (BZ activation)

**Target:** Parasite isolates obtained from patients submitted to BZ therapy: cured and non-cured.
2. Screening of candidates for Chagas disease treatment

- N’-[(5-nitrofuran-2-yl) methylene] substituted hydrazides

<table>
<thead>
<tr>
<th>IC50 (µM)</th>
<th>BZ</th>
<th>NF</th>
<th># 11</th>
<th># 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcI</td>
<td>29.16 ± 2.90</td>
<td>6.02 ± 0.32</td>
<td>4.75 ± 0.36</td>
<td>5.07 ± 0.28</td>
</tr>
<tr>
<td>TcII</td>
<td>40.40 ± 3.37</td>
<td>12.84 ± 1.30</td>
<td>3.10 ± 0.29</td>
<td>3.47 ± 0.20</td>
</tr>
<tr>
<td>TcV</td>
<td>30.63 ± 3.00</td>
<td>7.32 ± 0.76</td>
<td>4.41 ± 0.46</td>
<td>4.25 ± 0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EC50 (µM) against intracellular amastigotes (TcII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>BZ</td>
</tr>
<tr>
<td>NF</td>
</tr>
<tr>
<td># 11</td>
</tr>
<tr>
<td># 14</td>
</tr>
</tbody>
</table>

- Quinazoline and dimethoxibenzoate derivatives
Drug discovery for Chagas disease

Additional Research Priorities:

1. Why Benznidazole and Nifurtimox have low efficacy in the chronic phase of the disease (<30 - 50% cure)? Immunological response?

2. Development of diagnostic tests for early determination of therapeutic responses and cure (new biological markers).

Candido Portinari – Criança morta. 1944
Collaborations

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Support

FAPESP, CNPq
Muito obrigada!