

Drug discovery and the epidemiology of Chagas disease



Candido Portinari – Os retirantes. 1944

T. cruzi isolates differ in biological, pathological, immunological, molecular and ecoepidemiological criteria

Tissue tropism

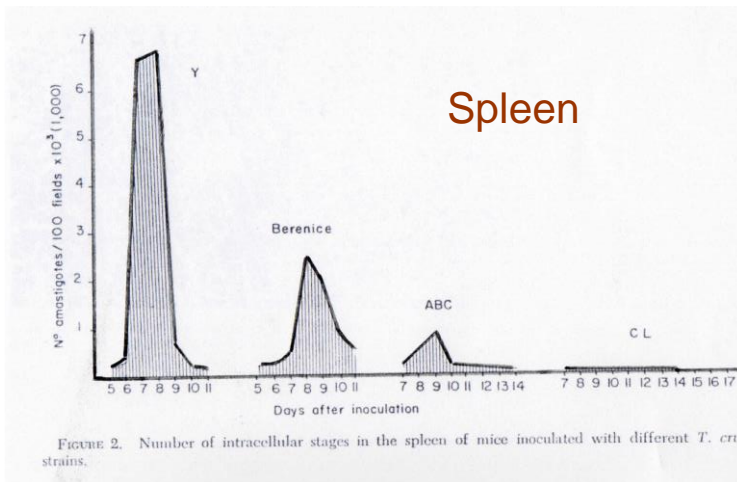


FIGURE 2. Number of intracellular stages in the spleen of mice inoculated with different *T. cruzi* strains.

Drug susceptibility

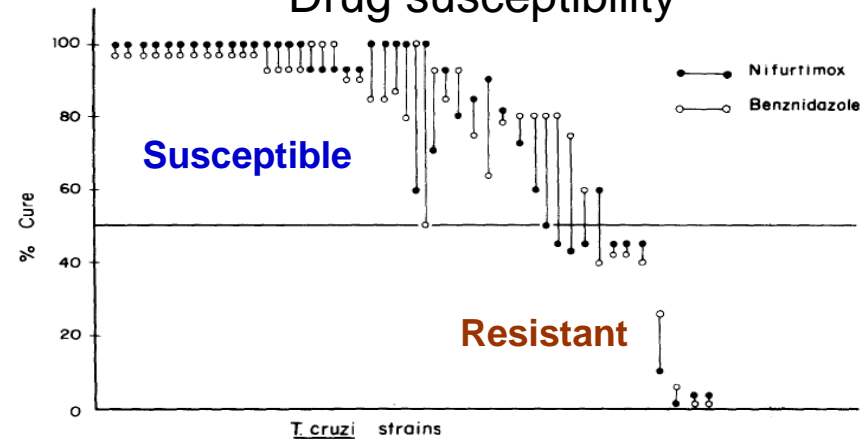
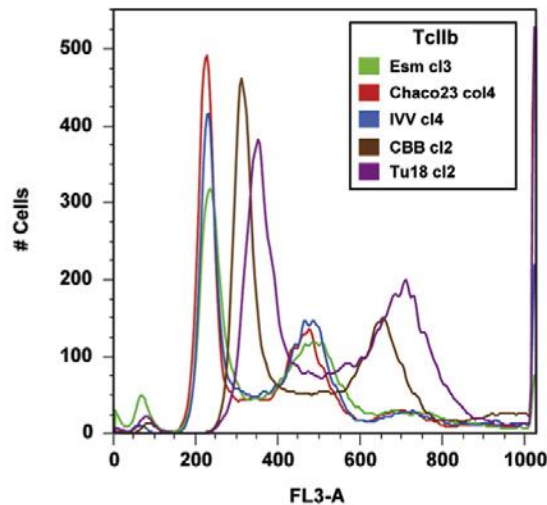


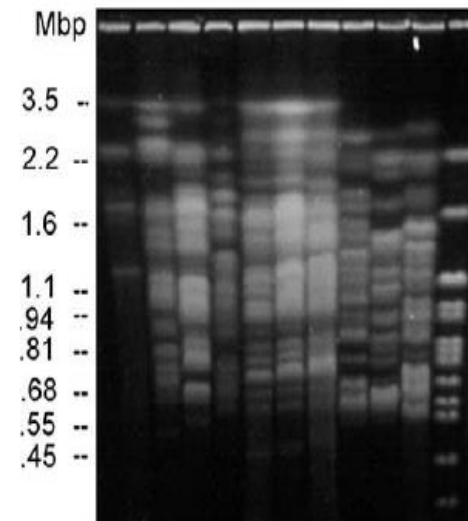
Fig. 2. Individual sensitivity of 47 *T. cruzi* strains to nifurtimox and benznidazole determined in experimentally infected mice.

DNA content



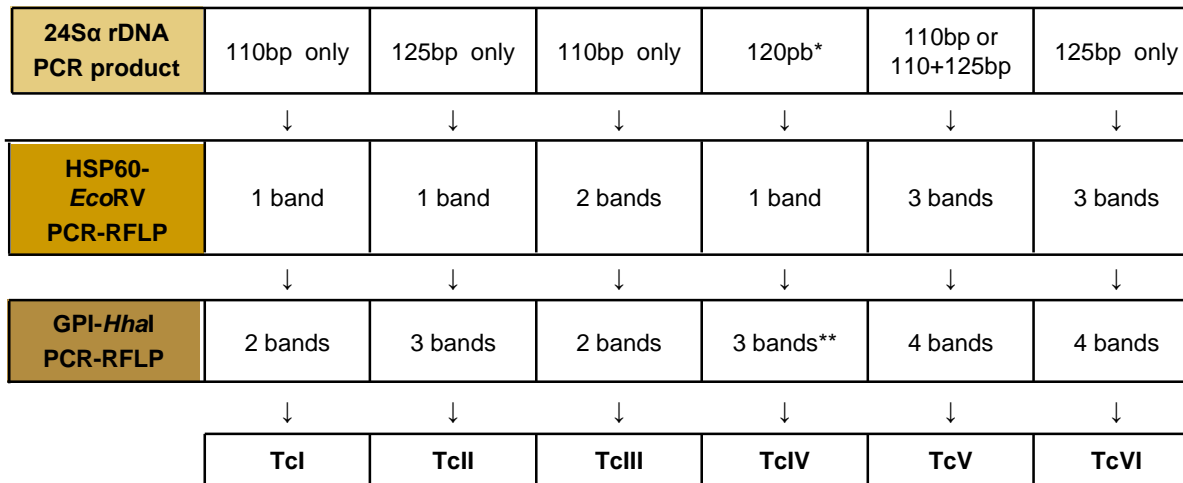
Genome size variation
80 to 120 Mbp

Chromosomes



At present, the taxon *T. cruzi* is divided into six lineages, named Discrete Typing Units (DTUs), TcI-TcVI

Protocol for genotyping the 6 DTUs



Zingales et al., 2009
Zingales et al., 2012

Association between DTUs and sylvatic reservoirs

TcI



opossums



anteaters



rodents

TcIII



armadillos

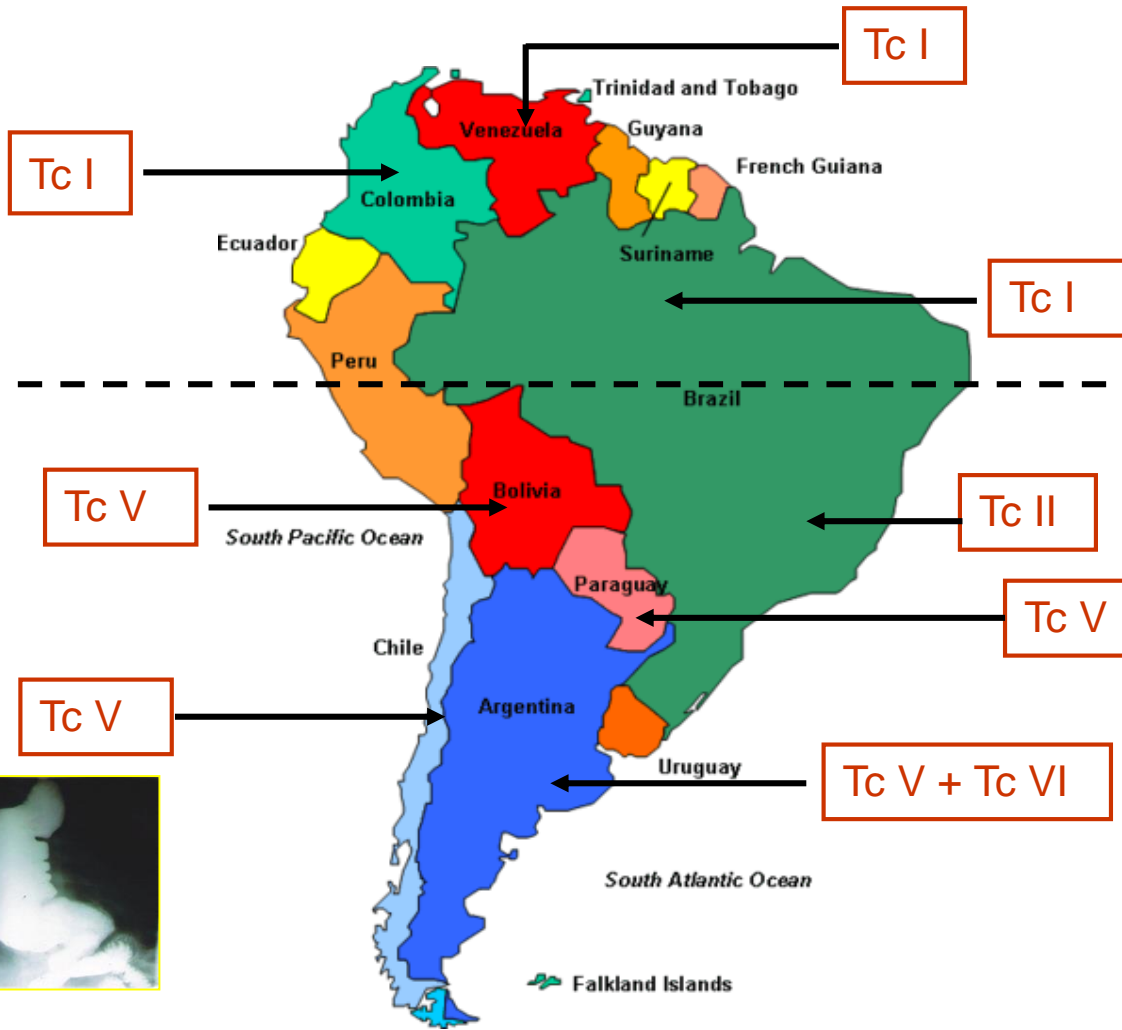
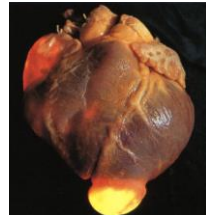
TcII, TcIV, TcV and TcVI rares

TcII



golden lion tamarins

DTU distribution in Humans (Prevalence)



Rhodnius



Triatoma

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

Differential drug susceptibility of *T. cruzi* strains

Nifurtimox and Benznidazole

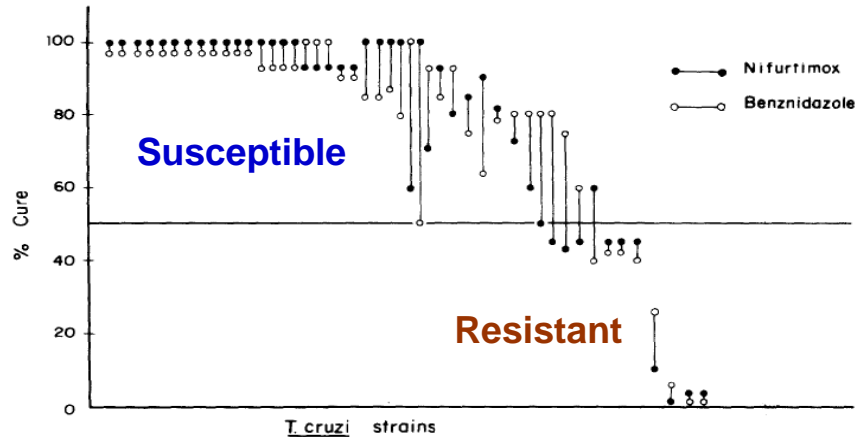


Fig. 2. Individual sensitivity of 47 *T. cruzi* strains to nifurtimox and benznidazole determined in experimentally infected mice.

Filardi & Brener, 1987

Drug Resistance is not associated to DTU group

Ergosterol biosynthesis inhibitors

Comparison of Bz and Ravuconazole – Cure in a murine model

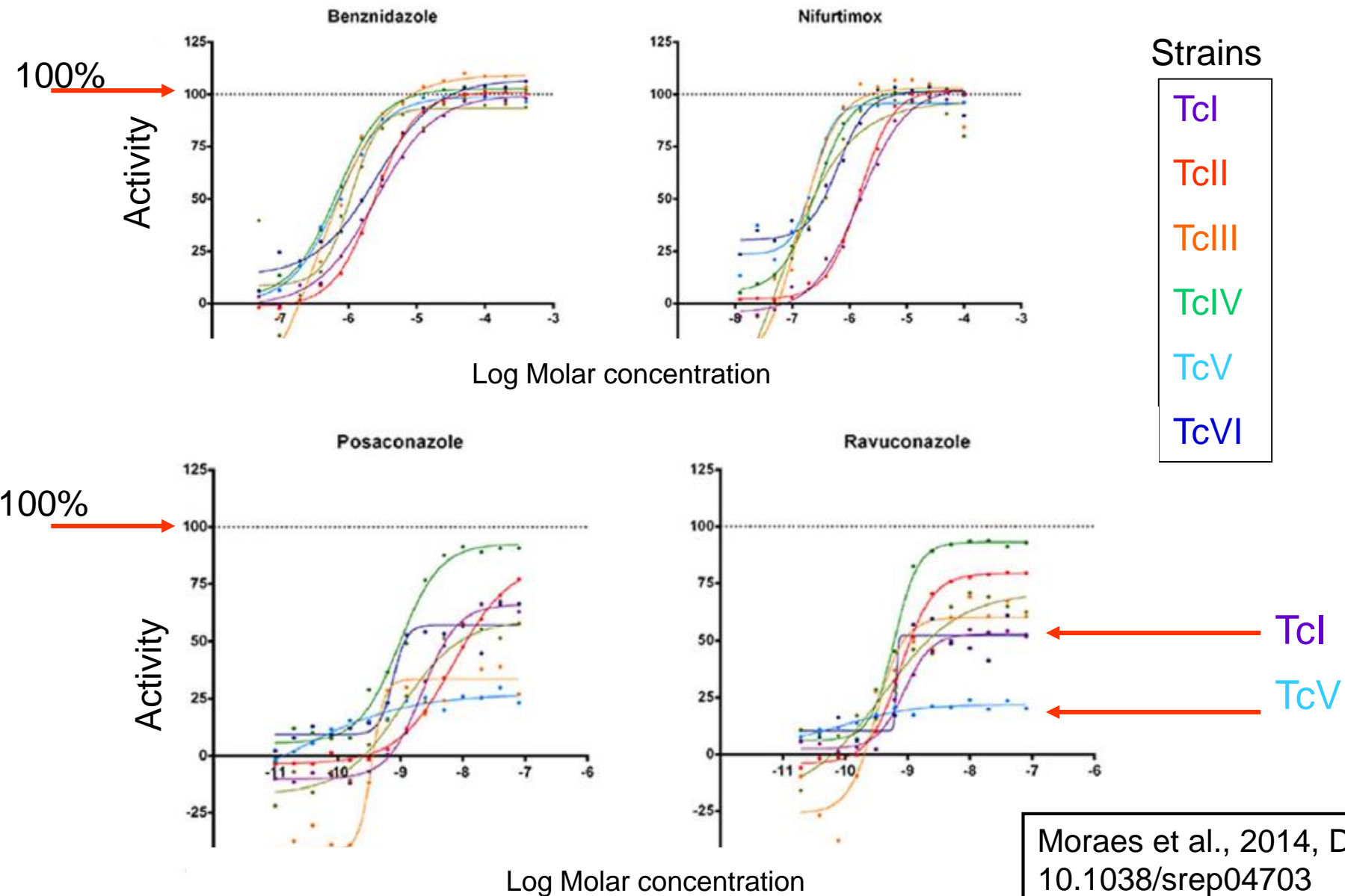
Strain	BZ (100 mg/Kg/20 doses)	Ravuconazole (15 mg/Kg/40 doses)
CL (TcVI) (BZ-susceptible)	12/12	12/12
Y (TcII) (BZ-intermediate)	9/12	7/12
Colombiana (TcI) (BZ-resistant)	4/12	0/10

Urbina et al., 2003



Colombiana (TcI) – resistant to both drugs

Activity of compounds against intracellular amastigotes



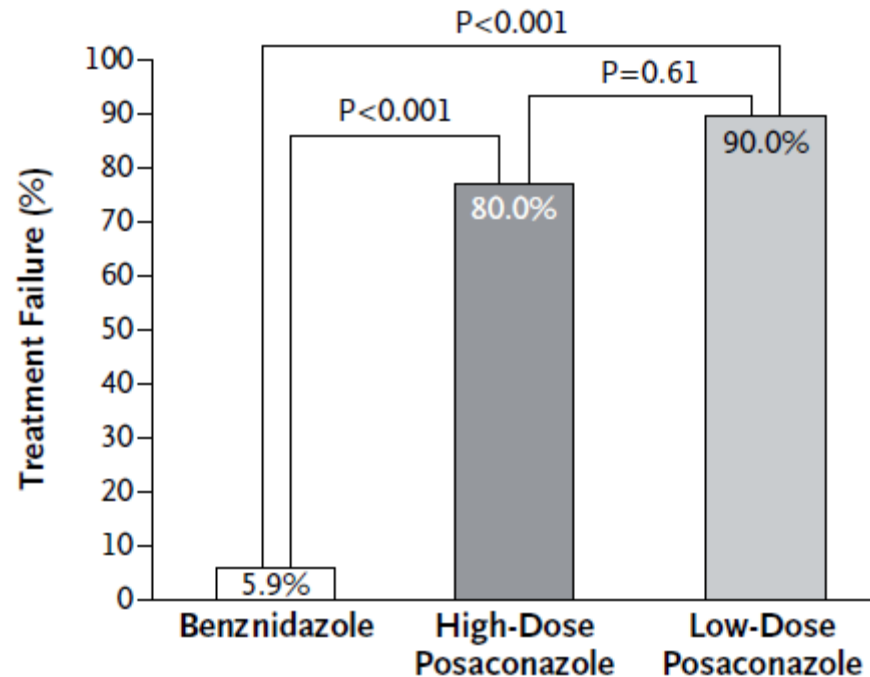
Moraes et al., 2014, DOI: 10.1038/srep04703

Posaconazole Phase IIa Trial (Barcelona)

Patients	Benznidazole (N = 26)	Posaconazole HD (N = 26)	Posaconazole LD (N = 26)
Bolivia (TcV)	24	25	26
Brazil (TcII)	1	0	0
Paraguay (TcV; TcVI)	1	1	0

Posa - Therapeutic failures

BZ still better



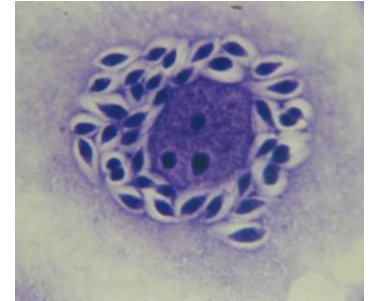
Drug discovery for Chagas disease: selection
of strains representing *Trypanosoma cruzi*
diversity

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

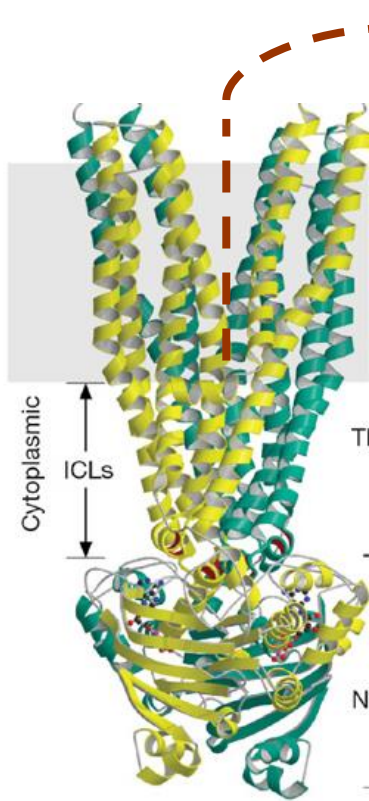


Note: Strains available upon request. Shipment problems

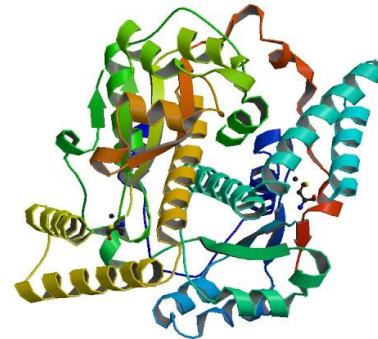
Research lines of my laboratory

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)



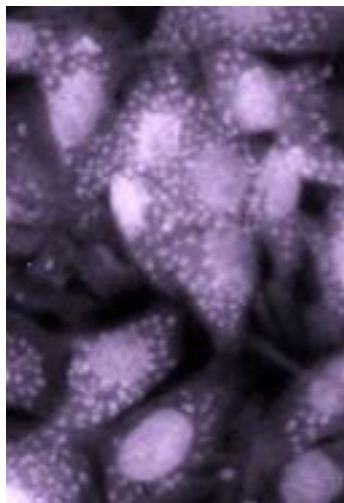
Target: Parasite isolates obtained from patients submitted to BZ therapy: cured and non-cured.

2. Screening of candidates for Chagas disease treatment

- N'-[(5-nitrofuranyl) methylene] substituted hydrazides

IC50 (μM)

Strains	BZ	NF	# 11	# 14
TcI	29.16 ± 2.90	6.02 ± 0.32	4.75 ± 0.36	5.07 ± 0.28
TcII	40.40 ± 3.37	12.84 ± 1.30	3.10 ± 0.29	3.47 ± 0.20
TcV	30.63 ± 3.00	7.32 ± 0.76	4.41 ± 0.46	4.25 ± 0.40



EC50 (μM) against intracellular amastigotes (TcII)

Compound	EC50 (μM)
BZ	2.0 ± 0.8
NF	0.9 ± 0.3
# 11	0.4 ± 0.1
# 14	0.9 ± 0.5

- Quinazoline and dimethoxybenzoate 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!