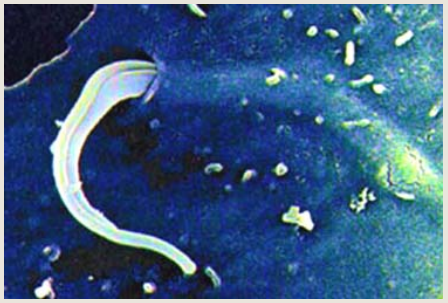
Chagas Disease Drug Discovery
Entering a New Era



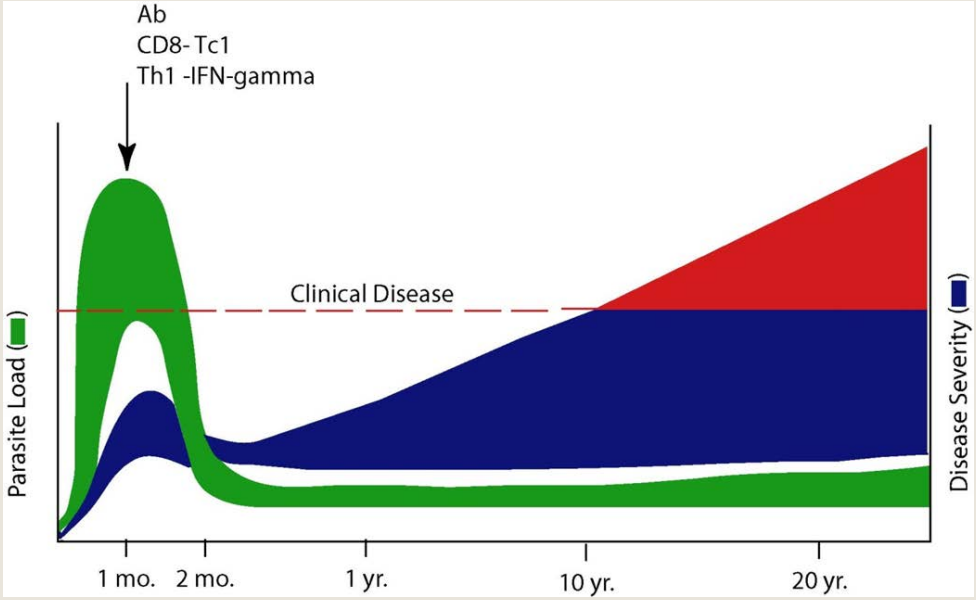
DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Eric Chatelain, PhD
Head of Drug Discovery

ICOPA Meeting, Mexico, 12th August 2014

Chagas Disease

- Effective immune responses provide control of the infection but do not prevent development of disease
- Very long time course for development of disease and evaluation of response
- 20-30% patients develop the disease



Tarleton R, 2003 in World class parasites vol 7 pp 107-15

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Chagas Disease – The TPP

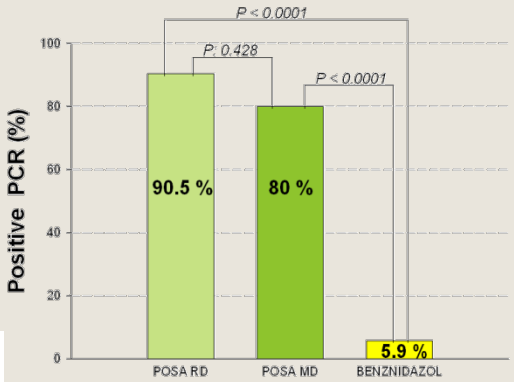
	Acceptable	Ideal
Target population	Chronic	Chronic and Acute (Reactivations)
Strains	TcI, TcII, TcV and TcVI (according to new 2009 classification)	All according to new classification (2009)*
Distribution	All areas	All areas
Adult/children	Adult	All
Clinical efficacy	Non inferior to benznidazole in all endemic regions (parasitological)	Superiority to benznidazole to different phases of disease (acute and chronic) (parasitological)
Safety	Superiority to benznidazole ** 3 CE plus 2 standard LE or ECG during treatment	Superiority to benznidazole or nifurtimox No CE or LE or ECG needed during treatment
Activity against resistant strains	Not necessary	Active against nitrofuran- and nitroimidazole-resistant *T. cruzi* strains
Contraindications	Pregnancy/lactation	None
Precautions	No genotoxicity; No pro-arrythmic potential	No genotoxicity; No teratogenicity; No negative inotropic effect; ; No pro-arrythmic potential
Interactions	No clinically significant interaction with anti-hypertensive, anti-arrythmic and anticoagulants drugs	None
Presentation	Oral	Oral
Stability	3 years, climatic zone IV	5 years, climatic zone IV
Dosing regimen	Comparable to systemic antifungal treatments	Once daily/ 30days

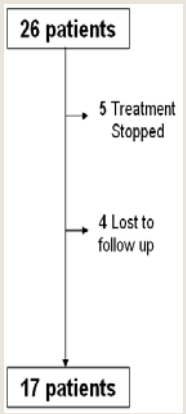
DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

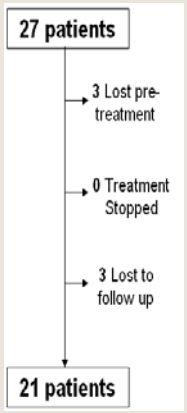
New Era for Chagas Disease Drug Discovery Why?

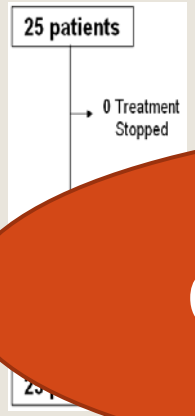
DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Results Clinical Trials NCT0116967 and NCT01489228

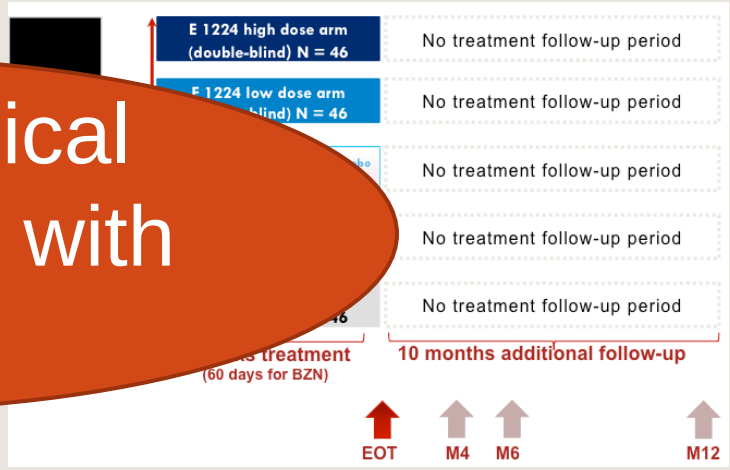








Parasitological Cure Failure with Azoles



Sustained clearance At 12 months	No	n (%)	**(N=47)** 43 (91.5)	**(N=48)** 44 (91.7)	**(N=46)** 41 (89.1)	**(N=45)** 32 (71.1)	**(N=45)** 8 (19.0)	**(N=231)** **168 (72.7)**
	Yes	n (%)	4 (8.5)	4 (8.3)	5 (10.9)	13 (28.9)	37 (81.0)	**63 (27.3)**

Implications for Delivery of Future Chagas Candidate

Why didn’t we predict these clinical outcomes?

What did we do wrong?

What don’t we understand?

Are we asking the right questions in our models?

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Chagas Translational Challenges

- Needs and way forward
  - 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
- Need for Biomarkers (Test of treatment efficacy)
- Integrate when available or generate MoA data: helpful to predict potential safety issues and monitor specific parameters during development (Toxicology studies, clinical trials)

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Chagas Disease Drug Discovery

- Major Advances
- New assays available

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

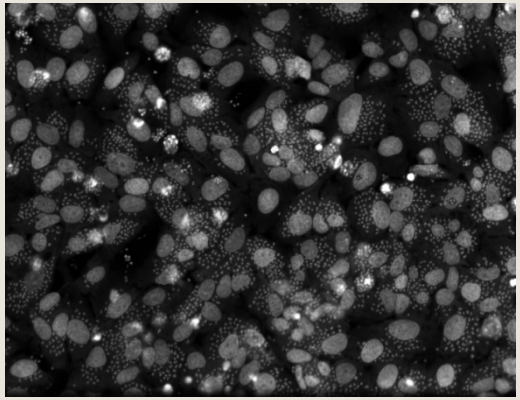
In vitro

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

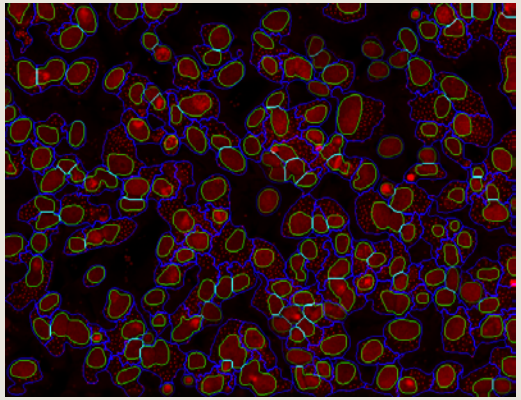
Phenotypic HTS for intracellular *T. cruzi*

- New research tools
  - Azoles

- New HTS assays for *T. cruzi (High content)*

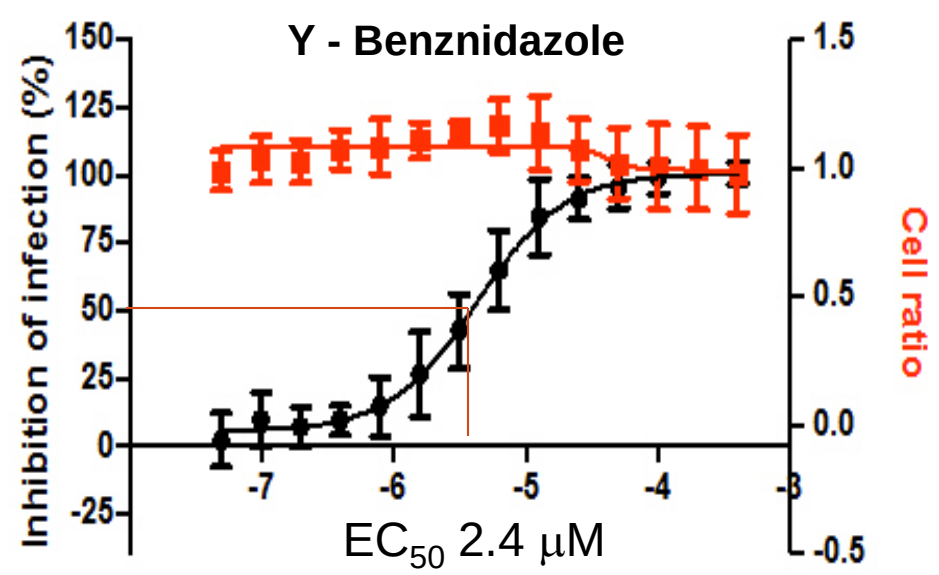


After software analysis: detection of host cell and intracellular parasites

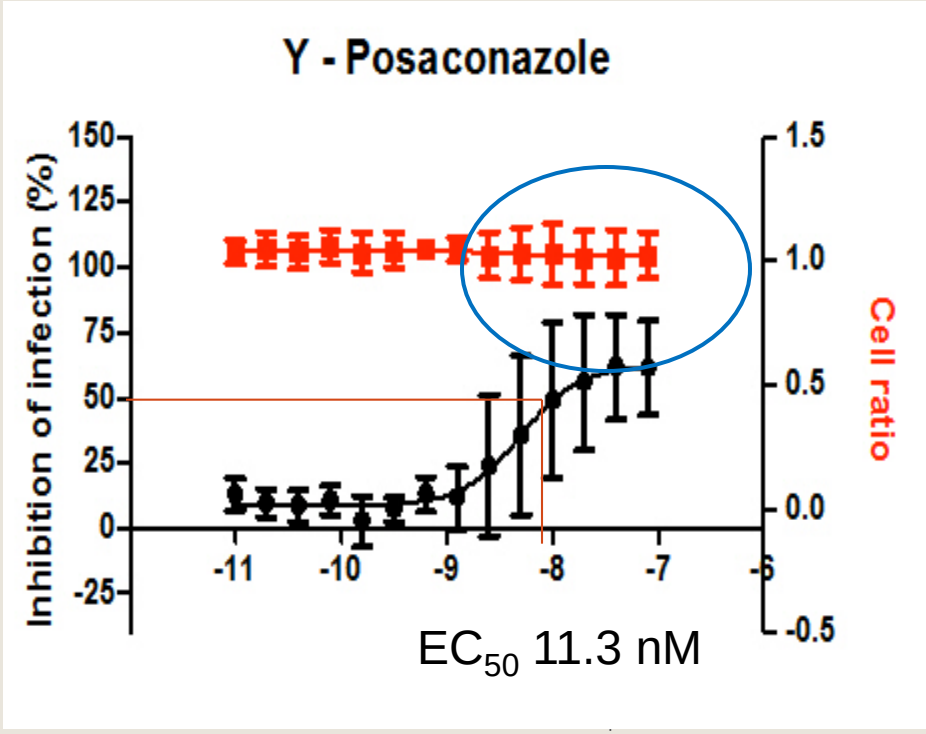


DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Primary Screening Assay
Potency vs Efficacy



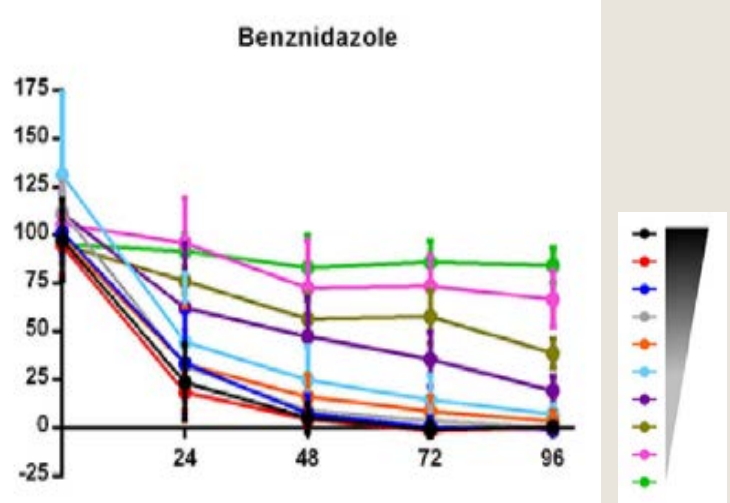
Potency always good but
Potency ≠ Efficacy

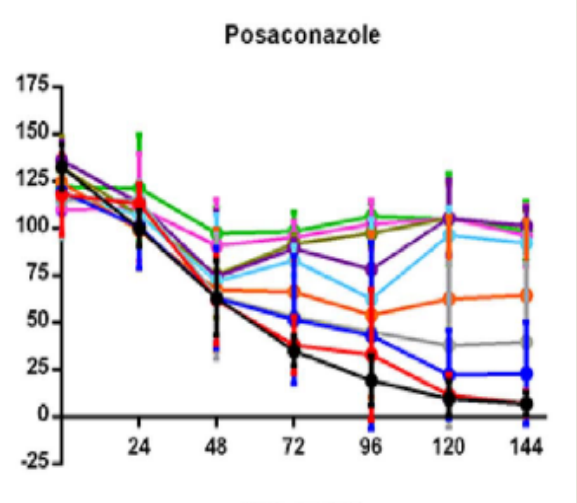


General Property of
CYP51 Inhibitors
(w. scaffolds tested so far)

Secondary Assays (1)
Compound Profiling

- Time and Concentration Dependencies
  - Fast vs slow killing





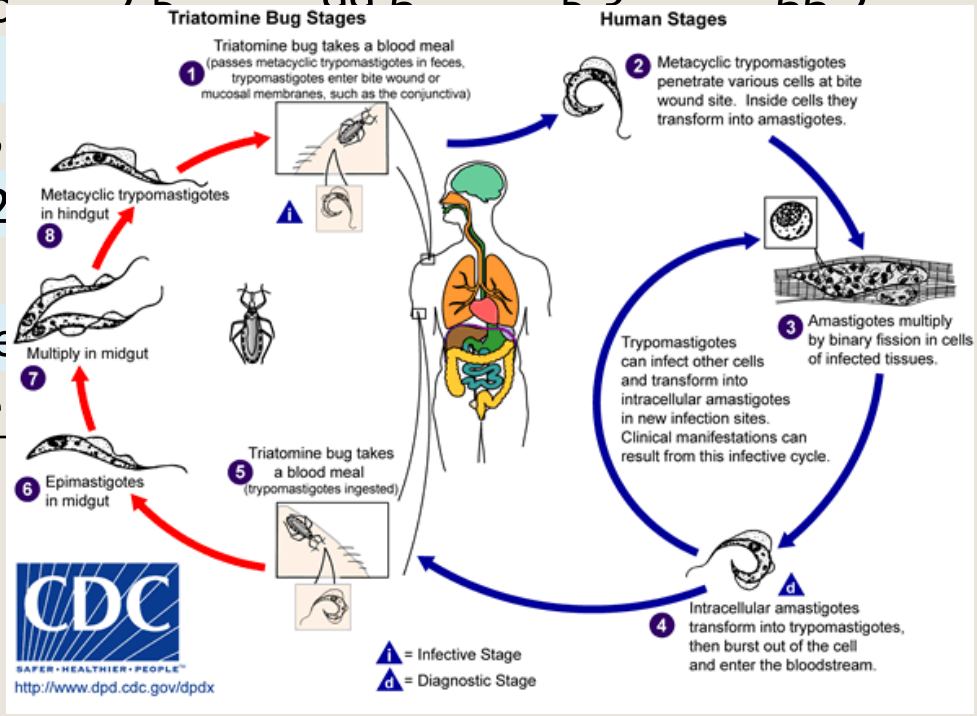
- Wash-out / Recovery assays
  - Static or cidal mechanism

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Secondary Assays (2)
Compound Profiling

- Drug potency and efficacy across *T. cruzi* groups

DTU	Strain or clone	Benznidazole		Posaconazole	
		EC_{50} (µM)	Max Activity (%)	EC_{50} (nM)	Max Activity (%)
I	DM28c	2.5	99.6	5.3	66.2
II	Y				
III	ARMA13				
IV	ERA cl2				
V	92-80 cl				
VI	CL Brene				
VI	Tulahue				



- Parasite stage-specific assay Trypomastigote/intracellular amastigote

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

In vivo

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Are the *in vivo* experimental models used predictive?

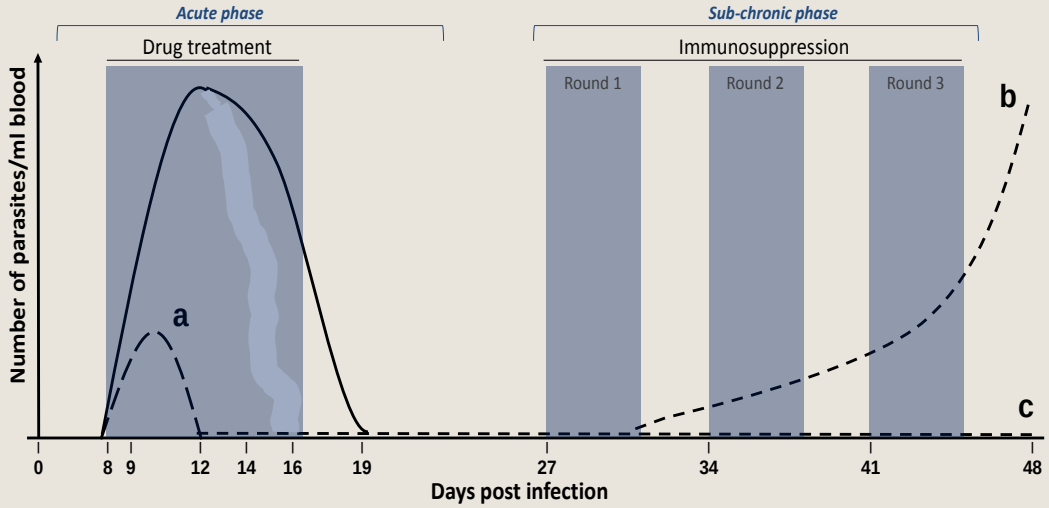
Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *105*(2): 233-238, March 2010

In vitro and in vivo experimental models for drug screening and development for Chagas disease

Alvaro José Romanha[1,2]/+, Solange Lisboa de Castro[1,3], Maria de Nazaré Correia Soeiro[1,3], Joseli Lannes-Vieira[1,3], Isabela Ribeiro[4], André Talvani[5], Bernadette Bourdin[4], Bethania Blum[4], Bianca Olivieri[1,3], Carlos Zani[1,2], Carmenza Spadafora[6], Egler Chiari[7], Eric Chatelain[4], Gabriela Chaves[8], José Eduardo Calzada[9], Juan Manuel Bustamante[10], Lucio H Freitas-Junior[11], Luz I Romero[6,9], Maria Terezinha Bahia[5], Michel Lotrowska[4], Milena Soares[12], Sonia Gumes Andrade[1,12], Tanya Armstrong[13], Wim Degrave[1,3], Zilton de Araújo Andrade[1,12]

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Evaluation of *in vivo* efficacy



_____ illustrates normal course of infection.
(Parasites eventually enter tissues/organs and are no longer detected in blood)
---------- indicates possible positive outcomes following drug treatment ie.,
a. Significant reduction in blood parasitemia ➔ If 100% efficacy is observed, animals are immunosuppressed.
b. Parasite rebound after immunosuppression ➔ Drug is tested in 20 day dosing model.
c. No parasite rebound after immunosuppression ➔ confirm cure with PCR

Model Compatible with a Discovery Program

On average 40-60% cure with posaconazole; variable but always > or = to that observed with Benznidazole (on average 40% cure)

No translation with clinical data using this model

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

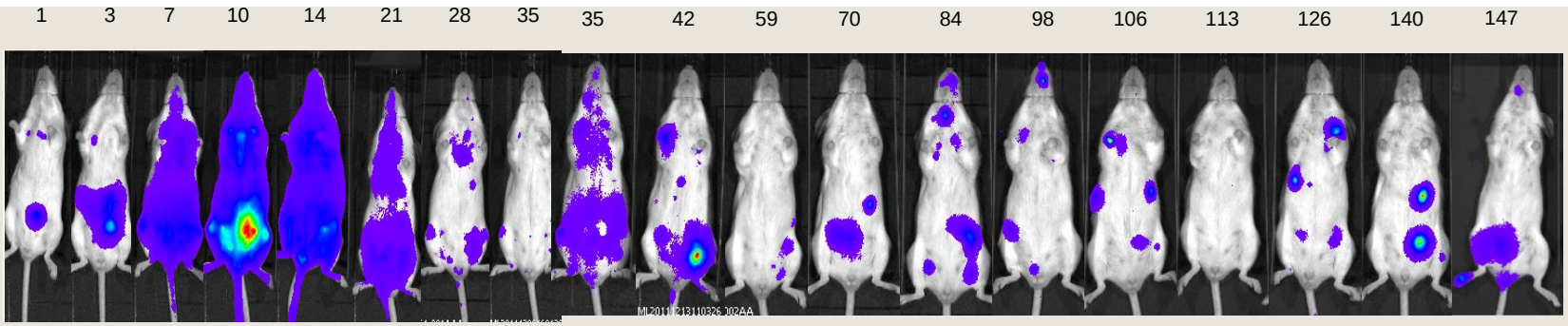
Challenge current animal models/Dogma
Generate systematic data

- Review of *in vivo* models and challenge their adequacy
- Design testing hypotheses to improve predictivity of models
- Assessment of new models or technologies and test hypotheses; generate systematic data
  - Bio Luminescent Imaging (BLI): Spatial diversity of chronic foci → impact on tissue PCR
- Assessment of the usefulness of identified markers from proteomic studies in animal models of Chagas disease

Increase the predictability of these models and their translation to human disease

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Testing hypotheses in BLI *in vivo* model



Protocol for investigating drug efficacy against chronic *T. cruzi* infection*

Infected mice (BALB/c) (Bioluminescent CL Brener)	
Benznidazole- treated	10
Posaconazole - treated	10
Posaconzole/ Benznidazole.Vehicle	10
Untreated	6
Untreated (non-bioluminescent CL Brener parasites)	3
Total mice	39

BLI Imaging – weekly/bi-weekly
Parasitemia monitoring

Day 60 p.i. — Day 80 p.i. — Day 100 p.i — Day 120 p.i.

daily drug treatment

Cyclophosphamide treatment (5 each of treated mice)

END POINT

BLI of organs
qPCR
histology

Protocol for investigating drug efficacy against acute *T. cruzi* infection

BLI Imaging – weekly/bi-weekly
Parasitemia monitoring

Infected mice (BALB/c) (Bioluminescent CL Brener)	
Benznidazole – treated	10
Posaconazole – (1)	10
Posaconazole – (2)	10
Untreated	6
Total mice	36

Day 14 p.i. — Day 34 p.i. — Day 54 p.i — Day 74 p.i.

daily drug treatment

Cyclophosphamide treatment (5 each of treated mice)

END POINT

BLI of organs
qPCR
Histology
Heart/spleen weights

New Chagas Screening Cascade

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

Acceptance criteria for a new chemical series

Screening on T. cruzi *Tulahuen* WT strain (TcVI)

IC_{50}	< 5 µM
Max. activity	> 90%

Cytotoxicity on host cell 3T3

SI	> 10

Towards PoP

Primary ADME characterisation

In silico predictions of Phys/Chem properties
→ no predicted absorption liabilities

Kinetic solubility (pH 2 & 6.5)	> 50 µg/ml
gLog D	< 4
CYP 3A4 inhibition (1 & 10 µM)	> 10 µM
In vitro metabolism (mouse LMs)	E_H < 0.5

Scale up

New series profiling

Panel of T. *cruzi* strains	→ Potency against all genotypes (priority to TcI, TcII, TcV and TcVI) or NO GO
CYP51	> 10 µM, or DE-PRIORITISATION
Trypomastigotes	→Potency or DE-PRIORITISATION
Time to kill	Fast-acting preferred

Intellectual Property assessment → FTO

PK in Balb/c mice
(PO 20 mg/kg and IV 1 mg/kg)

Pre- formulation (if needed)

Tolerability in Balb/c

***In vitro* validation against *T. Cruzi* CL Brener (TcVI)**

PoP efficacy *in vivo* – 5 days

Balb/c mice infected with CL Brener
(at the highest dose)

Potential candidate

Further profiling for a successful PoP

ADME
Plasma stability (mouse, rat & human)
Plasma protein binding (mouse, rat & human)
Permeability (Caco -2)

Safety & Toxicology
Panel of mammalian cells for cytotoxicity

CYP screening	> 10 µM
hERG	> 30 µM
Mini AMES	negative
In vitro Micronucleus	negative
CEREP profiling	
Preliminary CV test in rat	negative

Potency
Reversibility in *T. cruzi* Tulahuen assay

Entrance in LO

LO

In vivo efficacy
In vitro validation against *T. cruzi* CL Brener
Acute model
↓
Chronic model
↓
Dose – response in chronic model

In vivo ADME
PO exposure in Balb/c

Scale up

In vitro ADME
In silico predictions

KS	> 100 µg/mL
gLog D	< 3
In vitro metabolism	E_H < 0.3
CYP 3A4 inhibition	< 50%
PPB (mouse)	

In vitro efficacy – *T. cruzi*
Tulahuen WT strain in 3T3

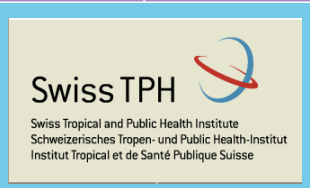
IC_{50}	< 1 µM
Max. activity	> 95%
SI	> 100

Conclusions

- Moving towards a better understanding of the characteristics needed for a compound to bring forward
- Translational Challenges are being tackled both *in vitro* and *in vivo*
- Recent major change/shaking of the Chagas drug discovery landscape
  - Scientific impact
  - New «players»: GNF, GSK/DDU, Broad/Eisai, and numerous academic groups and EU/FP7 consortia
- More confidence that candidates moving forward will be efficacious in the clinic but still need new series

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

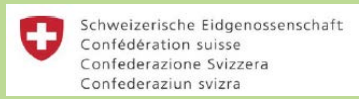
Acknowledgments



Libraries:

SANOFI

GSK


via the 4th Sector Health Project implemented by Abt Associates, Inc.

DNDi
Drugs for Neglected Diseases *initiative*
Iniciativa Medicamentos para Enfermedades Olvidadas

BY WORKING TOGETHER IN A CREATIVE WAY, PDPS, LARGE AND SMALL PHARMA, AND THE PUBLIC SECTOR CAN BRING INNOVATION TO NEGLECTED PATIENTS!

www.dndi.org

DNDi

Drugs for Neglected Diseases *initiative*

Iniciativa Medicamentos para Enfermedades Olvidadas