Chagas Disease Drug Discovery
Entering a New Era

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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
## Chagas Disease – The TPP

<table>
<thead>
<tr>
<th></th>
<th><strong>Acceptable</strong></th>
<th><strong>Ideal</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target population</strong></td>
<td>Chronic</td>
<td>Chronic and Acute (Reactivations)</td>
</tr>
<tr>
<td><strong>Strains</strong></td>
<td>TcI, TcII, TcV and TcVI (according to new 2009 classification)</td>
<td>All according to new classification (2009)*</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>All areas</td>
<td>All areas</td>
</tr>
<tr>
<td><strong>Adult/children</strong></td>
<td>Adult</td>
<td>All</td>
</tr>
<tr>
<td><strong>Clinical efficacy</strong></td>
<td>Non inferior to benznidazole in all endemic regions (parasitological)</td>
<td>Superiority to benznidazole to different phases of disease (acute and chronic) (parasitological)</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>Superiority to benznidazole ** 3 CE plus 2 standard LE or ECG during treatment</td>
<td>Superiority to benznidazole or nifurtimox No CE or LE or ECG needed during treatment</td>
</tr>
<tr>
<td><strong>Activity against resistant strains</strong></td>
<td>Not necessary</td>
<td>Active against nitrofuran- and nitroimidazole-resistant <em>T. cruzi</em> strains</td>
</tr>
<tr>
<td><strong>Contraindications</strong></td>
<td>Pregnancy/lactation</td>
<td>None</td>
</tr>
<tr>
<td><strong>Precautions</strong></td>
<td>No genotoxicity; No pro-arrythmic potential</td>
<td>No genotoxicity; No teratogenicity; No negative inotropic effect; ; No pro-arrythmic potential</td>
</tr>
<tr>
<td><strong>Interactions</strong></td>
<td>No clinically significant interaction with anti-hypertensive, anti-arrythmic and anticoagulants drugs</td>
<td>None</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td>Oral</td>
<td>Oral</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>3 years, climatic zone IV</td>
<td>5 years, climatic zone IV</td>
</tr>
<tr>
<td><strong>Dosing regimen</strong></td>
<td>Comparable to systemic antifungal treatments</td>
<td>Once daily/ 30days</td>
</tr>
</tbody>
</table>
New Era for Chagas Disease
Drug Discovery
Why?
Results Clinical Trials
NCT0116967 and NCT01489228

Parasitological Cure Failure with Azoles
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?
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)
Chagas Disease Drug Discovery
- Major Advances
- New assays available
In vitro
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*
Primary Screening Assay
Potency vs Efficacy

**Y - Benznidazole**
- EC$_{50}$ 2.4 µM
- Potency always good but Potency $\neq$ Efficacy

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

**Y - Posaconazole**
- EC$_{50}$ 11.3 nM
Secondary Assays (1)
Compound Profiling

- Time and Concentration Dependencies
  - Fast vs slow killing

- Wash-out / Recovery assays
  - Static or cidal mechanism
<table>
<thead>
<tr>
<th>DTU</th>
<th>Strain or clone</th>
<th>Benznidazole</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Max Activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(μM)</td>
<td>(%)</td>
</tr>
<tr>
<td>I</td>
<td>DM28c</td>
<td>2.5</td>
<td>99.6</td>
</tr>
<tr>
<td>II</td>
<td>Y</td>
<td>2.4</td>
<td>101.0</td>
</tr>
<tr>
<td>III</td>
<td>ARMA13 cl</td>
<td>0.7</td>
<td>109.4</td>
</tr>
<tr>
<td>IV</td>
<td>ERA cl2</td>
<td>1.1</td>
<td>93.3</td>
</tr>
<tr>
<td>V</td>
<td>92-80 cl2</td>
<td>0.7</td>
<td>98.8</td>
</tr>
<tr>
<td>VI</td>
<td>CL Brener</td>
<td>1.6</td>
<td>107.0</td>
</tr>
</tbody>
</table>

- Drug potency and efficacy across *T. cruzi* groups
- Parasite stage-specific assay: Trypomastigote/intracellular amastigote

*Secondary Assays (2) Compound Profiling*
In vivo
Are the *in vivo* experimental models used predictive?

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

Evaluation of *in vitro* efficacy

Model Compatible with a Discovery Program

No translation with clinical data using this model
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
Testing hypotheses in BLI \textit{in vivo} model

Protocol for investigating drug efficacy against chronic \textit{T. cruzi} infection*

- BLI Imaging – weekly/bi-weekly Parasitemia monitoring
- Infected mice (BALB/c) (Biotuminescent CL Brener)
  - Benzimidazole-treated: 10
  - Posaconazole treated: 10
  - Posaconazole/Benzimidazole Vehicle: 10
  - Untreated: 6
  - Untreated (non-bioluminescent CL Brener parasites): 10
- Total mice: 39

END POINT
- Cyclophosphamide treatment (5 each of treated mice)
- BLI of organs qPCR histology

Protocol for investigating drug efficacy against acute \textit{T. cruzi} infection

- BLI Imaging – weekly/bi-weekly Parasitemia monitoring
- Infected mice (BALB/c) (Biotuminescent CL Brener)
  - Benzimidazole – treated: 10
  - Posaconazole – (1): 10
  - Posaconazole – (2): 10
  - Untreated: 6
- Total mice: 36

END POINT
- Cyclophosphamide treatment (5 each of treated mice)
- BLI of organs qPCR Histology Heart/spleen weights

*Denotes protocol for chronic infections.
New Chagas Screening Cascade
Acceptance criteria for a new chemical series

New series profiling

Screening on T. cruzi *Tulahuen* WT strain (TcVI)
- IC$_{50}$ < 5 µM
- Max. activity > 90%

Cytotoxicity on host cell 3T3
- SI > 10

Panel of T. cruzi strains → Potency against all genotypes (priority to TcI, TcII, TcV and TcVI) or NO GO

CYP51 > 10 µM, or DE-PRIORITYISATION

Trypomastigotes → Potency or DE-PRIORITYISATION

Time to kill → Fast-acting preferred

Intellectual Property assessment → FTO

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

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)
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
  - Micronucleus: negative
  - CEREP profiling: negative
  - Preliminary CV test in rat: negative

**Potency**
- Reversibility in *T. cruzi* Tulahuen assay

**In vitro efficacy – *T. cruzi***
- Tulahuen WT strain in 3T3
  - IC$_{50}$: < 1 µM
  - Max. activity: > 95%
  - SI: > 100

**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

**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)

**Entrance in LO**

Potential candidate
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
Acknowledgments

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

DNDi
Drugs for Neglected Diseases initiative

Iniciativa Medicamentos para Enfermedades Olvidadas