Drug discovery against kinetoplastid diseases: the DNDi perspective

COST Action CM 1307 meeting
Belgrade, 27 October 2015

Jean-Robert Ioset
OUTLINE

- DNDi’s Model
- R&D landscape and portofolio
- DNDi’s Discovery strategy
- Selected discovery approaches: lessons learnt
- Identifying and addressing the challenges
- Achievements
DNDi’s Model
THE HISTORY OF DNDi

In the 1990s and after, MSF documented in the field that patients had no treatments for certain neglected diseases.

MSF used funding from the Nobel Prize and created the Drugs for Neglected Diseases working group, which then led to DNDi in 2003.
VISION & OBJECTIVES

DNDi VISION
A collaborative, patients’ needs-driven, virtual, non-profit drug R&D organisation to develop new treatments against the most neglected diseases.

DNDi OBJECTIVES
- Deliver 11 to 13 new treatments by 2018 for Sleeping sickness, Chagas disease, Leishmaniasis, Malaria, Paediatric HIV and specific Filarial infections
- Establish a robust pipeline for future needs
- Use and strengthen existing capacity in disease-endemic countries
- Raise awareness and advocate for increased public leadership

DNDi Nutshell - From bench to bedside
DNDi: PARTNERS & GLOBAL PRESENCE

FOUNDING PARTNERS
• Indian Council for Medical Research (ICMR)
• Kenya Medical Research Institute (KEMRI)
• Malaysian MOH
• Oswaldo Cruz Foundation, Brazil
• Médecins Sans Frontières (MSF)
• Institut Pasteur France
• TDR (permanent observer)
DNDi’s PRIORITY:
Neglected patients...

DNDi Nutshell - From bench to bedside
Dedicated Teams Worldwide
Over 660 People Committed to DNDi’s Vision
A Global Network to Leverage Resources
More Than 100 R&D Partners

Balance of public and private partnerships worldwide

- University/Research Inst.: 32%
- CRO: 28%
- Pharma/Biotech: 18%
- Int. Org/NGO: 9%
- MoH/Gov/Hosp: 9%
- PDPs: 4%

Utilizing and Strengthening Research Capacities in Disease-Endemic Countries

Major Role of Regional Disease Platforms:

- Defining patients’ needs and target product profile (TPP)
- Strengthening local capacities
- Conducting clinical trials (Phase II/III studies)
- Facilitating registration
- Accelerating implementation of new treatments (Phase IV & pharmacovigilance studies)
Overcoming Challenges in the Field
Thanks to Our Partners in Endemic Countries

In 10 years: >33,000 patients enrolled in >20 clinical studies in five disease areas
Neglected Diseases: Treatment Limitations 10 Years Ago

- Ineffective (resistance)
- Toxic
- Expensive
- Painful when delivered
- Difficult to use
- Not registered in endemic regions
- Restricted by patents

We Need Safe, Effective, Easy-to-Use Drugs
Fatal Imbalance Remains Despite Progress Over A Decade

- 10% of R&D dedicated to illnesses that affect 90% of global disease burden (‘10/90 gap’)
- 3.8% of new products for neglected diseases (reformulations, combinations)
- 1.2% of NCEs for neglected diseases
- Only 1.4% clinical trials
- Only 1% of global health investment for neglected diseases*

*Source ‘Mapping of available health research and development data: what’s there, what’s missing, and what role is there for a global observatory?’ Rottingen et al. Lancet, May 2013

Source
Fatal Imbalance: The Crisis in Research and Development for Neglected Diseases, MSF, 2001

1,393 total products approved
1975-1999

1.1%
16 new drugs for neglected diseases

1975-1999

Other diseases

2000-2011

756 products developed (excluding vaccines)

395
332
25
4

NCEs
Other products

for Neglected diseases
Human African Trypanosomiasis (HAT) or Sleeping Sickness

- 36 countries at risk in sub-Saharan Africa;
- *Trypanosoma b. gambiense* and *rhodesiense*
- Transmitted by the tsetse fly
- Estimated current cases: 20,000
- Difficult to diagnose; many patients go undiagnosed until late stage of disease
- Fatal if untreated
- 7 countries bear 97% cases (RDC = 2/3)
- Current drugs: melarsoprol, NECT
- Needs: a safe, effective, short-course and orally administered stage 2 treatment
By 2018? New Oral Treatments and Rapid Diagnostic Tests at Village Level

Fexinidazole
- A ‘rediscovered’ new chemical entity through compound mining
- Potential oral treatment
- Phase II/III in DRC and CAR

Oxaborole SCYX-7158
- New chemical entity from the Lead Optimization programme
- Potential oral treatment with a single pill
- Phase I completed; Entering Phase II/III soon

In partnership with Sanofi
Sleeping Sickness: From Unacceptable To Better, Towards Tools for Elimination

15 years ago:
- Eflornithine
- Melarsoprol

Since 2009:
- NECT

2018?
- Oral treatment
- & rapid diagnostic test

DNDi
Drugs for Neglected Diseases Initiative
Leishmaniasis

- 200 million at risk worldwide (in 70 countries but 90% in 5 countries)
- 500,000 cases in total with 51,000 deaths/year
- *Leishmania donovani*
- Transmitted by the sandflies
- 2 types of leishmaniasis:
  - **Visceral (VL): fatal without treatment**
  - Cutaneous (CL): has a spectrum of presentations; typically with self-healing or chronic lesions on the skin
- Current drugs: antimonials, Amphotericin B, AmBisome®, miltefosine, paromomycin
- **Needs: oral, safe, effective, low-cost and short-course treatment**
Chagas Disease

- 100 million at risk in Latin America
- 8 millions cases with 14’000 deaths/yea
- Trypanosoma cruzi
- Kills more people in region than malaria
- Patient number growing in non-endemic countries
- Transmitted by ‘kissing bug’, blood transfusion, organ transplantation, as congenitally or orally
- Majority of patients undiagnosed until late stage
- Current drugs: benznidazole, nifurtimox

Needs:
- paediatric drug (benznidazole)
- a new oral drug for early chronic stage
DNDi Portfolio-Building Model
Address Immediate Patient Needs & Deliver Innovative Medicines

- Long-term projects
  - New chemical entities (NCEs)
  - New formulations (fixed-dose combinations)
  - New indications of existing drugs

- Medium-term projects
  - Completing registration dossier
  - Geographical extension

- Short-term projects

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**Discovery**
- R
- LS
- LO

**Pre-clinical**

**Clinical**

**Implementation**
DNDi Portfolio June 2015

6 new treatments since 2003

**HAT**
- Leishmaniasis
  - Nitroimidazoles
  - Oxaleish
  - Amino pyrazoles

**Leishmaniasis**
- SCreE NeatinH H2L
- Chagas
  - Biomarkers
  - New Benz Regimens/Combos
- Chagas (CL)
- Cpg-D35
- Anfoleish (CL)
- New CL combos
  - New VL Treatments Latin America
  - New VL Treatments for HIV/VL

**Chagas**
- Biomarkers
- Fexinidazole
- New CL combos

**Filaria**
- Macro Filaricide 2
- Emodepsid

**Paediatric HIV**
- Two '4-in-1' LPV/r based FDC granules
- LPV/r pellets with dual NRTI FDC

**Mycetoma**
- Fosravuconazole

**Malaria**
- ASMQ FDC Artesunate-Amodiaquine Fixed-Dose Combination
- ASMO FDC Artesunate-Mefloquine

New Chemical Entity (NCE); Fexinidazole (for HAT, VL, and Chagas disease) = 1 NCE
DNDi’s Discovery strategy

An evolutive process
2003-2005: status of knowledge

- genome sequenced by TriTryp consortium (2005)
- little know about target validation -> lack of translation
- Whole cell (phenotypic) assay in place at low throughput
- Little known about kinetoplastid biology relevant to disease
- few drugs used in the clinics (MoA unknown, empirical use)

CREDIT: THE TRITRYP SEQUENCING CONSORTIUM http://tritrypdb.org/tritrypdb/
Discovery @ DNDi: an evolution process

- Started with academia/parasitology partners (networking)
- From individual projects
- Selection on opportunistic, scientific-based criteria
- Little H2L/LO capacity and expertise (project based)
- Small non-proprietary libraries (little related knowledge)
- Low screening capacity for all 3 protozoa

Adapt to changing R&D landscape
Discovery at DNDi: 2005-2008 lessons learnt

- success is the exception (fexinidazole)
- mostly library-based/chemistry-driven projects
  - individual projects
  - little known about developability of hits/hit series
  - little expertise and support (ADMET, PK, toxicity)
- in vitro to in vivo translation
  are we using pathology relevant models? Are we too stingent/too loose?
  Has chemistry to be questioned?
- literature mining
  lack of reproducibility
- chemical collections
  more opportunistic than rational selection
- Screening
  limited screening capacity: few hundred cpd/month
Discovery at DNDi: since 2008

- Increase phenotypic screening capacity
  strategic partnerships with Eskitis (HAT HTS assay development), Institut Pasteur Korea (VL and Chagas HCS development) and University of Dundee HCS assay development

- Review strategy re selection of compounds
  selection of compound libraries and partnerships (focus on Pharmas)

- Invest in fully integrated Lead Optimization consortia supported by DMPK/parasitology
  delink funding from research teams and series

- Develop of guiding/decisional tools
  Target Product Profiles, discovery cascades

- Invest in better understanding of assay models
  understand biology (drugs/clinical candidates as benmarkers, in vitro and in vivo -> translation, PK/PD, integrate secondary assays (HAT, VL, Chagas) into screening cascade
1. Literature mining (1-100)
   scientific litt and patent search (low hanging fruit)


2. Drug repurposing (100-1’000)
   drug candidates and approved drugs (preclinical to registration)

3. ADME/PK biased sets (1-3K)
   e.g. bioavailability, half-life, distribution, BBB…

4. Diversity screening core (10-150K) to larger decks (+500K)
   lead-likeness/diversity algorithms (and other filters)

5. Specific sets: anti-infective classes, orthology (100-1000)
   including target related and class related

6. Scaffold-hopping/analog mining (10-100)
   non-proprietary/published hits as templates

7. Others: NP, putative protozoan targets, activity predictive models, …

Selection of compounds for screening
screening/early Discovery - Process

Negotiation and contract

Sourcing of samples

- \textbf{HTS} screening
- \textbf{MTS} screening

novelty diversity alerts filters

\begin{itemize}
  \item Challenges: keep track on data/information
  \item Ensure timely communication (internal/external)
  \item Deal with more data/ more partners
\end{itemize}
Discovery - Tools

Target Product Profiles (TPPs)
• Defined in consultation with stakeholders:
  - Patients, Physicians, Regulators, Public health agencies

Drug Discovery manuals and screening cascades
• Objective values for:
  - Hits, Leads, Optimized leads, Drug candidates
• Surrogate markers to support the Discovery Manuals

Data Management
• Ensures rapid dissemination of data to partners (ScienceCloud)
### the TPP as a R&D guide
the example of Chagas Disease

<table>
<thead>
<tr>
<th>Target population</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic</td>
<td>Chronic</td>
<td>Chronic and Acute (Reactivations)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strains</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tcl, TcII, TcV and TcVI (according to new 2009 classification)</td>
<td>All according to new classification (2009)*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>All areas</td>
<td>All areas</td>
<td>All areas</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adult/children</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>All</td>
<td>All</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical efficacy</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<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>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Safety</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<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>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Activity against resistant strains</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not necessary</td>
<td>Active against nitrofuran- and nitroimidazole-resistant T. cruzi strains</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contraindications</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy/lactation</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precautions</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No genotoxicity; No pro-arrythmic potential</td>
<td>No genotoxicity; No teratogenicity; No negative inotropic effect; No pro-arrythmic potential</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No clinically significant interaction with anti-hypertensive, anti-arrythmic and anticoagulants drugs</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Oral</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stability</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 years, climatic zone IV</td>
<td>5 years, climatic zone IV</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dosing regimen</th>
<th>Acceptable</th>
<th>Ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparable to systemic antifungal treatments</td>
<td>Once daily/ 30days</td>
<td></td>
</tr>
</tbody>
</table>
Serum on T. cruzi Tulahuen strain (TcVI) IC₅₀ < 5 μM
Max. activity > 90%

Cytotoxicity on host cell 3T3 SI > 10

Acceptance criteria for a new chemical series

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)
  (> 10 μM)
- In vitro metabolism (mouse LMs) EH < 0.5

Pannel of cruzi strains ➔ potency against all genotypes (priority to TcI, TcII, TcV and TcVI) or NO GO
- CYP51 > 10 μM, or DE-PRIORITY
- Trypomastigotes ➔ potency or DE-PRIORITY
- 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

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
  - In vitro Micronucleus: negative
  - CEREP profiling: Preliminary CV test in rat: negative

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

**In vitro ADME**
- In silico: KS > 100 µg/mL
- gLog D: < 3
- In vitro met. (mouse LMs): EH < 0.3
- CYP 3A4 inhibition: < 50%
- PPB (mouse)

**In vivo ADME**
- PO exposure in Balb/c

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

**In vitro efficacy – T. cruzi Tulahuen strain in 3T3**
- IC₅₀: < 1 µM
- Max. activity: > 95%
- SI: > 100

**Entrance in LO**

**Potential candidate**
Selected discovery approaches: lessons learnt
Selection of compounds for screening

1. Literature mining (1-100)
   scientific litt and patent search (low hanging fruit)

2. Drug repurposing (100-1’000)
   drug candidates and approved drugs (preclinical to registration)

3. ADME/PK biased sets (1-3K)
   e.g. bioavailability, half-life, distribution, BBB…

4. Diversity screening core (10-150K) to larger decks (+500K)
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5. Specific sets: anti-infective classes, orthology (100-1000)
   including target related and class related

6. Scaffold-hopping/analog mining (10-100)
   non-proprietary/published hits as templates

7. Others: NP, putative protozoan targets, activity predictive models, …
Fexinidazole: data mining for low-hanging fruit was worth the effort!

DNDi’s First NCE to Reach Phase II/III Clinical Study (in DRC)

Objective: Drug candidate to become an oral, short course treatment for stage 1+2 sleeping sickness treatment, caused by either 
*T. b. gambiense* or *T. b. rhodesiense*

- Literature review of nitroimidazoles as a class
- Sourcing and screening of > 500 nitroimidazoles from various sources
- Identification of fexinidazole as drug candidate
- Preclinical development including ADME-PK, GLP-toxicology and safety pharmacology
- Phase I clinical trials in Paris - completed
- Agreement to co-develop with Sanofi
- Phase II/III with Sanofi in DRC and CAR

Drug repurposing: attractive but ...

- Screening of marketed drugs from commercially available collections
- Screening of (terminated) drug candidates from Pharmaceutical companies

### Outcome:
- several candidates identified
  - antidepressants for HAT
  - antihistaminics/antiallergics (anti-H1 inh.) for Chagas
  - clofazimine and disulfiram for VL
  - auranofin for HAT/VL/Chagas
  - disulfiram for VL...

### OUTCOME
- several candidates identified
- antidepressants for HAT
- antihistaminics/antiallergics (anti-H1 inh.) for Chagas
- clofazimine and disulfiram for VL
- auranofin for HAT/VL/Chagas
- disulfiram for VL...

**BUT**

### Key issues preventing further development
- unsuitable drug profile: lack of oral bioavailability, toxicity (safety marging)
- potency disconnect between primary (nM) and ND targeted (uM) indications
target drug classes: the added value of working with annotations

- Track record in R&D -> reduced attrition rate
- Access to analogs, data and expertise from Pharma
- Proceed quickly to PoC of in vivo efficacy
  - Oxaboroles (Anacor) -> SCYX-7158 for HAT
  - Nitroimidazoles (TB Alliance) -> VL-2098 and back-ups for VL
  - Other classes related to anti-infectives/oncology (macrolides, …)
  - Orthologs to targets relevant to parasites (Sanofi)

- DMPK collections (Sanofi, GSK, Abbvie)
- Inhibitors of putative targets
  trypanothione reductase, PDE, HDAC, adenosine A2a antagonists, fatty acid synthase, methionine aminopeptidase 2, DHFR, squalene synthase, protein farnesyltransferase, cystein protease,…
Compound collections biased for biological activities: enriched hit rates

Open Access Malaria Box
400 P. falciparum actives

HAT
55 hits
13.7% (0.45%)
30 X

Selectivity window

Chagas
21 hits
5.2% (0.55%)
11 X

Selectivity window

VL
8 hits
2% (0.06%)
33 X

Selectivity window

**MMV19017 is a slow-killer**

<table>
<thead>
<tr>
<th>Drug Exposure Time (h)</th>
<th>µM</th>
<th>1</th>
<th>6</th>
<th>24</th>
<th>72 Standard Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMV665961</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50</td>
<td>4.72±1.76</td>
<td>0.96±0.08</td>
<td>0.67±0.03</td>
<td>0.15±0.05</td>
<td></td>
</tr>
<tr>
<td>IC90</td>
<td>9.05±1.22</td>
<td>2.16±0.60</td>
<td>1.72±0.94</td>
<td>0.29±0.05</td>
<td></td>
</tr>
<tr>
<td><strong>MMV019017</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50</td>
<td>2.36±0.28</td>
<td>1.18±0.47</td>
<td>0.71±0.05</td>
<td>0.08±0.03</td>
<td></td>
</tr>
<tr>
<td>IC90</td>
<td>4.76±2.26</td>
<td>3.20±0.36</td>
<td>1.08±0.11</td>
<td>0.16±0.07</td>
<td></td>
</tr>
</tbody>
</table>

**Activity of MMV19017 is affected by increase in serum concentration**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50</th>
<th>IC90</th>
<th>IC50</th>
<th>IC90</th>
<th>IC50</th>
<th>IC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMV000498</td>
<td>0.336</td>
<td>1.84</td>
<td>0.145</td>
<td>0.285</td>
<td>0.217</td>
<td>0.394</td>
</tr>
<tr>
<td>MMV019017</td>
<td>0.002</td>
<td>0.013</td>
<td>0.084</td>
<td>0.165</td>
<td>0.251</td>
<td>0.429</td>
</tr>
<tr>
<td>MMV019746</td>
<td>0.602</td>
<td>1.13</td>
<td>1.83</td>
<td>2.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MMV19017 is not active in vivo**

<table>
<thead>
<tr>
<th>Treatment Period (days)</th>
<th>Dose (mg/kg/day)</th>
<th>Route</th>
<th>Cured / Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>0/4</td>
</tr>
<tr>
<td>MMV019017</td>
<td>4</td>
<td>60</td>
<td>0/4</td>
</tr>
</tbody>
</table>

## Chemical Diversity: outcome

<table>
<thead>
<tr>
<th>Partner</th>
<th>Size</th>
<th>Nature of library</th>
<th>Pathogen</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scynexis</td>
<td>100'008+</td>
<td>Scynexis Original Collection + few early collaboration (WEHI, Genzyme)</td>
<td>HAT</td>
<td>12 scaffolds, dropped</td>
</tr>
<tr>
<td>Eskitis</td>
<td>200'000</td>
<td>Natural Products (fractions)</td>
<td>HAT</td>
<td>2 scaffolds, dropped</td>
</tr>
<tr>
<td>WEHI</td>
<td>100’000</td>
<td>WEHI (commercial libraries)</td>
<td>HAT</td>
<td>10 scaffolds, H2L at WEHI</td>
</tr>
<tr>
<td>BioFocus</td>
<td>40’000</td>
<td>focused sets</td>
<td>HAT</td>
<td>3-4 scaffolds selected, not pursued</td>
</tr>
<tr>
<td>IPK</td>
<td>200’000</td>
<td>IPK (several commercial libraries)</td>
<td>VL</td>
<td>10 scaffolds, 1 selected for LO</td>
</tr>
<tr>
<td>GNF</td>
<td>700’000</td>
<td>GNF non-proprietary library</td>
<td>HAT, VL</td>
<td>10 scaffolds for VL, HAT (GNF and coll.), 2 Cha</td>
</tr>
<tr>
<td>Broad Institute</td>
<td>300’000</td>
<td>NIH library</td>
<td>Chagas</td>
<td>10 scaffolds in H2L/LO</td>
</tr>
<tr>
<td>Univ Dundee</td>
<td>100’000+</td>
<td>Various sets (commercial+HAT programme+VL programme)</td>
<td>VL</td>
<td>1 scaffold in H2L</td>
</tr>
</tbody>
</table>

Low yield (especially for VL) and high attrition rate
Lack of related R&D knowledge -> focus on Pharma collections
Reducing the odds: getting rid of the garbage

PAINS as Pan-Assay-INTerference compounds

PAINS are sets of chemical motifs likely to be promiscuous inhibitors (= frequent hitters = false positives) in an assay.

"Naivety about promiscuous, assay-duping molecules is polluting the literature and wasting resources" warn Jonathan Baell and Michael A. Walters

Reducing the odds: getting rid of the garbage

**PAINS filters**

- 480 substructures (filters)
- Most PAINS fall within 16 substructural motifs
- Publicly available as SMARTS
  http://blog.rguha.net/?p=850
- 5-12% of academic libraries
- Several substructures are found in NP

Saubern, S. et al. KNIME workflow to assess PAINS filters in SMARTS format. Comparison of RDKit and indigo cheminformatics libraries. Molecular Informatics, 2011, 30, 847 - 850
Reducing the odds: getting rid of the garbage toxicophores

List of reactive moieties related with toxicity effects (>100)

- can be applied as filters to rule out unwanted compounds
- might not always be an issue: is the presence of the toxicophore essential to maintain activity? can it be replaced?

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael_acceptors</td>
<td>[#6]C(=O)C=C([H1])[#6]</td>
</tr>
<tr>
<td>beta_hetero_substituted_carbonyl</td>
<td>C(=O)CC(C)[N;R0,O;R0]</td>
</tr>
<tr>
<td>N,O</td>
<td>[#6]N-O[#6]</td>
</tr>
<tr>
<td>N,S_(not_sulfonamides)</td>
<td>[#6][S;O0][N;H0]</td>
</tr>
<tr>
<td>non_ring_S-O</td>
<td>[S;R0][O;R0]</td>
</tr>
<tr>
<td>triphenylphosphines</td>
<td>P(c1aaaaa1)(c1aaaaa1)(c1aaaaa1)</td>
</tr>
<tr>
<td>diazonium</td>
<td>dN=Nc</td>
</tr>
<tr>
<td>polyene_chain_between_aromatics</td>
<td>cC=CC=CC=Cc</td>
</tr>
<tr>
<td>pyrene_fragments</td>
<td>c1c2ccccc3c2c4e(cc3)ccccc4c1</td>
</tr>
<tr>
<td>reactive_carbonyls_and_sulfonyls</td>
<td>C=[O,S][S,CF,CBr,CCl]</td>
</tr>
<tr>
<td>non_ring_S=N</td>
<td>[S;R0]=[N;R0]</td>
</tr>
<tr>
<td>thiourea</td>
<td>NC(=S)N</td>
</tr>
</tbody>
</table>
# Hit identification and prioritization

## Criteria of selection

<table>
<thead>
<tr>
<th>assay-related</th>
<th>compound-related</th>
</tr>
</thead>
<tbody>
<tr>
<td>relevance to the disease?</td>
<td>promastigote epimastigote trypomastigote axenic intracellular</td>
</tr>
<tr>
<td>relevance of end point?</td>
<td>IC50, IC90, Max %, hill slope, …</td>
</tr>
<tr>
<td>performance</td>
<td>controls, Z', heat maps, …</td>
</tr>
<tr>
<td>criteria of selection</td>
<td>Ref below</td>
</tr>
<tr>
<td>chemical characterization</td>
<td>purity</td>
</tr>
<tr>
<td>ID salt stereochemistry</td>
<td>QC (&gt;90/95%) resynthesis</td>
</tr>
<tr>
<td>drug-likeness</td>
<td>Ref below</td>
</tr>
</tbody>
</table>

---

**standardization - benchmarking**

**continuous optimization of assay protocols**

*better understanding of assays*

---

Identifying and addressing the challenges: VL
# Global Hit rates of kinetoplastid screens

<table>
<thead>
<tr>
<th>Assay</th>
<th>Compounds screened</th>
<th>Average Hit Rate</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. brucei</td>
<td>ca 1.3 M</td>
<td>0.45 %</td>
<td>0.1-1.2 %</td>
</tr>
<tr>
<td>Leishmania donovani (intracellular)</td>
<td>ca 500 K</td>
<td>0.06 %</td>
<td>0.02 - 0.09 %</td>
</tr>
<tr>
<td>T. cruzi (intracellular)</td>
<td>ca 500 K</td>
<td>0.55 %</td>
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</tr>
</tbody>
</table>

hit is defined as non-cytotoxic (SI>10) and IC$_{50}$ < 2µM (HAT), <10 µM (VL) and <5µM (Chagas)

**VL: 6 hits out of 10’000 compounds !**

before hit analysis (clustering, toxicity/reactivity, physchem properties)

Attrition stikes early on: hit confirmation rate

**Challenge: Deliver novel quality starting points for VL**

Evolution of drug chemical space for VL
Combining good drug-like properties and clinical efficacy

Ro5 space

Expanding clinical efficacy space into Ro5 space

Current clinical Efficacy space

S+logP model is based on artificial neural network ensembles (ANNE) constructed by our automatic model builder ADMET Modeler™ from almost 13,000 example compounds selected from the "StarList" of ion-corrected experimental logP values (Hansch, C. et al, 1995).
Adressing the challenges

• Develop and validated cost-effective higher throughput assays predictive of L. don. intracellular activity (cidal axenic assay)

• Screen more: highly diverse, novel and high quality collections

• Confirm activity in INMAC VL assay (Qced material)

• Move quickly to PoC of in vivo efficacy (build SAR, PK)

• Better understand drivers of in vivo efficacy: transition cidal axenic -> INMAC, INMAC to in vivo, PK/PD for novel series

• Obtain rights to develop (H2L/LO and beyond)

• Avoid duplication
**Leishmania cidal axenic assay**

compounds → LdBOB axenic amastigotes → 72 hours

in collaboration with University of Dundee and the Broad Institute

- good correlation of potencies between cAxenic and INMAC assay → predictivity
- screening of DOS collection (10K, Broad Institute): 2 novel series identified (INMAC activity confirmed)

Expand on diversity screening: a reasonable approach to generate novel active starting points

- **Pfizer global diversity research set (GDRS) 150’000**

- **Core diversity collection from Pharmas**
  typically 10-50K collections

- **GSK global 1.8 M collection screening** (DDW Tres Cantos)
  data and structures made public domain

- **Natural Product collections**
  focus on pure compounds (Pharma and beyond)

- **Commercial collections**
  novelty/diversity/quality and rights to develop/publish

Screening for more diversity

- access commercial compound libraries within but also outside of Pharmaceutical partnerships
  - complement on chemical diversity
  - de-risking IP restrictions/delays related hits identified from proprietary collections

access 0.5 Mio cpds to be screened against VL and secure screening capacity
  - library only include structurally characterized chemicals
  - access granted at no/low initial cost (apart from compound plating and material shipment)
  - no IP restriction with regard access to chemical structures of entire file
  - FTO related to hits (hits to be made public domain)

Status:
  - 420K compound secured (Axxam, Biofocus and SPECS)
  - contract in place at University of Dundee to screen 500K (started Q1 2015)
  - Screening to be completed by end Q1 2016 (confirmation in INMAC assay)
Identifying and addressing the challenges: Chagas
Sustained clearance of parasiteamia (rt- PCR for T. cruzi DNA) 10 months after treatment

Parasitological Cure Failure with Azoles

Sustained clearance of parasiteamia (rt-PCR for T. cruzi DNA)
10 months after treatment

Randomized trial of posaconazole and benznidazole for chronic Chagas’ disease
Parasitological Cure Failure with Azoles
Parasitological Response with Benznidazole

Sustained clearance of parasiteamia (rt-PCR for T. cruzi DNA)
10 months after treatment

Randomized trial of posaconazole and benznidazole for chronic Chagas' disease
Impact on Chagas discovery screening cascade

• Deprioritize azoles as a class

• Deprioritize any alternative CYP51 inhibitors

and likely

• Deprioritize any inhibitor of the Sterol biosynthesis pathway

Way forward:

- can we reproduce this observation at the preclinical level?

- can we develop suitable models to predict/rule out CYP51 inhibitors?
Learning from clinical data in vivo Chagas murine chronic models

untreated control

benznidazole

posaconazole (Noxafil)

posaconazole (HPMC-SV formulation)
Better Understanding of assay models: Chagas

*in vitro* potency doesn’t necessarily mean efficacy

Comparison of two T. cruzi assay read-outs CPRG (OD measurement) and microscopical counting of Giemsa stained cells.

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<tr>
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<th>CPRG</th>
<th>Giemsa</th>
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<tr>
<td></td>
<td>IC50</td>
<td>IC90</td>
</tr>
<tr>
<td>Benznidazole (n=5)</td>
<td>984 ± 163</td>
<td>3237 ± 388</td>
</tr>
<tr>
<td>Posaconazole (n=2)</td>
<td>0.4</td>
<td>na</td>
</tr>
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</table>

An in vitro assay to assess antichagasic candidates for sterile cure

Monica Cal, Jean-Robert Ioset, Matthias Fügi, Pascal Mäser, Marcel Kaiser, 9th ECTMIH meeting, Basel, 7-10 September, poster
### Global Hit rates of kinetoplastid screens

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Hit is defined as non-cytotoxic (SI>10) and IC\(_{50}\) < 2\(\mu\)M (HAT), <10 \(\mu\)M (VL) and <5\(\mu\)M (Chagas).

**Chagas**: 55 hits out of 10’000 compounds

Before hit analysis (clustering, toxicity/reactivity, physchem properties)

Hit confirmation: higher than VL

So far so good but ...
CYP51 inhibitors are prevalent...

...and enriched in T. cruzi hit list

...in compound collections


CYP51 inhibitors are prevalent…

…and enriched in T. cruzi hit list

…in compound collections

Use of secondary assays as part of the Chagas discovery cascade to prioritize non-CYP51 inhibitors

- CYP51 functional assay (University of Dundee)
- in vitro MoA assays (STPH, University of Dundee, LNBio)
- In vivo acute/chronic models (LSHTM)


Identifying and addressing the challenges: the NTD Booster
The NTD Drug Discovery Booster: an novel approach to address collaborative early drug discovery

DNDi is building a global consortium of companies for the Booster and requesting GHIT funding to support Booster screening & coordination (for Japanese contributions)

Short HTL stage after Booster needed to produce in vivo proof of concept before entering LO

Maximizing the potential of hardly won HTS hits (cheaper, faster, better)
Overview of NTD Booster

Objective: Delivering Novel Chemical Series for Leishmaniasis & Chagas disease:
Overall during the first two years the Booster project will rapidly expand at least 4 promising
hits/hit series each against Leishmania donovani and Trypanosoma cruzi, the causative agents
of Leishmaniasis and Chagas disease, respectively. This will provide series with well-
developed structure activity relationships (SAR) ready for immediate in vivo proof of concept
studies or, where necessary, focused medicinal chemistry optimization to provide improved
tools ready for in vivo studies. We aim that at least one novel chemical series will provide
promising in vivo activity for each of the two parasites.
First Iteration: S01 - Results

<table>
<thead>
<tr>
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<tr>
<td>Seed S01</td>
<td>1</td>
</tr>
<tr>
<td>DNDi (Historic)</td>
<td>27</td>
</tr>
<tr>
<td>Commercial*</td>
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<tr>
<td>Partner A</td>
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*(Scifinder 80% similarity search; used to represent possible chemical space)

Plot shows Coverage of "Chemical Space" around the starting seed. Axes are Principal Component Analysis dimensions of Chemical fingerprint (X) and Molecular Properties (Y, Z).
**First Iteration: S01 - Results**

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Plot shows Coverage of «Chemical Space» around the starting seed. Axes are Principal Component Analysis dimensions of Chemical fingerprint (X) and Molecular Properties (Y, Z).

Conclusion: For Seed S01 the Booster contributions are highly complementary and explore the chemical space well.
Main Challenges for Sustainable R&D for Neglected Patients

IP & Open Innovation Platforms

Overcoming Regulatory Barriers

Sustainable Financing & New Incentives for R&D
IP & Open Innovation Practices

- Access to compounds, know-how and knowledge
- Increase access to innovation
- Ensure equitable access to all patients & affordable treatment

⇒ Medicines Patent Pool
⇒ WIPO Re:Search
⇒ Open & equitable licensing
⇒ Open Innovation portal/CHEMBL

www.dndi.org/diseases-projects/open-innovation.html

• Open Access Drug Discovery
  Malaria Box, Pathogen Box, …
DNDi sharing discovery data via

**The Pathogen Box**
The Pathogen Box will contain ~400 diverse, drug-like molecules active against neglected diseases of interest and will be available free of charge at the end of 2015 (in collaboration with MMV)

**Antiprotozoal activity profiling of approved drugs: a starting point toward drug repositioning**
A set of 100 registered drugs with drug repositioning potential for neglected tropical diseases was assembled. The compound collection was systematically screened against protozoan parasites, *T. b. rhod.*, *L. donovani*, *T. cruzi* and *P. falciparum*

**Two series of fenarimols for the treatment of Chagas disease**
This second release includes data on a further 84 compounds from the two more advanced series of fenarimols which include the preclinical candidates EPL-BS0967 and EPL-BS1246, both of which are *T. cruzi* CYP51 inhibitors

**Source data from neglected disease R&D pipeline review**
In late 2013, DNDi and colleagues published an analysis in The Lancet Global Health looking at the R&D landscape over the last decade in terms of new therapeutic products for 49 so-called neglected diseases.
In the hope of promoting further research we have created a public data-sharing page with full datasets from the study freely accessible to all.

**Screening and lead optimization of new compounds for Chagas disease (>300,000 compounds)**
We have evaluated multiple hits generated from a high-throughput screen of over 300,000 compounds to identify inhibitors of *T. cruzi* (Broad Institute Screening of NIH collection). These studies have resulted in the discovery of two novel series currently in lead optimisation.

**iNTRODB - an Integrated system for searching drug-target proteins from parasitic protozoa genomes**
iNTRODB is an integrated system for searching drug-target proteins from parasitic protozoa genomes that cause neglected tropical diseases including leishmaniasis, Chagas' disease and human African trypanosomiasis. Japanese academic organizations and a pharmaceutical companies, including Tokyo Institute of Technology, University of Tokyo and Astellas Pharma Inc. [www.bi.cs.titech.ac.jp/introdb](http://www.bi.cs.titech.ac.jp/introdb)

**Screening identifies new compounds for HAT**
Identification of compounds against *Trypanosoma brucei brucei* BS427 by high-throughput screening of whole parasites (87,926 compounds, WEHI)

**DNDi Screening of the MMV Open Access Malaria Box for HAT, VL, and Chagas disease**
Screening of the MMV Open Access Malaria Box in the search for new drugs against Leishmaniasis, Chagas' disease and Human African trypanosomiasis

**SCYNEXIS Inc. as part of the DNDi HAT Lead Optimization Consortium**
Screening and optimization of specific chemical series against human African Trypanosomiasis (HAT): 4926 compounds
10-Year Results

- 2 new malaria treatments
- 1 new sleeping sickness combination
- 1 new visceral leishmaniasis combination for Africa
- 1 set of VL treatment modalities for Asia
- 1 Chagas paediatric dosage form
- Largest pipeline ever for the kinetoplastid diseases
- Clinical research platforms in Africa
- Over 350€M raised equally from public and private sources
- On track to deliver new treatments per business plan
6 New Treatments Developed Since 2007

☑️ Easy to Use  ☑️ Affordable  ☑️ Field-Adapted  ☑️ Non-Patented
Thank You to All Our Partners & Donors

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

DNDi Nutshell - From bench to bedside
THANK YOU

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