MEDCH/PCEUT 527

Drug Discovery in Academia for Neglected Tropical Diseases

Frederick S. Buckner, MD
Department of Medicine
March 13, 2013
Topics to be addressed

• What are NTDs?
• The process of drug discovery and development
• What are the pathways for developing drugs for “neglected tropical diseases”?
  – The role of Academia
• Synopsis of a “hit-to-lead” drug discovery project
## What are the neglected tropical diseases?

<table>
<thead>
<tr>
<th>Disease</th>
<th>Global prevalence</th>
<th>Population at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascariasis</td>
<td>807 million</td>
<td>4.2 billion</td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>604 million</td>
<td>3.2 billion</td>
</tr>
<tr>
<td>Hookworm infection</td>
<td>576 million</td>
<td>3.2 billion</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>207 million</td>
<td>779 million</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>120 million</td>
<td>1.3 billion</td>
</tr>
<tr>
<td>Trachoma</td>
<td>84 million</td>
<td>590 million</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>37 million</td>
<td>90 million</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>12 million</td>
<td>350 million</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>8-9 million</td>
<td>25 million</td>
</tr>
<tr>
<td>Leprosy</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Human African trypanosomiasis</strong></td>
<td>0.3</td>
<td>60 million</td>
</tr>
<tr>
<td>Dracunculiasis</td>
<td>0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Buruli ulcer</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Collectively NTDs affect 1.4 billion people worldwide

# DALYs Lost from NTDs

<table>
<thead>
<tr>
<th>Cause</th>
<th>DALYs Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/AIDS</td>
<td>64,970,667</td>
</tr>
<tr>
<td>Malaria</td>
<td>39,568,398</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>4,576,994</td>
</tr>
<tr>
<td>Trachoma</td>
<td>2,559,951</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>1,752,384</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>1,485,408</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>1,405,795</td>
</tr>
<tr>
<td><strong>HAT (trypanosomiasis)</strong></td>
<td><strong>1,335,075</strong></td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>803,111</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>604,002</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>574,644</td>
</tr>
<tr>
<td>Dengue</td>
<td>542,954</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>427,440</td>
</tr>
<tr>
<td>Leprosy</td>
<td>188,542</td>
</tr>
<tr>
<td>Hookworm disease</td>
<td>64,048</td>
</tr>
</tbody>
</table>

Data from [97].
doi:10.1371/journal.pntd.0000333.t001

DALY = disability adjusted life-year
Trypanosoma brucei

Tse-tse fly

Human African trypanosomiasis
“African sleeping sickness”
Distribution of human African trypanosomiasis

36 countries in sub-Saharan Africa

10,000 – 30,000 new cases per year

Lancet 375:148, 2010
Suramin for early stage HAT

Introduced in the 1920s

Other HAT drugs:
- Pentamidine
- Eflornithine/Nifurtimox
- Melarsoprol

Need better drugs!

Child with resolving exfoliative dermatitis (epidermal necrolysis) resulting from early stage *T.b. rhodesiense* HAT treatment with suramin

Fevre EM. PLOS NTD 2008
Target Product Profile for HAT Drug*

- Effective against stage 1 and stage 2 HAT
  - Must penetrate the blood brain barrier to be active in stage 2
- Effective against *gambiense* and *rhodesiense* strains
- Clinical efficacy >95% at 18 months follow up
- <0.1% drug related mortality
- Safe during pregnancy and lactation
- Stability in Zone 4 for > 3 years
- <30 € per course (only drug cost)

*Per Drugs for Neglected Diseases Initiative*
What is the process of drug discovery?

Chemical Universe $10^{40}$-$10^{100}$

Lead identification

Chemical Synthesis

Universe

10

40

100

Natural Products

Extraction & purification

Compounds

Synthetic Libraries & Natural Product collections

Screening

a) Biochemical assay
b) Cell assays
c) In silico screens
d) Animal screens

PK

Large animals

Toxicity testing

Acute, Chronic, Large animal

Lead compounds

Lead optimization

Chemical synthesis

Compound design

- SAR
- Structure based

Clinical Drug candidates

Clinical Trials

1 2 3

Drugs

IND

NDA

Preclinical

Clinical

Optimization

Lead identification

Preclinical
Drug discovery pipeline

Source: www.ncats.nih.gov
What does the complexity and high cost of drug development mean for tropical diseases?


1233 licensed drugs

13

1220

Tropical diseases

Non-tropical diseases

“10/90 gap”

Pecoul (1999) JAMA 281:361
• The problem:
  – Drug development is high-risk and expensive
  – Drug development primarily takes place in the private sector, for profit
  – Corporations view tropical disease drug development as a poor investment risk

Result: Little R&D for diseases affecting billions

• Possible solutions:
  – Try to lower the risks/costs
  – Early drug development in Academia with support through private-public partnerships (PPPs)
  – Involve Pharma later when risks are lower
Opportunities are available to lower risk

• The field hasn’t been “picked over” yet
  – “Low hanging fruit”?  
• Basic science on NTD pathogens is fairly advanced
  – Genomes are sequenced: *Plasmodium*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania*, *Toxoplasmosis*, *Cryptosporidium*, *Brugia malayi*, etc.
  – Decades of basic science reveal the cell biology and biochemistry
• Good animal models exist for many NTDs
• Tools of modern drug discovery can be brought to bear
  – Bioinformatics  
  – Robotics  
  – High-throughput screening  
  – Diverse compound libraries
Model of drug development of NTDs

- Private-Public Partnerships (PPPs), examples
  - Medicines for Malaria Venture (MMV) - 1999
  - Global Alliance for TB Drug Development (GATB) - 2000
  - Drugs for Neglected Diseases Initiative (DNDi) – 1999
Role of Academia in anti-parasitic drug development

- Target identification and validation
- Assay development for screening
  - Reporter assays of parasite growth inhibition
  - Enzyme assays for screening compound libraries
- Hit-to-lead drug development
  - Medicinal chemistry
  - Structural biology
- Animal models for efficacy testing
- Early pharmacology and toxicology
Four projects (Buckner group)

(represent different strategies)

Target-based drug discovery:
1. tRNA synthetase inhibitors for treatment of HAT
2. Sterol biosynthesis inhibitors for Chagas disease
   - Supported by NIH

Phenotypic screen against *T. brucei*
3. High-throughput screen (300k cmds) against parasite growth followed by hit-to-lead drug development
   - Supported by Novartis institute and a PPP

Drug repurposing:
4. Generic drug screen against *T. cruzi*
   - Supported by NIH and DNDi
Project #3: Phenotypic screen for HAT

• Start with compounds identified in a cell-based screening assay
• Biochemical targets are not known
• Perform “hit-to-lead” drug discovery campaign
“How were new medicines discovered”

Phenotypic vs. target-based screening

Distribution of new drugs discovered between 1999 and 2008 according to the discovery strategy.

Note: there were 75 new first-in-class drugs in the 10 year period.

Phenotypic screening cannot be ignored as a strategy for drug discovery even in the post-genomics era.
Rationale for phenotypic screen on \textit{T. brucei}

- The hurdle of identifying compounds with good BBB permeability is high
  - Most hits from screens will have poor permeability into the CNS
  - Thus, we need a lot of diverse hits to identify the subset with BBB permeability
- A phenotypic screen ensures
  - A diversity of hit scaffolds
  - Proof of cellular permeability
GNF* phenotypic screen

- GNF library of 300,000+ compounds screened on *T. brucei* BSF cultures
  - 1009 confirmed hits with IC$_{50}$ <4 µM and selectivity index >10 (Huh7 cells)
- Mike Gelb (UW Dept. of Chemistry) met with scientists at GNF to select scaffolds for further evaluation (Oct., 2009)
  - Compounds selected for oral bioavailability and potential for permeability through BBB

*Genomics Institute of the Novartis Research Foundation (San Diego, CA)
Selection Criteria

• Lipinski rules*
  • Further restriction with MW <450 for CNS penetration
• Structural alerts (avoid alkylating agents, etc.)
• Select for molecules with $IC_{50} < 2 \, \mu M$
• Avoid molecules with >1 chiral center
• Chemical tractability

* Lipinski’s Rule of Five
  • MW <500
  • Log $P$ not >5
  • Not more then 5 H-bond donors
  • Not more than 10 H-bond acceptors
Fifteen Scaffolds Selected

- Representative hit compounds purchased (repurified by HPLC) or resynthesized
- Activity confirmed:
  - Bloodstream forms of *T. brucei* (IC$_{50}$ values within 2X of GNF value)
  - Cytotoxicity $>20$ μM on lymphocytic cell line
Testing for CNS Penetration

• 15 compounds tested in mice
  • Dosed IP, collected plasma and brains at 3 time points, measured levels by LC/MS-MS
  • Controls: diphenhydramine (Benadryl) and Cetirizine (Zyrtec)

• 8 compounds with brain:plasma ratio >0.15
  • Selected for further characterization and medicinal chemistry
Hit-to-Lead Strategy on 8 scaffolds

Medicinal chemistry to improve potency, PK (1-2 chemists per scaffold)

\[ T. brucei \text{ EC}_{50} \]
Mammalian cell cytotoxicity
Solubility

Better than parent compound

Liver microsome stability to assess metabolic stability
MDR1-MDCKII assay to assess potential for BBB penetration
Mouse PK studies

Efficacy testing in acute mouse model of \textit{T. brucei} infection

Define SARs
Hit-to-Lead GNF Scaffolds

Scaffold 1
CPD-022-10
$EC_{50}$ 0.22 uM
ACTIVE
(Gelb lab: Hari Babu Tatipaka)

Scaffold 2
CPD-090-10
$EC_{50}$ 0.48 μM
ACTIVE
(Boykin lab: Jennifer Draper)
(Tidwell lab: Donald Patrick)
Hit-to-Lead GNF Scaffolds

Scaffold 3
CPD-035-10
EC$_{50}$ 0.57 µM

Scaffold 4
CPD-028-10
EC$_{50}$ 0.44 µM

DISCONTINUED
>100 analogs made
No progress in EC$_{50}$

ACTIVE
(Gelb lab: Andriy Buchnyskyy
Tidwell lab: Donald Patrick)
Hit-to-Lead GNF Scaffolds

Scaffold 5
CPD-009-10
EC\textsubscript{50} 0.66 \textmu M

DISCONTINUED
(Solubility problems, Poor mouse PK)

Scaffold 6
CPD-365-11
EC\textsubscript{50} 0.47 \textmu M

DISCONTINUED
(>35 analogs made; Flat SAR)
Hit-to-Lead GNF Scaffolds

Scaffold 7
CPD-146-10
EC$_{50}$ 0.55 uM

ACTIVE
(Gelb lab: N. Chennamaneni )

Scaffold 8
CPD-150-10
EC$_{50}$ 0.18 μM

ACTIVE
(Gelb lab: N. Chennamaneni )
Scaffold 1 - Summary

Site II: 46 analogs
Best activity: CPD-569-12
*Trypanosoma brucei* EC$_{50}$ = 0.08 µM
Cytotoxicity IC$_{50}$ = 73 µM
Mouse microsome $t_{1/2}$ = 0.8 min

Site I: 9 analogs
Best activity: CPD-155-10
*Trypanosoma brucei* EC$_{50}$ = 0.08 µM
Cytotoxicity IC$_{50}$ = 99.7 µM
Mouse microsome $t_{1/2}$ = 0.7 min

Site IV: 4 analogs
Complete loss of potency

**CPD-022-10**
*Trypanosoma brucei* EC$_{50}$ = 0.22 µM
Cytotoxicity IC$_{50}$ = 116 µM
Mouse microsome $t_{1/2}$ = 0.8 min

MDR1-MDCK:
- Papp (-918): 312 nm/sec
- Papp (+918): 293 nm/sec

Site V: 6 analogs
Best activity: CPD-566-12
*Trypanosoma brucei* EC$_{50}$ = 0.105 µM
Cytotoxicity IC$_{50}$ = 86 µM
Mouse microsome $t_{1/2}$ = 0.7 min

Site III: 58 analogs
Best activity: CPD-568-12
*Trypanosoma brucei* EC$_{50}$ = 0.05 µM
Cytotoxicity IC$_{50}$ = 224 µM
Mouse microsome $t_{1/2}$ = 0.8 min

Combinations of sites I, II, and III
Best activity: CPD-619-12
*Trypanosoma brucei* EC$_{50}$ = 0.004 µM
Cytotoxicity IC$_{50}$ = 24 µM
Mouse microsome $t_{1/2}$ = 9 min

Human microsome $t_{1/2}$ = 30 min
MDR1-MDCK:
- Papp (-918): 401 nm/sec
- Papp (+918): 503 nm/sec
Metabolite identification

Parent compound
CPD-022-10

Incubation with
mouse microsomes

Main metabolite
MW 245.0
LKZ605 had high clearance in the microsomal stability assay in mouse and human liver microsomes and moderate in rat.
One major oxidative (M+16) metabolite was observed in all species
GSH adduct observed to furan

Major metabolic soft spot:
Modification of hit compound

Furan \rightarrow \text{Hit} \rightarrow \text{Pyrroldidine} \rightarrow \text{“Lead”}

\text{T.b.b EC}_{50}: \ 0.220 \ \mu\text{M} \\
\text{Mouse microsome t½: 0.8 min.} \\

\text{T.b.b EC}_{50}: \ 0.004 \ \mu\text{M} \\
\text{Mouse microsome t½: 10 min.}
## Microsome & CYP data

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse $t_{1/2}$ (min)</th>
<th>Human $t_{1/2}$ (min)</th>
<th>CYP3A4 IC50 (nM)</th>
<th>T. brucei IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPD-022-10</td>
<td>0.8</td>
<td>6.4</td>
<td>&gt;5000</td>
<td>220</td>
</tr>
<tr>
<td>HB-175</td>
<td>10.1</td>
<td>30.6</td>
<td>1460</td>
<td>4</td>
</tr>
<tr>
<td>HB-178</td>
<td>13.7</td>
<td>8.3</td>
<td>1358</td>
<td>10</td>
</tr>
<tr>
<td>HB-180</td>
<td>8.6</td>
<td>7.5</td>
<td>568</td>
<td>15</td>
</tr>
<tr>
<td>HB-181</td>
<td>11.9</td>
<td>3.4</td>
<td>730</td>
<td>9</td>
</tr>
<tr>
<td>HB-182</td>
<td>&gt;60</td>
<td>&gt;60</td>
<td>70,127</td>
<td>11</td>
</tr>
<tr>
<td>HB-183</td>
<td>10.4</td>
<td>14</td>
<td>2668</td>
<td>8</td>
</tr>
<tr>
<td>HB-184</td>
<td>4.7</td>
<td></td>
<td>1175</td>
<td>5</td>
</tr>
<tr>
<td>HB-185</td>
<td>10.5</td>
<td>60</td>
<td>2196</td>
<td>6</td>
</tr>
<tr>
<td>HB-186</td>
<td>4.9</td>
<td></td>
<td>1239</td>
<td>28</td>
</tr>
<tr>
<td>HB-187</td>
<td>7.0</td>
<td></td>
<td>3055</td>
<td>21</td>
</tr>
<tr>
<td>HB-189</td>
<td>8.3</td>
<td></td>
<td>1224</td>
<td>4</td>
</tr>
</tbody>
</table>
MDR1-MDCK Permeability Assay

Figure 1

Transwell insert
Cell monolayer
Permeable membrane

A
B
B

Apical chamber
Basolateral chamber

Figure 2

Sources:
Figure 1 http://media.wiley.com/wires/WNAN/WNAN53/mfig003.jpg
Figure 2 http://www.krackeler.com/graphics/0083/jpg/3401-HR.jpg
Summary of MDR1-MDCKII Papp Values, Mass Balances, and Absorptive Quotients.

Average Papp (nm/sec) (-918; +918) 44; 304
Average Mass Balance (%) (-918; +918) 76; 84

*Average AQ

-918 Papp's (nm/sec)  37; 262
+918 Papp's (nm/sec) 0.85

-918 Papp's (nm/sec)  559; 585
+918 Papp's (nm/sec) 0.02

Brain Uptake Potential:
High
Moderate
Poor

*Rapp (nm/sec)
Brain Penetration Data:
60 min after 5 mpk IP Injection

![Graph showing brain penetration data for HB175 and HB185 compounds.]

- **HB175**
- **HB185**
GNF-HB175
Mouse PK
50 mg/kg PO
(Dosing solution:)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ ($\mu$M)</td>
<td>4.33</td>
<td>1.36</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>30.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Elimination Rate Constant (1/min)</td>
<td>0.0041</td>
<td>0.0018</td>
</tr>
<tr>
<td>Elimination Half-Life (min)</td>
<td>203.98</td>
<td>108.90</td>
</tr>
<tr>
<td>AUC(0-$\infty$) ($\mu$M-min)</td>
<td>1125.29</td>
<td>86.79</td>
</tr>
<tr>
<td>Clearance (mL/min)</td>
<td>2.32</td>
<td>0.01</td>
</tr>
</tbody>
</table>
GNF-HB185 Mouse PK 50 mg/kg PO (Dosing solution:)

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µM)</td>
<td>2.76</td>
<td>1.27</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (min)</td>
<td>120.00</td>
<td>103.92</td>
</tr>
</tbody>
</table>

Lipinski Properties

- Molecular weight: 402.424 g/mol
- log P: 2.76
- H-bond donors: 2
- H-bond acceptors: 4
- Lipinski Rule of 5: Satisfied

4 of 4 within desirable range
GNF-HB175

- *T. brucei* efficacy expt #1:
  - Mice infected with *T. b. rhod* (STIB900), day 0
  - Group size: 5 mice
  - Treat days 2, 3, 4, 5, 6 with:
    - HB175 50 mg/kg PO twice per day
    - HB175 20 mg/kg PO twice per day
    - HB175 5 mg/kg PO twice per day
    - HB175 50 mg/kg PO once per day
    - Pentamidine 10 mg/kg IP once per day (check on # of doses)
    - Vehicle twice per day
  - Monitor parasitemia to day 60 post-infection
  - Collect plasma levels of HB175 at mid-point of expt
**Acute *T. brucei* infection in mice**  
(Results through day 60 post-infection)

December, 2012

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Dose</th>
<th>Cure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>BID x 5 d</td>
<td>0/5</td>
</tr>
<tr>
<td>HB175</td>
<td>20 mpk QD</td>
<td>5/5</td>
</tr>
<tr>
<td>HB175</td>
<td>5 mpk BID</td>
<td>5/5</td>
</tr>
<tr>
<td>HB175</td>
<td>5 mpk QD</td>
<td>2/5</td>
</tr>
<tr>
<td>HB175</td>
<td>2.5 mpk BID</td>
<td>2/5</td>
</tr>
<tr>
<td>HB175</td>
<td>1 mpk BID</td>
<td>0/5</td>
</tr>
<tr>
<td>BA332</td>
<td>50 mpk BID</td>
<td>4/4*</td>
</tr>
<tr>
<td>BA332</td>
<td>50 mpk QD</td>
<td>4/5</td>
</tr>
</tbody>
</table>

- Infect with *T. b. rhod* (STIB900), day 0
- Dose days 2-6

---

*50 mpk BID Mouse FDIC day 4 due to experimental complications.*
HB175 Efficacy study in mice #1

- Mice (n=5 per group) infected with *T. brucei rhodesiense* on day 0
- Treated with HB175 or vehicle starting 48 hr post-infection for 4 days

Parasitemia

No parasites have been observed at any time point in any of the HB175-treated groups! Finalized at 60 days.
GNF-HB175  Rat PK

20mpk Oral dosing

5mpk IV dosing
# GNF-HB175 Rat PK

**HB-175**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV administration</th>
<th>Oral Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg)</td>
<td>Rat 3</td>
<td>Rat 4</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>0.825</td>
<td>0.825</td>
</tr>
<tr>
<td>Apparent t½ (min) steady state</td>
<td>119.7</td>
<td>101.9</td>
</tr>
<tr>
<td>Apparent t½ (min) terminal phase</td>
<td>118.6</td>
<td>101.5</td>
</tr>
<tr>
<td>Plasma CL (ml/min/kg)</td>
<td>13.27</td>
<td>16.35</td>
</tr>
<tr>
<td>Vz (L/Kg)</td>
<td>3.48</td>
<td>3.76</td>
</tr>
<tr>
<td>VSS (L/Kg)</td>
<td>2.33</td>
<td>2.38</td>
</tr>
<tr>
<td>AUC (min*umol/L)</td>
<td>992.6</td>
<td>862.7</td>
</tr>
<tr>
<td>Cmax (uM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA(%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Posaconazole**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV administration</th>
<th>Oral Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>Rat 3</td>
<td>Rat 4</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>3.8</td>
<td>3.9</td>
</tr>
<tr>
<td>Apparent t½ (min)</td>
<td>738</td>
<td>696</td>
</tr>
<tr>
<td>Plasma CL (ml/min/kg)</td>
<td>3.2</td>
<td>3</td>
</tr>
<tr>
<td>Vz (L/Kg)</td>
<td>3.5</td>
<td>3</td>
</tr>
<tr>
<td>VSS (L/Kg)</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>AUC (min*umol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (uM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA(%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Scaffold 1 (cont.)

*T. brucei* EC50: 0.0065 uM; 0.004 uM
CRL-8155 cytotox EC50: >50 uM
HepG2 cytotox EC50: 33 uM

Liver microsome T½
- Mouse: 10.1 min
- Human: 30.6 min

MDR1-MDCK
- 401 nm/sec (-918)
- 503 nm/sec (+918)

PROTEIN BINDING: 98.9%

Solubility
- pH 7.4: 5.9 uM; 8.5 uM
- pH 2.0: 29.5 uM; 28.7 uM

Potential concerns

**GNF-HB175**

MW 401.4
logP 3.98
TPSA 73.9
pKa 10.7
Plans with Scaffold 1

• Test in chronic mouse model:
  • Does the compound cure CNS infection?

• Further toxicity testing:
  • We know the compounds series is clean on Ames and hERG
  • Rat tox
  • Large animal (dog) tox

• Address activity against
  
  *T. cruzi*
  
  *Leishmania*
## Summary of progress on scaffolds from GNF screen

<table>
<thead>
<tr>
<th>Scaffold</th>
<th># analogs made</th>
<th>Best <em>T. brucei</em> EC$_{50}$</th>
<th>Reason for stopping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>190+</td>
<td>4 nM</td>
<td>Work ongoing</td>
</tr>
<tr>
<td>2</td>
<td>158+</td>
<td>18 nM</td>
<td>Work ongoing</td>
</tr>
<tr>
<td>3</td>
<td>109</td>
<td>520 nM</td>
<td>No progress on EC$_{50}$</td>
</tr>
<tr>
<td>4</td>
<td>75+</td>
<td>21 nM</td>
<td>Work ongoing</td>
</tr>
<tr>
<td>5</td>
<td>102</td>
<td>30 nM</td>
<td>Low solubility; bad mouse PK</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>300 nM</td>
<td>Flat SAR; synthesis difficult</td>
</tr>
<tr>
<td>7</td>
<td>126+</td>
<td>16 nM</td>
<td>Work ongoing</td>
</tr>
<tr>
<td>8</td>
<td>59+</td>
<td>78 nM</td>
<td>Work ongoing</td>
</tr>
<tr>
<td>Total</td>
<td>751</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Assays for hit-to-lead drug development

- Enzyme assays
- Whole cell assays
  - *T. brucei EC*$_{50}$
- Cytotoxicity on mammalian cells
- CYP enzyme inhibition
- Genotoxicity (Ames test, etc.)
- hERG channel
- Receptor profiling
- Animal toxicity (rodents)

- Aqueous solubility
- Protein binding
- Liver microsome stability
- Metabolite identification
- Permeability assays
  - MDR1-MDCK cells
- Mouse pharmacokinetics
- Cannulated rats
- Brain penetration in mice
Where we go from here…

Academia

Private-Public-Partnership

Contract labs

Contract clinical developers. Drug company.

Diagram:
- Discovery: R, LS, LO
- Pre-clinical
- Clinical
- Implementation

Steps:
- Screening
- Lead selection
- Lead optimization
Acknowledgements

- Buckner lab
  - Robert Gillespie
  - Ranae Ranade
  - Joy Laydbak
  - Matthew Hulverson
  - Sharon Creason
  - Jennifer Arif
  - Noah Smith
  - Sarah Hale
  - Nicole Duster
- Erkang Fan and lab
  - Sayaka Shibata
  - Zhongsheng Zhang
  - Jian Teng-Yue
- Christophe Verlinde
- Wim Hol and lab
  - Cho Yeow Koh
  - Jessica Kim
  - Allan Wetzel
  - Will devanderSchueren
  - Frank Zucker
- Wes Van Voorhis and lab
  - Angela Gillespie
  - Alberto Napuli
  - Lynn Barrett
Acknowledgements (cont.)

• Michael Gelb and lab
  – Haribabu Tatipaka
  – Andriy Buchynskyy
  – Naveen Chennameneni
  – Pendem Racherla
  – Amit Thakkar
  – Neil Norcross

• Richard Tidwell and lab (U. North Carolina)
• David Boykin and lab (U. South Florida)
• Richard Glynne and lab (GNF, Novartis)
• Peter Hodder, Laura Pedro-Rosa (Scripps Florida Research Institute)

Funding:
• National Institutes of Health
• Consortium for Parasitic Drug Development (CPDD)
• Drugs for Neglected Diseases Initiative