Fexinidazole: a rediscovered nitroimidazole drug candidate moving into clinical development for HAT

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Senior Project Manager
Drugs for Neglected Diseases initiative

Annual Conference of the ASTMH,
8-12 December 2008, New Orleans

Compound mining to create innovation for neglected diseases

- Megazol:
  - Existing compound (1968) from the nitroimidazole family
  - Shown to have potent oral trypanocidal activity in vivo
    - Several publications in 1980s-1990s
  - Toxic (mutagenic)
- Other anti-infective drugs exist in this family:
  metronidazole, tinidazole, benznidazole,…

Can we identify existing compounds with a better activity/toxicity profile?
Over 600 nitroimidazoles obtained and assessed as drug leads during 2005-7

Pharma
- sanofi-aventis, France - Germany
- Roche, CH
- Novartis (NITD), USA - CH -Singapore
- Alkem, India

Academics
- Swiss Tropical Institute
- Fiocruz, Brazil
- Glasgow Univ, UK
- Univ of Alberta, Canada
- ENH Research Institute, USA
- Tehran Univ of Medical Sc., Iran
- Silesian Univ of Technology, Poland
- LaSpienza Univ, Italy
- Univ of Auckland, New Zealand
- Univ of Dundee, UK
- Univ of Parma, Italy
- Univ of Tennessee, USA
- Tokushima Univ, Japan

Other
- TB Alliance
- retired pharma chemist, India

Fexinidazole

- 5-nitroimidazole
- in preclinical development by Hoechst in 80s as broad-spectrum anti-protozoal
  - Not progressed

\[
\text{O}_2\text{N} \quad \text{N} \quad \text{CH}_2 \quad \text{SMe}
\]

- DNDi profiling
  - Literature + Hoechst reports (s-a)
  - New studies: pharmacology – ADME/PK – toxicology
**2-step synthesis of fexinidazole**

```
1-methyl-2-hydroxymethyl-5-nitroimidazole
SOCl₂ → chloro intermediate (not isolated)

Fexinidazole
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2 steps purification process, developed by Centipharm

- Pure fexinidazole


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**Pharmacology (1)**

- Selective *in vitro* anti-trypanosomal activity

<table>
<thead>
<tr>
<th></th>
<th><em>T. b. rhodesiense</em></th>
<th><em>T. b. gambiense</em></th>
<th>cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC₅₀</strong></td>
<td>STIB 900</td>
<td>STIB 754</td>
<td>L-6 (rat skeletal myoblast cells)</td>
</tr>
<tr>
<td>Fexinidazole</td>
<td>0.72 (2.57)</td>
<td>0.32 (1.14)</td>
<td>&gt;90 (&gt;322)</td>
</tr>
</tbody>
</table>

**Reference compounds**

<table>
<thead>
<tr>
<th></th>
<th><em>T. b. rhodesiense</em></th>
<th><em>T. b. gambiense</em></th>
<th>cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megazol</td>
<td>0.02 (0.10)</td>
<td></td>
<td>57 (254)</td>
</tr>
<tr>
<td>Melarsoprol</td>
<td>0.004 (0.009)</td>
<td>0.0015 (0.004)</td>
<td>1.3 (3.3)</td>
</tr>
<tr>
<td>Eflornithine</td>
<td>0.90 (3.80)</td>
<td>0.40 (1.67)</td>
<td>12 (51)</td>
</tr>
<tr>
<td>Nifurtimox</td>
<td>0.41 (1.44)</td>
<td>0.31 (1.08)</td>
<td>25 (87)</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>0.003 (0.009)</td>
<td>0.002 (0.01)</td>
<td>3 (9)</td>
</tr>
</tbody>
</table>

Presented during DNDi / HAT Platform symposium on "Addressing HAT R&D Challenges" held during ASTMH 2008
### Pharmacology (2)

- **In vivo** activity in mouse model of acute infection (*T. b. rhodesiense* STIB900 - stringent model)

<table>
<thead>
<tr>
<th>Compound</th>
<th>#days x daily dose in mg/kg</th>
<th>route</th>
<th>Cured/infected</th>
<th>Mean survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td></td>
<td>0/4</td>
<td>7-8</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>4 x 25 po</td>
<td></td>
<td>0/4</td>
<td>15.75</td>
</tr>
<tr>
<td></td>
<td>4 x 50 po</td>
<td></td>
<td>1/4</td>
<td>&gt;34</td>
</tr>
<tr>
<td></td>
<td>4 x 100 po</td>
<td></td>
<td>4/4</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>4 x (2 x 12.5) (bid) po</td>
<td></td>
<td>0/4</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>4 x (2 x 25) (bid) po</td>
<td></td>
<td>2/4</td>
<td>&gt;39.5</td>
</tr>
<tr>
<td></td>
<td>4 x (2 x 50) (bid) po</td>
<td></td>
<td>4/4</td>
<td>&gt;60</td>
</tr>
<tr>
<td>1 x 200 po</td>
<td></td>
<td></td>
<td>3/4</td>
<td>&gt;51.75</td>
</tr>
</tbody>
</table>

- pentamidine and eflornithine are inactive in this model
- nifurtimox requires 4 x 200 mkd p.o (inactive at 4 x 100 mkd)
- melarsoprol cures at 4 x 8 mkd ip

### Pharmacology (3)

- **In vivo** activity in mouse model of chronic infection with CNS-involvement (*T. b. brucei* GVR35)

<table>
<thead>
<tr>
<th>Compound</th>
<th>dose: #days x mg/kg</th>
<th>mode</th>
<th>Cured/infected</th>
<th>MSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>1 x 40 ip</td>
<td></td>
<td>0/4</td>
<td>63</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>5 x 100 po</td>
<td></td>
<td>3/5</td>
<td>&gt;145</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>5 x (2 x 100) - bid</td>
<td>po</td>
<td>5/5</td>
<td>&gt;180</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diminazene diaceturate</td>
<td>1 x 40 ip</td>
<td></td>
<td>0/4</td>
<td>63</td>
</tr>
<tr>
<td>melarsoprol</td>
<td>1 x 10 ip</td>
<td></td>
<td>2/8</td>
<td>&gt;122</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>5 x 50 po</td>
<td></td>
<td>0/8</td>
<td>63.8</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>5 x 100 po</td>
<td></td>
<td>2/8</td>
<td>&gt;107</td>
</tr>
<tr>
<td>fexinidazole</td>
<td>5 x 200 po</td>
<td></td>
<td>7/8</td>
<td>&gt;169</td>
</tr>
</tbody>
</table>
**In vitro ADME fexinidazole**

- Good intestinal permeability (Caco-2)
  - no limiting factor for absorption
- Good potential for BBB permeability (MDR1-MDCK)
- High plasma protein binding
  - 95% (human); 93% (mouse)
- *In vitro* hepatocyte metabolism: < 1% remaining after 1h

<table>
<thead>
<tr>
<th>Species</th>
<th>$t_{1/2}$ (min)</th>
<th>$\text{In vitro } \text{CL}_{\text{int}}$ (mL/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1.4</td>
<td>4312</td>
</tr>
<tr>
<td>Rat</td>
<td>1.2</td>
<td>2890</td>
</tr>
<tr>
<td>Dog</td>
<td>1.1</td>
<td>5042</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.6</td>
<td>6467</td>
</tr>
<tr>
<td>Human</td>
<td>13.4</td>
<td>124</td>
</tr>
</tbody>
</table>

- 2 major metabolites: sulfoxide (M1) and sulfone (M2)

**Fexinidazole metabolism / PK**

<table>
<thead>
<tr>
<th>pH</th>
<th>Solubility (aqueous sol) (µg/mL)</th>
<th>LogD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>5.69</td>
<td>2.51</td>
</tr>
<tr>
<td>7.4</td>
<td>2.55</td>
<td>2.83</td>
</tr>
<tr>
<td>1.2</td>
<td>84.1</td>
<td>0.52</td>
</tr>
<tr>
<td>7.4</td>
<td>51.6</td>
<td>0.74</td>
</tr>
<tr>
<td>1.2</td>
<td>&gt;5000</td>
<td>0.19</td>
</tr>
<tr>
<td>7.4</td>
<td>942</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Plasma levels following 5-days oral administration of 200 mg/kg fexinidazole to mice.
**Fexinidazole distribution**  
*(after oral administration)*

- Plasma levels & AUC metabolites >> fexinidazole  
  - Similar profiles in mouse – rat – dog  
- Fexinidazole / metabolites are widely distributed over all tissues  
  - Including brain

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>fexinidazole</th>
<th>sulfone</th>
<th>sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1136</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>30</td>
<td>267</td>
<td>1105</td>
<td>156</td>
</tr>
<tr>
<td>60</td>
<td>254</td>
<td>1624</td>
<td>394</td>
</tr>
</tbody>
</table>

- Elimination of fexinidazole and metabolites is rapid (about 90% within 48h) and mainly with feces (~60%)
- No/limited accumulation over repeated administration

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**Toxicology**

**Completed regulatory toxicology studies**

- GLP safety pharmacology  
  - hERG (CVS)  
  - Irwin screen (CNS)  
  - Respiratory function  
  - Dog telemetry (CVS)
- Secondary pharmacology (receptor screen)
- *In vitro* phototoxicity
- Repeated dose toxicology, incl toxicokinetics  
  - 7d dose range and MTD in rat  
  - 4-weeks repeated dose toxicokinetics in rat - GLP  
  - 7d dose range and MTD in dog  
  - 4-weeks repeated dose toxicokinetics in dog – GLP
- Genetic toxicology (details below)
Safety pharmacology

- **In vitro hERG:**
  - Concentrations tested: 1, 5, and 30 µM
  - Fexinidazole and the sulfoxide do not affect hERG peak tail current
  - Fexinidazole sulfone showed a significant decrease (-33%) on hERG peak tail current at the 30 µM concentration only

- **In vivo cardiovascular parameters in the Beagle Dog (dog telemetry):**
  - Dose levels: 100, 300 and 1000 mg/kg oral
  - NOEL CV parameters and ECG intervals ≥1000 mg/kg
  - Overrules positive hERG signal

- **Irwin test in rat:** general behavior and body temperature
  - Dose levels: 100, 300 or 1000 mg/kg oral
  - NOEL ≥ 1000 mg/kg.

- **Respiratory Parameters in unrestrained Conscious Male Rat**
  - Dose levels: 100, 300 and 1000 mg/kg oral
  - NOEL ≥1000 mg/kg.

4-week repeated dose Toxicokinetics – Rat & Dog

**Doses:** 0, 50, 200 and 800 mg/kg/day, oral

- For both species, the dose of 200 mkd is considered the **NOAEL (No-observed-adverse-effect-level)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Sex</th>
<th>fexinidazole AUC0-24h mcg/h/mL</th>
<th>sulfoxide AUC0-24h mcg/h/mL</th>
<th>sulfone AUC0-24h mcg/h/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat</td>
<td>200</td>
<td>M</td>
<td>2,320</td>
<td>122,100</td>
<td>341,333</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>4,437</td>
<td>138,333</td>
<td>342,333</td>
</tr>
<tr>
<td>dog</td>
<td>200</td>
<td>M</td>
<td>0.395</td>
<td>42,200</td>
<td>268,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0.377</td>
<td>33,800</td>
<td>277,000</td>
</tr>
</tbody>
</table>

- For both species, the top dose of 800 mkd is considered as “**well-tolerated**”:
  - In rat: slightly decreased body weight (M) and increased liver weight (F) with minimal/slight hypertrophy of hepatocytes
  - In dog: slight reduction in food intake and decreased body weight (mainly F) but no histological findings

- **No particular issues identified**
**Genetic Toxicology: new GLP-studies**
(to confirm previous evidence from Hoechst-DNDi studies)

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Top dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>In vitro</em> Bacterial Ames test</td>
<td>Positive*</td>
<td>1000 µg/plate</td>
</tr>
<tr>
<td><em>In vitro</em> Human lymphocyte micronucleus test</td>
<td>Negative</td>
<td>220 µg/mL</td>
</tr>
<tr>
<td><em>In vivo</em> Rat Liver UDS</td>
<td>Negative**</td>
<td>2000 mg/kg</td>
</tr>
<tr>
<td><em>In vivo</em> Mouse micronucleus</td>
<td>Negative**</td>
<td>2000 mg/kg</td>
</tr>
</tbody>
</table>

*Decreased in NR-deficient strains
**with PK to confirm fexinidazole and both metabolites are being formed

Conclusion: fexinidazole and metabolites are unlikely to pose a genotoxic risk to volunteers or patients

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**Fexinidazole is promising drug candidate for clinical development for HAT**

- Complies with ideal Target Product Profile:
  - Oral treatment for stage 2 HAT
    - Ideally useful for both stage 1 and 2 (if practical and very safe)
  - Active on *T.b.gambiense* + *T.b.rhodesiense*
  - Short course, affordable

- Decision to progress to First-in-Human phase I trials
  - Objective: assess bioavailability/PK and tolerability (MTD) in healthy volunteers (SAD, MAD, food effect)
    - Assess prototype tablets
  - Planned to start Q2-2009

- Registration by 2014 if successful development
A multidisciplinary team effort to advance fexinidazole into clinical development

DNDi project team
- Els Torreele: DNDi project manager
- Michael Bray: pharmaceutical project management consultant
- Bernadette Bourdin: chemistry project support & documentation
- Guy Mazue: toxicology, preclinical development
- Jean-René Kiechel: CMC, formulation, pharmacology
- Pierre-Etienne Bost: chemistry, preclinical development
- David Tweats: toxicology, in particular genetic toxicology
- Daniela Sassella: clinical development
- François Chappuis: clinical HAT expert
- Matthias Dormeyer: regulatory advise, IMPD
- Eloa Pinheiro: formulation and manufacture expert
- Christian Burn, Gabriele Pohlig: HAT clinical trials experts

Operational partners
- **Main preclinical package**
  - **Accelera**, Italy: preclinical formulation, regulatory toxicology, safety pharmacology, pharmacokinetics, bioanalytics
  - **Covance**, UK: genotoxicology
- **Disease models**
  - **STI**, Swiss Tropical Institute, Switzerland: mouse models
  - **TRC**, Trypanosomiasis Research Centre, Kenya: monkey model
- **CMC**
  - **Axyntis** (ex Orgasynth), France: chemistry, GMP-production
  - **Aptuit**, UK: clinical formulation development and batch supply

Financial support
- **Project specific funding**:  
  - Private Swiss Foundation
  - French Ministry of foreign Affairs (MAEE)
- **DNDi core funding**:  
  - MSF
  - UK DFID
Thank you!
Addressing the R&D challenges in making new drugs available for human African trypanosomiasis (HAT) are potential in the pipeline and recent clinical results.

- HAT Platform - Success to date, and challenges/opportunities ahead in overcoming difficulties in clinical research of HAT drugs & in developing regional research platform
  Fred Kansulima, HAT Platform, Coordination Office for Control of Trypanosomiasis in Uganda, Kampala, Uganda

- Phase III results of multi-center study evaluating nifurtimox-eflornithine combination for treatment (NECT) of Stage 2 HAT
  Gerardo Prinetti, Epicentre, Paris, France

- Research results evaluating the diamidine class for the treatment of HAT
  Carol Olson, Immunex Pharmaceuticals, Vernon Hills, IL, United States

- Fenamidazole: a rediscovered nitroimidazole drug candidate moving into clinical development for HAT
  Elsa Torrelle, Drugs for Neglected Diseases Initiative, Geneva, Switzerland

Interventions by Christian Barfot, Swiss Tropical Institute, Basel, Switzerland, and Constantin Mba N Engle, HAT National Control Program, Kinshasa, Democratic Republic of the Congo (DRC). Followed by a Q&A session.

DNDi's HAT symposium will be followed by film broadcast on HAT: "Sleeping sickness: the deadliest disease", BBC series "Survival", produced by Reckhoper TV. At 1:15 pm, Room 150 BOC