New tools for monitoring drug resistance and treatment response in Visceral Leishmaniasis in the Indian subcontinent

KALADRUG-R

J.C. Dujardin

Paris, NID conference, 24/09/10
Leishmania donovani

Phlebotomus argentipes

Homo sapiens sapiens

The dogma
Visceral leishmaniasis (Indian sub-continent)

- 200 millions at risk
- 100,000 cases/yr

- disease lethal if untreated: 40,000 deaths/yr
- but, 9 asymptomatic carriers/1 clinical case*

* Ostyn et al. 2010, submitted
First regional programme for elimination of VL, start in 2007 (India, Nepal, Bangladesh)

Aim: to reduce the annual incidence of VL to less than 1 per 10,000 by 2015

Detecting patients with simple tools (rk39) and treating them with simple drug (miltefosine, oral drug): reducing parasite biomass

Vector control (insecticide spraying)
Chemotherapy: available arsenal

- Antimonials (SSG): used for decades, recently replaced in first line (Indian sub-continent)
- Miltefosine (MIL): oral drug, recently introduced in KAEP
- Amphotericin B (AmB): second line
- Paromomycin (PMM): latest antileishmanial drug on the market, phase IV trial
- Combination therapy: trials underway
Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas

Jean-Claude Dujardin¹,², Dolores González-Pacanowska³,⁴, Simon L. Croft⁵,⁶, Ole F. Olesen⁷ and Gerald F. Späth⁸,⁶
Aim of Kaladrug-R

Develop, evaluate and disseminate:

• New tools for the assessment of drug resistance in *L. donovani* for the treatments with Miltefosine (MIL), Sodium Stibogluconate (SSG), Paromomycin (PMM)

• Innovative methodologies for monitoring VL treatment effectiveness under routine conditions
**Disease and Health services:** Clinical and epidemiological monitoring of treatment effectiveness

**Disease and Parasites (India and Nepal):** clinical samples and isolates from 2 cohorts of patients treated with SSG and MIL

**Phenotyping:** unique collection of parasites with variable susceptibility to SSG and MIL

**Drug resistance markers**
- Targeted studies: candidate markers
- Global studies: genome diversity

**Epidemiological dynamics**
- Population genetics & fitness studies
- Mathematical modelling of drug resistance

**Translation** of knowledge into practical tools for surveillance

**Dissemination:** health authorities
Drugs considered

- **Antimonials (SSG):** used for decades, recently replaced in first line (Indian sub-continent)
- **Miltelifosine (MIL):** oral drug, recently introduced in KAEP
- **Amphotericin B (AmB):** second line
- **Paromomycin (PMM):** latest antileishmanial drug on the market, phase IV trial
- **Combination therapy:** trials underway
SSG: understanding the past

SSG treatment failure rates

<table>
<thead>
<tr>
<th>(Bihar, 1994-7)(^1)</th>
<th>(Nepal, 2001-4)(^2)</th>
<th>(Bihar, 2008)(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>209 patients, 6 months f.u.</td>
<td>210 patients, 12 months f.u.</td>
<td>68 patients, retrospective study at PHC level</td>
</tr>
<tr>
<td>65 %</td>
<td>11.4 %</td>
<td>25 %</td>
</tr>
</tbody>
</table>

SSG replaced

SSG: understanding the past

Role of parasite resistance

20 definite cure

7 SSG-S

13 SSG-R

5 non responder

0 SSG-S

5 SSG-R

Same type of resistance?

Rijal et al. 2007, Microbes and Inf., 9(4):529-35
Features of SSG-R parasites

- Resistance emerged several times and independantly (pop. genetics)
- Targeted molecular approaches indicate different ways to become ‘resistant’: thiol metabolism, fluidity of membranes. Are all these mechanisms clinically relevant? Single markers for surveillance?
- Higher fitness (even in absence of SSG!!!) of resistant strains: metacyclogenesis, in vivo infectivity, IL-10 production by infected Mfs

See posters 5, 6, 9
Features of SSG-R parasites (new approaches)

Full genome sequencing

Metabolomics

SNPs  aneuploidy

See posters 9, 15 + extra
MIL: tracking the present

MIL treatment failure rate (relapses)

<table>
<thead>
<tr>
<th>Study Location and Time Period</th>
<th>Number of Patients</th>
<th>Follow-up Duration</th>
<th>Treatment Failure Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bihar, 1999-2000)</td>
<td>299 patients</td>
<td>6 months f.u.</td>
<td>6%</td>
</tr>
<tr>
<td>(Nepal, 2009-10)</td>
<td>110 patients</td>
<td>6 months f.u.</td>
<td>11%</td>
</tr>
<tr>
<td>(Bihar, 2008)</td>
<td>43 patients</td>
<td>Retrospective study</td>
<td>16%</td>
</tr>
</tbody>
</table>

MIL introduced

See posters 11, 12
MIL: tracking the present

• 656 patients in f.u. (6 months), 452 clinical isolates + tissues

• Promastigote assay available for susceptibility testing

• Preliminary results of in vitro susceptibility: 3 post-tx isolates more tolerant to MIL, but
  BHU-872: relapse after 6 months
  BHU-814 and -782: clinical cure (6 months!)

• Need for active follow-up of treatment effectiveness (also in periphery)

See posters 4, 14
• Importance of monitoring tx effectiveness in the field: clinical and epidemiological tools

• Possible with higher commitment of staff and patients

• Results suggest the need for follow-up to 12 months!

See poster 8
Features of MIL-R parasites

• Emergence of MIL-resistance? Or heritage of SSG-resistance (cross-tolerance to all drugs)?
• Induction made on 5 Indian/Nepalese lines SSG-S or -R: up to 112 x more tolerant to MIL
• Targets available on induced Sudanese *L. donovani* strain: (i) increase in drug efflux (LMDR1) and (ii) decrease in drug uptake caused by inactive MIL uptake machinery (LdMT and LdRos1); *Perez-Victoria et al.*
• ‘omic studies in progress

See posters 4, 14
PMM: preparing the future

- No patients cohort in present project
- Promastigote assay in evaluation
- Observation of a few ‘cross-tolerant’ isolates (PMM, MIL and SSG): further supports the ‘SSG heritage’ hypothesis
- 1 line PMM-R induced so far, ‘omic analysis pending

See posters 4, 10, 13
Modeling the future

Time for replacement by R-strains, *uncritical*: takes very long

- Treatment fails in *all* patients who have resistant parasites
- Treatment fails in 50% of patients who have resistant parasites

See poster 7
Modeling the future

Fitness of parasites is 15% higher (causes 15% more symptomatic cases)

Time for replacement by R-strains, critical: fitness of R-strains

Treatment fails in all patients who have resistant parasites

See poster 7
Take-home messages

- The past may strongly influence the present and the future
- Need for continuity in research and surveillance: understand the past, track the present and anticipate the future
- Diversity of parasitic scenarios: also remind for drug discovery
- Some parasites are more tolerant to several drugs and already circulate in India and Nepal... clinical relevance?