Treatment failure, drug resistance and VL control in the Indian subcontinent

lessons learnt from a multidisciplinary research project

Manu Vanaerschot & Jean-Claude Dujardin
on behalf of the Kaladrug-R consortium
Kaladrug-R objectives & FP7 context

To develop, evaluate and disseminate:

- innovative methodologies for monitoring Kala-azar treatment effectiveness in routine conditions
- new tools for evaluation of drug resistance in *L. donovani*
Kaladrug-R work themes

**Disease and Health:**
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 & metabolome diversity

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

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

**Dissemination:** health authorities

Institute of Tropical Medicine
Generated knowledge (Pentav. Antimonials or SSG)

- SSG-R not always associated with treatment failure

Rijal et al. 2007, Microb Infect
SSG-R not always associated with treatment failure

SSG-R emerged several times, 1 specific genetic group of parasites with clinical relevance:

- ISC005 (SSG-R group):
  - 9/11 tested: *in vitro* SSG-R
  - 3/3 with SSG tx outcome: non-response

- other strains:
  - 15/38 tested: *in vitro* SSG-R
  - 2/30 with SSG tx outcome: non-response*

Downing et al. 2011, Genome Res unpublished results
1) Blue curve
(default: "No SSG-resistance"): 5% treatment failures expected, independent of time, when there is no SSG-resistance.

2) Green curve
Bihar observations cannot be explained, even when assuming that all patients infected with SSG-resistant parasite strains will be treatment failures.

3) Red curve
Additional assumptions needed to reproduce the Bihar observations.

- are resistant parasites better transmitted?
- do more humans with resistant parasites become sick?
- other?
• SSG-R *L. donovani* produce more infectious promastigotes *in vitro* & cause higher *in vitro/in vivo* infection levels

Ouakad et al. 2009, Parasitol
Vanaerschot et al. 2010 & 2011, PLoS ONE

• SSG-R *L. donovani* manipulate host immune system, but this might be reverted by imipramine & quercetine

Mukherjee et al. 2013, PNAS;
Mukhopadhyay et al. 2011, Int J Parasitol;
Mukherjee et al. 2012, PLoS NTD

• majority of clinical samples isolated now are still SSG-R, despite low SSG-pressure

Mukhopadhyay et al. 2011, Int J Parasitol;
unpublished

**SSG-R parasites are fitter parasites?**
Generated knowledge (MIL)

- relapse in up to 20% of MIL-treated patients\(^1\)

- underdosage of children & men at risk\(^2\)

- no MIL-resistance in natural populations so far; PKDL strains show higher tolerance\(^3\)

- *in vitro* induced MIL-R strains: different mechanisms targeting the same gene (LdMT)\(^4\)

---

1 Sundar et al. 2012, CID; Rijal et al. 2013, CID
2 Dorlo et al. 2014, JID; Ostyn et al. 2014, PLoS One
3 Bhandari et al. 2012, PLoS NTD
4 Carter et al., unpublished
- MIL-relapse parasites also ‘superparasites’?

heritage of SSG era? partially...

Rai et al. 2013, MBio
Generated knowledge (PMM)

- some strains naturally resistant to PMM\(^1\)

- PMM-resistance very easily induced *in vitro*\(^2\)

- molecular adaptations of *in vitro* induced PMM-R identified... what about natural strains?\(^3\)

---

\(^1\) unpublished results
\(^2\) Hendrickx et al., 2014, Parasitol Res
\(^3\) Bhandari et al. 2014, AAC
Generated knowledge (epidemiology)

- *L. donovani* genome deciphered, 203 isolates sequenced, several populations identified \(^1\)

- evolution tracked since DDT campaign in 1960s \(^2\)

- mathematical model of VL \(^3\):
  - chemotherapy alone will not control the disease (asymptomatics)
  - integrated vector control management likely to reach threshold required for elimination

---

\(^1\) Downing et al. 2011, Genome Res & unpublished
\(^2\) unpublished
\(^3\) Stauch et al. 2011 & 2012 & 2014, PLoS NTDs
Generated tools (for health authorities)

- standardised clinical tools to follow drug effectiveness ¹
- standardised biological & molecular tools for tracking SSG- & MIL-resistance ²
- molecular tools to track *L. donovani* populations in (post-) elimination phase ³
- mathematical model of VL: contextualising interventions ⁴

¹ Ostyn et al. 2013, TMIH
² Prajapati et al. 2013, AJTMH; Kulshrestha et al. 2013, Parasitol Res; Vanaerschot et al. 2012, JID; Roy S et al. unpublished
³ Dujardin et al., unpublished
Generated tools (for research)

- unique strain collection resistant to SSG, MIL and PMM (screening!)

- genome of 203 isolates + metabolome of 17 isolates: unique resources for drug development
Take-home messages

• monitoring, monitoring, monitoring !!!
  – treatment efficacy
  – drug resistance & parasite fitness
  – drug quality, access ...
  – drug dosage: adapting treatment for children (<12 yrs)
  – spread of parasite genotypes in post-elimination phase

• importance of vector control

• real-time field-lab collaboration
www.leishrisk.net/kaladrug

धन्यवाद