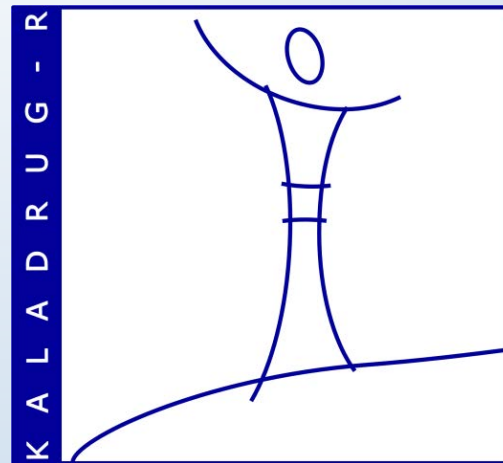# Treatment failure, drug resistance and VL control in the Indian subcontinent

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
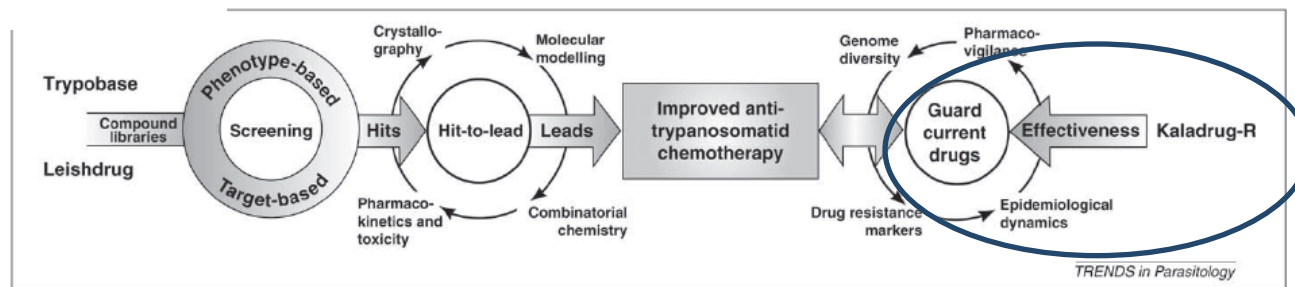- new tools for evaluation of drug resistance in *L. donovani*

Review

Cell PRESS

Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas

Jean-Claude Dujardin[1,2], Dolores González-Pacanowska[3,4], Simon L. Croft[5,6], Ole F. Olesen[7] and Gerald F. Späth[8,6]

Trends in Parasitology Vol.26 No.8

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

Epidemiological dynamics

Targeted studies: candidate markers

Global studies: genome & metabolome diversity

Population genetics & fitness studies

Mathematical modelling of drug resistance

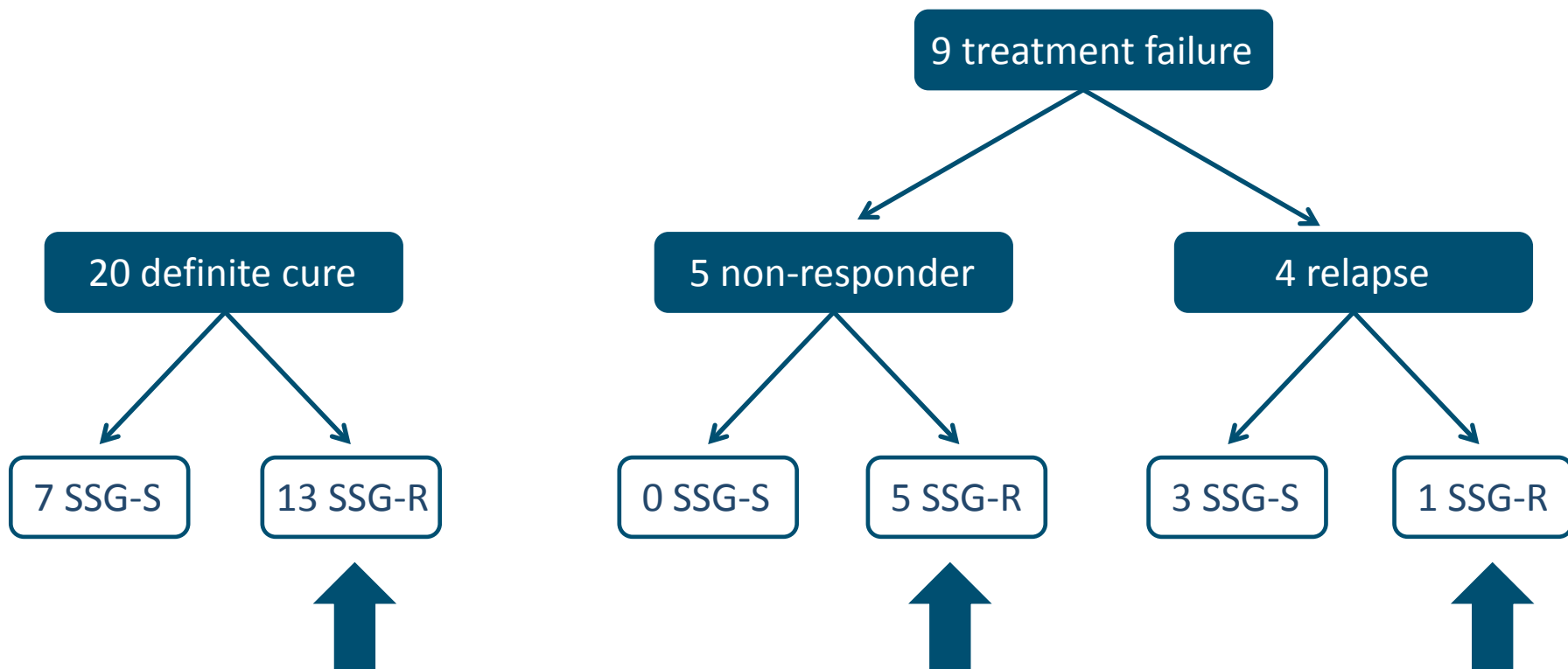**Translation** of knowledge into practical tools for surveillance

Dissemination: health authorities

Generated knowledge (Pentav. Antimonials or SSG)

- SSG-R not always associated with treatment failure

Rijal et al. 2007, Microb Infect


```
                              9 treatment failure
                          ┌──────────┴──────────┐
20 definite cure      5 non-responder          4 relapse
  ┌────┴────┐           ┌────┴────┐           ┌────┴────┐
7 SSG-S  13 SSG-R    0 SSG-S  5 SSG-R      3 SSG-S  1 SSG-R
```

Generated knowledge (SSG)

- 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
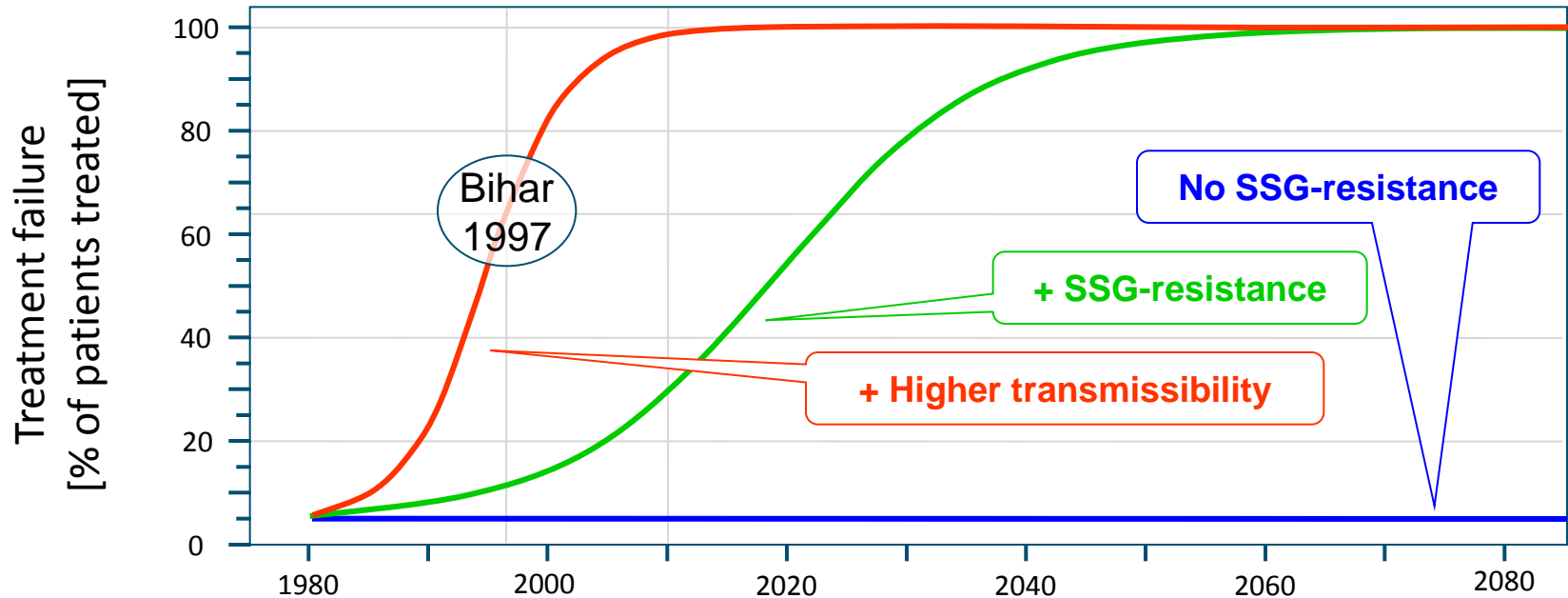   - 2/30 with SSG tx outcome: non-response*
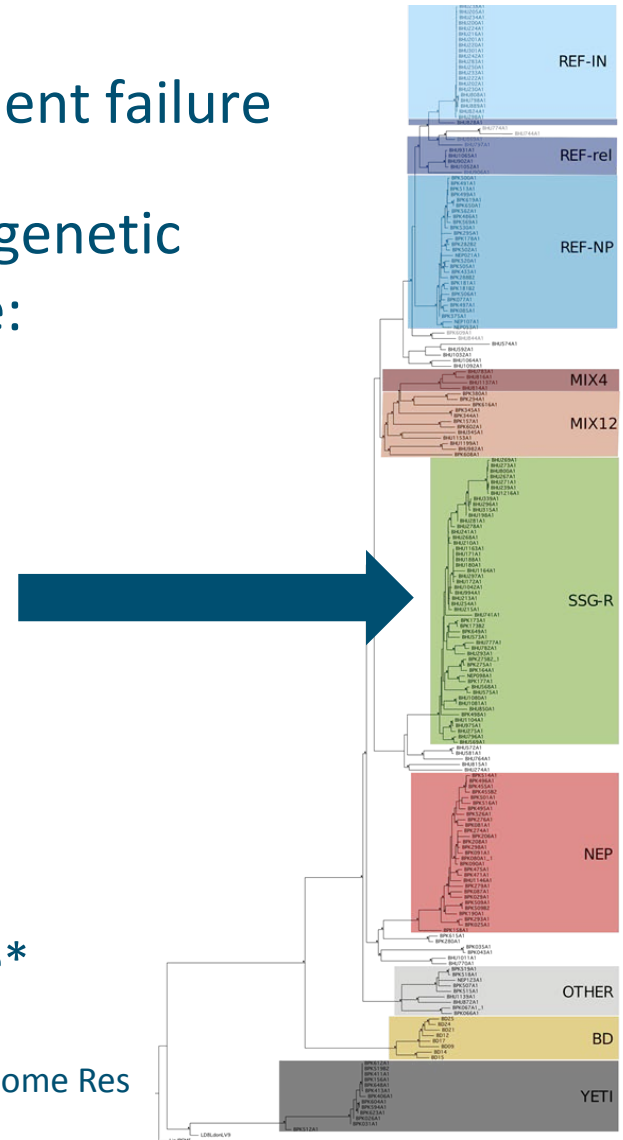
Downing et al. 2011, Genome Res
unpublished results

Generated knowledge (SSG)

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?

Generated knowledge (SSG) - superparasites?

- 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
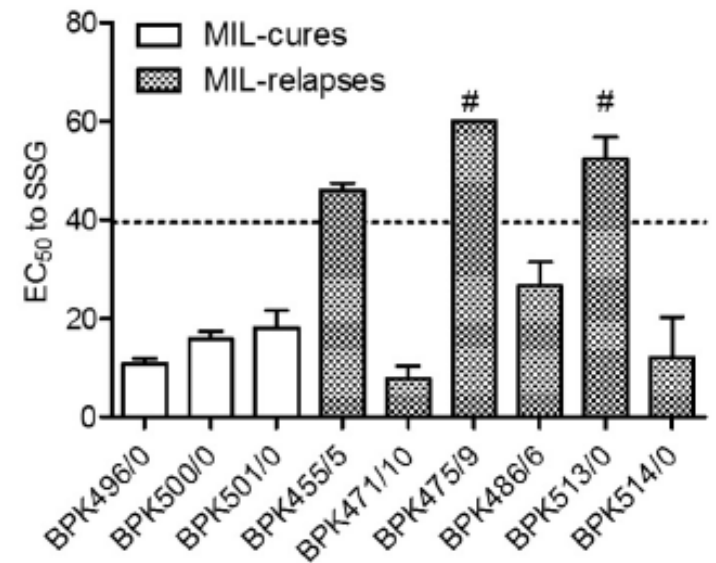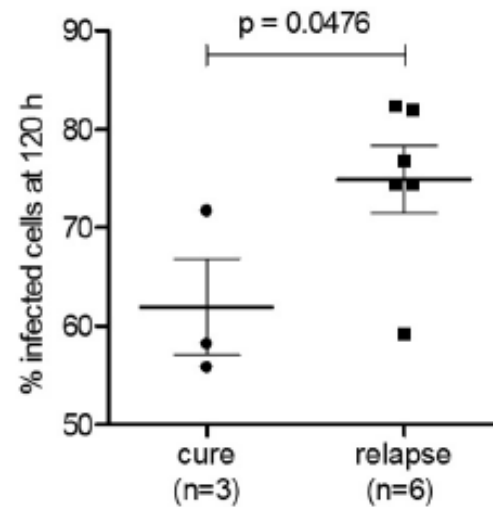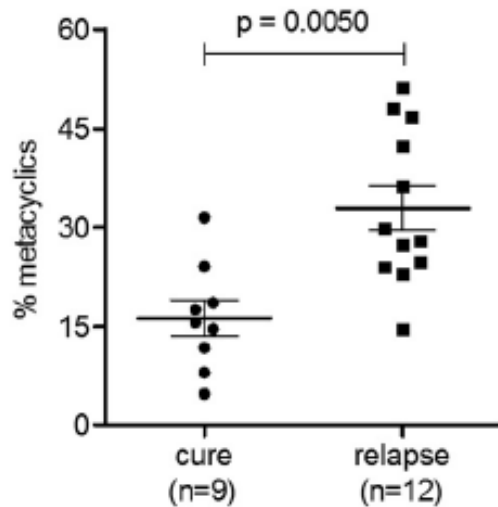[3] Bhandari et al. 2012, PLoS NTD
[4] Carter et al., unpublished

Generated knowledge (MIL)

- 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 adaptions 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
[4] Stauch et al. 2011&2012&2014, PLoS NTD

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