Assessment of artemisinin resistance of *Plasmodium falciparum* malaria in Suriname

Stephen G.S. Vreden, MD PhD
Foundation for Scientific Research
Suriname (SWOS)

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Malaria in Suriname

- Numbers are continuously decreasing
  - (2015 so far only 6 locally transmitted cases (4 P.v, 2 P.f))

- Threat of resurgence due to decreasing sensitivity?
- Assessing efficacy by traditional efficacy studies currently virtually impossible
Challenges for efficacy studies in Suriname

- Low number of cases, virtually only gold miners
- Population of gold miners is not available for 28 days follow up
- Assessing day 3 parasitaemia, difficult but feasible
Working definition of artemisinin resistance

- Discussed during the GPARC process and at the Fogarty Internal Center and NIH meeting in November 2010
- WHO is using **working definition** as below:
  - an increase in parasite clearance time, as evidenced by greater than 10% of cases with parasites detectable on day 3 following treatment with an ACT (suspected resistance); or
  - a treatment failure as evidenced by presence of parasites at day 3 and either persistence of parasites on day 7 or recrudescence after day 7 of parasites within 28/42 days, after treatment with an oral artemisinin-based monotherapy, with adequate blood concentration (confirmed resistance).
Figure 7 The proportion of patients with fully artemisinin sensitive *P. falciparum* infections who are slide positive on day 3 are shown with 95 and 99% confidence intervals. From Stepniewska et al with permission [45].
Assessment of Day 3 Parasitaemia in patients treated with Coartem

<table>
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<tr>
<th></th>
<th>2005/2006 ($n = 45$)</th>
<th>2011 ($n = 48$)</th>
<th>$^a p &lt; 0.001$</th>
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<tbody>
<tr>
<td><strong>Day 2 Parasitaemia</strong></td>
<td></td>
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<tr>
<td>Number of positive cases (percentage)</td>
<td>9 (20 %)</td>
<td>36 (75 %)</td>
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<td><strong>Day 3 Parasitaemia</strong></td>
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<tr>
<td>Number of positive cases (percentage)</td>
<td>1 (2.2 %)</td>
<td>15 (31.3 %)</td>
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$^a$ Fisher’s Exact test

Note: All patients followed until day 28 had cleared their parasites.
Parasite clearance rate

- Parasite clearance rate: $\frac{d(\text{parasite density})}{d(t)} = C$; linear association

Figure 1 Two *P. falciparum* parasite clearance curves with identical therapeutic responses illustrating the dependence of the parasite clearance time on pre-treatment parasite density.
Parasite Clearance Estimator (PCE)

Figure 2 The effect of lag phase and tail exclusion on the calculation of the clearance rate constant.

Flegg et al. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator Malaria Journal 2011, 10:339
More than 10% of patients with a parasite clearance half-life of > 5 h.

WHO definition for resistance to artemisinins assessed by P.C.E.
Protocol for Parasite clearance study 2013/14

• Our study in 2011 was conducted with Coartem (artemether/lumefantrine)

• Artemether is not available as a single agent.

• Therefore we used artesunate
Patients with *P. falciparum* mono-infection.

Parasitaemia: 200 - 10 000/µl

Artesunate 4 mg/Kg OD for 3 days, followed by mefloquine and primaquine after day 3.

Assessment of parasitaemia every 8 h until clearance of parasites, thereafter on day 7, 14, 21, 28 (if still available for the study).
Results study 2013/14

• 45 Patients enrolled

• 38 Patients evaluable

• Withdrawn: 7 patients (wrong inclusion, protocol violation, refusal to continue)
Characteristics of enrolled subjects: Origin

- Fr. Guyana: 36 patients
  - Eau Claire 14,
  - Sophie 15,
  - Pedi Limao 3,
  - Cacao 2,
  - Marrodeira 2.
- Guyana: 4 patients (3 Elash, 1 Aramu)
- Suriname: 3 patients (Benzdorp)
- Unknown: 2 patients
Characteristics of enrolled patients:
age/sex

- All adults (>18 years)
- Males: 25   females: 20
Follow up

- Follow up beyond day 3: 38

- Follow up until day 28: 8 (All ACPR)
Results (c’td)

- 22 Patients parasitaemic on day 2 (57.9 %)
- 3 Patients parasitaemic on day 3 (7.9 %)

- All patients followed until day 28 had cleared the parasite

- Mean initial parasitaemia: 9.635,62 par./μL
- (In study of 2011: 10.003.92 par./μL)
Parasitaemia half-life using WWARN parasite clearance estimator

- 20 patients ≤ 5.5 h
- 19 patients > 5.5 h (48.7 %)
- 7 patients > 7 h (17.9 %)
- 2 patients > 10 h
Figure 1: Stylised graphs showing the distribution of parasite clearance half lives for two populations: population B shows evidence of prolonged clearance, when compared to population A.
Distribution of slope half life

Figure 4: Distribution of slope half life
Conclusions artemesunate study:

- Day 3 parasitaemia 7.9%
  - (Coartem study in 2005 2% and in 2011: 31%)

- Day 2 parasitaemia 57.9%
  - (Coartem study in 2005: 20% and in 2011: 75%)

- >5 h parasite clearance half-life: 48.7%
  - (WHO threshold 10%)

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A molecular marker of artemisinin-resistant Plasmodium falciparum malaria

Frédéric Arieux\textsuperscript{1,2}, Benoit Witkowski\textsuperscript{3}, Chanaki Amaratunga\textsuperscript{4}, Johann Beghain\textsuperscript{1,2}, Anne-Claire Langlois\textsuperscript{1,2}, Nimol Khim\textsuperscript{3}, Saorin Kim\textsuperscript{3}, Valentine Duru\textsuperscript{3}, Christiane Bouchier\textsuperscript{5}, Laurence Ma\textsuperscript{5}, Pharth Lim\textsuperscript{3,4,6}, Rithea Leang\textsuperscript{6}, Socheat Duong\textsuperscript{6}, Sokunthea Sreng\textsuperscript{6}, Seila Suon\textsuperscript{6}, Char Meng Chhou\textsuperscript{6}, Denis Mey Bout\textsuperscript{7}, Sandie Ménard\textsuperscript{8}, William O. Rogers\textsuperscript{9}, Blaise Genton\textsuperscript{10}, Thierry Fandeur\textsuperscript{1,3}, Olivo Miotto\textsuperscript{11,12,13}, Pascal Ringwald\textsuperscript{14}, Jacques Le Bras\textsuperscript{15}, Antoine Berry\textsuperscript{8}, Jean-Christophe Barale\textsuperscript{1,2}, Rick M. Fairhurst\textsuperscript{4}, Françoise Benoit-Vical\textsuperscript{16,17}, Odile Mercereau-Puijalon\textsuperscript{1,2} & Didier Ménard\textsuperscript{3}
Figure 4 | Parasite clearance half-lives.  

a, Correlation of parasite clearance half-lives and K13-propeller alleles for parasite isolates in Pursat and Ratanakiri in 2009–2010. Wild-type parasites have shorter half-lives (median 3.30 h, IQR 2.59–3.95, n = 72) than C580Y (7.19 h, 6.47–8.31, n = 51, $P < 10^{-6}$, Mann–Whitney U test), R539T (6.64 h, 6.00–6.72, n = 6, $P < 10^{-6}$) or Y493H (6.28 h, 5.37–7.14, n = 21, $P < 10^{-6}$) parasites. The half-life of C580Y parasites is significantly longer than that of Y493H parasites ($P = 0.007$). 

b, Correlation of
Assessment of ‘K13’ mutations in isolates from the 2013/14 study in Suriname

- Carried out by CDC, Atlanta

- In none of the isolates the K13 mutation has been detected.
Summary
Artesunate mefloquine study 2013/’14

- This combination therapy is still highly efficacious in the treatment of *P. falciparum* malaria in our region.

- Day 3 parasitaemia rate is lower than 10 %.

- The 48.7 % rate of parasite half life > 5.5 h suggests a reduced sensitivity to artesunate.

- K13 mutation was not found in our samples.

- Molecular studies looking for other mutations are underway.
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Study collaborators and volunteers

- Prof. Malti R. Adhin, ADEK University, Suriname
- Mr. Jeetendra K. Jitan, Bsc, Ministry of Health, Suriname
- Dr. Pascal Ringwald, WHO, Geneva
- Lab technicians, fieldworkers, administrative staff
- The patients, who volunteered to participate

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Thank You!