Kevin Baird, Global Technical Strategy, Steering Committee member
Relapse Behaviors in Assessing Hypnozoitocidal Activity

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Hypnozoitocidal therapy matters

• >80% of incident acute attacks observed in endemic areas derive from hypnozoites
• Hypnozoite-borne illness not diagnosable or prevented by ACT or LLIN/IRS measures
• In most areas a single infectious mosquito bite often results in 5 or more attacks within a year or so, and attacks may persist for as long as 4 years
• Each attack as virulent as the primary but often in a patient not yet fully recovered – illness deepens with succeeding attacks
• Each attack an opportunity for delayed or inappropriate therapy and risk of poor clinical outcomes and onward transmission
Plasmodium vivax
Hypnozoitocidal therapy

• In a patient, always presumptive, we treat against a probability of a future event and usually while also treating an acute attack with blood schizontocides

• Assessment principles and analytical hazards wholly distinct from blood schizontocidal therapy and more nuanced

• Biology of latency profoundly more complex, more difficult to assess, and very poorly understood compared to patency

But assessing hypnozoitocides CAN be done
Relapse behaviors

Geographical variation in Plasmodium vivax relapse

1. North American
2. Central America
3. South American
4. Mediterranean + North Africa
5. Sub-Saharan Africa
6. Monsoon Asia
7. South-East Asia
8. Northern Europe and Asia
9. Melanesia

Time to first relapse (days)

Ecological zone
Terminology

- Tachyzoites
- Bradysporozoites

**Frequency, timing, and multiplicity of relapses vary by geographic zones**
Intrinsic latency period polymorphisms

Sporozoite Phenotype and Number in Epidemiology

**TEMPERATE**

**SUB-TROPICAL**

**TROPICAL**

[Graphs showing relative frequency of phenotypes in different regions with varying sporozoite inoculum and month post-inoculation.]

- *tachyzoite* (dark red triangle)
- *bradyzoite* (yellow triangle)

**Legend:**
- ▲ sporozoite inoculation
- ▲ primary attack
- ▲ secondary attack
Lysenko’s postulates

The first postulate: exoerythrocytic (EE) schizogony is a direct (non-cyclic) process

The second postulate: sporozoites are polymorphic, and the duration of EE development of the progeny of an individual sporozoite is a polymorphic characteristic

The third postulate: the duration of EE development is controlled by several loci

The fourth postulate: the progenies of sporozoites belonging to different phenotypes form independent lines of erythrocytic schizonts

Population studies of Plasmodium vivax.
1. The theory of polymorphism of sporozoites and epidemiological phenomena of tertian malaria

A. Ja. Lysenko, A. E. Beljaev, & V. M. Rybalka

Fig. 2. Life cycle of Plasmodium vivax. EES = exoerythrocytic schizogony; D = dormancy; ES = erythrocytic schizogony; G = gametogony; S = sporogony.

<table>
<thead>
<tr>
<th>Type of sporozoite</th>
<th>Designation of the infection</th>
<th>Clinical Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>pure TS infection</td>
<td>short incubation without late relapses</td>
</tr>
<tr>
<td>BS</td>
<td>pure BS infection</td>
<td>long incubation</td>
</tr>
<tr>
<td>TS + BS</td>
<td>mixed infection</td>
<td>short incubation with late relapses</td>
</tr>
</tbody>
</table>
Intrinsic latency period polymorphisms

Fig. 1. The phases of tertian malaria in different strains of *Plasmodium vivax*. A = the moment of the infection; B = primary manifestations after short incubation period; C, D, E, F, G, H, & K = relapses; J = primary manifestations after long incubation period. *Periods of latency*: B-C, B-G, & J-K = pre-relapse periods; C-D, D-E, E-F, & G-H = inter-relapse periods. *Incubation periods*: A-B = short incubation period; A-J = primary long latency (long incubation period).

Population studies of *Plasmodium vivax*.
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Fig. 3. The course of development of *Plasmodium vivax*, after different theories.
Intrinsic latency period polymorphisms

Relapses of *Plasmodium vivax* Infection
Result from Clonal Hypnozoites Activated at Predetermined Intervals

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**Clonality and genetic diversity of *P. vivax* samples.** Single alleles were found in 85 of the 86 *P. vivax* samples (collected from 71 ADF personnel) genotyped at the *pvmsp1* and *pvama1* (and, in some cases, *pvcs*) loci. This indicates that 99% of samples were obtained from patients with clonal infections.

A nonspecific trigger would have been expected to activate both clones of hypnozoites, resulting in a mixed allelic infection in the relapse samples. This led us to hypothesize that the hypnozoites were activated according to a genetically determined biological clock (i.e., hypnozoites initiated blood-stage infections at definite, genetically predetermined intervals). This hypothesis about a biological clock was tested using a mathematical simulation model. The simulation output replicated the observed temporal pattern of malaria episodes and the proportion of mixed infections, which suggests that the hypothesis is feasible.
Intrinsic latency period polymorphisms

Primary attack

primary relapse

first sequel relapse

second sequel relapse

third sequel relapse

Time Since Sporozoite Inoculation

Legend

- Hepatic Latency
- Hepatic Schizogony
- Blood Schizogony
Distinct strains had unique TS/BS ratios
Distinct strains had unique TS/BS ratios

**Table 1**

*Plasmodium vivax: Summary of dimensions of hypnozoites and pre-erythrocytic schizonts*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Biopsy day</th>
<th>Hypnozoites (μm)</th>
<th>Schizont sections with AMD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>&lt;30 μm</td>
</tr>
<tr>
<td>Chesson</td>
<td>7-day</td>
<td>4.9 x 4.5 4.7</td>
<td>28.4 x 22.8</td>
</tr>
<tr>
<td>(CH)</td>
<td></td>
<td>5.4 x 4.0 4.7</td>
<td>24.7 x 23.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8 x 6.0 6.4</td>
<td>26.9 x 22.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8 x 5.4 5.7</td>
<td>31.8 x 21.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.4 x 5.2 5.3</td>
<td>34.0 x 20.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.2 x 5.2 5.2</td>
<td>33.0 x 25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>26.6 x 25.8</td>
</tr>
<tr>
<td>means</td>
<td>5.6 x 5.1 5.3*</td>
<td>29.3 x 22.9</td>
<td>26.1*</td>
</tr>
<tr>
<td>10-day</td>
<td></td>
<td>6.7 x 5.6 6.2</td>
<td>19.4 x 13.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.9 x 4.7 5.3</td>
<td>31.7 x 21.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>26.1 x 22.6</td>
</tr>
<tr>
<td>means</td>
<td>6.3 x 5.2 5.8*</td>
<td>25.7 x 19.4</td>
<td>22.6*</td>
</tr>
<tr>
<td>N. Korean</td>
<td>7-day</td>
<td>6.5 x 4.7 5.6</td>
<td>—</td>
</tr>
<tr>
<td>(NK)</td>
<td></td>
<td>5.5 x 5.0 5.2</td>
<td>—</td>
</tr>
<tr>
<td>means</td>
<td>6.0 x 4.8 5.4*</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Average mean diameter.
Implications of TS/BS biology

• TS and BS may never be of the same clonal identity

• A primary infection will almost always be represented by >4 clonal identities, none of which may appear in subsequent relapses

• A relapse will usually be clonal and heterologous from primary attack

• Distinct relapses may always be heterologous if intrinsic latency period phenotype is genetic rather than epigenetic or extrinsically triggered

• A recurrence of the same clonal identity as the primary event may only be a recrudescence
Hypnozoitocidal assessment principles

- No reliable means of distinguishing a primary attack from a relapse except by CHMI or other isolation from reinfection.
Hypnozoitocidal assessment principles

• No reliable means of distinguishing a primary attack from a relapse except by CHMI or other isolation from reinfection
• Must know local relapse risk and timing to accurately measure efficacy, a placebo group
• Acknowledge possibility of DDI with partner blood schizontocicide (concurrent or consecutive therapy)
• Acknowledge interference with relapse by partner blood schizontocicide
• Therapeutic failure may require months to occur
• Recognize failure to relapse after therapy is not necessarily therapeutic success
• Recognize impaired CYP2D6 as a potentially important confounder
Randomized trial of primaquine hypnozoitocidal efficacy when administered with artemisinin-combined blood schizontocides for radical cure of *Plasmodium vivax* in Indonesia

Emi J. Nelwan¹, Lenny L. Ekawati², Bagus Tjahjono³, Rianto Setiabudy³, Irrojo Sutanto³, Kikin Chand⁴, Tyas Ekasari⁵, Dwi Djoko⁶, Rhaan Bari⁷, W. Robert Taylor⁸, Stephen Duparc⁷, Decy Sudiri⁹, Iqbal Elyazar⁵, Rintis Nusiyanti⁵, Herawati Sudoyo³ and J. Kevin Baird¹⁰

CHMI or this for unambiguity
Endemic population approach

Tease out impact of reinfection from relapse:

No PQ = relapse + reinfection

Radical cure = relapse + reinfection

No PQ – radical cure = reinfection
Chemotherapeutics principles

- **No primaquine for efficacy denominator**
- **“Therapeutic success” without primaquine**
- **Blood schizontocide interfering with relapse**
- **Therapeutic failure may take months**
- **Therapeutic failure by impaired CYP2D6**
- **Freedom from reinfection**
Rapid relapse without drug interference

DIAGNOSIS OF RESISTANCE TO CHLOROQUINE BY *PLASMODIUM VIVAX*:
TIMING OF RECURRENCE AND WHOLE BLOOD CHLOROQUINE LEVELS

J. KEVIN BAIRD, BUDI LEKSANA, SOFYAN MASBAR, DAVID J. FRAYAUF, M. AWALUDIN SUTANIHARDJA, SURADI, F. STEPHEN WIGNALL, AND STEPHEN L. HOFFMAN

*U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Naval Medical Research Institute, Bethesda, Maryland*
Slower relapse with drug interference

*Plasmodium vivax* Recurrence Following Falciparum and Mixed Species Malaria: Risk Factors and Effect of Antimalarial Kinetics

Nicholas M. Douglas,1,2 François Nosten,3,5,6 Elizabeth A. Ashley,3,5,6 Lucy Phiangsun,7 Michele van Vught,3,5 Pratap Singhasivanon,8 Nicholas J. White,3,5 and Ric N. Price1,2

Clinical Infectious Diseases 2011:52(5):612–620
A 30-day follow up?

Some trials have offered PQ efficacy estimates with 28-day follow-up. They point to this 28-day risk of relapse as justification.
Therapeutic failure may take months!

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DOI: 10.1186/s12916-015-0355-9

Nelwan et al. BMC Medicine 2015;13:24
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Therapeutic failure may take months!
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Some drugs “slowed down” hypnozoites but did not kill them.

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**Fig. 2.** First relapses following treatment of the initial attacks with certain 8-aminquinoline compounds, plus quinine, in volunteers infected with the Chesson strain of *vivax* malaria. Figures in brackets indicate days from end of treatment to release where interval was greater than 100 days.

**STUDIES IN HUMAN MALARIA**

XXXI. Comparison of Primaquine, Isopentaquine, SN-3883, and Pamaquine as Curative Agents Against Chesson Strain *Vivax* Malaria

W. Clark Cooper, Albert V. Myatt, Thomas Hernandez, Geoffrey M. Jeffery, and G. Robert Coatney

Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health, Bethesda 14, Maryland
The neglect of primaquine science

- Primaquine has been the only available therapy against relapsing malaria for past 65 years
- We do not know how it is metabolized or how it works against hypnozoites
- We do not know how it is toxic in G6PD deficient patients
- We do not know if it still works in most patients
- We know most patients don’t complete the 14 doses, is prohibited in pregnant/lactating women and young infants, and cannot work in CYP2D6 null metabolizers

The hypnozoite reservoir will need better than this to kill it. Tafenoquine a profoundly important and overdue improvement to this state of affairs – let’s not neglect its science
Summing up

• Guiding rationale and principles of chemotherapeutic assessment of hypnozoitocides fundamentally and profoundly distinct from those of blood schizontocides assessment

• No WHO guidance or standards for assessing hypnozoitocides

• These very important assessments can be done, but are difficult and broadly neglected

Photo by Pearl Gan at Alor in eastern Indonesia 2016
See Malaria in Asia Project -- http://www.asiamalariaimages.com