Selection of Drug Combinations for Resistant Malaria

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Current Issues with ACTs

- Artemisinin derivative combined with an existing drug
- Existing drug = Existing resistance
- Empirical selection of partner drugs
- Recrudescence
- Pharmacokinetics/Pharmacodynamics
  - Matched or unmatched?
- ACT = Antimalarial Combination Therapy
Agenda

• Artemisinin resistance and recrudescence
• Methods to identify and evaluate combinations
• What is the way forward?
Duration of Antimalarial Activity Following a 6 Hr Drug Exposure of Young Rings to Dihydroartemisinin

- Control
- DAR 0.2 ng/ml
- DAR 2.0 ng/ml
- DAR 20.0 ng/ml

TIME (Hr)  PARASITEMIA (%)

0  24  48  72  96  120  144  168  192
Duration of Antimalarial Activity Following a 6 Hr Drug Exposure of Young Rings to Quinine

PARASITEMIA (%) vs TIME (Hr)

- Control
- QUIN 100 ng/ml
- QUIN 1000 ng/ml
Figure 6-8: Effect of drug on rings (A-N) and trophozoites (O-T) of *P. falciparum* in vitro. Synchronous cultures of ring stage parasites (A) were exposed to compound C (100 nM) for 6 hr, washed three times, and placed into culture. Thick and thin smears were prepared every 24 hr during the study. Panels B-D = 24 hr, E = 48 hr, F = 72 hr, G = 96 hr, H = 120 hr, I = 144 hr, J-K = 170 hr, and L-N = 192 hr. Panels O-T present the results from experiments with trophozoites (O-P = 6 hr SHAM controls, Q = 24 hr, and R-T = 50 hr.)
Exposure of schizonts to DHA (70 nM)

- Schizonts less susceptible to DHA
- Second generation dormant rings
Morphology of *P. falciparum* following treatment of Aotus monkeys with artemisone
TOTAL PARASITES

Artesunate + Mefloquine

Detection limit

\( t_{1/2} \beta = 2 \text{ weeks} \)

Drug Concentration

0 1 2 3 4  

WEEKS

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TOTAL PARASITES

Artesunate + tetracycline
azithromycin
chlorproguanil-dapsone

Detection limit

$t_{1/2} \beta = < 1$ day
# Dormant Rings are Refractory to Subsequent Drug Exposure

<table>
<thead>
<tr>
<th>Regimen (1&lt;sup&gt;st&lt;/sup&gt;)</th>
<th>Regimen (2&lt;sup&gt;nd&lt;/sup&gt;)</th>
<th>Dormant Rings</th>
<th>Time to Recrud</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA (100 nM)</td>
<td>-</td>
<td>Yes</td>
<td>120 Hr</td>
</tr>
<tr>
<td>DHA (100 nM)</td>
<td>DHA (100 nM)</td>
<td>Yes</td>
<td>120 Hr</td>
</tr>
<tr>
<td>DHA (100 nM)</td>
<td>MFQ (80 or 400 nM)</td>
<td>Yes</td>
<td>120 Hr</td>
</tr>
<tr>
<td>DHA (100 nM)</td>
<td>TQ (700 nM)</td>
<td>Yes</td>
<td>96 Hr</td>
</tr>
</tbody>
</table>
Selection of Optimal Combination Drug Partners for ACT

- These data suggest that a successful companion drug must have activity against dormant forms or have a long enough half-life to remain active in the blood when dormant forms begin to emerge and grow.
Discontinuous exposure to AL or QHS *in vitro* produces AL and QHS resistant progeny of *P. falciparum*

- Clones W2 and D6 were included from the beginning of the study, whereas TM91-C235 was introduced at the 10 ng/ml AL level (left arrow)
- Parasites that were adapted to grow in 80 ng/ml of AL were then transferred to pressure with a different artemisinin derivative to examine the degree of cross-resistance induced.
  - The dashed line shows the point at which drug pressure was switch from 80 ng/ml AL to QHS (right arrow)
Cross Resistance between AL and Other QHS Derivatives

- As AL drug pressure increased, drug susceptibility decreased to other artemisinin drugs and mefloquine, whereas parasites under AL pressure became more susceptible to chloroquine.
- In the W2 clone, a significant reduction in sensitivity to AL and QHS was observed when parasites became adapted to 40 ng/ml AL drug pressure and this trend continued through the 200 ng/ml AL level.
Pfmdr1 gene amplification under artelinic acid (AL) or artemisinin (QHS) pressure in vitro

Copy number $N = 2^{X_{\text{aver}} \pm t^*SE}$, CI 95% (n=5-7)
Cross Resistance to QHS Derivatives

• Adaptation to tolerate QHS derivatives
  – AL (200 ng/ml)
  – QHS (200 ng/ml)
  – DHA (200 ng/ml)

• Discontinuous pressure versus continuous
  – $10^8$ Parasites exposed to DHA (100 nM)
  – Increased to 400 nM
    • Recrudescence after 7 days continuous exposure
Models to Evaluate Drugs and Drug Combinations

- In vitro drug susceptibility assays
  - Robust, relevant drug resistance genotypes and phenotypes
  - Standardization issues
  - Resistance threshold issues
  - Cidal versus static?
  - Underestimates QHS resistance
## Induction of *P. falciparum* Resistance to Artelinic Acid *In Vitro*

<table>
<thead>
<tr>
<th>Parasite Isolate Clone</th>
<th>IC₅₀ (nM)</th>
<th>Resistance Index</th>
<th>IC₉₀ (nM)</th>
<th>Resistance Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>W2</td>
<td>4.73 (± 0.19)</td>
<td>4.9</td>
<td>7.45 (± 0.40)</td>
<td>6.9</td>
</tr>
<tr>
<td>W2.AL50</td>
<td>23.30 (± 3.95)</td>
<td></td>
<td>51.31 (± 1.31)</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td>9.81 (± 1.41)</td>
<td>3.5</td>
<td>21.83 (± 3.04)</td>
<td>3.7</td>
</tr>
<tr>
<td>D6.AL50</td>
<td>34.00 (± 3.14)</td>
<td></td>
<td>81.26 (± 8.67)</td>
<td></td>
</tr>
<tr>
<td>TM91-C235</td>
<td>11.08 (± 1.68)</td>
<td>3.0</td>
<td>22.99 (± 2.60)</td>
<td>2.3</td>
</tr>
<tr>
<td>C235.AL50</td>
<td>33.72 (± 1.64)</td>
<td></td>
<td>53.51 (± 1.12)</td>
<td></td>
</tr>
</tbody>
</table>
In Vitro Drug Interactions

- Isobolograms
  - Qualitative indicator of synergy, additiviry, or antagonism
  - Data often ‘over-interpreted’
  - Most combinations of antimalarial drugs are additive
  - Can be a useful indicator with clinical correlates
Interaction of Artemisinin Drugs and Mefloquine
ACT Drug Combinations Additive In Vitro

- Mefloquine plus DHA, artemisinin, or artesunate
- Pyronaridine plus artesunate
- Piperaquine plus DHA
- Amodiaquine plus artesunate
Interaction of Artemisinin Drugs and Methylene Blue

- Artelinic Acid
- Artemisinin
- Dihydroartemisinin
- Methylene Blue
High Throughput Screen for Drug Interactions

- Carefully define the quantitative dose response for drug of interest (e.g., QHS)
- Combine QHS at $\text{IC}_{5}-\text{IC}_{10}$ with test substances
- Compare inhibition of test substance alone and in combination with subinhibitory concentrations of QHS
Reversal Phenotypes are linked to *Pfcrt* allelic types
Optimization of Artemisinin Combinations
Models for Combinations In Vivo

• Rodent Models
  – See Peters et al
  – Mechanisms of resistance may differ
  – Metabolism differences
  – 24 vs 44 hr life cycle

• Aotus – *Plasmodium* model
  – Schmidt, Rossan, and Obaldia
  – Excellent clinical correlates
Recrudescence following Treatment with AS

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P. falciparum: Aotus Model for Drug Combinations

Table 5: Summary of results obtained with 8 non-immune monkey groups in Panama given artemisone, amodiaquine and/or clindamycin.

<table>
<thead>
<tr>
<th>GML group</th>
<th>Drug</th>
<th>Dosage (mg/kg)</th>
<th>Treatment days</th>
<th>Total dosage (mg/kg)</th>
<th>Parasite clearance</th>
<th>Monkeys cured</th>
<th>Recrudescence (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Artemisone + amodiaquine</td>
<td>30</td>
<td>1</td>
<td>30</td>
<td>3/3</td>
<td>0/3</td>
<td>12, 12, 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Artemisone + amodiaquine</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>3/3</td>
<td>3/3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Amodiaquine</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0/3</td>
<td>0/3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Artemisone</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>3/3</td>
<td>0/3</td>
<td>17, 17, 19</td>
</tr>
<tr>
<td>5</td>
<td>Artemisone + amodiaquine</td>
<td>30</td>
<td>2</td>
<td>60</td>
<td>3/3</td>
<td>2/3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Artemisone</td>
<td>30</td>
<td>3</td>
<td>90</td>
<td>3/3</td>
<td>1 / 2 *</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>Artemisone + clindamycin</td>
<td>30</td>
<td>3</td>
<td>90</td>
<td>3/3</td>
<td>3/3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td>300</td>
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</tbody>
</table>

* One monkey in group 6 died of peritonitis 11 days after start of treatment.
Way Forward...

• Current demands require development of combinations with old drugs
• Proactively identify unique drug interactions
• Use available models, particularly Aotus, to evaluate new drugs and combinations thereof
• Better PK/PD required to select optimal combinations
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