Molecular epidemiology of antimalarial drug resistance

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Global Burden of Malaria

- 300-500 million clinical cases and 1-2 million deaths/year
- Control hindered by increasing drug resistance
Burden of Malaria in Africa

- Nearly all malaria in Africa caused by *P. falciparum*
- African children account for 75% of morbidity and 90% of mortality
- One African child dies of malaria every 30 seconds
Chemotherapy of malaria

- Treatment of severe malaria
  - Quinine
  - Artemisinins will probably replace quinine
- Treatment of uncomplicated malaria
  - Complicated by resistance to older drugs
  - Artemisinin-based combination therapy
- Prevention of malaria
  - Travelers
  - Intermittent preventive tx
    - IPTp
    - IPTi
Available antimalarial drugs – Developing countries

- Chloroquine
- Amodiaquine
- Sulfadoxine/pyrimethamine (SP, Fansidar)
- Chlorproguanil/dapsone (Lapdap)
- Quinine
- Primaquine
- Artemisinin-based combination therapy (ACT)
  - Artemether/lumefantrine (Coartem)
  - Artesunate/amodiaquine (ASAQ)
  - Artesunate/SP
  - Artesunate/mefloquine
  - Dihydroartemisinin/piperaquine
  - Artesunate/chlorproguanil/dapsone (CDA)
Field-based studies of antimalarial drug resistance

• What is the extent of drug resistance?
• What are the molecular predictors of drug resistance?
• Are modern antimalarial regimens selecting for resistant parasites?
Means of identifying emerging drug resistance

• Clinical trials
• Association of resistance-mediating parasite polymorphisms with subsequent drug failure
• Selection of parasites with resistance-mediating mutations or altered in vitro sensitivity by prior therapy
Resistance to CQ

- Principally mediated by mutations in pfcrtr
  - 76T is the key mediator of resistance
  - Other pfcrtr mutations appear necessary to maintain fitness of resistant parasites
- Mutations in pfmdr1 and other genes likely contribute to resistance
Resistance to CQ in Africa

- The horse is out of the barn
- Uganda - Prevalence of pfcrt 76T nearly 100%
- Burkina Faso - Prevalence 76T lower, but increasing
- Malawi - Prevalence 76T and clinical resistance have decreased with elimination of CQ use in early 1990s
What about amodiaquine?

- AQ resistance is much less common than CQ resistance, but mechanisms of resistance are probably similar.
- AQ resistance not well studied clinically
- Does AQ select for any mutations of interest?
  - Selects for key mutations in pfcrt (76T) and pfmdr1 (86Y)
### Antifolates in widespread use

<table>
<thead>
<tr>
<th>Drug</th>
<th>DHFR inhibitor</th>
<th>DHPS inhibitor</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMP/SMX</td>
<td>Trimethoprim</td>
<td>Sulfamethoxazole</td>
<td>Antibacterial; Prophylaxis in AIDS patients</td>
</tr>
<tr>
<td>SP (Fansidar)</td>
<td>Pyrimethamine</td>
<td>Sulfadoxine</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>Lapdap</td>
<td>Chlorproguanil</td>
<td>Dapsone</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>Malarone</td>
<td>Proguanil (+ Atovaquone)</td>
<td></td>
<td>Antimalarial</td>
</tr>
</tbody>
</table>
Why do we care about resistance to SP?

- SP is no longer recommended for tx in most countries.
- SP is still quite heavily used.
- SP is the only proven drug for IPT, and is recommended and heavily used for this purpose.
- Chlorproguanil-dapsone (Lapdap) is a new approved drug and CDA is under advanced development. Resistance to SP may predict resistance to CD.
DHFR mutations and resistance

• Step-wise progression:
  – S108N → N51I → C59R
  – This “triple mutant” is now common in Africa.
  – The triple mutant predicts moderate resistance to SP, but parasites remain sensitive to cycloguanil.

• Additional mutations
  – S108T+A16V; I164L; E30”Bolivia repeat”; C50R
  – Common in parts of Asia and South America
  – Mediate higher-level resistance to multiple antifolates.
DHPS mutations and resistance

• Sulfas and sulfones are relatively poor antimalarials, but are valuable components of combination therapies.
• Step-wise progression less clearly delineated than for DHFR
• Key mutations in Africa: A437G; K540E
• Other mutations seen in Asia and/or South America: S436A; A581G; A613T/S
Selection of polymorphisms by SP and AQ in Burkina Faso

Dokomajilar, et al., 2006, AJTMH 75:162
Importance of dhps 540E in activity of SP-containing regimens- Uganda

Dorsey, et al. AJTMH 2004, 71:758
What will be the impact of TMP/SMX?

- Prophylaxis with TMP/SMX becoming standard in HIV-infected children in Africa
- Does TMP/SMX select for SP-resistant parasites?
- Does TMP/SMX offer protection against malaria?
TMP/SMX prophylaxis in AIDS pts
What is the impact on malaria?

Oct. 2005

561 healthy children
None taking TMP/SMX
6% report ITN use

May - June 2006
All children given ITN

519 children remaining
100% ITN use

300 HIV-infected children
All taking TMP/SMX
88% report ITN use
(remainder given ITNs)

August 2006

290 children remaining
100% ITN use

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>HIV-infected children (n=300)</th>
<th>Healthy children (n=561)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>162 (54%)</td>
<td>266 (47%)</td>
</tr>
<tr>
<td>Mean age yrs (SD)</td>
<td>5.6 (2.6)</td>
<td>6.5 (2.6)</td>
</tr>
<tr>
<td>Parasite prevalence (enrollment)</td>
<td>0 (0%)</td>
<td>113 (20%)</td>
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<tr>
<td>% CD4</td>
<td></td>
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<tr>
<td>Median (IQR)</td>
<td>21% (15-28%)</td>
<td>N/A</td>
</tr>
<tr>
<td>&lt; 15%</td>
<td>74 (25%)</td>
<td></td>
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<td>15-20%</td>
<td>64 (21%)</td>
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<td>&gt;20%</td>
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<tr>
<td>ARV use</td>
<td>35 (12%)</td>
<td>N/A</td>
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## Effect of TMP/SMX and ITN use on malaria incidence

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<tr>
<th>Exposure Group</th>
<th>IRR (95% CI)</th>
<th>P-value</th>
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<tr>
<td>No TMP/SMX, No ITN</td>
<td>Reference group</td>
<td></td>
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<tr>
<td>TMP/SMX prophylaxis alone</td>
<td>0.65 (0.27-1.57)</td>
<td>0.34</td>
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<tr>
<td>ITN alone</td>
<td>0.56 (0.45-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Both TMP/SMX and ITN</td>
<td>0.03 (0.01-0.11)</td>
<td>&lt;0.001</td>
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Did TMP/SMX use select for dhfr/dhps polymorphisms?

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<th>HIV-infected</th>
<th>Community-based</th>
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<tr>
<td></td>
<td>( n = 9 )</td>
<td>( n = 440 )</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>6.8 (2.6)</td>
<td>6.8 (2.7)</td>
</tr>
<tr>
<td>Infection with <em>P. falciparum</em></td>
<td>9 (100%)</td>
<td>419 (95%)</td>
</tr>
<tr>
<td>Geometric mean parasite density</td>
<td>2769/µL</td>
<td>11791/µL</td>
</tr>
<tr>
<td>Mean temperature °C (SD)</td>
<td>37.3 (1.0)</td>
<td>37.7 (1.3)</td>
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<tr>
<td>Prevalence of dhfr/dhps mutations(^b)</td>
<td></td>
<td></td>
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<tr>
<td><em>dhfr</em> 511</td>
<td>9/9 (100%)</td>
<td>79/80 (99%)</td>
</tr>
<tr>
<td><em>dhfr</em> 59R</td>
<td>9/9 (100%)</td>
<td>65/80 (81%)</td>
</tr>
<tr>
<td><em>dhfr</em> 108N</td>
<td>9/9 (100%)</td>
<td>80/80 (100%)</td>
</tr>
<tr>
<td><em>dhfr</em> 164L</td>
<td>1/9 (11%)</td>
<td>0/80 (0%)</td>
</tr>
<tr>
<td><em>dhps</em> 437G</td>
<td>9/9 (100%)</td>
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Do antiretrovirals have antimalarial activity?

- HIV protease inhibitors block activity of an aspartic protease of HIV
- Malaria parasites express a family of aspartic proteases known as plasmepsins
- Plasmepsins I-IV and the HIV protease are biochemically quite similar
- Do HIV protease inhibitors inhibit plasmepsins and exert antimalarial activity?
# In vitro antimalarial activity of HIV PIs

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<tr>
<th>Drug</th>
<th><em>P. falciparum</em> IC$_{50}$ (μM)</th>
<th>Serum concentration with standard dosing (μM)</th>
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<tr>
<td></td>
<td>HB3</td>
<td>D6</td>
<td>Dd2</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>5.6</td>
<td>4.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>4.7</td>
<td>7.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Indinavir</td>
<td>5.8</td>
<td>15.6</td>
<td>31.2</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>15.2</td>
<td>23.0</td>
<td>19.1</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>51.9</td>
<td>25.0</td>
<td>17.4</td>
</tr>
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<td>Lopinavir</td>
<td>1.4</td>
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Parikh, et al., AAC 49:2983, 2005
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Parikh, et al., AAC 49:2983, 2005
Effect of lopinavir on cultured *P. falciparum* parasites

A. 0h control  12h control  24h control  48h control

B. Lopinavir  12h lopinavir  24h lopinavir  48h lopinavir

Parikh, et al., AAC 49:2983, 2005
HIV PIs have antimalarial activity

- PIs might be lead compounds for new antimalarial drugs?
- An advantage of a PI based antiretroviral regimen might be prevention of malaria.
Artemisinins

- Extracted from *Artemisia annua*
- Used as herbal remedy for fevers in China for thousands of years (Qinghao)
- Active ingredient purified 1972 (Qinghaosu)
- Artemisinin and derivatives extensively tested in China beginning in late 1970s
- Used widely to treat malaria by 1980s in China, 1990s in other Asian countries
- Very rapid-acting
- Well-tolerated, minimal toxicity
- Short half-lives necessitate combination therapy
Artemisinin-based combination therapy

- Artemisinins very potent
- Short half-life of artemisinins helps to prevent selection resistant parasites
- Partner drugs have longer half-lives, and eliminate small numbers of remaining parasites
How should we treat malaria?  
WHO recommendations

The following ACTs are currently recommended (alphabetical order):

- Artemether-Lumefantrine (Coartem)
- Artesunate + Amodiaquine
- Artesunate + Mefloquine
- Artesunate + Sulfadoxine–Pyrimethamine
- Amodiaquine + sulfadoxine–pyrimethamine

► Amodiaquine + sulfadoxine–pyrimethamine may be considered as an interim option where ACTs cannot be made available, provided that efficacy of both is high.

Guidelines for the Treatment of Malaria  
WHO, 2006
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Guidelines for the Treatment of Malaria
WHO, 2006
Resistance to artemisinins

- Reports of decreased sensitivity of field isolates to artemisinins in S. America & Africa
- Resistant parasites have mutations in PfATP6, a Ca^{++} ATPase and putative drug target
- Resistant parasites have not been successfully cultured
- Stable artemisinin resistance has been selected in *P. chabaudi*
Resistance to artemisinin partner drugs- a key concern?

• Failures of mefloquine-artesunate seen in Thailand & Cambodia
• Amodiaquine- resistance already common in some areas
• Lumefantrine- no known resistance, but usage selects for Pfmdr1 polymorphisms associated with decreased sensitivity to halofantrine
• Piperaquine- resistance seen with monotherapy in China
Antimalarial efficacy of combination therapies in Kampala
(28-day outcomes from a longitudinal study)

- Recurrent parasitemia
- Treatment failure (recrudescence)

Dorsey, et al. JAMA, 2007
AQ + AS versus AM/LM at a very high transmission site: Tororo, Uganda
(28-day outcomes)

Antimalarial efficacy of combination therapies in Bobo-Dioulasso, Burkina Faso (28-day outcomes)

- Recurrent parasitemia
- Treatment failure (recrudescence)

- AQ/SP: 6% recurrent parasitemia, 4% treatment failure
- DHA/PQ: 2% recurrent parasitemia, 2% treatment failure
- AL: 20% recurrent parasitemia, 3% treatment failure

Zongo, et al., CID, in press
Are ACT partner drugs selecting for resistant parasites?

- AS/AQ
- Artemether-lumefantrine (AL)
- Dihydroartemisinin-piperaquine (DP)
Selection by AQ, SP, and lumefantrine- Burkina Faso

Selection by AS/AQ, Tororo, Uganda

Nsobya, et al. AAC 2007, 51:3023
Selection by AL: Tororo, Uganda

Dokomajilar, et al., AAC, 2006, 50:1893
Reciprocal drug resistance

- AQ selects for pfmdr1 86Y
  - This mutation leads to decreased AQ sensitivity
- MQ, related drugs (LU), and artemisinins select for pfmdr1 N86
  - This mutation probably leads to decreased LU sensitivity
- New combination therapies contain both classes AS/AQ↔AL
Selection for decreased in vitro drug sensitivity by AQ

Prior AQ: tx within prior 12 weeks
What about piperaquine?
Bobo-Dioulasso, Burkina Faso
(28-day outcomes)

- Piperaquine is chemically similar to CQ and AQ
- Piperaquine monotherapy was common in China in the 1980s
- Reports of high-level piperaquine resistance in China in 1980s-90s
- Preliminary studies- selection of pfmdr1 mutations seen with AQ *not* seen with DP (but numbers small)
Summary and Conclusions

• New ACTs are becoming the standard to treat malaria
• In general, the antimalarial efficacy of ACTs is currently outstanding
• Selection of resistance to artemisininins may be occurring
• Selection of resistance to ACT partner drugs is clearly occurring, and threatens the utility of these regimens
• Additional antimalarial regimens, including non-ACT regimens, are needed
# Research Team

<table>
<thead>
<tr>
<th><strong>UCSF</strong></th>
<th><strong>Makerere University, Uganda</strong></th>
<th><strong>IRSS, Burkina Faso</strong></th>
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</thead>
<tbody>
<tr>
<td>Grant Dorsey</td>
<td>Moses Kamya</td>
<td>Jean-Bosco Ouedraogo</td>
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<tr>
<td>Sarah Staedke</td>
<td>Fred Kironde</td>
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