The PMI-supported Antimalarial Resistance Monitoring in Africa (PARMA) Network

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Presentation outline

• Therapeutic efficacy study background
• PARMA background
• Molecular markers background and PARMA results (preliminary)
• Moving forward
• Discussion
Therapeutic Efficacy Studies (TESs)

- **Routine monitoring of antimalarials**
  - All PMI countries should have ongoing or recent TES
  - WHO recommends testing every 2 years
  - Number of sites depends upon country-specific epidemiology
  - Often test 1\textsuperscript{st} and 2\textsuperscript{nd} line drug
## TES schedule of follow-up activities

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical assessment</td>
<td>0</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
</tr>
<tr>
<td>Blood slide for parasite count</td>
<td>2</td>
</tr>
<tr>
<td>Urine sample</td>
<td>3</td>
</tr>
<tr>
<td>Blood for:</td>
<td>7</td>
</tr>
<tr>
<td>genotyping</td>
<td>14</td>
</tr>
<tr>
<td>haemoglobin or haematocrit</td>
<td>21</td>
</tr>
<tr>
<td>molecular markers</td>
<td>28</td>
</tr>
<tr>
<td>in vitro test</td>
<td>35</td>
</tr>
<tr>
<td>antimalarial blood concentration</td>
<td>42</td>
</tr>
<tr>
<td>Treatment</td>
<td>Any other</td>
</tr>
<tr>
<td>Medicine to be tested</td>
<td>X</td>
</tr>
<tr>
<td>Rescue treatment</td>
<td>(X)</td>
</tr>
</tbody>
</table>

Parentheses denote conditional or optional activities. For example, treatment would be given on days 1 and 2 only for 3-day dosing. On day 1, the patient should be examined for parasitaemia if he or she has any danger signs. Rescue treatment could be given on any day, provided that the patient meets the criteria for treatment failure. Extra days are any days other than regularly scheduled follow-up days when the patient returns to the facility because of recurrence of symptoms. On extra days, blood slides may be taken routinely or at the request of the clinical staff.
Decision-making process based on TES results

Day 3: % patients parasitemic

Day 28 or 42: % treatment failures

Interpretation

Response

< 10%

< 10%

≥ 10%

No evidence of resistance to artemisinin Partner drug is effective

No change in treatment policy required

≥ 10%

≥ 10%

≥ 10% or < 10% but increasing over time

< 10%

≥ 10%
Countries with recent or upcoming PMI-funded TESs

- 2016
- Ongoing or planned for 2017

[Map of Africa highlighting countries with recent or upcoming PMI-funded TESs]
PARMA Network
Objectives

1) To assist PMI countries in testing malaria samples from TESs for genetic markers associated with antimalarial resistance

2) To support training and capacity building of African collaborators who possess the sufficient infrastructure:
   • Laboratory (e.g., real time PCR, thermocyclers, gel electrophoresis)
   • Bioinformatics (e.g., computer with sufficient memory and processing power)
PARMA Network

• PMI follows WHO technical guidance:
  • To incorporate resistance testing into existing monitoring
• k13 and other resistance testing at the CDC
• Technology transfer
• Results shared with NMCPs, who are encouraged to share the results with WHO and other interested partners in a timely manner
Using phenotypic and genotypic data together

Country example: Angola

After excluding re-infections, out of 81 subjects in the Zaire artemether-lumefantrine arm:

- 3 early failures
- 8 late failures

- **k13** (artemisinin): all wildtype
- **mdr1** (lumefantrine): majority with NYD and NFD haplotypes, associated with resistance
Current PARMA sites

• Currently in discussions
  • DRC
  • Mozambique
  • Madagascar

• Outside partners testing K13
  • Liberia (WHO)
  • Uganda

• Testing within the country
  • Nigeria
  • Ghana

• To be determined
  • Zimbabwe
Over 2200 samples evaluated to date

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Study type</th>
<th>Markers assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angola</td>
<td>2015</td>
<td>TES</td>
<td>$k13$ (n=389), $mdr1$ (n=528)</td>
</tr>
<tr>
<td>Guinea</td>
<td>2015</td>
<td>TES</td>
<td>$k13$ (n=371), $mdr1$ (n=401)</td>
</tr>
<tr>
<td>Malawi</td>
<td>2014</td>
<td>TES</td>
<td>$k13$ (n=128), $pfdhfr$ (n=128), $dfdhps$ (n=128)</td>
</tr>
<tr>
<td>Mali</td>
<td>2016</td>
<td>TES</td>
<td>$k13$ (n=310), $mdr1$ (n=302)</td>
</tr>
<tr>
<td>Senegal</td>
<td>2011-2014</td>
<td>observational surveillance</td>
<td>$k13$ (n=249), $mdr1$ (n=249), $pfcrt$ (n=249)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2016</td>
<td>TES</td>
<td>$k13$ (n=387), $mdr1$ (n=365)</td>
</tr>
<tr>
<td>Zambia</td>
<td>2016</td>
<td>TES</td>
<td>$k13$ (n=216), $mdr1$ (n=216)</td>
</tr>
</tbody>
</table>

As of May 2017 and counting each sample only once regardless of number of mutations analyzed
Preliminary results

Many countries are still analyzing their data for publication and presentations at the upcoming ASTMH 2017 Meeting

(5 abstracts submitted)
Artemisinin resistance and $k13$

- Defined as delayed parasite clearance
- A molecular marker, $k13$, has been identified
- Suspected endemic resistance
  - Phenotypic or genotypic evidence
- Confirmed endemic resistance
  - Phenotypic and genotypic evidence
- High treatment failure rates in the Mekong associated with concomitant resistance to partner drug
Which \( k_{13} \) mutations matter?

- In SE Asia, distinct alleles originating from multiple independent events of emergence have been observed.
- In Africa, non-synonymous mutations are rare and diverse.

<table>
<thead>
<tr>
<th>K13 Mutation</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>E252Q</td>
<td>Not associated</td>
</tr>
<tr>
<td>P441L</td>
<td>Candidate</td>
</tr>
<tr>
<td>F446I</td>
<td>Candidate</td>
</tr>
<tr>
<td>G449A</td>
<td>Candidate</td>
</tr>
<tr>
<td>N458Y</td>
<td>Candidate</td>
</tr>
<tr>
<td>Y493H</td>
<td>Validated</td>
</tr>
<tr>
<td>R539T</td>
<td>Validated</td>
</tr>
<tr>
<td>L543T</td>
<td>Validated</td>
</tr>
<tr>
<td>P553L</td>
<td>Candidate</td>
</tr>
<tr>
<td>R561H</td>
<td>Validated</td>
</tr>
<tr>
<td>V568G</td>
<td>Candidate</td>
</tr>
<tr>
<td>P574L</td>
<td>Candidate</td>
</tr>
<tr>
<td>A578S</td>
<td>Not associated</td>
</tr>
<tr>
<td>C580Y</td>
<td>Validated</td>
</tr>
<tr>
<td>A675V</td>
<td>Candidate</td>
</tr>
</tbody>
</table>

*Other less frequent variants were reported associated with in vivo or in vitro tests, or both: M476I; C469Y; A481V; S522C; N537I; N537D; G638V; M579I; D584V; H719N.
• No confirmed or candidate markers of artemisinin resistance (i.e., known SNPs in the \(k13\) gene) were detected

• Low level of other polymorphisms were observed (e.g., A578S)
  • Not associated with resistance
  • Frequently found in Africa
pfmdr1 gene

• *P. falciparum* multidrug resistance transporter 1
• Mutations (NYD, NFD) and copy number variation associated with susceptibility/resistance to many ACT partner drugs...
  • Lumefantrine
  • Amodiaquine
  • Mefloquine
• ...as well as other antimalarials
  • Chloroquine

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*Treatment of uncomplicated *P. falciparum* malaria*

Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended artemisinin-based combination therapies (ACT):
- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artesunate + sulfadoxine–pyrimethamine (SP)

*Strong recommendation, high-quality evidence*
• Mutations in the \textit{pfmdr-1} gene associated with resistance to lumefantrine (partner drug)
• Important haplotypes associated with resistance
  • NYD
  • NFD
Other molecular marker associations

- *P. falciparum* chloroquine resistance transporter (*pfcrt*)
  - amodiaquine, chloroquine
- dihydrofolate reductase (*pfdhfr*)
  - pyrimethamine
- dihydropteroate synthase (*pfdhps*)
  - sulphadoxine
- *plasmepsin* 2 and 3
  - piperaquine
What if a concerning result is found?

• Notify NMCP, WHO, and other relevant parties
• Plan follow-up confirmatory studies in consultation with WHO
  • Consider intensifying the number of TES sites
  • Continue to utilize molecular testing in future studies
  • Emphasize timely turnaround of testing results
• Engage with NMCP and WHO in reviewing treatment policy and options for first-line ACT (e.g., Angola)
Moving forward

• Disaggregate the results
  • Only Day 0 → point prevalence
  • Only Day of Late Treatment Failure → focus on failures

• Encourage countries to incorporate genetic results in their TES publications

• Better define significance of certain haplotypes and single nucleotide polymorphisms

• Next round of TES samples and technology transfer
  • Ethiopia, Rwanda, Benin, Mozambique, and DRC

• Increase collaboration and communication

• Emphasize sustainability
Increase collaboration and communication
Emphasizing sustainability

Step 1: Training at CDC (Atlanta)
Step 2: Reinforce concepts in home country
Step 3: Establish regional hubs

Guinea
Kenya
Malawi
Mali
Senegal
Tanzania
Zambia
More to come

Senegal
Tanzania
More to come

Country A
Country C
Country A
Country B
Country D
To be determined
Regional Hubs

Country A ➔ Country B

Country A ➔ Country C

Country C ➔ Country D

Qualifications

• Africa-based
• Adequate infrastructure and personnel
• Demonstrated ability to independently operate
• Desire to work collaboratively

Services offered at the hub

• Testing
  • All samples
  • Confirmatory (e.g., 10%)
• Training
• Technical support
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