Antimalarial Drug Resistance

Where are we and where do we go from here?

Professor PL Chiodini
Dedication

To the memory of

Dr WHG (Harry) Richards PhD

He died in 2018 aged 89 years, having devoted his scientific career to antimalarial chemotherapy
WHO World Malaria Report 2017
WHO World Malaria Report 2017

- 216 million reported cases in 2016
  - Increased by 5 million from 2015
- 445,000 deaths
- Malaria case incidence has fallen since 2010
  - But rate of decline has stalled/reversed since 2014
World Malaria Report app
Launched January 2018
Plasmodium spp
Kirchner et al. Genome Medicine 2016; 8: 92

- Eukaryotes
- Sexual and asexual reproduction
- Genome
  - 14 linear chromosomes
  - Aggregate size c 22 megabases
  - >5000 protein-encoding genes
Schizonticides

Transmission blocking

Causal prophylactics

Sites of drug action

Life cycle of *Plasmodium* spp.

Adapted and redrawn from CDC.
A brief history of antimalarial chemotherapy
Quinine

- Ancient fever remedy
- Peruvian bark
- To Europe in the mid 17th Century
- Mainstay of Rx until 20th Century
Synthetic antimalarials

- Pamaquine (8-aminoquinoline) 1920s
- Mepacrine [quinacrine] (Atabrine™) 1930s
- The 4-aminoquinolines
  - Chloroquine 1930s and 1940s
  - Amodiaquine 1948
- The antifolates
  - SP (sulfadoxine-pyrimethamine) 1970s and 1980s
Synthetic antimalarials

- Mefloquine 1970s and 1980s
- Halofantrine 1970s
- Atovaquone-proguanil 2000
The relentless progression of antimalarial drug resistance

• Chloroquine resistance
  – Arose in Thailand 1957; Colombia 1959
  – Spread through SE Asia and India
  – Reached East Africa from Asia 1978
  – Almost all falciparum areas by end of the 1980s
The relentless progression of antimalarial drug resistance

• SP resistance
  – Triple mutation in dihydrofolate reductase gene
    • 51, 59 and 108
  – Also arose and spread from South East Asia
  – Dihydropteroate synthetase
    • 540 E correlates with trends in SP efficacy

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>SP use (estimated %)</th>
<th>dhps540E prevalence (estimated %)</th>
<th>Year</th>
<th>SP use (estimated %)</th>
<th>dhps540E prevalence (estimated %)</th>
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</thead>
<tbody>
<tr>
<td>Malawi</td>
<td>1993</td>
<td>58</td>
<td>46</td>
<td>2007</td>
<td>13</td>
<td>96</td>
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<tr>
<td>Kenya</td>
<td>1998</td>
<td>41</td>
<td>31</td>
<td>2004</td>
<td>9</td>
<td>91</td>
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<tr>
<td>Zimbabwe</td>
<td>2000</td>
<td>&lt; 1</td>
<td>14</td>
<td>2004</td>
<td>1</td>
<td>26</td>
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<tr>
<td>Burundi</td>
<td>2001</td>
<td>2</td>
<td>41</td>
<td>2003</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>DRC</td>
<td>2001</td>
<td>&lt; 1</td>
<td>10</td>
<td>2005</td>
<td>2</td>
<td>33</td>
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<tr>
<td>Tanzania</td>
<td>2001</td>
<td>52</td>
<td>30</td>
<td>2004</td>
<td>23</td>
<td>71</td>
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<tr>
<td>Cote d’Ivoire</td>
<td>2003</td>
<td>2</td>
<td>2</td>
<td>2005</td>
<td>2</td>
<td>3</td>
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</tbody>
</table>

*The year of policy change to sulphadoxine-pyrimethamine (SP) and later to artemisinin combination therapy (ACT) are both given, along with the estimated SP usage and dhps540E prevalence in each of these years. The countries are listed in order of the year of policy change to SP. DRC refers to the Democratic Republic of Congo.*
Quinine resistance
Blasco et al. Nature Medicine 2017; 23(8): 917-928

- Still only partial
  - pfcr
  - pfmdr1
  - Possibly also protein ubiquitination pathway
Artemisia annua
(qinghao or sweet wormwood)
in Oxford Botanic Garden ©
Artemisinin (a sesquiterpene lactone)

The power of the artemisinins

- Reduce parasite load by a factor of ~10,000 per asexual cycle

- Factor is 100 to 1,000 fold for most other antimalarials
  WHO 2006

ACT (artemisinin combination therapy)

- Introduced in the mid 1990s
- WHO treatment of choice for uncomplicated falciparum malaria since 2006
ACT (artemisinin combination therapy)

- Artesunate-mefloquine
- Artesunate-sulfadoxine-pyrimethamine
- Artesunate-amodiaquine
- Artemether-lumefantrine
- Dihydroartemisinin-piperaquine
- Artesunate-pyronaridine
ACTs (artemisinin combination therapy)

But…….

• From 2006 onwards
  – Reports of declining efficacy of artesunate monotherapy and also ACT in western Cambodia
Dondorp et al. NEJM 2009; 361: 455-467

- Pailin, western Cambodia
  Artemisinins in use >30 years

- Wang Pha, northwestern Thailand
  ACT in use since 1994
Artemisinin resistance

• Associated with mutation in the Kelch-like protein K13
  – Strong association between parasite clearance half-life >5h and single point mutations in K13
  – Multiple mutants, but C580Y mutation predominant
  – Likely due to improved fitness over other mutants
Kirchner et al. Genome Medicine 2016; 8:92

Genetic factors contributing to ART resistance

- PI3K
- ART resistance
- FD, ARPS10, MDR2, CRT
- K13
Possible promoters of artemisinin resistance in SE Asia
Woodrow & White. *FEMS Microbiology Reviews* 2017; 41: 34-48

- **Drugs**
  - Monotherapy
  - Fake or substandard
  - Incomplete course
  - Dosing regimen (either component)

- **The parasite**
  - Resistant to partner drug
  - Hyperparasitaemia
  - Genetic background

- **Low transmission**
  - Lower immunity
  - Single clone infections
  - Higher proportion symptomatic

- **Host factors**
  - Nutritional state
  - Immunosuppression
Partner drugs

- Increasing prevalence of K13 mutants means more and more parasites are exposed to the partner drug acting alone.
- Partner drug resistance and treatment failure will select for higher levels of artemisinin resistance.
Resistance to partner drugs
Blasco et al. Nature Medicine 2017; 23(8): 917-928

- *pfcrt*
  - Digestive vacuole membrane protein
  - Chloroquine selected for mutants, especially K76T
  - Active H+ dependent CQ efflux from digestive vacuole
  - Other antimalarials also affected via altering local concentration at site of action
  - Cambodian strains have Cam734; CQ resistance with no fitness cost
Resistance to partner drugs
Blasco et al. Nature Medicine 2017; 23(8): 917-928

- *pfmdr1*
  - An ATP-binding cassette transporter
  - Affects many antimalarials
  - Mefloquine and lumefantrine both select for increased copy number
  - N86Y can augment CQ resistance due to mutant *pfcrt*
  - Many new mutants since ACTs introduced
Chloroquine resistance in non-falciparum malaria

- *Plasmodium malariae*
- *Plasmodium ovale*
  - AMR currently not a public health problem
  - Siswantoro *et al.* AAC 2011. 55: 197-202
Chloroquine resistance in *Plasmodium vivax* malaria (WWARN)
Molecular targets and mechanisms of resistance
Blasco et al. Nature Medicine 2017; 23(8): 917-928
Other ways to use existing drugs

• Longer courses of ACT?
• Multiple first line therapies?
• Single dose primaquine
Other ways to use existing drugs

- Combination therapy
  - Trials underway for
    - Artemether-lumefantrine-amodiaquine
    - Dihydroartemisinin-piperaquine-mefloquine
Before it is too late....

Eliminate malaria in artemisinin resistance-affected areas before resistance can become locally more severe or spread further.
Elimination Strategy for the Greater Mekong Sub-region
Dondorp et al. Trends in Parasitology 2017; 33: 353-363

• Community-based
  – Primary Health Care
    • VMW; VHW; Private Sector

• Surveillance
  – Mobile phones
  – Dried blood spots on filter paper
Elimination Strategy for the Greater Mekong Sub-region
Dondorp et al. Trends in Parasitology 2017; 33: 353-363

• Early effective treatment
  – ACTs plus gametocytocidal dose of primaquine
  – Drug rotation but difficult to administer
  – Trials of triple agent ACTs
  – 5 or 7 day ACT regimens
  – Sequential Rx with 2 different ACTs
Elimination Strategy for the Greater Mekong Sub-region
Dondorp et al. Trends in Parasitology 2017; 33: 353-363

• Vector control
  – Insecticide-treated bed nets
    • But in GMS 60% of infected bites occur before bed time
  – Indoor residual spraying
    • But outdoor biting
    • Architecture of rural homes
Elimination Strategy for the Greater Mekong Sub-region
Dondorp et al. Trends in Parasitology 2017; 33: 353-363

• Asymptomatic parasite carriers
  – Targeted mass drug administration
    • With the addition of ivermectin?
  – Mass screening and treatment
    • High-throughput LAMP?
    • Ultrasensitive HRP2-based RDT?
Elimination Strategy for the Greater Mekong Sub-region

Dondorp et al. *Trends in Parasitology* 2017; 33: 353-363

- Case and focus investigation and response?
  - Less applicable to GMS
  - Target individuals co-exposed with index case?
- Vaccination?
  - In combination with other measures
- Chemoprophylaxis of high-risk groups?
  - Eg forest workers
Clinical Module
Clinical responses of malaria patients treated with various drugs
Pharmacology Module
Precise antimalarial drug concentrations and pharmacokinetic parameters for key target populations

In vitro Module
In vitro drug susceptibility results from malaria parasites collected from infected patients

Molecular Module
Molecular markers for drug resistance in malaria parasites
What is in the pipeline?
https://www.mmv.org/

Medicines for Malaria Venture
Artefenomel (synthetic peroxide)-Ferroquine (3\textsuperscript{rd} generation 4-AQ)  
https://www.mmv.org/

<table>
<thead>
<tr>
<th>Product vision</th>
<th>• Novel agents in combination for treatment including resistant strains, single-dose potential</th>
</tr>
</thead>
</table>
| MoA            | • Artefenomel (OZ439): novel, synthetic trioxolane  
• Ferroquine (FQ): inhibition of heme detoxification |
| Key features   | Artefenomel  
• Fast killing of parasites  
• Active against artemisinin-resistant parasites  
• 800mg human dose stays above Minimal Parasitocidal Concentration for >8 days  
• Transmission-blocking activity in a Standard Membrane Feeding Assay  
Ferroquine  
• Long duration of plasma exposure  
• Active against chloroquine, mefloquine and piperaine-resistant \textit{P. falciparum} |
| Challenges     | • OZ439 food effect/formulation |
| Status         | • Phase Ib combination study ongoing |
| Next milestone | • Phase Ib study completion in 2019 |
| Previously     | • OZ439: discovery partnership with Monash University, University of Nebraska and Swiss Tropical Institute  
• Ferroquine discovered by CNRS Lille |
| MMV Project Director | • Dr Florian Wartha (interim) |
Imidazolopiperazine: KAF156 targets liver, asexual blood stages and gametocytes
https://www.mmv.org/

<table>
<thead>
<tr>
<th>KAF156/ Lumefantrine</th>
<th>Novartis</th>
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<tbody>
<tr>
<td><strong>Legend</strong></td>
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<td><img src="image" alt="Chemical Structure" /></td>
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<tr>
<th><strong>Product vision</strong></th>
<th>• Novel agents in combination for treatment including resistant strains; single-dose potential</th>
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<tbody>
<tr>
<td><strong>MoA</strong></td>
<td>• Not yet determined. Decreased susceptibility to KAF156 is associated with mutations in three <em>P. falciparum</em> genes, CARL (cyclic amine resistance locus), UDP-galactose and Acetyl-CoA transporters</td>
</tr>
<tr>
<td><strong>Key features</strong></td>
<td><strong>KAF156</strong> \n• Novel mechanism of action – activity against parasites that are resistant to current drugs \n• Rapid killing of parasites (PCT &lt;48 hours) \n• 800mg human dose stays above Minimal Parasitocidal Concentration for &gt;8 days \n<strong>Lumefantrine</strong> \n• New once-daily formulation \n• Transmission-blocking activity in a Standard Membrane Feeding Assay</td>
</tr>
<tr>
<td><strong>Status</strong></td>
<td>• Phase IIb combination study ongoing</td>
</tr>
<tr>
<td><strong>Next milestone</strong></td>
<td>• Phase IIb study completion in 2019</td>
</tr>
<tr>
<td><strong>Previously</strong></td>
<td>• Previous name: GNF156. Discovery partnership with Novartis and STPHI</td>
</tr>
<tr>
<td><strong>MMV Project Director</strong></td>
<td>• Dr Florian Wartha (interim)</td>
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### Spiroindolones: KAE609, Cipargamin
Upsets parasite sodium homeostasis; very rapid clearance

https://www.mmv.org/

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<tr>
<th>Cipargamin</th>
<th>Novartis</th>
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<tr>
<th><strong>Legend</strong></th>
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<tr>
<th><strong>Product vision</strong></th>
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<tr>
<td>• Part of single-exposure radical cure</td>
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<tr>
<td>• Potential for use in severe malaria</td>
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<tr>
<th><strong>MoA</strong></th>
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<tr>
<td>• ( P/\text{ATP4} ) inhibitor</td>
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<th><strong>Key features</strong></th>
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<tr>
<td>• First validated new molecular target in 20 years; very rapid killing of parasites</td>
</tr>
<tr>
<td>• 75mg human dose stays above Minimal Parasitidal Concentration for &gt;8 days</td>
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<tr>
<td>• Potential for transmission-blocking activity in a Standard Membrane Feeding Assay</td>
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<tr>
<th><strong>Challenges</strong></th>
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<tbody>
<tr>
<td>• Safety profile to be further characterized in malaria patient study</td>
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<th><strong>Status</strong></th>
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<tr>
<td>• Completed first phase Ila (short-duration monotherapy PoC in patients)</td>
</tr>
<tr>
<td>• Phase II study in patients started in February 2018</td>
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<tr>
<th><strong>Next milestone</strong></th>
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<tbody>
<tr>
<td>• Phase II study completion in 2019/20</td>
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<tr>
<th><strong>Previously</strong></th>
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<tbody>
<tr>
<td>• Names NITD609, KAE609: discovery partnership with Novartis and STPHI</td>
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<tr>
<th><strong>MMV Project Director</strong></th>
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<tr>
<td>• Dr Wiweka Kaszubska (interim)</td>
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Inhibitors of dihydroorotate dehydrogenase in mitochondrion: DSM265 acts against liver and blood stage schizonts

https://www.mmv.org/

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<tr>
<th>Product vision</th>
<th>DSM265</th>
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<td>Takeda</td>
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<thead>
<tr>
<th>MoA</th>
<th>Plasmoidal dihydroorotate dehydrogenase (DHOOH) Inhibitor</th>
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<tbody>
<tr>
<td>Key features</td>
<td>- Novel mechanism of action</td>
</tr>
<tr>
<td></td>
<td>- 400mg human dose gives concentrations above Minimal Parasitocidal Concentration for &gt;8 days</td>
</tr>
<tr>
<td>Challenges</td>
<td>- Cost of goods for API and formulation</td>
</tr>
<tr>
<td></td>
<td>- Reduced relative activity against <em>P. vivax</em></td>
</tr>
<tr>
<td></td>
<td>- Single enzyme target; potential for resistance</td>
</tr>
<tr>
<td>Status</td>
<td>- Phase IIa in Peru in patients with <em>P. falciparum</em> or <em>P. vivax</em> malaria completed</td>
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<td>- Controlled Human Malaria Infection Study of combination with OZ439 completed</td>
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<tr>
<td>Next milestone</td>
<td>- Confirm suitability of new formulation</td>
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<tr>
<td>Previously</td>
<td>- Discovery partnership with University of Texas Southwestern, Washington University and Monash University</td>
</tr>
<tr>
<td>MMV Project Director</td>
<td>- Dr Jörg Mührle</td>
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</table>
# Inhibitors of PfPI4K (phosphatidylinositol 4-kinase)

https://www.mmv.org/

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<tr>
<th><strong>MMV048</strong></th>
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<tr>
<th><strong>Legend</strong></th>
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<tr>
<th><strong>Product vision</strong></th>
<th>Part of a single-exposure radical cure</th>
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<tr>
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<td>Potential for chemoprotection</td>
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<tr>
<th><strong>MoA</strong></th>
<th>PfPI4K inhibitor</th>
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<tr>
<th><strong>Key features</strong></th>
<th>80mg dose predicted to give coverage above Minimal Parasitidal Concentration for ≥8 days</th>
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<tr>
<td></td>
<td>Good prophylactic activity against <em>P. cynthiae</em>, <em>in vivo</em> after single dose</td>
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<td>Long half-life in human</td>
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<td>Potential for transmission-blocking activity in a Standard Membrane Feeding Assay</td>
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<thead>
<tr>
<th><strong>Challenges</strong></th>
<th>Preclinical safety limiting further dose escalation in patients</th>
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<tr>
<th><strong>Status</strong></th>
<th>Phase I and human volunteer challenge model completed (including with new formulation)</th>
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<tr>
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<td>FFPV Phase Ila in Ethiopia (end 2017)</td>
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<thead>
<tr>
<th><strong>Next milestone</strong></th>
<th>De-risk safety margin in preclinical species to allow further dose escalation in patients</th>
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<thead>
<tr>
<th><strong>Previously</strong></th>
<th>Name MMV390049: Discovery and Phase I partnership with H3D, University of Cape Town</th>
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<thead>
<tr>
<th><strong>MMV Project Director</strong></th>
<th>Dr Cristina Donini</th>
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“Malaria response at a cross-roads”

“The choice before us is clear. If we continue with a “business as usual” approach – employing the same level of resources and the same interventions – we will face near-certain increases in malaria cases and deaths.”

Dr Tedros Adhanom Ghebreyesus
Director-General, World Health Organization