Relapsing malaria: the burden and impact

Half the world’s population lives at risk of infection from the Plasmodium vivax parasite that causes relapsing malaria. Each year, an estimated 8.6 million people suffer from this debilitating disease, with around 85% of the burden concentrated in just five countries: Afghanistan, Ethiopia, India, Indonesia and Pakistan (see also map of global endemicity in 2010). In the Americas, P. vivax is the predominant parasite where it causes 64% of malaria cases. In South-East Asia and the Eastern Mediterranean region the burden of this tiny parasite also weighs heavily.

Of the two most prevalent malaria parasite species, only P. vivax can lie dormant within the liver, hidden from standard antimalarial medicines, and reawaken at regular intervals causing the symptoms of malaria. In addition, while previously viewed as the more ‘benign’ of the two major species, P. vivax has been reported to cause some of the more severe complications of malaria. Over time, relapses lead to persistent anaemia (a decrease in red blood cells), which weakens individuals, making them more susceptible to other diseases such as those caused by bacteria. In some cases, particularly for infants and children, this anaemia can lead to coma and death. Furthermore, each malarial episode keeps a child from school and an adult from work for 3 or more days. Then for those that survive, there is evidence that P. vivax malaria could significantly impede their physical and cognitive development.

Due to its relapsing nature, the human and economic cost of P. vivax is high. An estimated 4% of the world’s malaria cases are attributed to P. vivax. As gains are made in tackling P. falciparum, this proportion will inevitably increase. But research for new medicines to prevent relapse has lagged behind that to treat the symptoms of malaria. Tackling P. vivax can contribute to achieving both the Sustainable Development Goals (SDGs) and malaria elimination targets set by the World Health Organization (WHO).
**P. vivax** malaria: a challenge to prevent and treat

Female *Anopheles* mosquitoes that transmit the *P. vivax* parasite often bite during the day, making bednets largely ineffective protection. Furthermore, in contrast to *P. falciparum* (the species of malaria that causes the majority of deaths in Africa), the transmissible form of *P. vivax* is present in an infected person before they become symptomatic. This provides ample time for transmission to occur before patients are treated.

*P. vivax* parasites lie dormant in the liver of the human host as ‘hypnozoites’ – a Greek name meaning ‘sleeping animals’. These hypnozoites can burst back into life at any time from 3 weeks to several years after the initial infection, leading to the periodic and debilitating malarial relapses that *P. vivax* sufferers endure.

The initial blood-stage infection of relapsing malaria, just like an infection of *P. falciparum*, can be treated with artemisinin combination therapies (ACTs). In some countries, chloroquine is still active, although chloroquine-resistant vivax is becoming more and more common. The issue, however, is how to stop the relapse. The only medicine approved for preventing relapse until now was primaquine, an 8-aminooquinoline compound that was first launched in the 1950s. Primaquine itself can cause haemolysis in any patient with lower than normal levels of the enzyme glucose-6-phosphate dehydrogenase (G6PD), which is required for normal red blood cell functioning. On average, 8% of people in malaria-endemic countries are G6PD deficient.

Furthermore, the WHO recommends that primaquine be taken daily for up to 14 days – a lengthy treatment regime that many patients fail to complete once they start to feel better. New, better tolerated and more convenient approaches to the management of relapsing malaria were urgently needed to address the unmet medical needs of patients. Helping to control relapsing malaria will also support the achievement of the SDG targets for the region as well as national targets for malaria elimination in countries where *P. vivax* predominates.

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12. Haemolysis occurs when red blood cells burst and haemoglobin, the part that carries oxygen, is released into the rest of the blood.
Pring’s story

Pring Chon is a soya bean and cassava farmer from Oslev Village, Cambodia, where he lives with his wife and children. He has suffered with malaria more than 12 times in his life; on one occasion it became severe and he was hospitalised.

“I feel bad with this illness. When I’m infected, I can’t work and my wife can’t work,” Pring explained.

In Cambodia, artemisinin-based combination therapies (ACTs) are used to treat blood-stage malaria infections, leading to quick relief of symptoms. However, in view of concerns over G6PD deficiency in the country, primaquine is currently not routinely used to prevent the relapse of P. vivax.

Without treatment to eliminate the dormant liver parasite, people like Pring continue to suffer the debilitating effects of relapsing malaria.

Renewed focus on relapsing malaria

MMV has strengthened its network of partnerships with researchers and funders to discover and develop new medicines against P. vivax and address challenges in P. vivax patient management. MMV has adopted a multi-pronged approach to achieve this:

1. Working with GSK to develop single-dose tafenoquine, a simpler alternative to primaquine, for use in combination with a point-of-care G6PD test.
2. Working with partners to identify new anti-relapse compounds targeting the liver-stage hypnozoite.
3. Screening potential anti-relapse drug candidates to select those without G6PD liability to enable their community-wide use.
4. Testing new drug candidates against P. vivax using the Volunteer Infection Study (VIS) platform, to de-risk and expedite their development.
5. Expanding clinical trial capability in Peru and Ethiopia for the development of new P. vivax medicines.

Tafenoquine: enhancing convenience in the management of relapsing malaria

Tafenoquine, a single-dose treatment to target P. vivax hypnozoites developed by GSK and MMV, was approved by the US Food and Drug Administration and the Australian Therapeutic Goods Administration in July and September 2018, respectively. With these approvals, tafenoquine became the first medicine for this indication in more than 60 years marking a major milestone on the road to malaria eradication.

The compound, an 8-aminoquinoline, was originally discovered by scientists at the Walter Reed Army Institute of Research in 1978. In 2008, MMV and GSK began a joint project to develop the compound for relapsing malaria.

The next step, to ensure widespread patient access, is to seek registration in P. vivax-endemic countries. Meanwhile, a paediatric formulation of tafenoquine is also in development.

The support and advice we receive from the MMV team and its Expert Scientific Advisory Committee, as well as access to MMV’s network, is key to the success of the tafenoquine project, particularly given the global nature of the challenge of P. vivax malaria.”

Dr Justin Green, Director, tafenoquine clinical team lead, GlaxoSmithKline
Looking out for G6PD patients

Tafenoquine, like primaquine, is an 8-amino quinoline. As such, it also bears the risk of causing haemolysis in patients who have severe G6PD deficiency. GSK is working with PATH\(^{17}\) to develop a G6PD test that will enable practitioners to determine, at the point of treatment, if either of drug can be offered safely. (Currently for patients with G6PD deficiency, a different course of primaquine can be considered, under close medical supervision.) The next 5 years will be critical; MMV and partners will work closely with the WHO and countries striving for malaria elimination to roll out the “tafenoquine proposition” (tafenoquine with a point-of-care G6PD test).

The availability of the tafenoquine proposition will facilitate greater acceptance, effectiveness and safety in the treatment of \(P.\) vivax relapsing malaria. This approach will also make malaria elimination more feasible in areas where most people are G6PD-normal and can therefore be treated with tafenoquine or primaquine.

However, in areas where G6PD-deficiency is more prevalent, the inability of some patients to receive a radical cure will leave a reservoir of infection that will undermine elimination efforts. In addition, given the ever-present risk of drug-resistance, the discovery and development of \(P.\) vivax radical cures is imperative.

New assays and new compounds

MMV is part of a global research consortium committed to improving technologies to facilitate research into this parasite. For the first time, this consortium has made it possible to test compounds \textit{in vitro} on the dormant liver-stage hypnozoites of \(P.\) vivax.

Historically, it has been very difficult to develop these \textit{in vitro} liver-stage assays, because it is neither easy to obtain reliable samples of the infectious form of the parasite, the ‘sporozoite’, nor keep the liver cells ‘alive’ in culture for long periods. Three original approaches have found a way forward.

First, the use of a laboratory model of mouse malaria that allows us to look at compounds that prevent liver infection and the eventual escape of the parasite from the liver cell. This approach enables us to identify compounds that could potentially offer protection from malaria but does not study the dormancy. Dr Elizabeth Winzeler and her team at the University of California San Diego, ‘micro-miniaturised’ their assay enabling the screening of hundreds of thousands of compounds and thereby their prioritization for screening against hypnozoites.

The other two assays work more directly on studying the dormant forms. Prof. Dennis Kyle at the University of Georgia has developed a system that uses human primary liver cells, the most physiologically relevant test, and draws on their preference for growth in a confined space (in the well of a screening plate) enabling them to be studied for longer. Prof. Jetsumon Sattabongkot of Mahidol University in Thailand has worked on developing a special human cell line,\(^ {18}\) which retains the ability to be infected by \(P.\) vivax, but has low metabolic activity, in principle meaning that it could be more sensitive.

Thanks to these new assays, we not only have the possibility to screen directly on the dormant liver stages of \(P.\) vivax, but also in larger numbers than before. This opens up the possibility to screen large novel chemical libraries and ‘go fishing’ for new compounds. To date, MMV and partners have screened more than 500,000 compounds on the mouse model and more than 15,000 on hypnozoites. As a result, the first new compounds with activity against the hypnozoite have been identified and are being explored – marking the first anti-hypnozoite series to be discovered in over 70 years.

For their pioneering research, the three groups were jointly awarded the MMV Project of the Year 2016. Furthermore, these new assays are part of a pragmatic test cascade and broader MMV discovery strategy to identify new anti-relapse medicines. Once activity is identified \textit{in vitro}, we can work with a number of partners to “close the loop” and confirm their activity \textit{in vivo} laboratory models.

\(^{17}\) PATH is an international, nonprofit global health organization: https://www.path.org/\n
\(^{18}\) A cell culture that is derived from one cell or set of cells of the same type and in which under certain conditions the cells proliferate indefinitely in the laboratory. Cell lines are often used in place of primary cells to study biological processes.
Screening candidate drugs for G6PD liability

MMV tests every late lead and candidate compound (i.e. those selected to progress further in drug discovery research) in a laboratory model in which human blood cells from volunteers with low G6PD activity are engrafted. This model, set up at the Upstate Medical University, Syracuse, NY, in collaboration with MMV and the Non-Haemolytic 8-aminoquinoline Consortium, USA, and now run at the University of Denver, was validated by testing various compounds blinded, including those known clinically to cause haemolysis. Only those known to cause haemolysis (e.g. primaquine, tafenoquine, dapsone etc.) were positive. So far, no candidate compound that we have tested has shown to cause haemolysis. This criterion is important in the context of delivering an anti-relapse cure because, as explained earlier, it is a specific liability with existing medicines.

Using the VIS platform to progress P. vivax drug candidates

Over the last several years, MMV has supported the development of the Volunteer Infection Study (VIS) platform, a cutting-edge research innovation used to test the effect of new drugs against the blood stage of the malaria parasite lifecycle in human volunteers. This approach offers major opportunities in the R&D process to reduce risks, timelines and costs. While the VIS platform was initially created for P. falciparum, MMV’s partners at the QIMR Berghofer Medical Research Institute of Queensland have started to develop the model to allow drugs to also be tested for their activity against P. vivax, thereby accelerating their development.

Developing clinical trial capacity for P. vivax

MMV is also working with partners to develop clinical trial capability to facilitate the timely development of novel P. vivax drugs; for instance, clinical trial sites have been constructed/refurbished in Iquitos, Peru, and in Gondar and Jimma, Ethiopia.

In addition, MMV has supported the efforts of the Eijkman Oxford Clinical Research Unit (EOCRU) in Indonesia by providing specialized equipment. MMV and EOCRU are supporting a clinical trial in Indonesian soldiers diagnosed with malaria to investigate the effectiveness of tafenoquine when used in tandem with Eurartesim® (dihydroartemisinin-piperaquine), an ACT co-developed by MMV and its partner, Alfasigma) instead of chloroquine. The trial is a critical next step to support the use of this new radical cure treatment in the country and in the development of this clinical trial platform, which could then be used to develop future anti-relapse medicines.

Malaria liver-stage biology and assays to identify compounds to kill it

**HUMAN LIVER-STAGE**

- **1 HOUR**
  - Transmission to Man
  - Sporozoite
  - Hepatocyte

- **5–7 DAYS**
  - Nucleus
  - Schizont
  - Merozoite

- **≥ 8 DAYS**
  - Erythrocyte
  - Ring

**HUMAN BLOOD STAGE**

In vitro liver-stage assay:

Hepatocytes infected with sporozoites are incubated with a compound for 1–6 days. The viability of the parasite developing into schizonts and/or hypnozoites is then assessed.

In vivo relapse assay:

Patients/subjects infected with P. vivax, P. ovale or P. cynomolgi malaria are treated with a blood-stage agent together with an experimental anti-relapse agent. Parasitemia in the patients’/subjects’ blood is then monitored over time to determine whether hypnozoites (e) remain and reactivate.

Number of parasites by lifecycle stage:

- 10–100 sporozoites injected in blood stream following a mosquito bite
- 10–100 liver schizonts
- Number of liver hypnozoites unknown
- 10,000 to 50,000 merozoites per schizont

Following an infective mosquito bite, sporozoites travel via the blood to the liver. Here they develop into schizonts, which then burst and release merozoites into the blood, leading to the clinical symptoms of malaria. In some species of parasite, particularly P. vivax, some sporozoites become hypnozoites. This form lies dormant in the liver and can reactivate leading to schizont formation and the ensuing symptoms of malaria in the absence of an infectious mosquito bite.
Volunteer Infection Study (VIS) platform

One cohort of 8 volunteers per dose

Inoculate parasites

Administer drug candidate

Count parasites in the mosquito

Mosquito feeds (direct and indirect)

End of study

* Rescue drug administered at the end

* Monitor parasitaemia in volunteers

Malaria elimination efforts in the Asia-Pacific

Leaders throughout the Asia-Pacific region, where more than 80% of the P. vivax burden is concentrated, have committed to eliminating malaria by 2030 in their countries. Malaria elimination would save more than a million lives, as well as create cost savings and social benefits of almost US$300 billion, thereby strengthening regional economic prosperity.19

In support of these efforts, MMV and partners are working in Papua New Guinea on the Lihir Malaria Elimination Project (LMEP). In 2011, MMV began a partnership with Newcrest Mining Ltd to establish a roadmap for improved malaria control throughout the communities where the company works.

In 2014, the Newcrest/MMV collaboration took the additional step of commissioning a study to assess the feasibility of eliminating malaria on Lihir Island – the site of one of Newcrest’s larger mines.

Based on this study, Newcrest and MMV jointly created the LMEP in collaboration with health authorities in PNG. The project includes improving the standards of care and access to malaria prevention and treatment throughout the island as well as improving routine surveillance and disease monitoring.

Eventually, pending national authorization, multiple rounds of mass drug administration could help accelerate the elimination drive on the island. PNG health authorities recently described LMEP as a project of national interest to advance malaria control and elimination in the country.

Conclusion

MMV recognises that developing novel and better tolerated ways of preventing the relapse of P. vivax is essential to achieving malaria elimination. We have thus prioritised the hunt for safer, more convenient medicines which can completely clear the liver of the dormant parasite, the hypnozoite. The development and approval of tafenoquine is a big step forward in these endeavours. The team is now focused on the next critical step of getting this important drug to the patients that need it.

In parallel, MMV is putting in place ground-breaking new tools as well as the capability and know-how to discover and develop the next-generation of anti-relapse medicines. Such new medicines would support malaria elimination and the ambitious global goals to end the disease for good.

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