The goal to eradicate malaria cannot be achieved with current medicines. To determine and exactly define the medicines that are needed for eradication, the Malaria Eradication Research Agenda (malERA) initiative drew on the knowledge of malaria experts from around the world and defined two key Target Product Profiles (TPPs): Single Exposure Radical Cure and Prophylaxis (SERCaP), the silver bullet malaria cure, and Single Exposure Chemoprotection (SEC). All of these clinical attributes will, however, not be found in one molecule and so MMV has defined five Target Candidate Profiles (TCPs) (see last two columns of Table 1), corresponding to the necessary attributes of the TPPs. With partners, MMV has identified and is developing numerous molecules to meet these.

**OZ439**

**Patient exploratory**

**Target indication:** Acute uncomplicated malaria  
**TPP:** Single-dose cure  
**TCPs:** 1 (fast clearance) & 3b (transmission blocking)

**Features:**
- Fast reduction of parasites, similar to artemisinin
- Potential for a one-dose cure and therefore improved patient adherence
- Potential to block transmission
- Potential to treat artemisinin-resistant strains of malaria

**Project Leader:** Dr Marc Adams, MMV  
**OZ439/4-aminoquinoline development partner:** Sanofi  
**Discovery partners:** University of Nebraska Medical Center, USA; Monash University, Australia; Swiss Tropical and Public Health Institute, Switzerland

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**KAE609**

**Patient exploratory**

**Target indication:** Acute uncomplicated malaria  
**TPP:** Intermittent chemotherapy  
**TCPs:** 1 (fast clearance); 2 (long duration) & 3b (transmission blocking)

**Features:**
- Potential for a one-dose cure and therefore improved patient adherence  
- Highly potent and rapid antimalarial action  
- Potential to treat artemisinin-resistant strains of malaria  
- Potential to block transmission

**Project Leader:** Dr Roger Waltzman, Novartis Pharma AG  
**MMV Project Director:** Dr Jörg Möhrle, MMV  
**Development partners:** Novartis Pharma AG  
**Discovery partners:** Novartis Institute for Tropical Diseases, Singapore; the Wellcome Trust, UK; Swiss Tropical and Public Health Institute, Switzerland; Biomedical Primate Research Institute, the Netherlands and Genomics Institute of the Novartis Research Foundation, USA

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**KAF156**

**Patient exploratory**

**Target indication:** Acute uncomplicated malaria, treatment and prevention  
**TPP:** Intermittent chemoprevention  
**TCPs:** 1 (fast clearance); 2 (long duration) & 4 (chemoprevention)

**Features:**
- In vitro activity against liver schizonts and potential for chemoprophylaxis  
- Potential for a one-dose cure and therefore improved patient adherence

**Project Leader:** Dr Thierry Diagana, Novartis Institute for Tropical Diseases, Singapore  
**MMV Project Director:** Dr Jörg Möhrle, MMV  
**Development partners:** Novartis Institute for Tropical Diseases, Singapore  
**Discovery partners:** Genomics Institute of the Novartis Research Foundation, USA; Novartis Institute for Tropical Diseases, Singapore; the Wellcome Trust, UK; Swiss Tropical and Public Health Institute, Switzerland; and the Netherlands
<table>
<thead>
<tr>
<th>Table 1</th>
<th>TPP and attributes</th>
<th>Key actions</th>
<th>TCP and lifecycle stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td><strong>SERGaP</strong> (Single Exposure Radical Cure and Prophylaxis)</td>
<td><strong>Control of resistant blood-stage parasites</strong></td>
<td><strong>TCP1</strong> Blood stage</td>
</tr>
<tr>
<td></td>
<td>➔ Single dose</td>
<td>➔ Fast clearance</td>
<td>➔ Blood stage</td>
</tr>
<tr>
<td></td>
<td>➔ Radical cure – kills parasite at all lifecycle stages</td>
<td>➔ Long duration of action/Post-treatment prophylaxis</td>
<td><strong>TCP2</strong> Blood stage</td>
</tr>
<tr>
<td></td>
<td>➔ Treatment for all five species to infect humans</td>
<td>➔ <strong>Relapse prevention</strong></td>
<td>➔ <strong>TCP3a</strong> Hypnozoites</td>
</tr>
<tr>
<td></td>
<td>➔ High barrier to resistance</td>
<td>➔ Transmission prevention</td>
<td>➔ <strong>TCP3b</strong> Gametocytes</td>
</tr>
<tr>
<td></td>
<td>➔ Post-treatment prophylaxis</td>
<td>➔ <strong>Chemoprevention</strong></td>
<td>➔ <strong>TCP4</strong> Liver schizonts</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td><strong>SEC</strong> (Single Exposure Chemoprevention)</td>
<td><strong>Blood stage (slow-onset activity)</strong></td>
<td><strong>TCP4</strong> Liver schizonts</td>
</tr>
<tr>
<td></td>
<td>➔ Single dose</td>
<td>➔ <strong>Chemoprevention</strong></td>
<td>➔ <strong>blood stage</strong></td>
</tr>
<tr>
<td></td>
<td>➔ Suitable for mass administration</td>
<td>➔ <strong>Blood stage</strong></td>
<td>➔ <strong>TCP4</strong> Liver schizonts</td>
</tr>
<tr>
<td></td>
<td>➔ Chemoprevention for all five species to infect humans</td>
<td>➔ <strong>Relapse prevention</strong></td>
<td>➔ <strong>TCP4</strong> Liver schizonts</td>
</tr>
<tr>
<td></td>
<td>➔ Different mechanism of action to treatment</td>
<td>➔ <strong>Transmission prevention</strong></td>
<td>➔ <strong>TCP4</strong> Liver schizonts</td>
</tr>
</tbody>
</table>

**DSM265**

**Target indication:**
Acute uncomplicated malaria

**TCPs:** 3 (long duration) & 4 (chemoprevention)

**Features:**
- Novel mechanism-of-action and highly selective for the parasite with the potential to treat artemisinin-resistant strains of malaria
- Potential for a one-dose cure and therefore improved patient adherence
- Long duration of action and potential for causal and post-treatment prophylaxis

**Project Leader:** Dr Thomas Rückle, MMV

**Partners:** University of Texas Southwestern, USA; University of Washington, USA; Monash University, Australia

**MMV390048**

**Target indication:**
Acute uncomplicated malaria

**TCPs:** 3 (fast clearance) & 3b (transmission blocking) & 4 (chemoprevention)

**Features:**
- Highly potent against *P. falciparum* blood stage
- Good prophylactic activity against *P. cynomolgi* (surrogate for *P. vivax*) in vivo after single dose

**Project Leader:** Dr Cristina Donini, MMV

**Partners:** University of Cape Town, South Africa

**(+)-SJ557733**

**Target indication:**
Acute uncomplicated malaria

**TCPs:** 3 (fast clearance) & 3b (transmission blocking) & 4 (chemoprevention)

**Features:**
- Novel chemotype and validated pathway
- Rapid parasite clearance
- Potential for a one-dose cure and therefore improved patient adherence
- Potential to block transmission

**Project Leader:** Dr David Floyd, Rutgers University, NJ, USA; Dr R. Kip Guy, Department of Chemical Biology and Therapeutics, St Jude Children’s Research Hospital, CA, USA

**MMV Project Director:** Dr Lidija Bebrevska

**DDD107498**

**Target indication:**
Acute uncomplicated malaria

**TCPs:** 3 (fast clearance) & 3b (transmission blocking) & 4 (chemoprevention)

**Features:**
- Novel mechanism of action
- Comparable activity across multiple stages of the malaria parasite lifecycle including inhibition of development of all liver stages and outstanding transmission blocking potential

**Project Leader:** Prof. Ian Gilbert and Dr Kevin Read, Drug Discovery Unit, University of Dundee, UK

**MMV Project Director:** Dr Lidija Bebrevska
Developing a single-dose malaria cure

<table>
<thead>
<tr>
<th>ISSUE</th>
<th>SOLUTION</th>
<th>ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current treatments must be taken over 3 days and so patient adherence cannot be guaranteed. This can lead to incomplete cure and exacerbate the development of drug resistance.</td>
<td>Develop antimalarials that are easier to take and can assure patient adherence.</td>
<td>MMV and partners identify and develop molecules that are fast-acting and have a long duration of action for combination into a single-dose cure and first-generation Single Exposure Radical Cure and Prophylaxis (SERCaP).1</td>
</tr>
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</table>

In 2014, we saw the progress of several compounds able to meet the requirements of our TCPs with single-dose potential and therefore able to form the building blocks of a first-generation SERCaP.1

The most advanced compound, the aromatic trioxolane OZ439, is a molecule that MMV has taken from discovery right up to Phase IIb, where its efficacy in patients will now be determined. This will be the first time malaria patients will receive treatment with OZ439 in combination with a partner drug. Encouragingly, in vitro data from the first artemisinin-resistance assay indicates the compound is active against resistant strains at clinically relevant concentrations.2

Meanwhile, a spiroindolone, KAE609, and an imidazolopiperazine, KAF156, are in Phase IIA clinical trials to identify suitable dosages to take forward. DSM265 – a triazolopyrimidine-based highly selective inhibitor of Plasmodium falciparum’s enzyme, dihydroorotate dehydrogenase (DHODH), is in Phase I to determine its safety in man. MMV390048, the first antimalarial compound to be researched on African soil, has potent activity against multiple stages of the malaria parasite’s lifecycle, and the potential to block malaria transmission is set to enter Phase I in 2014. (+)-SJ557733, a completely novel molecule, was identified as a drug candidate in 2013 and is undergoing preclinical development. DDD107498, a compound from Dundee University, has activity across multiple stages of the parasite’s lifecycle affording potent in vitro transmission-blocking activity and was approved for preclinical development in 2013.

Dr Marc Adamy

MMV Director, Product Development for our front-runner compound, OZ439, explains the development plans to speed its progress to patients.

Q Given the emergence of artemisinin resistance and now treatment failures with ACT, next-generation antimalarials are urgently needed. What strategies have been employed to accelerate the development of OZ439?

In drug development, patient safety must be a top priority and so the process is highly regulated. With that in mind, the team is looking at innovative strategies to speed things up. For example, the typical route to drug development is to gather all the evidence in adults at least up to Phase II and then to start the paediatric development. Given the huge burden of malaria in children, we will combine adults and children within the same Phase IIb programme. By adopting a staggered approach, starting first with adults, we will have safety results before moving to younger age groups and overall, will be able to expedite the process.

Given the high level of unmet need presented by drug resistance and that OZ439 has demonstrated ex vivo efficacy against resistant parasites, the US Food and Drug Administration might consider it for fast track and/or breakthrough medicine designation. Either of these FDA statuses would help to bring an OZ439 combination therapy to patients in less time.

Q What are the next steps for the development of an OZ439 combination therapy?

The next step is to run two Phase IIb trials together, where we will test the OZ439/4-aminoquinoline combination. We will investigate the safety and efficacy of OZ/PQP and OZ/FQ in typical dose-ranging studies to select the optimal dose for Phase III. The trial should be complete around mid-2015. Phase III is scheduled to begin in 2016.

References:
1 SERCaP: a medicine to cure patients (targeting the blood stage) and eliminate the human reservoir of parasites (targeting the sexual stages). In addition, for radical cure of Plasmodium vivax malaria, the medicine would need to eliminate all blood-stage forms as well as hypnozoites in the liver. Finally, it would need to prevent reinfection of the treated individual for at least 1 month.
2 Witkowski B et al., unpublished data.

Blood stages in the lifecycle of P. falciparum.
Source: Benedict Campbell, Wellcome Images
New tools to accelerate drug development

**ISSUE**
Drug resistance is emerging to current first-line artemisinin-based combination therapies (ACTs), both to artemisinin and the partner drug and in some cases leading to treatment failure.1

**SOLUTION**
Drug resistance monitoring and containment efforts are underway, greatly supported by the recent identification of a molecular marker for artemisinin resistance.2 At the same time, we must be prepared with alternative antimalarials that are easier to take and effective against resistant strains of the parasite.

**ACTION**
Draw on innovative new tools to accelerate the development of a Single Exposure Radical Cure and Prophylaxis (SERCaP).

To help expedite the development of promising compounds, MMV is employing innovative new tools such as the Challenge Model and pharmacokinetic/pharmacodynamic (PK/PD) modelling. The Challenge Model enables us to test candidate medicines in volunteers inoculated with a small dose of malaria in a tightly controlled environment. Using PK/PD modelling, we are able to take raw data, such as that generated in the Challenge Model, to determine relationships between concentration and efficacy.

Taken together, these approaches have provided a granularity of data that was previously inaccessible, enabling us to understand quickly and affordably whether a compound will work in man and provide guidance on dose selection for subsequent studies. For example, with DSM265, we have been able to link safety, efficacy and dose together in 6 months to determine the optimal dose; versus the 2 years it would take using conventional Phase I and Phase II trials.

Prof. James McCarthy
Queensland Institute of Medical Research, Berghofer Medical Research Institute, explains how the Challenge Model is helping to accelerate antimalarial drug development.

**What are the advantages of the Challenge Model?**
First, it has the potential to reduce the size of Phase IIa clinical trials in malaria-endemic countries, which can be very difficult and time consuming to conduct. The second thing is that when you test an experimental antimalarial drug there is always a risk that if the drug doesn’t work people could come to harm. In the Challenge Model, patients have very low levels of parasites in their blood so we know that they won’t be harmed.

**What has it been like to work with MMV on developing and using the Challenge Model?**
There has been continuous communication and open sharing of information. MMV’s product development partnership model is particularly suited to collaborative relationships between academia, industry and pharma. MMV’s strength is its strong relationships and flexibility. Also, its viewpoint is different from that of a pharma partner, as they are not just looking at one drug but the overall need for malaria drug development and so their strategy supports that. It’s a more holistic approach. On the other hand, MMV obviously has budgetary constraints; a big drug company can throw large amounts of money and manpower behind one drug, while MMV is more constrained by the money that’s available.

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“MMV’s strength is its strong relationships and flexibility.”
Targeting the relapse

Relapsing *Plasmodium vivax* malaria results in around 70–80 million clinical infections each year.¹ Primaquine is the only medicine available to cure it. It has been in use for 60 years, has a very long treatment regimen and potentially fatal side effects in some patients.

*P. vivax* is associated with appreciable mortality. Fatal cases have been reported from several endemic countries. The most common clinical manifestation is severe anaemia and this is associated with additional respiratory or diarrhoael infection. The consequences can be disastrous. What we don’t know is how many more people are dying directly from *P. vivax* or indirectly from co-associated morbidities.

We lack a reliable treatment for *P. vivax* malaria. Primaquine has been the only available radical cure of *P. vivax* malaria for the last 60 years. WHO recommends a 14-day course, which is difficult to deploy, often not implemented by malaria control programmes, and rarely adhered to by patients. Also, in susceptible individuals, it can cause haemolysis, making anaemia worse. Addressing this issue is perhaps the greatest challenge in the management of *P. vivax*.

In addition, the *P. vivax* parasite has become resistant to chloroquine. This was first described in Papua New Guinea in 1989, but appears to have spread across much of Asia and South America. However, chloroquine continues to be used as the first-line treatment in nearly all *P. vivax* endemic countries.

**Q** What are the limitations of the current tools for the treatment and management of *P. vivax* malaria? We lack a reliable treatment for *P. vivax* liver stages. Primaquine has been the only available radical cure of *P. vivax* malaria for the last 60 years. WHO recommends a 14-day course, which is difficult to deploy, often not implemented by malaria control programmes, and rarely adhered to by patients. Also, in susceptible individuals, it can cause haemolysis, making anaemia worse. Addressing this issue is perhaps the greatest challenge in the management of *P. vivax*.

**Q** Do you believe *P. vivax* malaria can be eradicated? Absolutely! It has already been eliminated in the UK, Italy, USA, Russia and many other countries. Sri Lanka is the latest country close to elimination. So we know we can tackle it, the question is how: through repeated blood stage treatment or expedite the process by reliably dealing with the hypnozoite.

**Q** Why do you consider *P. vivax* malaria to be a research priority? *P. vivax* causes a huge burden globally, and outside Africa accounts for almost 40% of the world’s malaria. In some communities, young children have bouts of *P. vivax* every 3 to 4 weeks leading to a huge burden of disease in individuals, families and communities. As *P. falciparum* malaria declines, there has been a rise in the proportion of malaria attributable to *P. vivax*, both relative and, at times, absolute. In many regions, *P. vivax* is now the predominant species; its ability to relapse from dormant liver stages makes it much harder to eliminate.

**Q** What tools do we need to achieve *P. vivax* eradication? As for any major public health campaign, health systems need to be strengthened. For malaria, the priorities are vector control and access to early diagnosis and treatment. But we desperately need a safe and reliable radical cure for *P. vivax*. This means either rethinking how we deploy primaquine or developing alternative options.

Exciting data, published at the end of last year, highlights the potential of tafenoquine, a drug under development with GSK and MMV, to provide radical cure with a single dose. If subsequent clinical trials can confirm its safety and comparative efficacy against current treatment options and it can be deployed widely, then it has potential to transform the management of *P. vivax* and become a major tool in the ultimate elimination of malaria. Like primaquine, however, the drug causes haemolysis in susceptible individuals and would need to be rolled out in conjunction with better diagnostics.

Significant resources are now being brought to bear in tackling these issues and in the coming years we hope to be able to ensure that goals for elimination and eradication include *P. vivax* as well as *P. falciparum*.

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Discovering new molecules to target the relapse

To identify safe, new molecules active against the dormant liver form of *P. vivax* (the hypnozoite) MMV has put in place a pragmatic cascade of tests. Compounds known to be active against blood-stage parasites are screened first against a rodent malaria liver assay to test them for activity against liver-stage schizonts. Active compounds are then progressed to a primate malaria *in vitro* assay followed by an *in vivo* model to test for activity against the hypnozoites.

The limitation of the current test cascade, however, is that we are not testing against parasites that infect humans (*P. vivax*) and so might miss some molecules. Additionally, the throughput of our current assays that look at the hypnozoite is limited. To overcome this, MMV and the Bill & Melinda Gates Foundation are working with different research groups to develop a cost-effective *P. vivax* cell assay.

In a major step towards that goal, a team led by Massachusetts Institute of Technology (MIT) researchers has now developed a system to grow liver tissue that can support the liver stage of both *Plasmodium falciparum* and *P. vivax* malaria.1

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**Prof. Sangeeta Bhatia**

Director, Laboratory for Multiscale Regenerative Technologies, MIT, USA, explains her interest in this area of research and how the system works.

As a professor of electrical engineering specializing in computer science, how did you come to be involved in malaria research?

A lot of our work is about leveraging engineering tools for medicine. Many tools for computer chip manufacturing have been very useful for manipulating cells. We had been using these chips to manipulate liver cells and grow implantable livers for patients. About 5 years ago, after the renewed call for malaria eradication, the Gates Foundation hosted a liver-stage meeting. They had seen our work and invited us. That’s where it all began. It was a combination of opportunism, experience and interest in making a global impact.

What progress has been made in the development of a *P. vivax* (hypnozoite) cell-based assay to identify new anti-relapse medicines?

We knew it would be a big challenge to grow the hypnozoite in the lab; everyone we spoke to in the malaria field was understandably sceptical. Sanaria, a biotechnology firm, was able to provide us with cryopreserved parasites, which means we were not dependant on fresh supplies. As the project progressed, led by Sandra March, we were able to get full liver-stage maturation of *P. falciparum* parasites.

We then started the *P. vivax* experiments with the help of many different groups, including Sanaria. Eventually we were able to see a sub-population of liver forms progress, while a sub-population persisted and became dormant. It is the latter population that we termed “persistent small forms”. Before we can really call them hypnozoites we need to see reactivation. The other thing we are looking at is differential drug sensitivity, which is work planned for next year. It has been really gratifying to get a glimpse at what we believe is the hypnozoite. Of course, there will be lots more to do if we can really reproduce it in this way. We’re really excited!

How do the assays work?

The assay we have working at the moment is really for *P. falciparum*; for *P. vivax* we have only achieved feasibility. For both assays, you start with a well plate of microcultured human livers, with hepatocytes in colonies of 250 cells, on islands of collagen surrounded by feeder cells that help to support their differentiation. We then add parasites directly onto these media and essentially allow them to progress through the liver stages. First, they glide and traverse several hepatocytes and then they will choose to set up shop in one. You are then left with a well plate of infected liver cells, to which you add the drug. You then stain and count the forms that remain to determine if the drug worked.

What has it been like to work in the malaria community?

Once we were introduced to the community and they embraced the potential of our assay, their input was transformative. We have had access to expertise, reagents, parasites and decades of research that wouldn’t have been available to us otherwise. Credit goes to the Gates Foundation and MMV for being visionary. They really took a chance on us, a group of complete outsiders to the malaria community, and we hope to live up to their expectations.
Tafenoquine, a potential next-generation anti-relapse medicine for *P. vivax* malaria, successfully completed a Phase IIB trial in 2013. In that trial, a 300 mg single dose of tafenoquine plus chloroquine provided better protection from *P. vivax* relapse than chloroquine alone. In addition, the US FDA granted tafenoquine Breakthrough Therapy designation – one of its newest initiatives aimed to accelerate the development and review times of drugs for serious or life-threatening diseases.

Tafenoquine belongs to the same chemical family as primaquine and thus is associated with haemolytic side effects in patients who are deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD). As a result, it will need to be deployed alongside a point-of-care G6PD-deficiency diagnostic test. The tafenoquine team are working together with PATH, whose Diagnostics Group received a grant from the United Kingdom’s Department for International Development (DFID) to accelerate the development of a G6PD test to help achieve safe and effective use of medicines for radical cure of patients infected with *P. vivax*.

Thanks to the success of the Phase IIB trial, tafenoquine entered Phase III in April 2014, taking it closer to becoming the only new medicine approved for the treatment of relapsing malaria in over 60 years. In recognition of the team’s dedicated work to advance the project this far and in view of the promise tafenoquine holds, MMV’s Expert Scientific Advisory Committee (ESAC) has nominated tafenoquine the MMV Project of the Year 2013.

Representatives of the GSK/MMV project team talk about the challenges, the partnership and what the future holds.

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**Q** How did you ensure patients would return for follow-up in the Phase IIB trial?

By drawing on MMV’s network we were able to select appropriate clinical sites able to run the trial. GSK’s local presence ensured we had the operational support we needed. We placed a great deal of focus on the importance of patient retention and ensured everyone involved clearly understood this. Our efforts were certainly rewarded as 97% of patients returned for their 6 month visit. It was a remarkable MMV/GSK team achievement and really key to the successful completion of the Phase IIB trial.

**Q** MMV and GSK have been working together for a number of years on antimalarial research projects. What makes the partnership work so well?

Both GSK and MMV share the same view that malaria is one of the world’s deadliest diseases and that overcoming it requires a joint effort between the public and private sector. The partnership initially began in 2003 to work on a drug discovery “mini-portfolio”. At the time, it was a unique and ground-breaking agreement and it set the tone for collaboration on many other projects, including tafenoquine.

There is a lot of synergy between the partners; both bring very different skills to the table. At GSK, we have the open lab in Tres Cantos, Spain, focused on drug discovery and a clinical development group in London, UK. But it’s really MMV that oversees the entire portfolio of investigational antimalarials. MMV has established governance and oversight committees of malaria and drug development experts that simply don’t exist anywhere else. Our senior review committees at MMV always take ESAC (page 53) advice into consideration. Additionally, the expertise and network at MMV, spanning the public and private sectors as well as malaria-endemic regions, has been crucial to the progress of our efforts to tackle malaria. For tafenoquine, we look forward to continuing the successful partnership during the Phase III trial, which began in April 2014.
Dr Wiweka Kaszubska

Vice President, Head of Product Development and current MMV Project Sponsor for tafenoquine.

What is the significance of the US FDA Breakthrough Therapy designation?

This designation offers us the possibility to get tafenoquine to patients quicker. First of all, the FDA will review the marketing application in 6 months’ time instead of the standard 10 months; and provide guidance to ensure an efficient development programme. Second, we will then be able to progress earlier with registrations in endemic countries or additional studies to support these registrations.

What are the next steps for tafenoquine?

The Phase III trial began in April 2014 and is expected to run until the end of 2015. We will thereafter complete the submission of the regulatory dossier. In the meantime, MMV/GSK’s access and product management team are laying the groundwork for endemic-country access, where we will see the real impact of the medicine. This includes ensuring enough evidence is generated to support the inclusion of tafenoquine as well as an appropriate G6PD diagnostic test in the WHO malaria treatment guidelines. The access team is also evaluating how to improve the supply chain in disease-endemic countries to ensure the availability of tafenoquine together with a G6PD test.

While the current formulation is expected to be used by adults and adolescents, we are also progressing our plans to develop a formulation for children.

Dr Jörg Möhrle

Head of Translational Medicine, MMV and former Project Director for tafenoquine.

JP mentioned that the biggest challenge in the development of tafenoquine was ensuring patients return for follow-up. What challenges stand out for you?

To help speed the development process we designed an innovative, seamless Phase IIb/Phase III protocol. This concept has been employed for cancer but never for malaria so it was challenging to explain it to some of the regulatory agencies, particularly as the historical perception of P. vivax malaria is that of a benign disease. Before starting the Phase IIb study there were only a few patients that had been treated with a single dose of tafenoquine to eliminate P. vivax hypnozoites, so embarking on the development programme required a great deal of confidence.

Another challenge was deciding where to conduct the trial. First, as we chose to treat the P. vivax blood stage with chloroquine (standard of care against chloroquine-sensitive strains of P. vivax) we could only go to countries which use chloroquine as first-line treatment. Second, different strains of P. vivax around the world, leading to different rates of relapse, would imply different follow-up times for the trial. Unfortunately, data on relapse rates from around the world are limited. We made our selection based on available data and on the advice of the investigators from the countries with the greatest burden of P. vivax disease.

What is the significance of the Phase IIb results?

We now know from the Phase II trial that a single dose of 300 mg of tafenoquine protected around 90% of patients from relapse. For the purpose of statistical analyses, before the trial, we decided that the efficacy of tafenoquine combined with chloroquine would have to be 30% better than chloroquine alone, in the end it was 50% better. As a consequence, the level of confidence that we will be successful in Phase III, both among the team and the wider malaria community, is much higher than before. So now, we have a huge amount of momentum and a clear pathway to complete development, and if successful, registration with a stringent regulatory authority and in endemic countries.
Blocking transmission

**ISSUE**

To reduce the overall burden of malaria we need to be able to stop transmission from person-to-person. Primaquine is the only medicine able to do this, but few studies have been conducted to determine its efficacy and safety.

**SOLUTION**

Discover and develop new, safe and easy-to-take transmission-blocking medicines that can be combined into a Single Exposure Radical Cure and Prophylaxis (SERCaP).

**ACTION**

MMV and partners have developed and are using a test cascade to identify new and in-development molecules with transmission blocking activity.

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**The WHO recommends a single low dose of primaquine to be taken alongside ACTs to block malaria transmission.**

This is particularly valuable in preventing transmission of drug-resistant parasites emerging in south-east Asia. However, there have been no suitably powered clinical trials to confirm the efficacy and safety of single, low-dose primaquine, though these are now in progress. Safety is of particular concern given the known haemolytic side effects in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency treated with primaquine, especially since the deficiency can occur in up to 32.5% of the population in some malaria-endemic countries. Consequently, there is a need to identify alternative medicines that treat patients and block transmission.

In addition to their established blood-stage activity, OZ439 and KAE609 have both demonstrated transmission-blocking potential in the laboratory. The key now is to investigate this potential in malaria-infected people, by carefully monitoring the development of the parasite from humans to mosquitoes after patients have been treated with either OZ439 or KAE609. Working with scientists in Tanzania, MMV has established an insectary and proof-of-concept transmission-blocking model, which is currently being used to see whether the laboratory findings are confirmed in patients.

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**Dr Didier Leroy**

Director of Drug Discovery at MMV.

**Q** What has been the biggest challenge in the hunt for transmission-blocking molecules?

The biggest challenge is that historically the most informative assay, known as the Standard Membrane Feeding Assay (SMFA), was pretty labour intensive. Mosquitoes feed on malaria-infected blood – with or without the addition of a test drug – and after a week we then dissect the mosquito to see whether or not the parasite developed in its midgut. Dissecting individual mosquitoes is laborious, so we were initially only able to fully characterize the activity of one or two molecules a year with this assay.

**Q** How did you scale-up the standard membrane feeding assay to increase the number of molecules that can be characterized?

TropQ, a spin-off company from Radboud University in the Netherlands, developed a rigorous, and reproducible approach, which enabled us to characterize the activities of more than 10 molecules in the last year. We have now been able to determine the potency of all the molecules in late-stage development and all of our preclinical candidates in the SMFA.

The next step is to scale-up the assay to an industrial level, enabling around 40–50 molecules a year to be tested. In 2012, we collaborated with GSK to establish an insectary at Tres Cantos, Spain, where mosquitoes are bred. In this way, we now have an autonomous unit where molecules can be tested against falciparum gametocytes to determine their impact on transmission to the mosquitoes after feeding.

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**Q** What progress has been made since the 2012 publication to identify a new transmission-blocking medicine?

The most potent transmission-blocking molecule we have so far is DDD107498, (page 25) a new compound from the Drug Discovery Unit at the University of Dundee. DDD107498 is in preclinical development and is extremely potent in the standard membrane feeding assay. In addition to DDD107498 over half of our recent preclinical candidates also show some potential to block or significantly reduce transmission.
Figure 1: Malaria transmission biology and the assays to identify compounds to block it

MMV’s strategy is to identify compounds able to kill the asexual blood stages and gametocytes, thus curing the patient and blocking transmission. To do so, we have a cascade of tests in place. Blood-stage active compounds are screened against a gametocyte assay, followed by a male and female gamete formation assay followed by the Standard Membrane Feeding Assay (SMFA).

**Gametocyte Assay**

Gametocytes are incubated with a compound for 24–48 hours and their viability assessed.

**Dual Male & Female Gamete Formation Assay**

Late-stage gametocytes are incubated with a compound for 24–48 hours and their viability and fertility assessed.

**Standard Membrane Feeding Assay**

Gametocytes in human blood are incubated with a compound for 24–48 hours. Mosquitoes are fed on the blood. Ten days later the mosquitoes are dissected to determine if there are oocysts present in their midgut.

**Number of Parasites by Lifecycle Stage:**

- ≈ 5,000 Gametocytes in a blood meal
- ≈ 500 Gametes
- ≈ 100 Ookinetes
- ≈ 5–10 Oocysts
- ≈ 10^3 Sporozoites
Open source drug discovery

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**ISSUE**

20,000 molecules active against malaria have been identified through MMV and partners’ extensive screening campaign and released into the public domain. They now need to be followed up.

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**SOLUTION**

Initiate drug discovery programmes to explore the viability of these compounds.

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**MMV ACTION**

Pioneer open source initiatives using active compounds to catalyse antimalarial and neglected disease drug discovery.

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In 2011, MMV launched an Open Source Drug Discovery (OSDD) programme in a bid to bring as many of the world’s best scientific minds together to solve some of the complex scientific challenges posed by malaria. Working with scientists initially in Australia and then in India, it was the first attempt by a Product Development Partnership to facilitate open sharing of information, data and ideas in real time among fellow researchers.

In 2013, MMV signed a memorandum of understanding with the Royal Society of Chemistry, UK, and the Drugs for Neglected Diseases initiative (DNDi), to build on the networks established through OSDD to create a global community of open source drug discovery researchers for diseases of poverty.

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**Prof. Mat Todd**

University of Sydney, who leads Open Source Malaria (OSM),1 explains how the project works and what he thinks the future holds for open science.

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**Q** How does open source drug discovery work?

The day-to-day work is very similar to that of any research project. You have a lab book and jot down everything that is done. The critical difference is that everything is in the public domain. It sounds trivial but it’s actually a big mind shift. In addition to describing everything that has happened, the project also makes clear what is planned for the weeks ahead. The idea is that no one is behind the curve; people can provide input ahead of time.

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**Q** What are the advantages of open source drug discovery?

You open up to the possibility of working with experts even if you don’t know them. Whenever you look at a scientific problem, you think, “I want to solve this, but not if someone else already has, or if someone else is better placed to do so”. Open source is not for the faint-hearted though. It creates an extremely challenging environment: if you make a mistake it becomes “public”; if someone is better at what you are doing they might take over. We must work in an environment where the best people work on the right problems. Open source allows you to do that.

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**Q** With the contribution of a new chemical series to OSM in September 2013, how is the project progressing?2

The new series, the triazolopyrazines, have already performed well in vivo. Promising new compounds are now emerging. There have been synthetic contributions to the series from Scotland and Stockholm, as well as advice and informatics support from various industrial and academic laboratories around the world.

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**Q** What is the advantage of working with MMV?

It’s amazing to have funders who have the courage to support an initiative that has never been attempted before. One of the main advantages is scientific expertise. Working with Paul Willis has been sensational. He is extremely knowledgeable about medicinal chemistry and so his contributions drive the project forward. He is also happy to accept scientific questions live over the internet, something many scientists would be far more reticent to do.

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1 Open Source Malaria: http://opensourcemalaria.org/


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“We must work in an environment where the best people work on the right problems.”
The Malaria Box and Pathogen Box

The Malaria Box was launched in December 2011, in response to the need for scientists to have access to physical samples of molecules to initiate drug discovery programmes for malaria and neglected diseases.1,2,3

The Malaria Box contains 400 diverse molecules active against blood stage *P. falciparum* malaria, available free of charge on request. To date, more than 160 boxes have been despatched to 27 countries catalysing numerous drug discovery programmes.

Based on the success of the Malaria Box, MMV was awarded a grant from the Bill & Melinda Gates Foundation in 2013 for a follow-on project, the Pathogen Box.4 This new box will contain up to 400 molecules for distribution to scientists, but this time they will be active not just against malaria, but also against one of a range of neglected diseases.5

**Dr Thomas Spangenberg**

**MMV Research Scientist**, tells us how the Malaria Box is being used today and what’s in store for the Pathogen Box.

**Q** More than 160 Malaria Boxes have been distributed, how are they being used today?

Two thirds of the Malaria Boxes distributed are being used for malaria research and the other third on neglected diseases, such as sleeping sickness and Chagas disease. For malaria, the focus is on understanding the mechanisms of action of the molecules. While those working on other diseases are looking to test the molecules for activity against their organisms of choice.

**Q** Why do you believe it has been successful?

It is the first initiative of its kind, where a set of bioactive chemicals are given away for free to catalyse neglected disease drug discovery. More than that, the Malaria Box bridges the worlds of biology and chemistry to initiate new drug discovery programmes. The box breaks down the financial and technical barriers of accessing the most promising molecules.

**Q** How successful has the Malaria Box been so far?

There have been many exciting findings already and this is just the beginning. For example, researchers from the University of Vermont tested the Malaria Box compounds against *Cryptosporidium*, a parasite that causes diarrhoea and is from the same family as *Plasmodium*. They identified three active molecules, which led to a publication6 and funding to begin a medicinal chemistry programme. There are similar stories for *schistosomiasis*7 with the Swiss Tropical and Public Health Institute (TPH) and the London School of Hygiene & Tropical Medicine; and collaboration between the University of Antwerp, Swiss TPH and DNDi on human African trypanosomiasis.

**Q** What are the next steps for the Malaria Box and the Pathogen Box?

For the Malaria Box we are focused on gathering, mining and encouraging recipients to share their data and initiate new programmes. For the Pathogen Box we are in the process of determining which molecules to select. This is where we need input from the whole neglected diseases community, as MMV’s experience is of course focused on malaria. We aim to launch the Pathogen Box in 2015.

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1 The Malaria Box website: [www.mmv.org/malaria/](http://www.mmv.org/malaria/)
3 The Pathogen Box website: [www.pathogenbox.org](http://www.pathogenbox.org)
4 The Pathogen Box will contain compounds active against the following neglected diseases: ascarisis, Buruli ulcer, Chagas disease, cryptosporidiosis, hookworm, human African trypanosomiasis (HAT, sleeping sickness), leishmaniasis (kala-azar), lymphatic filariasis (elephantiasis), malaria, onchocerciasis (river blindness), schistosomiasis (bilharzia), trichuriasis and tuberculosis.
5 Basco K et al. “Identification of *Cryptosporidium* panum active chemical series by repurposing the Open Access Malaria Box.” Antimicrob Agents Chemother. Feb 24 (2014), [Epub ahead of print]
7 ChEMBL, Malaria Data website: [www.ebi.ac.uk/chembl/malaria/](http://www.ebi.ac.uk/chembl/malaria/)