To make malaria eradication a real possibility we need medicines that do more than cure patients. Clearly, it is critical to develop curative medicines that kill the parasite replicating in the blood, but we also need medicines that target the parasite at several other stages in its lifecycle.

To complement existing blood-stage assays (that identify curative molecules), MMV and partners have developed new high-throughput assays to screen for activity at other key lifecycle stages, notably liver stages (for chemoprotection capability) and gametocyte stages (for transmission-blocking capability). In addition, we have developed assays against *Plasmodium vivax* liver stages (for anti-relapse activity; pages 28–29). Active compounds that meet the Target Candidate Profiles (page 8) are then selected for further research.

In support of these endeavours, in 2016, MMV established new drug discovery collaborations with support from the Government of the Netherlands. In one example, MMV is collaborating with Dutch research partners including TropIQ Health Sciences, Radboud University Medical Centre and Pansynt on a new series of targeted compounds (pantothenamides) that are active against *P. falciparum*.

Since 1999, there has been a five-fold increase in the number of validated drug targets for malaria; from five targets in 1999 to 26 in 2016, 19 of which were discovered with MMV’s support. Furthermore, since 2010 MMV and partners have brought 17 drug candidates into preclinical development (13 are still active). Each of the active candidates has the potential to form part of a next-generation combination medicine to support malaria eradication. Meanwhile, given the high rate of attrition in drug development, sustaining a steady stream of new candidates from drug discovery remains a priority for the future.

Figure 2: Growth of the MMV portfolio since 2000
The novel PIP4K inhibitor, UCT943, was selected as a promising new preclinical drug candidate by MMV’s independent Expert Scientific Advisory Committee (ESAC) in 2016. The compound is being developed in partnership with scientists at the University of Cape Town (UCT)’s Drug Discovery and Development Centre, H3D, in South Africa, as a back-up¹ to MMV048 (page 18). From the same chemical family as MMV048, UCT943 has shown impressive potency and solubility in the preclinical setting. Importantly, it is active against both P. falciparum and P. vivax – across all stages of the parasite lifecycle – and has the potential to be used for chemoprotection and to block transmission of the parasite from person to person. Given the need for new compounds for treatment as well as prophylaxis, there may also be an opportunity to take both compounds forward.

Dr Tanya Paquet, project lead at UCT, tells us how the two compounds compare and talks about the next steps for UCT943.

As UCT943 and MMV048 are from the same family, how are they similar and how do they differ?

Both compounds inhibit the intracellular development of the malaria parasite at each stage of its lifecycle, by targeting the same key enzyme. However, small differences in the molecular structure of UCT943 increase its potency and significantly improve its solubility compared with MMV048. This has important implications for drug development, since a molecule with high solubility is less likely to require a complex formulation and can be administered at a lower dose without compromising its efficacy. Such potential benefits will be evaluated against the emerging data for MMV048.¹

What are the next steps in the development of UCT943?

The aim is to complete all preclinical safety and toxicity assessments by year end 2017. At that point, the team, led by the translational medicine team at MMV of which we are part, will review all the available data and decide whether to progress the compound to first-in-human studies.

What has it been like to work with MMV on this project?

Quite incredible. Before we started out on this journey, we were a group of university academics who knew very little about malaria or the drug discovery process. Our team back then consisted of only four chemists! Because of this, we had no choice but to outsource a lot of our work. Since partnering with MMV, however, we have grown into a team of around 50 interdisciplinary scientists, and most of our research is now done in-house. Throughout this process, we’ve had the opportunity to learn from experts in the field and work with inspiring and motivational project directors. Their support has been invaluable and the overall experience has been really beneficial for us. We hope the strong collaboration between UCT and MMV will continue to grow over the coming years."
In recent years, the CHMI model has become a very valuable tool for accelerating the evaluation of promising new drugs. In 2010, MMV and Prof. James McCarthy from QIMR Berghofer Medical Research Institute, Australia, assessed the safety and feasibility of using the CHMI model to test for blood-stage activity. In a tightly controlled environment, the level of parasitaemia is closely monitored in volunteers inoculated with a low number of drug-sensitive parasites who, around 7 days later, receive the drug candidate (Figure 3). In this way the model allows us to understand quickly whether a compound will be efficacious and guides dose selection for subsequent clinical studies. New drug candidates tested in this experimental model include artefenomel, KAE609, ferroquine, DSM265, ACT840,1 MMV048 and SJ733 (pages 12–18). In 2015, we began looking at how molecules work in combination, initially exploring how artefenomel and DSM265 act together. This type of study informs the selection of optimum partner drugs, the potential interactions between them and their dosages.

More recently, the model was adapted to explore compound activity against the sexual (gametocyte) stages of the malaria parasite to assess transmission-blocking activity (Figure 3). After completion of a pilot study with artefenomel in 2016, the model is being used to assess the transmission-blocking activity of new molecules coming through the pipeline from drug discovery.

Dr Katharine Collins explains how volunteer safety is assured and how the CHMI model has been adapted to explore transmission-blocking activity.

How do you ensure that volunteers are not at risk?

During all the studies, when volunteers are inoculated with parasites they are monitored very frequently via visits to the clinic or telephone follow-up and, if needed, treatment is promptly initiated. When the test drug is administered, the volunteers remain in the clinic for 72 hours so we can readily detect if the drug is not working and deliver a rescue therapy, if needed.

The only difference for evaluating transmission-blocking activity is that a small cup of mosquitoes feed on volunteers’ arms once or twice during the study. At the end of all our studies the participants receive an antimalarial to clear any remaining parasites and ensure they are completely free of malaria. The model has been shown to be very safe.*

How was the original blood-stage model adapted to look at the transmission-blocking potential of new molecules?

A transmission-blocking drug could either kill or sterilize the mature gametocytes (which are the form of the malaria parasite that are transmitted to mosquitoes), thereby preventing transmission; or it could kill the early forms of the gametocyte and prevent them from maturing and becoming transmissible.
The simplest way to assess transmission-blocking activity is to evaluate if a drug can kill the early-stage developing gametocytes at the same time as it clears the asexual parasites. In these studies, volunteers are inoculated with a low number of drug-sensitive parasites and when a certain threshold of parasitaemia is reached, they are treated with the experimental drug. We then closely monitor the clearance of the asexual parasites and the development of gametocytes, to determine if the drug can kill the early-stage gametocytes.

A second more complex method is currently under development to evaluate a drug’s action against the mature gametocytes. In this model, volunteers are inoculated with blood-stage parasites as before, but are then treated with a drug known to clear asexual parasites while allowing early-stage gametocytes to continue developing. When mature gametocytes are detected we administer the experimental drug and evaluate their clearance. We also feed mosquitoes with the test subject’s blood containing the gametocytes to see if the drug can prevent transmission.

What are the advantages of the CHMI model compared with other tools to assess transmission-blocking activity, such as laboratory assays?

These models will enable us to learn what actually happens in humans and help us determine an effective dose to assess in the field. The ability to tightly control the infection process and frequently monitor subjects also means that accurate determinations can be made from a relatively limited number of test subjects. The laboratory assays are extremely valuable for looking at the drug’s ability to kill or sterilize the gametocytes, or block transmission, and to select the most promising drug candidates. But we don’t know how well they predict what will actually occur in humans.

What has it been like to work with MMV on this project?

It’s been an extremely challenging project but MMV has understood this and has been incredibly supportive and enthusiastic in helping us succeed. I would say it’s MMV’s supportive approach that has actually made the project possible.

Working with scientists led by Prof. Peter Kremsner and Dr Benjamin Mordmüller at the Institute of Tropical Medicine at the University of Tübingen in Germany, the model is being used to explore compound activity against liver stages of the malaria parasite and chemopreventive/chemoprotective ability (Figure 4).

Volunteers receive a dose of the experimental drug before being inoculated with sporozoites – the infective form of the parasite transmitted from mosquito to man. The length of time between drug administration and parasite inoculation can vary to determine activity and also to predict the frequency of administration required for chemoprevention. Following the inoculation of sporozoites, parasitaemia is monitored from day 6 onwards and rescue medication is given as needed, or on day 28. The model is currently being used to explore which drugs could be used as part of a once-weekly chemoprotection regimen (page 8 for more information on chemoprotection).
Seeding drug discovery with open initiatives

While data is available on some compounds known to be active against malaria and neglected diseases, scientists lack access to the physical compounds to begin drug discovery research. While open access to publications and data sets has become increasingly common, providing free access to a small library of physical compounds with associated data and advice had never been attempted until MMV’s Malaria Box was launched in 2011. By freely providing 400 diverse molecules active against malaria, the project showed there was a real appetite from researchers for compounds to evaluate. Based on this success, MMV launched the Pathogen Box with 400 different compounds active against malaria or one of a range of neglected disease pathogens.

To support research on these two boxes of compounds, particularly in disease-endemic countries, MMV awarded seven challenge grants to researchers working on each of them. In 2016, all of the Pathogen Box grant recipients, together with four additional runners-up, were invited to attend a 2-day drug discovery workshop organized by MMV, the Cape-Town based H3D Drug Discovery Centre and the South African Medical Research Council at the International Conference on Pure and Applied Chemistry 2016 in Mauritius. Invites included Dr Fidelis Cho-Ngwa (facing page) who assisted with the compilation of the Pathogen Box compounds by helping to confirm their activity. Through these initiatives we aim to not only provide access to compounds but also create an open and collaborative forum for researchers.

Dr Benoît Laleu explains the progress of research spurred by these initiatives as well as the key learnings.

To date 180 Pathogen Boxes have been dispatched; how is research with the molecules progressing?

Excitingly, we saw results just one month after the box was launched. Prof. Robin Gasser at the University of Melbourne screened the compounds against parasitic worms and identified one compound in particular that was highly active. Thanks to funding from the Wellcome Trust and through the Australian Research Council Linkage Project initiative, the compound is undergoing further research.

To date, 23 groups have shared their findings with us and two articles have been published.1,2 We have also been able to link different groups working on the same diseases as well as those with complementary results. This helps them to learn from each other’s findings.

By providing not only compounds, but medicinal chemistry advice and further information on the compounds, we have been able to support several research groups to apply for independent funding. This is particularly exciting for those diseases that have been severely neglected such as eumycetoma – a fungal infection with only a 35% cure rate using antifungals and surgery.*

How are these initiatives helping to seed endemic-country research capacity?

Of the 180 Pathogen Boxes dispatched, 50 have gone to countries where malaria and/or other neglected diseases are endemic.

The challenge grants for the Malaria Box and Exploiting the Pathogen Box have really helped to build capacity. For example, Dr Fabrice Boyom in Cameroon received further funding from the British Society for Antimicrobial Chemotherapy to continue his research on toxoplasmosis, thanks to the quality of his findings researching the Malaria Box with the grant he received. This is also an example of how the next generation is getting trained and involved. Fabrice is now working with seven young researchers including PhD students.*

What key learnings have MMV made through these projects that could be applied to similar initiatives in the future?

Based on the demand for the two boxes, it is clear that researchers are interested in receiving active compounds for their work. Other key learnings are that:

→ It’s important to include non-commercially available compounds in the boxes, as they provide more novelty versus those commercially available (which have been previously researched).
→ Researchers often require additional copies and so we need a reasonable stock of compounds.
→ Most of the researchers that request the boxes are biologists and so require medicinal chemistry advice and input to be able to progress their research.
→ As we learnt early on with the Malaria Box, compounds can be repurposed against different diseases.

With these learnings in mind, our next initiative is the Pandemic Box, which will include antiviral and antibiotic compounds, again at no cost to researchers. The goal here is to provide chemical starting points for drug discovery for diseases like Zika and Ebola as well as to repurpose compounds for malaria and neglected diseases. This will be a joint project between MMV and the Drugs for Neglected Diseases initiative.*

1 Preston S et al. “Screening of the Pathogen Box identifies an approved pesticide with major anthelmintic activity against the barber’s pole worm.” Int J Parasitol Drugs Drug Res. 03:329-334 (2016).
Dr Fidelis Cho-Ngwa explains the challenges of his research and how the Pathogen Box and grant are helping to overcome them.

What is the main focus of your research?

“The focus of my research is to find a cure for onchocerciasis, also known as river blindness. River blindness is a neglected eye and skin disease prevalent in the tropics. It is caused by worms transmitted by flies that breed in fast-flowing streams and rivers and can lead to skin lesions and blindness, hence the name. The current treatment, ivermectin, kills only the juvenile worms and not the adults, which then keep reproducing and so patients must take medicines for up to 15 years to cover the lifespan of the adult worms.”

What was your initial reaction when you heard about the Pathogen Box initiative?

“We were really excited and eager to get involved and start to use the compounds in our assays and models. For diseases of the poor, like onchocerciasis, open and collaborative research has to be the way forward.”

How are you using the Pathogen Box compounds and challenge grant?

“We have screened all the compounds against the microfilariae of bovine-derived Onchocerca ochengi, the closest relative and best model of the medically important Onchocerca volvulus. We are using the grant for fieldwork to get the parasite from the field sites to the labs for the assays. It also covers the staff costs, local transport, disposables and reagents.”

What has your research revealed?

“We got a handful of hits that inhibit the viability of the juvenile worms at 100%, which can be moved forward. We are now working to compile the results and share them with MMV to get more of the compounds to screen against the adult worms.”

The biggest challenge we face right now is getting our hands on quality compounds for research.”
Three discovery teams led by Prof. Dennis Kyle, Prof. Elizabeth Winzeler and Dr Jetsumon Sattabongkot Prachumsri are to jointly receive the MMV Project of the Year 2016 award for their impressive progress in developing new assay platforms to test compounds for activity against the liver stages of malaria. These new assays are now making it possible to screen and identify novel compounds that could stop the relapse and protect against malaria.

The relapse of *Plasmodium vivax* malaria is the cause of a significant burden of disease – WHO estimates it causes around 8.5 million clinical infections every year. Yet only one anti-relapse medicine, primaquine, is currently available with a second, tafenoquine, in development. Both these medicines are associated with potentially severe side effects in a small percentage of patients (on average 8% of people in malaria-endemic countries), who have a deficiency of the enzyme glucose-6-phosphate dehydrogenase.

Basic research on *P. vivax* has historically lagged behind that for *Plasmodium falciparum* partly because *P. vivax* parasites are difficult to access and maintain in laboratory assays. Today, thanks to a global research strategy and these new assays, this is set to change.³

**Prof. Dennis Kyle, Prof. Elizabeth Winzeler and Dr Jetsumon Sattabongkot Prachumsri talk about their achievements, the unique features of the assays they have developed and their plans for the future.**

**What can your team’s assay tell us and what’s unique about it?**

**EW:** Our assay uses the *P. berghei* (rodent) malaria parasite – we measure its ability to infect human hepatocytes (liver cells) in the presence of test compounds. We’ve shown that activity in this assay can predict whether or not a compound has chemoprotective activity. As the assay screens for activity against the liver stage it provides a kind of ‘filter’ to help prioritize compounds to be tested for anti-relapse activity.

Speed and efficiency are really the key features we have been able to contribute. We now have a very robust and reproducible assay that allows us to screen literally hundreds of thousands of compounds at low cost. So we’re able to cast a very wide net.⁴

**JSP:** We have developed an *in vitro* culture system that enables us to see the impact of compounds on the small (hypnozoite) and large (schizont) liver forms of *P. vivax* (Figure 5). The unique element is that it uses a homogenous cell line that can be cultured in the lab, which means we are not limited by the supply of cells from donors. This cell line has minimal metabolic activity and so doesn’t degrade compounds, which makes it easier to identify those that are active.⁴

**DK:** We have developed a 384-well plate assay and can routinely do *in vitro* radical cure screens and assess the activity of compounds against the hypnozoite. One of the unique features of our assay is that we’ve enabled primary human hepatocytes to maintain their physiology for over 30 days, allowing us to do experiments over a longer period, when required, which is key when looking at mature hypnozoites. We are then able to infect these cells with the sporozoites (Figure 5) from mosquitoes and get higher infection development rates than we’ve ever seen, meaning we have a robust and reliable assay for screening campaigns.

**How many compounds have you been able to screen and what has the assay told us so far?**

**JSP:** We have tested 32 compounds while optimizing the 384-well plate format using two MMV compounds as a positive control for prophylactic mode. As our assay was the first to test compounds against *P. vivax* hypnozoites, it has helped us better understand how the ‘surrogate’ animal malaria assays that were being used before are predictive of the situation in human relapsing malaria. Once fully optimized, the 384-well plate assay will allow us to test up to 5,000 compounds this year in three concentrations, for their prophylactic and anti-relapse activity.
**Figure 5: Malaria liver-stage biology**

**EW:** We have screened over 750,000 compounds provided to us by MMV and an additional ~250,000 compounds from MMV partners. There are some promising prospects – I am personally interested in compounds that could be used to protect people from getting malaria. My vision is to try to find compounds that could be used as a ‘chemical vaccine’ or a ‘chemical bed net.’

**DK:** We have screened close to 1,500 compounds, mostly in the previous 4–5 months. The breakthrough came at the end of 2016 and since then we’ve already rescreened all of these compounds to reconfirm our findings. We’ve also conducted concentration-response assays to quantify the potency of the hits. The assay has allowed us for the first time to screen against true *P. vivax* mature hypnozoites and has produced lots of information on these forms of the parasite. We now plan to scale-up this assay to be able to screen up to 15,000 compounds in 2017.

**JSP:** Our team feels very honoured to be selected as MMV’s Project of the Year 2016. It indicates that MMV recognizes the difficulty of developing the assay and the great progress we have achieved in the past year.

**How did you feel when you found out your project had been selected for MMV’s Project of the Year 2016?**

**DK:** I was happy for our team because they put in a lot of work to get to this point. I feel quite strongly, with MMV, that it is critical for us to identify new anti-relapse drugs, and this assay is an important tool in that endeavour.

**EW:** We really appreciate the recognition. It makes everyone on the team feel like their work is that bit more worthwhile. It’s good for our team morale.

**JSP:** Our team feels very honoured to be selected as MMV’s Project of the Year for 2016. It indicates that MMV recognizes the difficulty of developing the assay and the great progress we have achieved in the past year.

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.