

New tools for resistance and elimination

Novel assays to identify anti-relapse compounds

ISSUE

Relapsing malaria contributes to a significant burden of disease (see p. 34) and is a considerable challenge to malaria eradication efforts. Next-generation medicines that are well tolerated in all malaria patients are needed to support these efforts.

ACTION

To identify new treatments for relapse prevention, several new assays to screen compounds against liver-stage *Plasmodium vivax* malaria have been developed by MMV's partners.

Only two anti-relapse medicines exist: primaquine, which has been available for more than 60 years, and tafenoquine, which is in development (pps. 34–35). Both medicines are associated with potentially severe haematological side effects in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. New treatments for relapse prevention without the G6PD liability are urgently needed. Identifying compounds active against the parasite in assays, however, has proved challenging, since these parasites are difficult to access and maintain in the laboratory. Owing to the clear, unmet medical and scientific needs, this area of research is a key focus for MMV and its partners. Today, the development and use of novel assays to identify next-generation anti-relapse compounds is ongoing at several sites.¹



University of Georgia

Dr Steve Maher
Assistant Research Scientist, Center for Tropical and Emerging Global Diseases, University of Georgia, USA, tells us about his work on a new high-throughput assay.

How did you develop the assay platform?

We have been developing this and other assay platforms for the past 8–9 years. Several reports in the literature describe how liver cells prefer to be contained in a 3D space. However, we have been able to keep the liver cells viable for over 30 days by confining them in a commercial multi-titre plate, allowing fast analysis with standard equipment. This simple invention now allows us to screen over 300 compounds at a time against the dormant form of the parasite.

What challenges did you face and how did you overcome them?

There is a perception in scientific research that complexity is needed to overcome challenging problems, such as how to maintain liver cells in culture. We spent our first few years developing a 3D culture model, but found such a model difficult to reproduce and share. By going back and re-analysing the root cause of difficulty in culturing liver cells, we found simple, systematic solutions, while maintaining model simplicity. We were then able to develop a highly detailed protocol that would allow for easy implementation of the culture in nearly every lab worldwide.

Another challenge was the collection of *P. vivax* parasites to test drugs against. These parasites only exist in malaria-endemic countries where pharmaceutical companies, together with all the infrastructure necessary to perform high throughput screening, are typically not present.

To source the parasites, our team made numerous trips to partner laboratories in endemic areas of Asia to collect blood donated by infected individuals. We are grateful to be working with fantastic collaborators, including Dr François Nosten from the Shoklo Malaria Research Unit in Thailand and Dr Benoit Witkowski from the Pasteur Institute in Cambodia.

What has been achieved through the platform in terms of screening?

The MMV portfolio contains compounds active against most stages and species of malaria parasites, but little was known about their anti-hypnozoite activity. This is the first platform capable of generating such data on a large scale. In the past 2 years, we have screened thousands of compounds and portfolio drugs, and have identified new compounds with activity against *P. vivax* hypnozoites. These hits represent the first anti-hypnozoite series discovered in over 70 years.

What are the next steps?

The next steps are to confirm that the activity of this series is specific to malaria parasites and will not adversely affect humans.

We would also like to dramatically expand our screening capacity and are looking to work with partners in other malaria-endemic countries such as India and Brazil.

How does working with MMV help to further innovation in this exciting area?

MMV's project directors have been extremely supportive, first by clearly laying out the goals of developing such a platform, then providing honest feedback as we developed and characterized the platform to meet those goals. I have really enjoyed working with the MMV team. They are objective and understanding as well as pragmatic when obstacles are encountered. ●

¹ University of Georgia (USA), Shoklo Malaria Research Unit (Thailand), Pasteur Institute, Cambodia, Mahidol Vivax Research Unit (Thailand) and National Centre for Biological Sciences (India).

New molecule to protect vulnerable and migratory populations

ISSUES

As more countries move towards elimination of malaria, natural immunity will decline and there will be an increasing need for new chemoprotective medicines to ensure populations remain malaria free. Such medicines would also help in the control of potential malaria epidemics, and could be used to protect vulnerable populations such as pregnant women and children.

ACTION

MMV is working with Janssen Pharmaceuticals to develop long-acting injectable chemoprotective medicines that could be administered monthly or even less frequently.

In 2016, MMV and Janssen Pharmaceuticals formed a new partnership to help develop better medicines to treat and protect vulnerable populations from malaria. The partnership focuses on the development of new medicines suitable for reduced dosing regimens, in particular, long-acting injectable formulations for chemoprotection that meet target product profile 2 (p.10).

The MMV/Janssen team has begun this work with the compound known as P218, originally discovered through an MMV partnership with BIOTEC (Thailand), Monash University (Australia) and the London School of Hygiene and Tropical Medicine (UK). P218 is currently being developed as a potential long-acting injectable chemoprotective medicine that would deliver a regimen with less frequent dosing.

What is interesting about P218?

Currently, there are few well-defined, clinically validated malaria drug targets against which we can direct our drug discovery efforts. The best known such target is *Plasmodium falciparum* dihydrofolate reductase (PfDHFR). The P218 molecule was designed based on three-dimensional structures of PfDHFR to hit this target and circumvent mutations that have led to resistance to pyrimethamine, a well-tolerated and previously effective drug for the treatment of malaria.

P218 also has activity against liver-stage parasites. The malaria parasite enters the liver before it multiplies in the blood, causing the symptoms of malaria. Killing the parasite at this liver stage can therefore prevent malaria infection and stop the progression of the disease before the symptoms emerge.

How is the development plan progressing?

The phase I study for P218 is now complete. We have also performed various modelling studies to investigate the type of profile needed for P218 as a long-acting injectable.

We are working with MMV to assess P218 in a sporozoite volunteer infection study (VIS) (p. 29). This study will assess P218's chemoprotective activity and will also inform the concentration needed for P218 to achieve chemoprotection. This is being done in parallel with the development of long-acting injectable formulations that need to meet cost, stability, release and drug-loading requirements for use in malaria-endemic regions.

What is great about this is that all the study data are published in journals that are freely accessible to everyone, so all scientists can use these data to further understand and refine their own protocols, advancing the science together.

What did Janssen and MMV each bring to the partnership? How has working with MMV added to this process?

The MMV team is a great group of people to work with. Within Janssen Global Public Health, we have interest and experience in infectious diseases, such as tuberculosis (TB), HIV and malaria, as well as in the development of long-acting injectables. Throughout our collaboration, we have seen that MMV is passionate about what it does and is dedicated and focused on delivering treatments to those most in need.

One of the strengths of MMV is its network of leading scientists in all aspects of antimalarial drug discovery and development. When a programme is being set up, MMV invites experts who have a tremendous amount of experience and understanding of the field to work together.

MMV also has a stringent drug selection process, augmented by its Expert Scientific Advisory Committee, adding further scrutiny and rigour.

Of the three big infectious diseases in the world – TB, HIV and malaria – malaria probably has the biggest chance of being eradicated. MMV has played a huge role in tackling the multiple issues and obstacles that must be overcome to achieve this. This has been an extremely productive relationship, we have learned a lot and really enjoy working with them. ●



Janssen
Global Public
Health

Dr Alfred Tonelli
Head of External
Innovation,
Janssen Global
Public Health R&D,
USA, talks about
chemoprotection and
the development plan
for P218.

New tools to identify optimal drug combinations

ISSUE

Developing a new medicine that can successfully treat patients and combat drug resistance ideally requires at least two drugs in combination, each with a different mechanism of action or different mechanism of resistance. At any one time, there are 14 compounds in MMV's translational portfolio, and the resulting number of new combinations to sort through and prioritize is potentially over 100. Selecting the optimal combination is a complex scientific challenge.

ACTION

MMV and partners have developed and implemented a series of platforms to gather data to feed into a tool that enables every compound pair to be compared in a similar manner. This allows unbiased prioritization of optimal drug combinations for further research. We call this the Combo tool.

Today, in antimalarial drug development, we are at a juncture that offers many challenges and opportunities. Owing to the threat of resistance, we are aligned with the World Health Organization's (WHO) recommendation to develop combination therapies to treat malaria, so that one compound in the combination can kill any remaining parasites if resistance to the other compound is generated. Today, we have 14 compounds in preclinical and early translational development. Given the number of possible combinations this provides, a significant amount of data must be sifted through in order to select the best. Ideal combinations should have matched pharmacokinetics (PK; duration of activity in the body), different molecular targets and different paths of resistance. They must not interact in a negative way, for example, by increasing each other's metabolism or showing additivity in safety signals, but ideally show additivity in their pharmacological activity. In addition, each time we take a combination forward, the owners of each molecule must consent.



MMV

Dr Nicole Andenmatten
Project Manager,
Translational Science,
MMV, explains
how new tools are
being employed to
determine which drug
combinations should
progress further.

How were drug combinations selected traditionally?

In the past, the first time a combination would be evaluated would be in a clinical setting once the efficacy of the individual compounds had been confirmed in patients. This is a very expensive and time-consuming process. It's okay if you only have one combination to consider, but once you have many, we need a way to prioritize them. We need to give our clinical colleagues two or three options that will work; not too many, and not too few.

What tools are being used to develop drug combinations at MMV?

Today, we have an *in silico* combination tool (see figure on adjacent page), which began as a manual tool to develop combinations. We fondly call it the Combo tool. It was originally developed by Dr Wesley Van Voorhis from the University of Washington when he came to MMV on sabbatical 3 years ago.

We gather extensive data on each compound to determine which to take into development. This tool helps us organize those data. But the tool is only as good as the data that we enter into it. Those data come from a range of different studies and analyses. Using SCID mouse studies and volunteer infection studies (VIS)¹ we look at the efficacy of individual compounds against the parasite that infects humans. As part of the standard drug development process we also evaluate the PK, safety, resistance potential and chemical characteristics of each compound.

Fed with all these data on individual compounds, the Combo tool can then generate a matrix of the compounds that form the best combinations. It's a little bit like an online dating platform that collects data on each compound and then sorts them by compatibility, and suggests the best possible match.

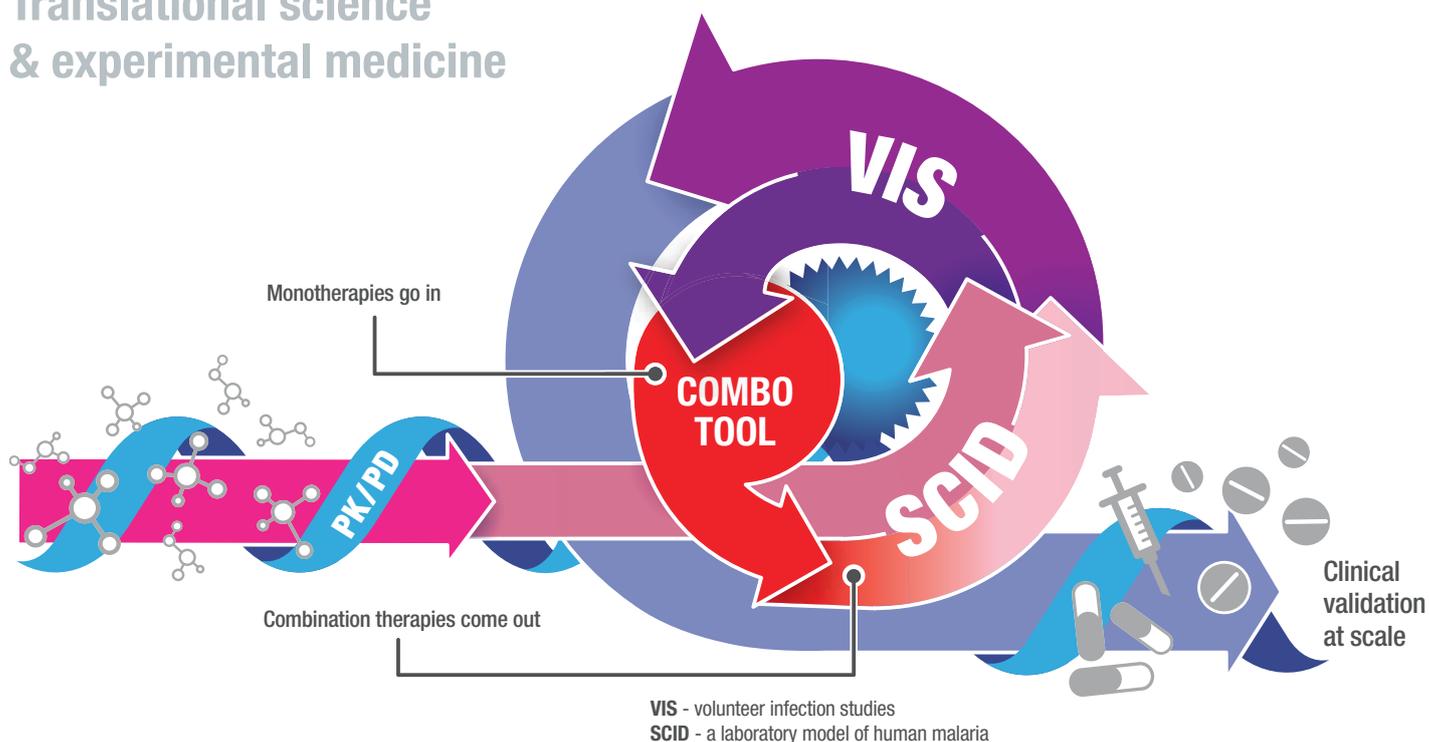
We then select a subgroup of the most promising, and perform SCID combination studies to look at the combined action of these compounds. Using these data and human pharmacodynamics (PD)/PK information, we can predict how the combination might work in humans. From this, a decision to move forward into combination VIS and clinical trials can be made.

What are the advantages of these tools?

By evaluating the combination more thoroughly before phase IIb in patients, we "de-risk" the research programme, as we have more information on how the compounds will behave together, and so stand a much higher chance of choosing an optimal and efficacious combination to progress. It also means that we may be able to conduct a clinical development plan with fewer trials in fewer patients.

Ultimately, by evaluating combination therapies earlier, we aim to bring down development timelines and reduce the costs of bringing a product to market.

Translational science & experimental medicine



What have these tools helped us achieve?

Using the Combo tool, we've been able to analyse the compounds post candidate selection in MMV's portfolio and rank them in terms of their compatibility. In collaboration with numerous partners, the SCID platform is helping us assess the efficacy of 16 of the most compatible combinations. We are in the midst of analysing the data.

Who have been the key players in this effort and what has it been like to work with them?

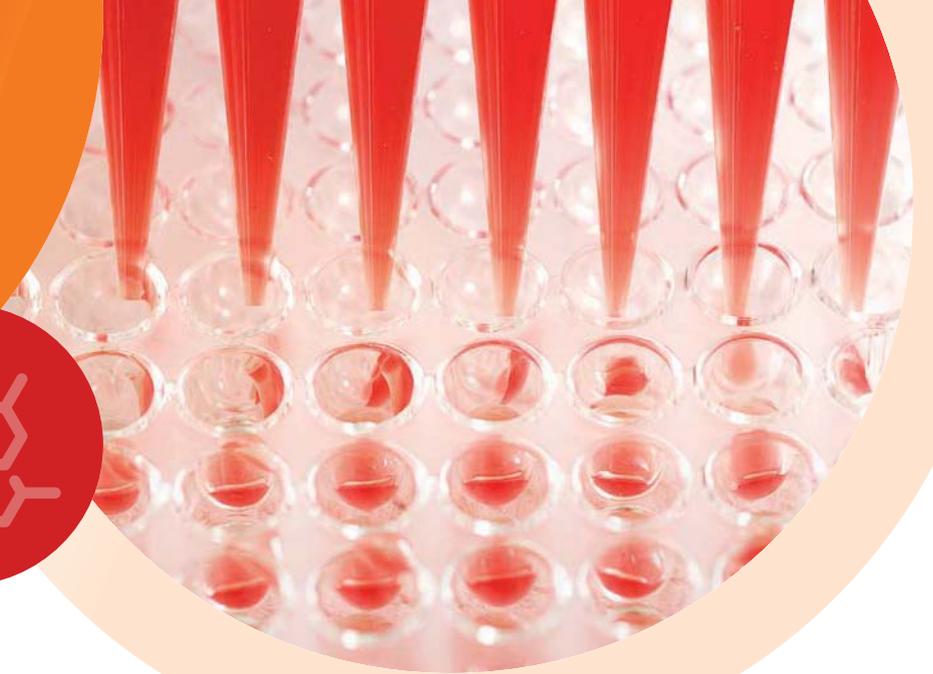
Externally, we have worked with Merck, Novartis and Sanofi on their respective compounds. They have been very happy and supportive of the work we have done with the Combo tool. For the SCID combo studies, we worked closely with two groups in Spain: The Art of Discovery, Bilbao and GSK's Diseases of the Developing World 'open lab' in Madrid. The great thing is that both groups follow the same protocols, so the data can be compared between different project teams.

Internally at MMV, it's also been a real cross-departmental effort. The discovery team, together with partners, developed the tools for assessing compounds as monotherapy. The translational medicine team then took these compounds to see how they work in combination – applying the expertise from the discovery team. The medical team provides support for the safety assessment of these different compounds. Important modelling work is

also being conducted by the pharmacometrics team, who use the data to predict efficacy in humans. Preclinical studies support the clinical development path and so there is a really close interaction with the clinical science team as well, to ensure the studies answer the right questions for clinical development. Subsequently, the IT team provided substantial support to automate the Combo tool. The work also involved an extensive effort from MMV's legal and business development teams, who ensured all appropriate contractual agreements were quickly put in place: some of these molecules are owned by MMV, but many of them have pharmaceutical companies as their guardians. It has been a stimulating collaboration between all partners, internal and external, to develop this preclinical combination approach and I look forward to continuing the exciting work. ●

¹ Volunteer infection studies (VIS) involve the inoculation of human volunteers with a low level of malaria parasites in a tightly controlled environment. Parasitaemia is closely monitored and the volunteers are administered a study drug 7 days later to assess its activity.

MMV Project of the Year 2017



Using biology to help guide the search for new molecules

The discovery team led by Dr Alain Pellet at Sanofi and Dr Didier Leroy at MMV receives MMV's 2017 Project of the Year award for the identification and development of SAR441121 (shortened to SAR121). The team applied a smart approach to screening guided by biology, which led to the accelerated discovery of this promising molecule.

SAR121, the first antimalarial candidate molecule to be delivered by the team, kills the malaria parasite very quickly, and so may also rapidly reduce malaria symptoms. It has been very difficult to generate parasites that are resistant to SAR121 in the laboratory, which, if replicated in a real-world setting, means the molecule could be one of our strongest weapons in combatting parasite resistance.

The current focus of the team is to show that the molecule is sufficiently well tolerated to be tested in humans. The goal will then be to test it initially in human volunteer infection studies (VIS) in 2018. In addition, the team is working to identify the best partner molecule for a combination therapy.

“At the peak of infection, there can easily be more than a trillion parasites in the body... The faster the parasites are killed, the sooner the patient will recover.”



Alain Pellet
Pharmaceutical
Chemistry Team
Leader, Sanofi

Sanofi

How does it feel to be a part of the team selected to receive the MMV Project of the Year 2017?

AP We feel honoured to receive this recognition, which extends beyond the immediate team. This project was also recognized by Sanofi as one of the best R&D projects of 2017. I personally feel very proud, as combatting malaria was a motivation for me when I joined the pharmaceutical industry.

DL This molecule is the result of a long-standing collaboration between Sanofi and MMV. I am extremely pleased to have contributed to its discovery, and now to see the molecule being moved forward to trials in humans. I'm delighted to have achieved this in partnership with Sanofi.



Didier Leroy
Senior Director,
Drug Discovery,
MMV

MMV

What is exciting about SAR121 as a future antimalarial?

AP Based on the data we have from cell biology and mouse models, SAR121 is expected to be well tolerated, both fast and long acting, and promises to be part of a simpler therapy, perhaps a single dose. It has also been shown to have a very low potential to induce resistance in the field.

DL In the early stages of drug discovery, we put ourselves in the shoes of the physicians treating patients and ask what characteristics they would like to see: for example, a fast-acting drug.

At the peak of malaria infection, there can easily be more than a trillion parasites in the body, and these can kill the patient by blocking the capillaries in the brain, the lungs or simply killing the red blood cells, leading to anaemia. The faster the parasites are killed, the sooner the patient will recover.

The ideal antimalarial also needs to resist the human metabolism so it can be present in the bloodstream for more than a week. It also needs to be potent and easily administered – large tablets are extremely difficult for young children to swallow. SAR121 remains at a concentration that can kill the parasite in the blood for a week or more, from a single dose of 100 mg, and this could be sufficient to be curative.

Another potential advantage is that our attempts to raise resistance to SAR121, by challenging parasites with suboptimal doses over a 2-year period, was almost "mission impossible". The resistance that eventually appeared was extremely limited. This suggests SAR121 should be highly unlikely to induce resistance in the field.

Together, these factors make SAR121 exceptional in the current landscape of drug candidates.

What process was followed to discover this promising compound?

DL Sanofi took a different approach from everyone else, which they called 'orthology'-based screening. Usually we start by screening a large library of up to one million molecules against the parasite; this is like looking for a needle in a haystack. Sanofi instead picked out compounds from their in-house collection that were from families already known to hit targets from other therapeutic areas (and therefore potentially more likely to be active against malaria).

A total of 66 common targets were identified between *Plasmodium* parasites and human diseases. For each target, a variety of different compounds were picked, some potent against the human target, and others that were similar, but less potent. This gave a library of around 1,000 compounds, which were then tested on the parasite. Usual screening procedures give a 'hit rate' for activity of less than 0.1%. Following this orthology-based screening process, the hit rate was 15%.

Using medicinal chemistry, these hits were modified to make new compounds. The best-performing compounds were more specific for the parasite and less specific for human cells, giving a lower likelihood of side effects. Molecules were then optimized to increase their half-life¹ *in vivo* and to make sure the final candidate was as potent as possible.

How has the MMV and Sanofi partnership helped the drug discovery efforts?

DL Having the right team is important. Over the last 5 years we have also been fortunate to have Sir Simon Campbell working with us as a mentor. He brings over 50 years' experience in drug development. The team had exceptional motivation and commitment, were hard working, and understood and appreciated each other. This is important, since in the 9-year journey there were always difficult lessons to be learned along the way. One of our best insights came from an unknown compound that the team decided to identify: attention to detail and a willingness to explore the unknown, and to persevere, are critical in drug discovery.

AP Throughout the project there was super team spirit and enthusiasm, and the team benefited greatly from MMV's and Sanofi's wide network of experts. ●

¹ Half-life: the period of time required for the concentration or amount of drug in the body to be reduced by one-half