KEYWORDS
Plasmodium falciparum and vivax; malaria chemotherapy; MMV; chemoprophylaxis; drug discovery and development; public-private partnerships; uncomplicated malaria

Summary Choosing appropriate chemoprophylaxis and stand-by treatment for travelers will remain a problem for the near future because of resistant Plasmodium falciparum. For those who live in the malaria endemic regions of the world it is a matter of life and death, but the future looks bright for control of malaria because of the development of organizations like MMV and their ability to forge suitable partnerships to tackle really big problems. This would not be possible if it were not for the MMV Stakeholders who provide the funding necessary for the discovery and development of new drugs. Malaria is a difficult problem but even if only a few of the potential drugs in the MMV pipeline become drugs, the control of malaria may again become possible.

Introduction
There has never been a time when new antimalarial drugs were needed more, with an estimated 500 million cases of malaria per year and nearly 2 million deaths mainly in sub-Saharan Africa with the rest in South East Asia and South America. It is likely that more people are infected with malaria today than at any other time in history. Control of malaria has been severely compromised by the development of malaria parasites resistant to nearly all antimalarial drugs used for prophylaxis and treatment, particularly in Plasmodium falciparum. There is a compelling and urgent need for new antimalarials to save lives and reduce morbidity. Unfortunately, while a major medical need exists for new antimalarials, most of those afflicted are too poor and the global market too small to stimulate commercially driven R&D.1,2 Prospects for commercial profitability are further diminished when the complicated and costly activities required to make drugs accessible to consumers in developing countries are added. For the traveler to malaria endemic countries the decision as to potential treatments and prophylaxis is complex because of resistance, safety, tolerability, and availability of antimalarial drugs in the traveler’s country.

Medicines for malaria mission
The Medicines for Malaria Venture (MMV) was established in November 1999 in response to the failure of the market system to provide the required incentives for malaria drug R&D. The MMV mission is to discover, develop, and deliver safe, effective and affordable treatments for malaria through public-private partnerships.3 While, the public sector has long recognized the need for drug R&D, their competence is mainly in basic research.
Modern drug R&D requires considerable technological, managerial and regulatory inputs that are found in the private sector. MMV, by forming ‘public–private partnerships’ to discover, develop and deliver new drugs, has done what neither the public nor the private sector could do on their own to produce drugs which meet the same standards as drugs produced for the European or US markets.

MMV has defined product profiles mainly focused on uncomplicated malaria (Table 1), but other indications including radical cure of Plasmodium vivax are included in the present portfolio.

**Table 1** MMV product profiles.

<table>
<thead>
<tr>
<th>For uncomplicated malaria</th>
<th>Further indications of interest</th>
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<tbody>
<tr>
<td>Efficacy against drug resistant strains</td>
<td>Intermittent treatment in pregnancy</td>
</tr>
<tr>
<td>Cure within three days</td>
<td>Intermittent treatment in early infancy)</td>
</tr>
<tr>
<td>Low propensity to generate rapid resistance</td>
<td>Treatments suitable for emergency situations, e.g. single dose treatment for refugee camps</td>
</tr>
<tr>
<td>Safe in small children (&lt; 6 mos.)</td>
<td>P. vivax malaria (including radical cure)</td>
</tr>
<tr>
<td>Safe in pregnancy</td>
<td>Severe malaria</td>
</tr>
<tr>
<td>Appropriate formulations and packaging</td>
<td>Prophylaxis</td>
</tr>
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<td>Low cost of goods</td>
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Medicine for malaria venture portfolio

The MMV portfolio has grown to 21 projects since its inception over 4 years ago (Fig. 1). It has reached its optimal size of 20–25 projects as defined by the MMV business model, which was calculated to deliver one new antimalarial every 5 years with the first by 2010. In drug discovery and development many more projects are started than finished. It may appear as if there are more drug projects than would be needed, but in fact more projects will fail than succeed. Since the start of MMV four projects have already failed. There are several reasons for the high failure rate which include a poor biological target, lack of activity against the parasite, lack of oral bioavailability, metabolism issues, toxicity, tolerability, and cost of goods. Knowing that some of our present projects will fail, MMV will have a new call for proposals every other year with the fourth call for proposals already initiated in February 2004. In this way we can add new projects to replace the ones which are not successful.

MMV has set a number of criteria for candidate drug selection in order to meet the specific need of populations in which the drug is intended (Table 1). For example, short treatment course not exceeding three days, stable formulation, favorable tolerability and safety are all-important to ensure acceptability of new antimalarial drugs. MMV has eight completely new therapeutic targets represented within the 11 discovery projects and ten development projects in the pipeline. The MMV partners on all the projects are listed in Table 2.

**Table 2** MMV project partners.

<table>
<thead>
<tr>
<th>Pharma</th>
<th>Academia</th>
<th>Institutes</th>
<th>Organizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayer</td>
<td>UNC&lt;sup&gt;a&lt;/sup&gt;, US</td>
<td>LSHTM&lt;sup&gt;c&lt;/sup&gt;, UK</td>
<td>WHO/TDR&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>HKUST&lt;sup&gt;e&lt;/sup&gt;, HK</td>
<td>STI&lt;sup&gt;f&lt;/sup&gt;, CH</td>
<td>EDCTP&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>GSK&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Hughes, US</td>
<td>WRAIR&lt;sup&gt;i&lt;/sup&gt;, US</td>
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<tr>
<td>Roche</td>
<td>Liverpool, UK</td>
<td>NSTDAl, TH</td>
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<td>Novartis</td>
<td>Mahidol, TH</td>
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<td>Ranbaxy, IN</td>
<td>Monash, AU</td>
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<td>Paratek, US</td>
<td>Nebraska, US</td>
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<td>Jacobs, US</td>
<td>UCSF&lt;sup&gt;k&lt;/sup&gt;, US</td>
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<tr>
<td>Holley, PRC</td>
<td>Yale, US</td>
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<tr>
<td>Nguyen, Guangzhou, PRC</td>
<td>Oxford, UK</td>
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MMV is also using the services of several Contract Research Organizations (CROs) and consultants in some of the portfolio projects.

<sup>a</sup> University of North Carolina.
<sup>b</sup> London School of Hygiene & Tropical Medicine.
<sup>c</sup> World Health Organization/WHO Special Programme for Research and Training in Tropical Diseases.
<sup>d</sup> Bristol-Myer Squibb.
<sup>e</sup> Hong Kong University of Science & Technology.
<sup>f</sup> Swiss Tropical Institute.
<sup>g</sup> The European and Developing Countries Clinical Trials Partnership.
<sup>h</sup> GlaxoSmithKline.
<sup>i</sup> Walter Reed Army Institute for Research.
<sup>j</sup> National Science & Technology Development Agency.
<sup>k</sup> University California, San Francisco.
The clinical development projects are gaining momentum and several projects are now in Phase I and II clinical trials. This portfolio represents the largest investment in new antimalarial drugs ever. Despite 'market failure' from a public health perspective, new product development for malaria drugs has never been more promising because of the significant investment by the MMV donors. In addition to the MMV portfolio projects, a few other organizations have ongoing antimalarial drug development programs.

Single drugs vs. combination drugs

Chloroquine was a very successful single drug, and because of its good activity, ease of use and cost of treatment, malaria was kept under control for a long time. Resistance to chloroquine was slow to develop and the malaria community was not prepared for how rapidly resistance would develop to agents used to replace Chloroquine such as sulphadoxine/pyrimethamine. The loss of new drugs used as single agents to treat malaria is a lesson already learned in treating TB and AIDS. New antimalarial drugs will no longer be single agents, but combinations of at least two drugs. In April 2001, the Roll Back Malaria published a Report of a World Health Organization (WHO) Technical Consultation that concluded that, from now on, any country changing antimalarial drug policy should change to combination therapy containing an artemisinin (ACT). As a result of this most of the projects in the MMV portfolio which are in development are combinations of Artemisinins with another agent. The near future will see additional combinations with variations of the artemisinin pharmacophore, but other combinations will be possible as new target directed agents are discovered. MMV is already exploring the possibilities of new drug combinations, which are not ACT. Another question is: will a single agent therapy ever be possible in the future. This is unlikely as long as malaria remains a significant public health problem which must be treated empirically.

New drugs to meet the International Conference on Harmonization standards

New drugs to treat malaria will gradually make their way to the private market, as well as to the public sector through purchase or donation or low cost programs, e.g. those supported through the global fund and other agencies. MMV, through its public-private partnerships will provide drugs which meet the standard of drugs sold in the developed countries and at an affordable price. A major obstacle to the success of these agents will be the production of non-GMP versions of theses drugs by manufacturers who use profit as their only motivation. This practice is possible because most of the artemisinin-based combinations, which will become available in the near future are not covered by patents. This allows some companies to produce drugs with no assurance of the stability or bioavailability of their product. These companies, and often the countries where these drugs are marketed, do not have any system to monitor the safety and quality of the products they sell (pharmacovigilance). The majority of these companies are based in countries where malaria exists, while others produce these drugs in the developed world for sale in Africa and other developing countries.

Even more worrisome are the counterfeit drugs which appear in the private markets of South East Asia and Africa. These fake drug peddlers are experts at making their products look exactly like the real products. Fake brand name watches and clothing hurt the market but fake drugs kill people. Counterfeit drugs and sub standard drugs are a problem for local populations as well as travelers to malaria endemic countries where they may have to purchase these drugs for acute treatment and/or prophylaxis.

Clinical development projects in the MMV portfolio

Coartem® pediatric

Malaria is one of the leading causes of death in the developing world, killing more than one million people every year. The majority of victims are in Africa, where a child dies every 30s from the disease. In sub-Saharan Africa, 71% of deaths occur in children below the age of five. By the age of one, the average child in high-transmission areas of Africa has contracted three life-threatening malaria infections. However, less than half of all African children under five who are infected with malaria are treated with an antimalarial medicine.

Coartem, consisting of artemether and lumefantrine, is the first and only fixed artemisinin-based combination therapy, or ACT, for uncomplicated falciparum malaria available worldwide, which has been developed to international standards. Because the malaria parasite has become resistant...
to most traditional treatments, the WHO now recommends that countries adopt ACT when there is strong evidence that existing conventional medicines are no longer working. This combination of artemether and lumefantrine is on the WHO’s Essential medicines list. In order to improve access to this lifesaving therapy, Novartis is making Coartem available at cost to the WHO, for distribution in developing countries.

A pediatric formulation would be easier for children to take and would improve compliance, ease and accuracy of administration, ensuring the drug’s efficacy. MMV is working with Novartis on an aggressive schedule to deliver a pediatric dosage form suitable for children as small as 5 kg.

**Chlorproguanil-dapsone (Lapdap™)-artesunate**

Chlorproguanil-dapsone-artesunate (CDA) is a fixed-ratio three-drug combination being developed to treat uncomplicated malaria. The first two components are antifolates: chlorproguanil, a type 2 antifolate, and dapsone, a type 1 antifolate. This combination of chlorproguanil-dapsone (also known as Lapdap™), has been shown to exhibit less selection for resistance than the widely used antifolate combination sulfadoxine pyrimethamine (SP). Lapdap™ has been studied extensively and regulatory approval was granted for its use in patients with uncomplicated *P. falciparum* malaria.11,12 The addition of artesunate as a third component is expected to delay the development of resistance to Lapdap™ and provide a safe and effective first-line malaria treatment for sub-Saharan Africa. The project is being led by The University of Liverpool in collaboration with GSK and WHO/TDR.

**DB289**

DB289 is the dimethoxine prodrug of 2,5-bis (4 aminophenyl) furan (DB75) a dicationic molecule based on the activity of pentamidine to treat African sleeping sickness. It is currently under development for the treatment of *Pneumocystis carinii* pneumonia and Africa trypanosomiasis. It has shown good tolerance and activity against blood stage trypanosomiasis. It has also been used for up to 3 weeks in patients with *P. carinii* pneumonia and was well tolerated. DB75 is active in vitro against chloroquine-resistant *P. falciparum* and in mice against *P. chabaudi* and *P. berghei*.

A Phase II proof of concept study to evaluate the safety and efficacy of DB289 in patients infected by either *P. vivax* or *P. falciparum* was performed in Bangkok.13 A total of nine patients with *P. vivax* and 23 with *P. falciparum* were treated with 100 mg BID for 5 days. DB289 was well tolerated. All patients cleared parasites by Day 7 and malaria symptoms resolved rapidly. A cure rate of >90% was observed for *P. falciparum* patients after a 28-day follow-up. A few patients experienced *P. vivax* relapse suggesting that DB289 may lack tissue schizontocidal activity. Although these results are encouraging it appears that the onset of action of DB289 was relatively slow. Immtech International and University of North Carolina are conducting studies to evaluate the pharmacokinetics of a once daily dosing regimen for 3 days and to select a partner drug for combination therapy. DB289 could bring a new pharmacophore in the field of malaria in the very near future.

**Artemisone**

Fast-acting artemisinin derivates are the components of the ACT strategy. These compounds work against Plasmodium strains that are otherwise multi-drug resistant. Artemisone provides a potentially significant improvement to this vital class of drugs. Artemisone is a semi-synthetic artemisinin derivate selected based on lack of neurotoxicity using in vitro testing. From this standpoint alone, the compound could overcome a worrying potential toxicological liability described for artemisinin compounds.14 Its higher bioavailability and enhanced antiparasitic activity potentially makes artemisone an improved second-generation drug of the artemisinin class. Bayer Pharma in Germany is driving this project and has completed the regulatory preclinical work required for entry into man in the Phase I study which started in December 2003. The Phase II dose ranging studies in uncomplicated malaria will target the vital question of whether this compound does better than the artemisinin derivates currently available, such as artesunate. In addition, the question as to which other antimalarial drugs will be suitable partners in a drug combination will be approached.

**Pyronaridine/artesunate**

Pyronaridine was first synthesized in China in 1970. It is an acridine-type Mannich base with a ring system similar to quinacrine (mepacrine) efficacious against chloroquine-resistant parasites. Pyronaridine has been used extensively as monotherapy to treat malaria in the Hunan and Yunan Provinces, China, where it has been found to be safe and effective in the treatment of uncomplicated malaria.15,16 The addition of artesunate will provide
faster onset of action and should delay the development of resistance. This combination will be assessed for safety and efficacy in the treatment of uncomplicated acute \textit{P. falciparum} (and possibly \textit{P. vivax}) malaria in adults and children in South–East Asia and Africa.

Pre-clinical studies required for an IND have been completed. The lack of significant safety liabilities from these pre-clinical studies, all conducted according to International Conference on Harmonization (ICH) guidelines, justify progress into clinical development. A comprehensive Phase I program will be initiated in Korea immediately upon obtaining clearance of the IND by the Korean FDA, which is expected during the second quarter of 2004.

Shin Poong Pharmaceuticals Ltd, in partnership with MMV and the WHO/TDR are confident that the simplification of pyronaridine synthesis and improved stability of the new formulation of artesunate will result in an affordable, stable and high quality product.

**Preclinical development projects in the MMV portfolio**

**Synthetic peroxide RBx-11160**

Many synthetic antimalarial compounds have been prepared but most suffer from low oral activity, a defect shared in part by the current artemisinin derivatives that are sourced from plants. Therefore, a need exists to develop new peroxide antimalarial agents, especially ones that can easily be synthesized, are inexpensive, have high oral activity, and are devoid of neurotoxicity. Although currently available non-synthetic artemisinins act rapidly, they have very short half-lives and must be administered over 5–7 days, leading to non-compliance and recrudescence.

The goal of this project is to develop OZ277/RBx-11160 as an orally active low-cost peroxide that is more potent than any of the currently available artemisinin derivatives and has a treatment regimen of 3 days or less to ensure good patient compliance.\textsuperscript{17} This compound is watersoluble and retains good oral activity in the \textit{P. berghei} mouse model. This suggests that intravenous (IV) formulation for severe malaria is possible. These compounds generate longer-lasting activity than current artemisinin derivatives, suggesting that treatment courses of 3 days or less are feasible. Exploratory pharmacokinetics and toxicology shows superiority over artemisinins.

Activity against \textit{P. vivax} is being investigated. Back-up compounds with likely chemoprophylaxis potentials are also being identified. By moving this project faster we can get a better and less expensive drug with a proven pharmacophore to treat malaria. A group of investigators led by the University Nebraska discovered the compound. Ranbaxy (an Indian pharmaceutical company) is MMV’s development partner on the project.

**Intravenous artesunate**

There are an estimated 600,000–1,000,000 severe malaria cases a year, of which at least 120,000 end in death. IV quinine is used to treat severe malaria, except in the United States, where quinidine is used because of its availability and similar efficacy. Quinine and quinidine are not ideal antimalarial drugs. They can cause fatal toxic effects on the cardiovascular system, cinchonism, and painful local reactions after intramuscular administration. Because of a short half-life, the drugs must be administered two to three times a day. Even in non-resistant areas and in spite of quinine treatment, mortality is 20% and more. These developments have led to concern that in many places there will soon be no adequate treatment for severe malaria.

An IV formulation of soluble artemisinins could represent an improvement in efficacy and safety for the treatment of severe malaria. They are effective against multi-drug-resistant \textit{P. falciparum} and clear parasites from the blood more rapidly than other antimalarial agents in severe malaria. The Walter Reed Army Institute of Research in Washington DC focused on the development of an IV artesunate. Since both activity and safety data were obtained from the same test species (rats and rhesus monkeys), a therapeutic index for both drugs was established. Extensive experiments comparing the neurotoxicity of these compounds have been completed and careful analysis of the data lead the team to conclude that IV artesunate should be developed to international (ICH) standards, which is key to maximizing the potential public health impact. MMV is supporting this development and has the ‘public health’ rights in endemic countries. All preclinical work is complete and the IND is planned for third Q 2004. An IV formulation has been developed and a Phase I clinical trial is planned for 2004.

**Artekin (dihydroartemisinin/piperaquine)**

Dihydroartemisinin-piperaquine is an inexpensive, safe, highly efficacious fixed-dose antimalarial
combination treatment that could make an important contribution to the control of multi-drug-resistant falciparum malaria. Large clinical trials in Southeast Asia have demonstrated this repeatedly. The product has been registered in China and a few other Southeast Asian countries by Holleykin, a Pharmaceutical Company in Guangzhou China. Much of the supporting data does not conform to current ICH guidelines and is not manufactured to Good Manufacturing Practices. This is a major limitation to more widespread use of this potentially leading antimalarial drug and its acceptance by international agencies interested in supplying malaria medicines to developing countries. This project, besides bringing forward a potentially useful drug, has the added advantage of working with the private sector in China to bring their manufacturing and clinical development programs up to ICH guidelines.

Isoquine

Isoquine is a drug discovered at the University of Liverpool. It is an amodiaquine-like compound that has been redesigned and synthesized to remove the structural cause of toxicity of its class while retaining full antimalarial activity. Although old, this class of drugs has been the most successful antimalarial to date. Widespread resistance to currently used compounds of this class has rendered them useless in vast areas where malaria is endemic. Isoquine, a second-generation aminouquinolone, retains the easy synthesis of amodiaquine from inexpensive precursors, and promises a new generation of affordable, well tolerated, and effective antimalarials. At the same time, it is devoid of any cross-resistance to its chemical cousin chloroquine (and amodiaquine). GlaxoSmithKline and the University of Liverpool are examining the candidate in an extensive candidate confirmation program that is providing data on metabolism, genotoxicity and in vivo activity.

Discovery lead optimization projects in the MMV portfolio

Plasmodium falciparum-protein farnesyltransferase inhibitors

Several lines of evidence support the idea that P. falciparum-protein farnesyltransferase (PF-PFT) is a validated target for the development of a novel class of antimalarial drugs. PFT inhibitors may be selectively toxic to malaria because the parasite lacks protein geranylgeranyltransferase type I; genes for the two subunits of PF-PFT have been identified and are expressed in the erythrocyte form of P. falciparum; PF-PFT can be prepared from infected RBC for high throughput screening assays; there is an excellent correlation between PF-PFT inhibition and inhibition of parasite growth; pharmaceutical companies have created large libraries of PFT inhibitors that the malaria program will benefit from; and inhibitors that are non-selective (inhibit both human and PF-PFT) are probably suitable for use due to the short treatment regime required for a malarial drug and because inhibitors of human PFT are well tolerated in the clinic.

This project exemplifies how the 'piggyback' approach can facilitate drug development for neglected diseases. The antimalarial program is piggybacking on the human-PFT inhibitors that BMS are studying for cancer chemotherapy. In vivo animal work on efficacy, PK, absorption, distribution, metabolism and excretion (ADME), and early toxicology, has started. Exploration of other scaffolds is also in progress. The University of Washington Seattle and Yale University are MMV partners on the project.

4(1H)-Pyridones

The 4(1H)-pyridones program is directed at finding selective inhibitors of plasmodial mitochondrial function. Atovaquone blocks electron transport by inhibiting cytochrome b, a critical element in the ubiquinol:cytochrome c oxidoreductase complex, also known as respiratory complex III, or bc1 complex. The 4(1H)-pyridone compound was tested on isolated mitochondria side by side with atovaquone. Both compounds displayed the same pattern of inhibition of individual redox reactions, providing strong circumstantial evidence that the site of action of 4(1H)-pyridones is in complex III (cytochrome b), however, 4(1H)-pyridones are especially active against atovaquone resistant P. falciparum strains. Therefore, their binding mode in complex III appears not to be identical to that of atovaquone. This project is part of the MMV/GSK mini-portfolio projects. The rapid success in lead optimization for this project resulted in a decision by GSK and MMV to assign additional resources to the program early last year. The project is poised to select a development candidate and to start the preclinical animal toxicology program. The potential of these compounds to be used for P. vivax and for prophylaxis will be assessed. The public health impact is a new pharmacophore to treat malaria.
New dicaticonic molecules

Diamidine or dicaticonic type molecules demonstrated excellent antimicrobial activity since their discovery in the 1930’s. The only drug to result from this research is Pentamidine. Pentamidine has been used to treat P. carinii pneumonia, antimony-resistant leishmaniasis, and early stage African Trypanosomiasis, but its use has been limited by toxicity. Recent work from Dr Tidwell’s group in North Carolina has demonstrated good activity against malaria. The dicaticonic molecules concentrate selectively in P. falciparum infected red blood cells, entering the parasite and killing the organism. The exact mechanism of action of these compounds is not well understood but they are believed to interact with hemoglobin by binding to ferrprotoporphyrin IX (FPiX) and interfering with heme degradation pathway.20 Other targets for their activity have also been proposed. At present DB289 one of the compounds is being developed in Europe and a Phase II proof of concept study to treat malaria. Its demonstrated activity in humans is a good proof of concept for the new dicaticonic molecules and new more active compounds can be designed as future drugs to treat malaria. The University of North Carolina and other institutions are partnering with MMV on this programme.

Enantioselective 8-aminoquinolones

While not the cause of significant mortality P. vivax remains a significant health problem in many regions of the world. Outside Africa, P. vivax accounts for 50% of all malaria cases. Primaquine has been the only effective therapy for radical cure of P. vivax for over 40 years. However, it is used for treating P. vivax malaria and has been tested in a Phase III study. Its demonstrated activity in humans is a good proof of concept for the new dicaticonic molecules and new more active compounds can be designed as future drugs to treat malaria. The University of North Carolina and other institutions are partnering with MMV on this programme.

Novel tetracycline derivatives

Tetracyclines have been used as antimalarials for over 30 years.21 At present, doxycycline is used for chemoprophylaxis in areas with drug resistant P. falciparum malaria and doxycycline or tetracycline is used for treatment of P. falciparum in combination with other drugs. As antimalarial agents the tetracyclines have been under utilized because of the concerns with phototoxicity, the potential to stain teeth when used in children, and their antibacterial activity.22 Chemical modification of the core tetracycline nucleus has recently become possible allowing the creation of improved tetracyclines specifically for use against malaria. In addition to having defined a preliminary structure activity relationship (SAR) the project has demonstrated that new tetracyclines can be identified that have: improved potency against drug resistant P. falciparum in vitro; improved efficacy in a rodent model of malaria; improved efficacy with a compound having minimal antibacterial activity and improved pharmacokinetics. These new tetracyclines created by Paratek Pharmaceuticals have the potential to be non-antibacterial, safer, and better tolerated than present compounds. A new tetracycline could become an additional weapon in the war against malaria.

Discovery lead identification projects in the MMV portfolio

Dihydrofolate reductase inhibitors

Drugs which inhibit the folate pathway have been widely used for treatment of malaria but their use is
declining due to the emergence of drug resistance. Resistance develops through mutations in the targeted enzyme dihydrofolate reductase (DHFR). However, the folate pathway remains a good target for chemotherapy because resistance mutations depend on the structures of antifolates and the enzyme has limitations in mutation capability. Improving technology coupled with availability of the crystal structure of both wild and mutant DHFR means that we can now design and synthesize compounds that inhibit mutant (resistant) enzymes. The project goal is to develop new DHFR inhibitors that are inexpensive, active against multi-drug resistant strains of malaria, and can cure within three days.

The project is at the lead identification phase to identify lead compounds that are active in mutant enzymes and whole cells, and examine SAR. Public health impact of a resultant drug is replacement for pyrimethamine that can be taken in combination for \textit{P. falciparum} malaria.

**Falcipain (cysteine protease) inhibitors**

A number of proteases participate in \textit{P. falciparum} hemoglobin degradation, including cysteine-class proteases called falcipains. \textit{P. falciparum} has three falcipains, named falcipain-1, -2, and -3. Inhibitors must block both falcipain-2 and falcipain-3 to kill the parasite. This is best demonstrated using cultured \textit{P. falciparum} parasites in an in vitro system. Blocking of hemoglobin degradation by this mechanism results in a visible change in the parasites followed by death of the malaria parasites. Thus the falcipains are exciting potential malaria drug targets. The project goal is to identify novel compound(s) that are orally active and meet the criteria of the MMV product profile.

A proof of concept study using the initial leads in a humanized mouse model was successful and the project is now progressing to identify simpler scaffold with good pharmacokinetics and oral activity. The University of California San Francisco and GSK are MMVs partners on this project.

**Fatty acid biosynthesis**

The absence of fatty acid biosynthesis II (FAS II) in humans and the identification of specific malaria parasite inhibitors validate this pathway as an excellent potential target for the development of new antimalarial drugs. Triclosan, a known inhibitor of bacterial enoyl ACP reductase (ENR), also inhibits the \textit{P. falciparum} enzyme Pf-ENR, a key component of the \textit{P. falciparum} FAS II system. This and other chemical leads against •ENR support targeting \textit{P. falciparum} FAS II for novel antimalarial drugs. The project’s objective is to identify compounds that are efficacious against drug resistant strains of parasite, and orally bioavailable with the potential for a 3-day course of treatment.

This is a lead identification project that is supported by structural biology and virtual screening. The public health impact is potential new pharmacophore to treat malaria. The Texas A&M University and other partners work with MMV on the project.

**Manzamines**

The Manzamine Alkaloids were isolated from marine sponges abundant in the coastal areas in the IndoPacific. They were found to have activity against \textit{P. falciparum}. A significant advancement was made when the team identified a sponge-associated microbe that is responsible for the biosynthesis of these alkaloids. This microbe has been characterized by 16S RNA analysis and has been determined as a new undescribed \textit{Micromonospora} sp. This discovery further strengthens the potential value of these compounds as drugs through the use of fermentation technology to produce the compounds. There are a number of chemical and biological characteristics for this drug class, which makes them ideal candidates for further development. These compounds are very stable arene-alkaloids and can be isolated in high yield (5%) from the sponges. The project is at a lead optimization phase and will soon transition into preclinical development. This project could add a new pharmacophore from a natural product to the treatment of malaria. The University of Mississippi and other partners work with MMV on this project.

**Discovery exploratory projects in the MMV portfolio**

**Pf-Glyceraldehyde-3-phosphate dehydrogenase (PfGAPDH)**

Glyceraldehyde-3-phosphate dehydrogenase is a glycolytic enzyme that catalyses the \textit{NAD}⁺ dependent reversible oxidative phosphorylation of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in \textit{P. falciparum}. In malaria parasites, the obligate dependence on glycolysis for ATP production combined with the possible involvement of PfGAPDH in non-glycolytic functions like biogenesis
of apical complex renders this conserved, single copy gene product an attractive target to explore for malaria drug development. This target benefited from high throughput screening at F. Hoffman-La Roche that resulted in 527 good hits.

If successful this exploratory project will progress to lead identification in the next year with additional partners. The public health impact of this project is a possible new pharmacophore to treat malaria. The Swiss Tropical institute and Roche are MMV partners on this project.

**Enoyl-ACP Reductase (FabI)**

This project is in the exploratory stage and is focused on the same ENR enzymes described earlier. It is part of the GSK/MMV mini-portfolio.

**P. falciparum** peptide deformylase inhibitors (PF-PDF)

Available data shows that inhibitors of bacterial PF-PDF also inhibit the growth of P. Falciparum in culture. This has encouraged further exploration of this target for development of antimalarial drug. PF-PDF is a single enzyme in P. Falciparum with an established X-ray structure. The gene sequence is available but a recombinant expression system is being established to facilitate target assay development, compound screening target validation and hits/leads generation. This is part of the GSK/MMV mini-portfolio.

**Acknowledgements**

We thank all MMV project partners, and MMV colleagues for support. We thank Marion Hutt for assistance with the document. Medicines for Malaria is a not for profit. Swiss foundation was supported by Bill and Melinda Gates Foundation, ExxonMobil Corporation, Global Forum for Health Research, International Federation of Pharmaceutical Manufacturers Associations, Netherlands Minister for Development Cooperation, Rockefeller Foundation, Swiss Agency for Development and Cooperation, United Kingdom Department for International Development, World Bank, World Health Organization, Roll Back Malaria, UNDP/WHO Special Programme for Research and Training in Tropical Diseases, Wellcome Trust.

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