Glossary

Actinomycetes are a group of Gram-positive bacteria, which can be found in a wide range of aquatic and terrestrial environments.

Biodiversity is the measure of the variety of species in a given habitat.

Cytotoxicity is the state of being toxic to living cells.

Endophytic fungi are eukaryotic organisms of the kingdom Fungi that live within the body or cells of another organism.

Myxobacteria are a group of predominantly soil-dwelling bacteria.

Optimization is the process by which the properties of a chemical compound are improved towards those required for a potential drug candidate.

Subfractions are fractions generated by chromatographic separation of microbial fermentation broths.

Proliferation assay allows the determination of the number of cells growing in the absence or presence of certain proliferation affecting agents.

Medicines for Malaria Venture (MMV) is a not-for-profit organization created to discover, develop and deliver effective and affordable antimalarial drugs through public-private partnerships. Our vision is a world in which these innovative medicines will cure and protect the millions at risk of malaria and help to ultimately eradicate this terrible disease.

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MMV in natural products

Harnessing the power of nature in malaria drug discovery

Historically, drug discovery and development has greatly benefited from sourcing compounds from nature. In fact, between 1981 and 2002, 61 percent of new chemical entities brought to market can be traced back to, or were inspired by, natural sources.[1] Malaria drug discovery is no exception. Artemisinin, the current antimalarial therapeutic mainstay, is actually an endoperoxide-containing natural product isolated from the leaves of the sweet wormwood plant (*Artemisia annua*), native to temperate regions. The 1990s saw a demise in research into natural products for drug discovery, due in part to the emergence of high throughput screening (HTS) and combinatorial chemistry. Today, however, the current demand for novel compounds to tackle emerging resistance to antimalarials, has stimulated new interest in their potential. Alongside our academic and pharmaceutical partners, MMV is working to tap into the power of nature to discover, develop and deliver a future generation of natural-product-derived antimalarials.

Rutgers University – the "Wilson" project: back to the future

The antimalarial research work of Rutgers University, NJ, USA, has taken inspiration from an unexpected source. Following publication of a newspaper article outlining Dr Ilya Raskin's research on natural products at the University, Mrs Christine Malanga Wilson, a 94-year-old retired Merck & Co. scientist, contacted Dr Raskin describing a project she had been involved in during WWII.

The project, initiated by Merck & Co. in 1942 in response to US government appeals, set out to discover new plant-based antimalarial compounds to replace the then dwindling quinine supplies. Mrs Wilson had been part of the team of top Merck scientists and New York Botanical Garden botanists, that succeeded in describing nearly 80 plant species (of more than 600 tested), mainly from the USA, active against avian malaria. Unfortunately, however, as the war ended and quinine supplies returned to normal, the research was abandoned and the knowledge archived. To the dismay of Mrs Wilson, one of the last remaining stewards of this information, few of these active plant species had been investigated further. Fortunately, Dr Raskin and colleagues understood the significance of the work, particularly in the context of emerging drug resistance to current antimalarials.

The current MMV-supported team, combining expertise from Rutgers University, North Carolina State University, NC, USA, and the University of Cape Town, South Africa, have made it their mission to utilize the data entrusted to them by Mrs Wilson. The team plans to fully characterize, using modern methods, the antimalarial activity of those species deemed active by Merck. To date, the team has been able to validate the activity of nearly a dozen species prioritized in Merck's studies and have begun to isolate and characterize their putative antimalarial components. The goal is to isolate and optimize antimalarial compounds from one or more of the antimalarial plants initially discovered by Merck's scientists more than 60 years ago.

Mrs Christine M Wilson (left) and Dr Albert Seeler working with malaria-infected chicks in April 1944.

1. Newman DJ, Cragg GM and Snader KM. (2003) Natural Products as Sources of New Drugs over the Period 1981–2002. *J. Nat. Prod.* 66 (7) 1022-1037.

Medicines for Malaria Venture

The University of South Florida – no sponge left unturned

At the University of South Florida, FL, USA, Prof. Bill Baker has assembled a consortium of public and private partners from across the globe to focus on the identification of antimalarial agents from a variety of natural sources. The aim is to identify lead compounds from niche microbial diversity – from ice-laden Antarctica (marine invertebrate-associated eubacteria) to temperate South-east Asia (endophytic fungi), adding in a number of filamentous fungi from all around the world.

The strength of this approach lies in the breadth and complexity of the biodiversity being sampled – thereby increasing the potential to discover novel chemical compounds. Further, the cultivatable nature of the sources, the sheer number of specimens to be screened and the rapid *in vitro* assays each contribute to enhancing the likelihood of success.

Over 10,000 cultures have been grown to date. Bioassay results have identified several active extracts with low mammalian cytotoxicity and compounds active at nanomolar levels that hold great promise as novel antimalarial drugs.

Harvard Medical School – targeting mega-biodiversity

Prof. Jon Clardy and scientists at Harvard Medical School, MA, USA, are focusing their antimalarial drug discovery work on the mega-biodiverse countries of Madagascar and Costa Rica. Interestingly, the biodiversity of these two countries has arisen as a result of very different circumstances. Costa Rica, being a very narrow strip of land connecting North and South America, has, over time, become populated by migrants from these two huge pools of diversity. Madagascar, on the other hand, is an island that has been isolated for millions of years and therefore contains a number of endemic species found nowhere else on earth.

The team at the National Biodiversity Institute of Costa Rica (INBio) has been built-up through 20 years of collaborative research with the Harvard team and other organizations. INBio focuses its strategy on rare endophytic fungi, which have proven to be a rich resource in the past, can be easily cultured, are icreasingly genetically accessible and do not necessarily have to be re-collected in the wild. In such mega-biodiverse areas as Costa Rica and Madagascar, source organisms are relatively rare; consequently, collection of such species could have an adverse effect on the species and on bystander species. By using fungi that grow inside plants, sample collection has a minimal impact on the existing ecosystem.

The INBio team works with the Madagascar International Centre for Research and Training in the Valorization of Biodiversity (ValBio) to provide assistance with culturing, extraction and fractionation. This South-South collaboration is not the only unusual feature of this project, the majority of the extraction process, from sample collection to new chemical entity takes place *in situ* in Costa Rica or Madagascar. The results from the first big screening campaign are now being analyzed.

From sample collection to new chemical entity

1. Sample collection
2. Culture and isolation of microorganisms
3. Fermentation and subfractionation
4. Purification
5. Dispensing and high-thoughput screening
6. Structure elucidation of the new chemical entity

BioFocus DPI – cutting-edge technology

BioFocus DPI, a company headquartered in the UK, offers comprehensive target-to-candidate drug discovery services to the pharmaceutical industry. The company's subsidiary in Switzerland has established a cutting-edge natural product platform, based on the fermentation of microbial strains, to circumvent previous issues regarding the identification of active compounds and resupply of the source material. In collaboration with MMV, the team at BioFocus DPI uses the platform to identify and purify natural products with potential antimalarial activities.

Dr Peter Eckard and his team work with their unique strain collection, which consists of over 45,000 actinomycetes and 8,000 fungi collected from diverse habitats across the globe. To maximize discovery of novel chemistry, the team focus on actinomycetes and fungi that are rare and typically not easy to cultivate.

The BioFocus team has developed a technique to prepare subfractions from microbial culture broths for HTS. In total 140,000 subfractions have been screened for inhibitory activity against *Plasmodium falciparum*. Subfractions with identified activity have also been screened for cytotoxicity. Further analysis of active subfractions is ongoing.

Eskitis Institute, Griffith University – untapped resources Down Under

Starting in late 2007, Prof. Ron Quinn, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Australia, and his team have worked closely with their Australia-based project partners, including the Queensland Institute for Medical Research, the Centre for Drug Candidate Optimisation at Monash University, and the Australian Army Malaria Institute, in order to identify novel chemical compounds to target the malaria parasite.

With funding from MMV, Eskitis have screened their 'Nature Bank' – a unique resource of over 200,000 optimized natural product fractions, derived from a collection of over 40,000 samples of plants and marine invertebrates from Australia, China and Papua New Guinea. The diverse properties of the Nature Bank increase the probability of identifying a hit fraction, which is then processed to identify and characterize the bioactive compound(s) within that fraction.

Over 100 compounds have been isolated thus far and as the project moves forward, these will be further examined by the team for their potential as new drugs. This is an all-Australian effort that will fully characterize and profile the compounds in preparation for their development.

Novartis Natural Products Unit – collaboration is key

At Novartis, Dr Frank Petersen's Natural Products Unit (NPU) built – and continues to expand – a library of over 10,000 compounds extracted from a wide range of natural sources, including plants, myxobacteria and fungi as well as purchased fully synthetic compounds with structural features typically found in natural products.

To identify potential antimalarial drugs the NPU library was screened with a HTS *Plasmodium falciparum* proliferation assay under the leadership of Dr Esther Schmitt (NPU), in a highly collaborative effort including the Genomics Institute of the Novartis Research Foundation (GNF), the Swiss Tropical Institute (STI) and the Novartis Institute for Tropical Diseases (NITD).

From this screen, 17 hits were selected for further profiling and one series was eventually selected as a lead scaffold. Further optimization of this scaffold is ongoing at NITD and it is the project team's goal to select a preclinical candidate by late 2009/early 2010.

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