Summary

During the last two years, the goal of providing effective antimalarial therapy to people with *Plasmodium* infections throughout the endemic regions of the globe has suffered a serious setback - the emergence of parasites resistant to artemisinin on the Thai-Cambodian border, and near Mae Sod on the Thai-Myanmar border. In this section, we present a range of projects by members of the LSHTM Malaria Centre addressing the challenge posed by drug resistance, from improved implementation of combination therapy, to studies mapping, describing and seeking to elucidate markers for drug resistance, to work in the area of drug discovery – the search for and testing of new lead compounds as precursors of the antimalarial drugs of the coming decades. These projects are addressing the following questions:

- How can Artemisinin-based Combination Therapy (ACT) be effectively delivered to peripheral health systems, and its impact monitored?
- How can we assist country Malaria Control Programmes to maintain access to high quality, authentic drugs for malaria?
- How do the new combination malaria drugs now being heavily used across Africa interact with drugs used by HIV-infected individuals?
- Can emerging artemisinin resistance in Southeast Asia be contained through coordinated monitoring and control? How far has it spread?
- Does the emergence of artemisinin resistance in Southeast Asia threaten ACT efficacy in Africa?
- Do we have genetic markers of artemisinin resistance we can use for monitoring and surveillance?
- Can parasite genomics provide new approaches to these questions?
- Are preventive treatment-based interventions an important source of drug selection, exacerbating the spread of resistant parasites?
- Can our knowledge of important molecules such as protein kinases, essential for survival of the *Plasmodium* parasite, assist in finding new classes of therapeutic drugs?
- Do new candidate antimalarial compounds, tested on lab strains, also have demonstrated activity on *Plasmodium* parasites taken directly from malaria patients?

These projects thus begin with the task of ensuring effective delivery of combination anti-malarial drugs to those who need them, and then examine the potential threat to these drugs of developing resistance, in both Asia and Africa. Novel approaches to monitoring drug efficacy are described, and we track the spread of resistant parasites in different parts of the malaria-endemic world, in some places assisted by current digital mapping technologies. The impact of drug-based public health interventions, particularly Intermittent Preventive Treatment (IPT),

on developing parasite resistance is investigated in southern Tanzania, where IPT for malaria in infants was first trialled. Finally, we examine potential new therapeutic strategies using protein kinase inhibitors, and test new drugs and compounds for anti-parasite activity on *ex vivo* parasite isolates taken directly from our patients at the Hospital for Tropical Diseases in London.

In each of the studies described, Malaria Centre staff and students are working with a variety of partner institutions, including UK and endemic country government agencies, and European and endemic country academic institutions, hospitals and non-government organisations.

The ACT Consortium: a research consortium to optimize the delivery of effective anti-malarial treatment.

***LSHTM Investigators**: Evelyn Ansah, Daniel Chandramohan, Sian Clarke, Catherine Goodman, Harparkash Kaur, Toby Leslie, Seth Owusu-Agyei, Hugh Reyburn, Mark Rowland, Sarah Staedke, Jayne Webster, Virginia Wiseman, Clare Chandler, Bonnie Cundill, Kristian Hansen, Shunmay Yeung, Christopher Whitty & David Schellenberg.*

***External Investigators/Collaborators:** Salim Abdullah (Ifakara Health Research & Development Centre, Tanzania); Karen Barnes (University of Cape Town, South Africa); Anders Bjorkman (Karolinska Institutet, Sweden); Emma Davies, David Lalloo, Munir Pirmohamed & Steven Ward (Liverpool School of Tropical Medicine, UK); Facundo Fernandez (Georgia Institute of Technology, USA); Michael Green & Patrick Kachur (Centers for Disease Control & Prevention, USA); Martha Lemnge (National Institute for Medical Research, Tanzania); Pascal Magnussen & Lasse Vestergaard (University of Copenhagen, Denmark); Wilfred Mbacham (University of Yaounde, Cameroon); Anthony Mbonye & Richard Ndyomugyenyi (Ministry of Health, Uganda); Paul Newton (University of Oxford, UK), Obinna Onwujekwe (University of Nigeria, Nigeria), & Kamija Phiri (University of Malawi, Malawi).*

***Funding Body:** The Bill & Melinda Gates Foundation.*

The ACT Consortium (www.actconsortium.org) is an international research collaboration aiming to maximize the public health impact of Artemisinin-based Combination Therapy (ACT) through high-quality, policy-driven, multidisciplinary operational research. Active in 10 countries and with partners world-wide, we use a variety of study designs including complex evaluations to address questions about:

- How to improve access to good-quality ACT for those who most need them;
- How to target ACT to patients with malaria, through the deployment of Rapid Diagnostic Tests;
- Drug quality, by assessing the prevalence of sub-standard and counterfeit artemisinin drugs;
- The safety of ACT under operational conditions, especially in high risk groups and when used in combination with other drugs.

Answering these key questions will help give policy makers and programme managers the evidence they need to ensure effective malaria control programmes can be implemented.

A surveillance system and drug forensic network to monitor the quality and authenticity of artemisinin combination treatments in Africa.

***LSHTM Investigators**: Harparkash Kaur, Albert van Wyk, Naiela Malik, Caroline Lynch & Shunmay Yeung.*

***External Investigators/Collaborators:** Facundo Fernandez (Georgia Tech. School of Chemistry and Biochemistry, USA); Michael Green (Centers for Disease Control and Prevention, USA); Paul Newton (Centre for Clinical Vaccinology and Tropical Medicine, UK, Mahosot Hospital, Lao PDR & LSHTM, UK).*

***Funding Body:** The Bill & Melinda Gates Foundation though the ACT Consortium.*

The lack of reliable estimates of suspect antimalarial drugs – particularly that of Artemisinin-based Combination Therapy (ACT) in malaria-endemic countries poses an enormous threat to malaria patients. To contribute to the tackling of this problem, our project aims to provide robust estimates of sub-standard, counterfeit and degraded artemisinin containing drugs and to develop standardised methodologies for sampling. To assist in classifying whether the drugs are degraded due to environmental impact rather than manufacturing practices, we are carrying out the ageing of ACT in field and laboratory based studies.

We have used a range of sampling strategies to collect drugs from public and private healthcare providers in Rwanda, Cambodia, Ghana and Tanzania, with sampling in other locations currently underway.

All samples are logged onto a database, the packages scanned and tablets weighed and measured. Qualitative (mass spectrometry, near infrared and Raman spectroscopy) and quantitative (high performance liquid chromatography and high performance liquid chromatography-mass spectroscopy) content analyses are conducted.

Thus far around 5,000 drugs have been analysed. Preliminary content analyses indicate a number of samples fall below the internationally recommended thresholds for their stated Annual Parasitic Incidence (API) with variations found to occur both between and within batches of the same brand. Following cross verification between the three collaborating laboratories, the results will be shared with the national authorities and stored on the "Counterfeit Drug Forensic Network – CODFIN" database.

a) ACT placed in stability chamber at LSHTM

b) ACT placed in a malaria endemic country

Packets of ACT placed in the stability chamber and field clinic for up to four years.

Front and back of package of artesunate tablets.

Fake artesunate in Africa. A near death experience for an European tourist.

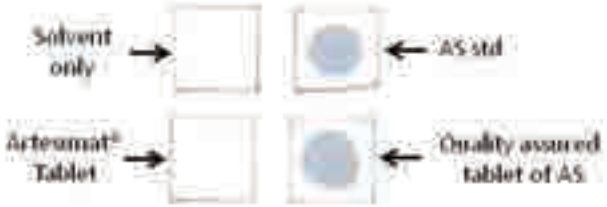
***LSHTM Investigators**: Harparkash Kaur, Albert van Wyk, Naiela Malik & David Mabey.*

***External Investigators/Collaborators:** Carlos Chaccour, Pozo Luis Del & Jose Luis Del (Clínica Universidad de Navarra, Spain).*

There is limited documented evidence directly linking malaria treatment failure to proven poor quality antimalarial medication. Herein we present a case of *Plasmodium falciparum* malaria treated with fake Artesunate purchased in West Africa.

In 2011, a Spanish citizen who visited Equatorial Guinea (EG) on a regular basis, attended a health centre in Malabo where she was diagnosed with slide-positive *falciparum* malaria and advised to buy artesunate and sulfadoxine-pyrimethamine (SP) from the local traders. She bought two boxes of "Artesunat®" 50 mg labeled as being manufactured by Mekophar, Vietnam, and took four tablets a day together with SP. After three days, the patient's symptoms persisted and on returning to Navarra (Spain) was admitted to hospital and treated with the hospital's supply of intravenous artesunate, leading to full recovery within three days.

The patient notified her doctor regarding her illness and subsequent treatment from EG. She supplied her physician with the remaining "Artesunat®". To confirm the cause of treatment failure, he forwarded the tablets to the LSHTM analytical facility. We tested the tablets for chemical content using our qualitative Rapid Field Test and then confirmed the result using high performance liquid chromatography. The tablets were not found to contain the stated (artesunate) active pharmaceutical ingredient.



Results of the field test showing the Prussian blue colour in the presence of the artemisinin reference standard (AS std) and the quality assured tablet of artesunate. The falsified drug (Artesunat®) taken by the patient did not give the expected Prussion blue colour of an authentic artesunate.

Treatment of asymptomatic malaria in HIV-positive and HIV-negative Nigerian adults using artemether-lumefantrine.

***LSHTM Investigators**: Ifeyinwa Chijioke-Nwauche, Mary Oguike, Albert van Wyck, Naiela Malik, Harparkash Kaur & Colin Sutherland.*

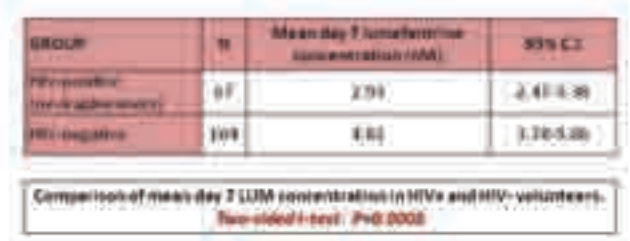
***External Investigators/Collaborators:** University of Port Harcourt, Nigeria.*

The co-infection of malaria and HIV has become a major challenge to public health in developing countries. The highly active anti-retroviral therapy currently used for the treatment of HIV patients has improved the prognosis of the disease, reducing mortality due to HIV infection. However the administration of these drugs poses a great challenge due to the potential drug-drug interactions as a result of multiplicity of drugs involved especially in co-morbid situations with malaria.

Using HPLC we assessed the bioavailability of lumefantrine based on the concentration in the capillary blood 7 days following treatment and also the impact of concomitant nevirapine treatment in subjects positive for *Plasmodium falciparum* infection.

In HIV-positive people, with and without PCR-positive parasitaemia, artemether-lumefantrine improved CD4+ counts by day 28. Day 7 peripheral blood levels of lumefantine were significantly higher in nevirapine-treated people; lumefantrine levels were also higher in parasite positive people (by PCR) than PCR negative, but this effect was weaker than the ARV effect.

Nevirapine, a non-nucleoside reverse transcriptase inhibitor, is known to stimulate live P450 enzymes and this activation may account for improved day 7 lumefantrine levels. We are investigating the impact of higher levels on parasitological outcomes.



HIV positive individuals receiving regular nevirapine ARV treatment were found to have significantly higher lumefantrine serum levels 7 days after receiving a full course of artemether-lumefantrine.

Tracking Resistance to Artemisinins Collaboration (TRAC)

***LSHTM Investigators**: Shunmay Yeung, Colin Sutherland, Phillipe Guyant & Richard Coker.*

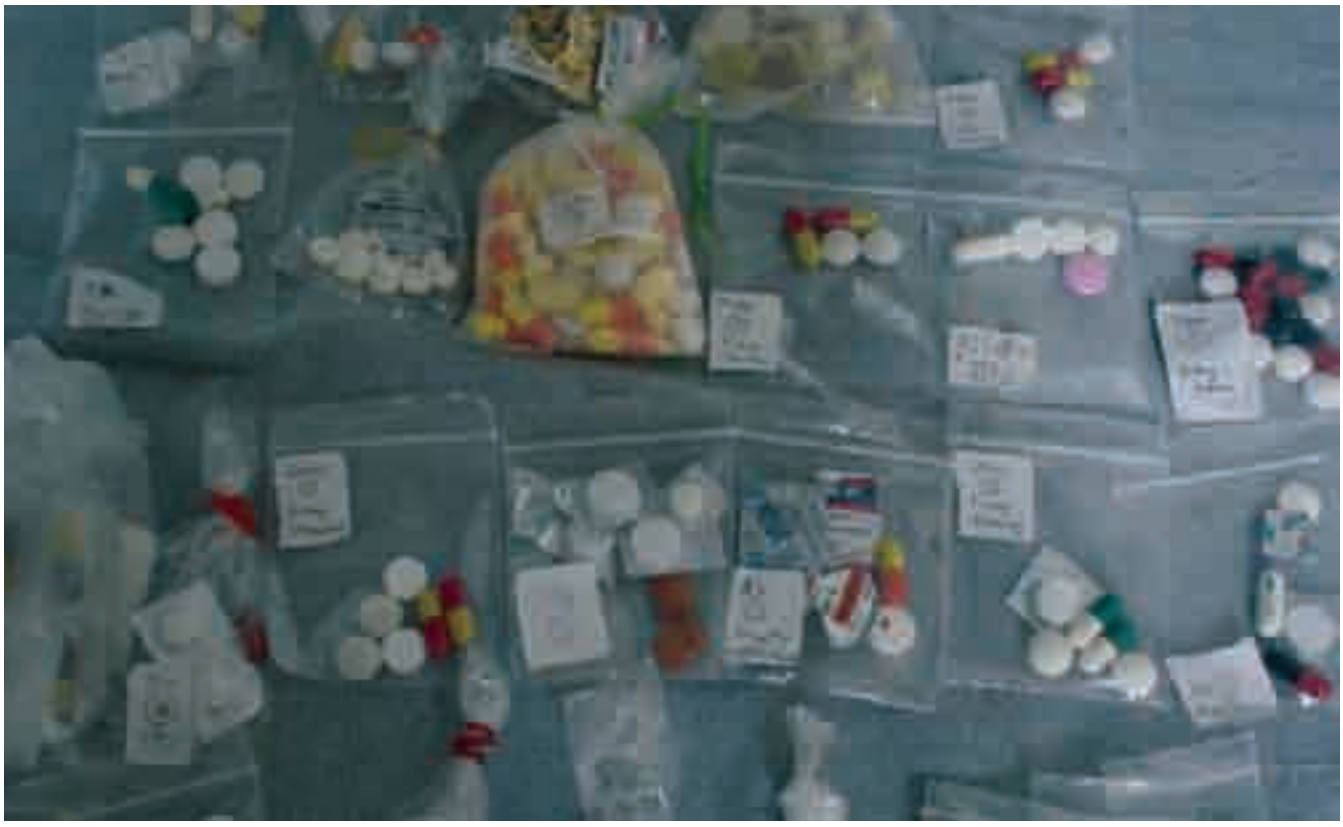
***External Investigators/Collaborators:** Rick Fairhurst (National Institutes of Health, USA); Pharath Lim & Yi Poravuth (Cambodian National Malaria Centre, Cambodia); Mayfong Mayxay (Wellcome Trust-Mahosot Hospital-Oxford Tropical Medicine, Laos); Francois Nosten (Shoklo-Malaria Research Unit, Thailand); Sasithon Pukrittanyakamee, (Mahidol University, Thailand); Abdul Faiz (Chittagong Medical College, Bangladesh); Olubenga Mokuolu (University of Ilorin, Nigeria); Neena Valecha (India); Steffan Boramman (KEMRI- Wellcome Trust Research programme Research Collaboration, Kenya); Ye Htut (Department of Medical Research, Myanmar); Tran Tinh Hien (Oxford University Clinical Research Unit, Vietnam).*

***Funding Body:** UK Department for International Development.*

The Tracking Resistance to Artemisinin Collaboration is a multi-country multi-disciplinary collaboration, led by the Mahidol-Oxford Research Unit, that was formed to inform artemisinin resistance containment efforts in response to a call for proposals by the UK Government Department for International Development. The initiative is funded for three years and has three main components:

- Clinical. A multicentre clinical trial at 15 sites in 9 countries, predominantly in Asia, to detect evidence of spread of artemisinin resistance. This work is coordinated by the Mahidol Oxford Tropical Medicine Research Unit in Bangkok with the support of WWARN and involves molecular and pharmacokinetic studies.
- Understanding demand factors. This component ,led by LSHTM, focuses on social and economic research including the demand for, use of and quality of drugs, by at-risk populations and the implications for the development and control of drug resistance using mixed methods. It is closely linked to the clinical studies in Cambodia, Thailand, Laos, Bangladesh and Nigeria, with additional operational research being conducted in Cambodia. Vector control. Researchers from the Liverpool School of Tropical Medicine plan to produce and evaluate innovative vector control strategies developed specifically to suit the Cambodian context.

The Global Malaria Programme of the World Health Organisation is another key partner and will review results generated by the 3 research arms.



Drug cocktails.

Measuring parasite clearance after ACT treatment using a newly developed qPCR method.

***LSHTM Investigators**: Khalid Beshir & Colin Sutherland.*

***External Investigators/Collaborators:** WANECAM group (West African Network for Clinical trials of AntiMalarial drugs): Malaria Research and Training Center; Université de Bamako, Mali; Centre National de Recherche et de Formation sur le Paludisme, Burkina Faso; Institut de Recherche en Science de la Santé, Burkina Fas; Centre National de formation et de Recherche en Santé Rurale, Guinée; Medical Research Council, The Gambia; de Recherche sur la Malaria, Université Claude Bernard, France; The malaria research unit at the Karolinska University Hospital, Sweden; Molecular-epidemiology of malaria group, University of Heidelberg School of Medicine, Germany.*

***Funding Body:** The European and Developing Countries Partnership (EDCTP) through the WANECAM Consortium.*

Parasite clearance, a measure of artemisinin response phenotype, is usually estimated using microscopic examination of thin and thick blood-smears.

However, a frequent closely-spaced venous sampling is required in order to see a significant difference in clearance time using microscopy. This approach may not be suitable in Africa where the majority of trial participants are children. In addition, some treatment failures have been reported after ACT treatments, yet no differences in parasite clearance were reported between patients who had recrudescent infection and those who were treated successfully.

We have developed a rapid and sensitive qPCR assay to measure parasite clearance in the first three days after treatment. The validation of the new qPCR method on samples derived from the venous blood of imported malaria cases in the UK has recently been published. The assay has now been tested on DNA derived from filter-paper bloodspots taken from a 2009 clinical trial of AL and DHA-PIP in western Kenya. The method identified a greater range of parasite clearance kinetics among children than did microscopy, allowing the analysis of phenotype-phenotype, phenotype-genotype and drug effect relationships with more statistical power. The new qPCR method will be used in WANECAM clinical trials of four ACT.

Identification and validation of candidate gene alleles associated with ACT resistant phenotypes of Plasmodium falciparum: part of the MALACTRES Consortium.

***LSHTM Investigators**: Colin Sutherland, Rachel Hallett, Nahla Gadalla, Khalid Beshir, Brighid O'Neill & Teun Bousema.*

***External Investigators/Collaborators:** Henk Schallig & Petra Mens (Royal Tropical Institute, The Netherlands), Umberto D'Alessandro (The Prince Leopold Institute of Tropical Medicine, Belgium), Aart van Amerongen (Biomolecular Sensing and Diagnostics, Agrotechnology & Food Innovations, The Netherlands), Chris Danks & Paul Meakin (Forsite Diagnostics Ltd, UK), Ehise Enato (Tropical Diseases Research Group, Nigeria), Halidou Tinto (Centre Muraz, Burkina Faso) & Seif Shekalaghe (Kilimanjaro Christian Medical Centre, Tanzania).*

***Funding Body:** The European Union Seventh Research Framework Programme through the MALACTRES Consortium.*

MALACTRES is a consortium of researchers aiming to tackle multi-drug resistance in malaria under combination therapy. With partners in Europe and Africa, the overall objective is to assess specific genetic markers in *Plasmodium falciparum* for associations with artemisinin combination therapy (ACT) resistance and to develop innovative, rapid and simple diagnostics for malaria.

Since the last Malaria Centre Report we have:

- Made progress in establishing SNP detection tests for new candidate genes *pfubp1* and *pfap2-mu.*
- Produced good quality genotyping results from isolates with in vitro drug susceptibility data from Burkina Faso.
- Applied a novel molecular parasite clearance time assay to samples from a clinical trial in Kenya.
- Shown good evidence that slow parasite clearance by PCR is linked to post-treatment parasite recurrence and carriage of gametocytes.
- Generated evidence that *pfmdr1* selection occurs in both dihydroartemisinin-piperaquine and artemether-lumefantrine (AL) treatment groups by day 3 after treatment and thus is being selected by the artemisinin component.
- Validated the *pfmdr1 N86Y* SNP as a useful marker of parasite response to both artemisinin and lumefantrine.
- Expanded our large external network of collaborators including groups running cross-sectional surveys and clinical trials from which parasite material can be used for a detailed analysis of known and candidate drug resistance markers.

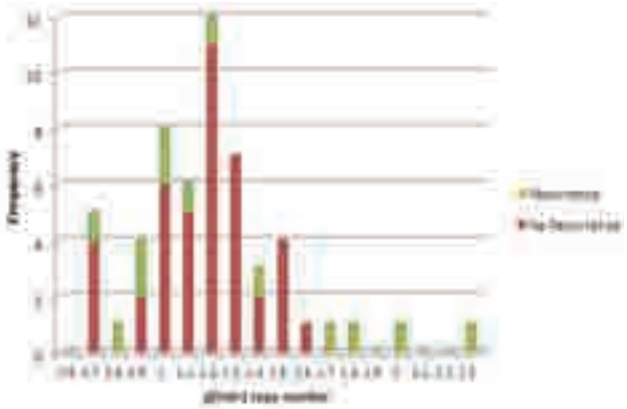
For more information see www.malactres.eu

Polymorphism in pfmdr1, pfatpase6 and pfubp1 in Plasmodium falciparum infections following treatment with artemether-lumefantrine in eastern Sudan.

***LSHTM Investigators**: Colin Sutherland, David Warhurst & Nahla Gadalla.*

***External Investigators/Collaborators:** Badria El-Sayed (Tropical Medicine Research Institute, Sudan); Ishag Adam (University of Khartoum, Sudan).*

***Funding Body:** WHO/TDR & IAEA.*



Frequency distribution of pfmdr1 copy number estimates. Estimates of pfmdr1 locus copy numbers obtained from 55 pretreatment isolates with complete follow-up data are grouped in bins of 0.1 copy units. The values shown represent the means of at least two independent experiments; each DNA sample in each experiment was run in duplicate. Red data represent pretreatment parasite isolates from patients without subsequent recurrent parasitemia. Green data represent pretreatment parasite isolates from patients with later recurrent parasitemia by microscopy and/or PCR. Isolates with copy number estimates of 1.8 and above were considered true duplications.

Molecular markers for surveillance of *Plasmodium falciparum* resistance to current artemisinins and their partner drugs are a public health priority. In Sudan, ACT have been recommended as first-line since 2004. A few reports of the clinical efficacy of artemether/lumefantrine (AL) from Sudan are available, but there is a lack of evaluation of molecular markers for AL in Sudan and elsewhere in Africa.

A 28-day antimalarial drug efficacy trial of artemether-lumefantrine was carried out in eastern Sudan in 2006. In this study 5 (n=100) patients failed treatment with recurrent infections detected by microscopy during the follow-up. In addition, 9 further individuals were found to harbour parasites by PCR at day 14. Polymorphisms in p*fmdr1, pfatpase6* and *pfubp1* were detected by DNA sequencing and *pfmdr1* copy number was estimated by qPCR. One individual carried parasites with a novel *pfmdr1* polymorphism (F1044L). *pfmdr1* copy number ranged from 0.73 to 2.33 (95% CI, 1.16 to 1.32) with an average copy number in pretreatment isolates of 1.24. *Pfmdr1* copy number estimates above 1.8 in at least two independent experiments were obtained for three pretreatment isolates. Interestingly, *pfmdr1*-amplified isolates in this study carried the 86Y allele instead of the N86 consistent with other African reports and in contrast to those seen in Southeast Asia. The NFD haplotype of *pfmdr1* was found to be selected by AL. There was genetic diversity in both *pfatpase6* and *pfubp1,* but a lack of association of either gene with treatment response.

The appearance of amplified *pfmdr1* warrants further investigation into the evolution and spread of this genotype in the study area despite the lack of mefloquine use.

Exploring the contribution of new genetic markers of drug resistance in human malaria parasites.

***LSHTM Investigators**: Gisela Henriques, Rachel Hallett, Colin Sutherland & Teun Bousema.*

***External Investigators/Collaborators:** Pedro Cravo (Universidade Federal de Goiás, Brazil); Paul Hunt (University of Edinburgh, UK); Halidou Tinto (Centre Muraz, Burkina Faso); Umberto D'Alessandro (Medical Research Council, The Gambia); Seif Shekalaghe (Kilimanjaro Christian Medical College, Tanzania).*

***Funding Body:** Portuguese Foundation for Science and Technology.*

Artemisinin derivatives form the cornerstone of anti-malarial drug therapy for the treatment of *Plasmodium falciparum* infection and recent evidence suggesting the possible development of resistance to this group of drugs is a significant public health concern. The main aim of this PhD project is to investigate the genetic basis of the malaria parasite's resistance to artemisinin derivatives. Using genome-wide strategies in the rodent malaria parasite *Plasmodium chabaudi* Pedro Cravo and Paul Hunt, our collaborating research partners, have identified a number of novel genetic markers of antimalarial drug resistance.

Now, using field isolates that were tested *in vitro* for their response to artemisinin derivatives as well as pre- and post- treatment samples from *in vivo* ACT trials, we are investigating the contribution that these new candidate genetic markers may be making to the development of artemisinin resistance in the human malaria parasite. Our preliminary results indicate that polymorphisms in a gene encoding the mu chain of the AP2 adaptor complex protein (*pfap2-mu*) are associated with varying degrees of *in vitro* and *in vivo* responses to artemisinin.

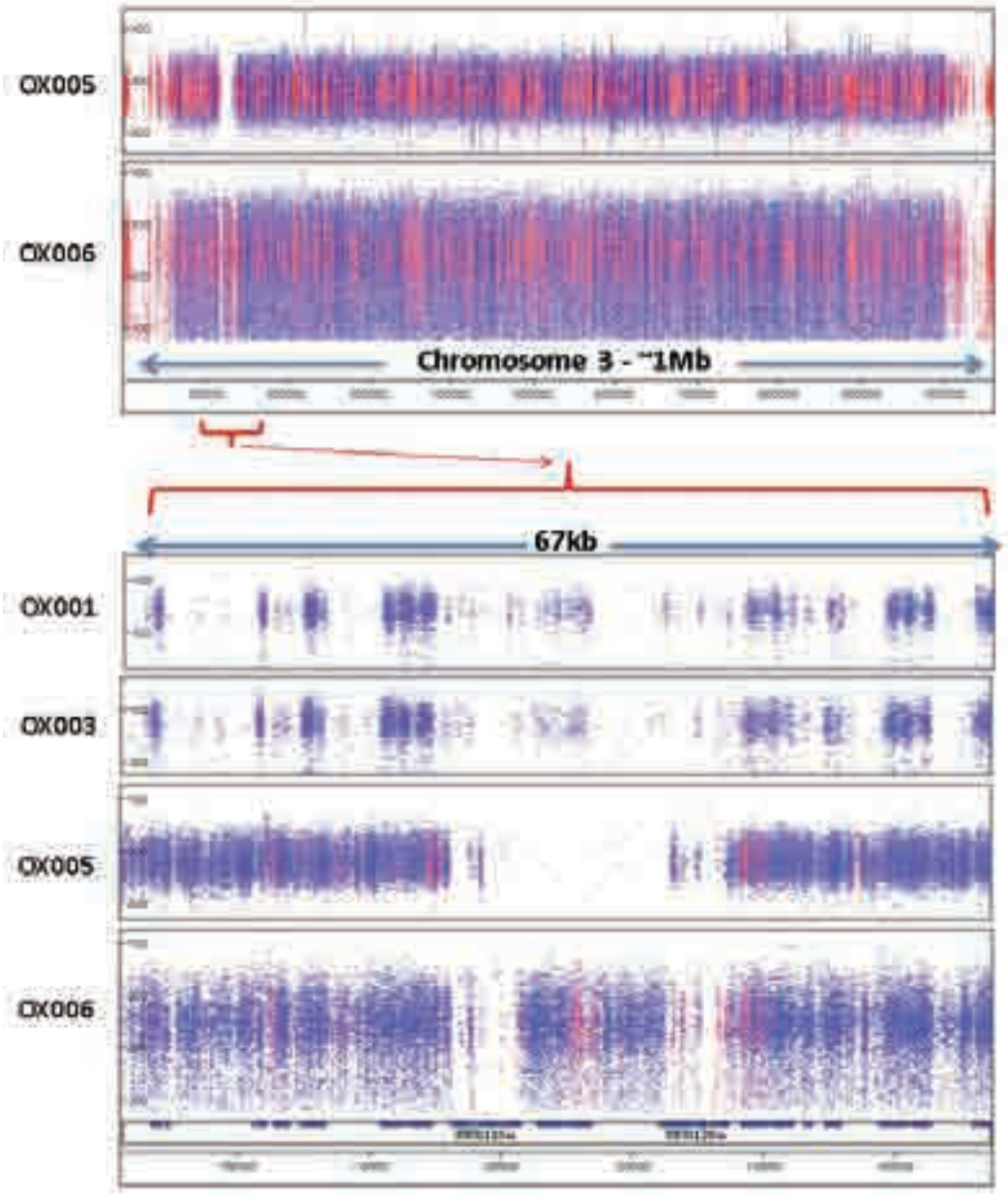
Analysis of copy number variation, drug-resistant genotypes, mitochondrial haplotypes and multi-clonality in Plasmodium falciparum by direct genome sequencing from patient peripheral blood.

***LSHTM Investigators**: Taane Clark & Colin Sutherland.*

***External Investigators/Collaborators:** Sanger Institute, UK.*

***Funding Body:** Wellcome Trust & UK Health Protection Agency.*

We are deploying next generation sequencing to derive genome data for non-propagated parasite isolates taken directly from patients treated for clinical *falciparum* malaria at the Hospital for Tropical Diseases. In a proof of principle study, all patients harboured at least 3 clones of *Plasmodium falciparum*, novel CNV were identified, mitochondrial polymorphism detected and robust analysis of full-length drug resistance-associated loci was successfully completed. We conclude that genome sequencing of peripheral blood *Plasmodium falciparum*, taken directly from malaria patients provides high quality data useful for studies of drug resistance, genomic structure, population genetics and clonal multiplicity. We are currently investigating differences between *Plasmodium falciparum* isolates taken from the same patient at different timepoints after initiation of antimalarial treatment.



Evidence of an apparent major deletion at the right end of chromosome 3 in Plasmodium falciparum, from a patient in HTD, London. Paired reads across the whole of chromosome 3 are presented in pile-up view for two isolates, OX005 and OX006 (upper panel). A detailed view of ~70kb around the clag3.2 and clag3.1 loci is also shown for 4 isolates (lower panel). The locus between PFC0110w and PFC0120w is a degenerate var gene lacking a full-length ORF in 3D7 and other parasite sequences in the available databases.

Drug-sensitive Plasmodium falciparum genotypes co-circulate with Plasmodium malariae, Plasmodium ovale and Plasmodium vivax in northern Angola.

***LSHTM Investigators**: Rachel Hallett & Colin Sutherland.*

***External Investigators/Collaborators:** Dina Gamboa, Cláudia Videira, Yuri Sebastião, Susana Vaz Nery (CISA – Health Research Center, Angola).*

***Funding Body:** Portuguese Institute for Development Assistance & Calouste Gulbenkian Foundation with the support of the Angolan Ministry of Health and the Bengo Provincial Government.*

The aims of this study were:

- To determine the prevalence of the different *Plasmodium* species in Northern Angola.
- To measure the prevalences of drug-resistance associated mutations in *Plasmodium falciparum* genes.

Angolan collaborators carried out a large cross sectional survey of women and children in a rural area of Bengo Province, north-western Angola, approximately 60 km north of the capital Luanda. Microscopy and the molecular method PCR were compared for their ability to detect 4 different *Plasmodium* species infecting humans. 541/3316 (16.3%) of the participants were found to carry parasites by PCR and all 4 species were identified, while in comparison, microscopy identified only 58.23% of the parasite positive people. *Plasmodium falciparum* parasites were present in 97% of the infections, and we examined mutations in 2 genes (*pfcrt* and *pfmdr1*) that are known to influence parasite sensitivity to the antimalarial drugs used in Angola. The haplotypes found varied significantly from those reported in a study in Luanda 4 years previously, perhaps reflecting the heterogeneous drug pressures that can occur across a relatively small geographical area. The use of molecular methods to monitor antimalarial drug resistance provides key information to policy makers as decisions regarding the use of artemisinin combination therapies are made.

Collection of samples during a cross-sectional survey in Bengo Province, north-western Angola.

Mapping the spread of drug resistance in African malaria.

***LSHTM Investigators**: Cally Roper & Inbarani Naidoo.*

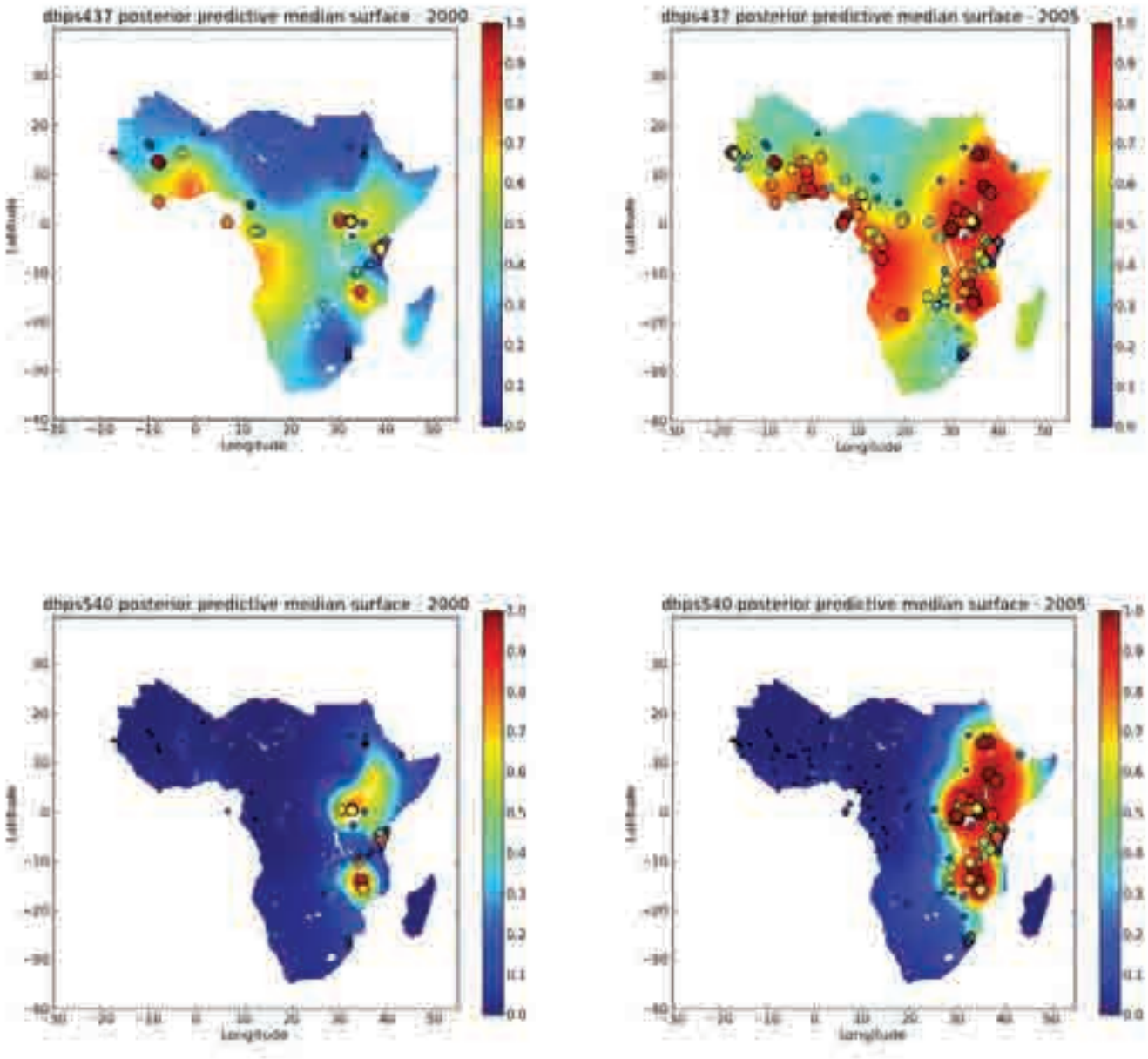
***External Investigators/Collaborators:** Jennifer Flegg & Philippe Guerin (WWARN and University of Oxford, UK); Simon Hay & Anand Patil (MAP and University of Oxford, UK).*

***Funding Body:** The Bill & Melinda Gates Foundation through WWARN.*

In this project we have developed a mathematical model of the prevalence of point mutations in the *dhfr* and *dhps* genes of *Plasmodium falciparum* that confer resistance to sulphadoxine and pyrimethamine. Resistance in *dhfr* and *dhps* genes emerged on just a few occasions, but then showed a remarkable capacity to disperse.

After collating, standardising and mapping all published surveillance data, we are using modelling to describe spatial and temporal patterns of resistance dispersal. Adopting a Bayesian model-based geostatistics approach, we converted spatio-temporal data to a continuous surface.

Models of resistance dispersal patterns in the past will help to inform policy on the management and containment of resistance in future.



Modelling the dispersal of resistance mutations at codons 437 and 540 of the dhps gene in 2000 and 2005.

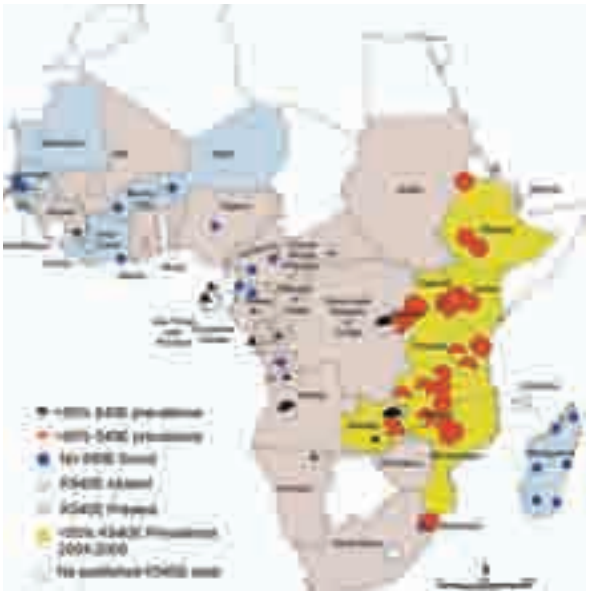
Drug resistance maps to guide intermittent preventive treatment of malaria in African infants.

***LSHTM Investigators**: Cally Roper & Inbarani Naidoo.*

***Funding Body:** The Bill & Melinda Gates foundation through the Gates Malaria Partnership and IPTi Consortium.*

We have created a geographical database of the prevalence of point mutations in the *dhfr* and *dhps* genes of *Plasmodium falciparum* that confer resistance to sulphadoxine and pyrimethamine. This was used to guide WHO policy recommendations on SP-IPTi.

The data is available as a publically available web-based resource (http://www.drugresistancemaps.org/).



Africa: The mapping is continent-wide.

In Focus: The evolution of malaria drug resistance.

Malaria Centre members have been involved in the development of a novel genetic approach for looking at the ancestry of drug resistance mutations and applied this to field populations of parasites in East Africa. Work has shown that pyrimethamine and sulphadoxine resistance genes were derived from only a few ancestral lineages resulting in a paradigm shift in our basic understanding of the evolution of drug resistance in Africa.

Analysis has been extended to Southeast Asian parasites in collaboration with a research groups there and in the USA and has shown that highly pyrimethamine resistant parasites in Africa were derived from an Asian mutation. This work has shown that international migration of drug resistant malaria infections were responsible for importation of drug resistance into Africa.

In highlighting the significance of international transportation of resistant parasites in the global spread of drug resistance this research has had a significant impact on policy and thinking on the future management of emerging drug resistance. There is recognition of the need for containment of emerging resistance in Asia to protect Artemisinin-based Combination Therapy in Africa. Containment is central to policy on management of artemisinin resistance in Southeast Asia today.

The analysis has also been extended to 20 countries across Africa and has shown that regional blocks of countries share common parasite genotypes. There is however a separation between east and west Africa as well as between the northern and southern populations in the east and west.

Development of an anti-malarial drug that targets the Plasmodium falciparum cGMP-dependent protein kinase.

***LSHTM Investigators**: David Baker, Paul Bowyer, Simon Croft & Lindsay Stewart.*

***External Investigators/Collaborators:** Katy Kettleborough & Andrew Merritt (MRC Technology, UK).*

***Funding Body:** Medical Research Council, UK.*

We are aiming to develop a new antimalarial drug that cures malaria by killing the asexual erythrocytic parasites but also attacks additional life-cycle stages. We have identified a *Plasmdoium falciparum* protein kinase (cGMP-dependent protein kinase, PfPKG) that is essential in multiple life-cycle stages. *PfPKG* is a novel target; a specific inhibitor could be used in combination with antimalarials that hit alternative targets. Selective inhibitors of *PKG* block *Plasmodium* blood-stage schizont, gamete and ookinete development. Recent data by others indicate an essential role for PKG in liver stages. Medicinal chemists at MRC Technology are synthesising new inhibitors which are being tested at LSHTM for their ability to block the growth of malaria parasites at various life cycle stages. Within the duration of the project we anticipate generating lead compounds with efficacy against the *Plasmodium berghei in vivo* model.

Drug sensitivity of wild Plasmodium isolates.

***LSHTM Investigators**: Sharan Atwal, Rebekah Burrow, Mary Oguike Colin Sutherland & Don van Schalkwyk.*

***External Investigators/Collaborators:** Ric Price (Menzies School for Health Research, Australia).*

***Funding Body:** Medicines for Malaria Venture.*

The goal of malaria eradication needs development of new antimalarial drugs that are demonstrably effective against parasites that are currently circulating in the second decade of the 21st century. Almost all laboratory-based drug development work utilises *in vitro* sensitivity testing against a stable of stock *Plasmodium falciparum* lines isolated in the seventh and eight decades of the 20th century.

New protocols developed in the laboratory of Ric Price have opened up the possibility that investigational compounds with demonstrated efficacy against *Plasmodium falciparum* laboratory line cultures can now be tested against *ex vivo* parasites of all human-infecting parasite species. Where sufficient high quality material is available, DNA will be extracted for genomic sequencing.

The proposed *ex vivo* approach provides two major advances on current testing protocols for investigational drugs:

- Broad spectrum activity against non-*falciparum* species will be evaluated.
- Testing against 21st century *Plasmodium falciparum* isolates, with their varied genetic background and a contemporary history of drug exposure, can be performed early in the drug development process.

A schizont and gametocyte of Plasmodium ovale curtisi after 48 hours of culture in vitro.

To date, *ex vivo* cultures have been attempted on two isolates of *Plasmodium ovale curtisi*, one of *Plasmodium malariae,* and on five *Plasmodium falciparum* isolates taken directly from malaria patients in London.